

# Production of Cells Without Deoxyribonucleic Acid During Thymidine Starvation of *lexA*<sup>-</sup> Cultures of *Escherichia coli* K-12

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When thymidine-requiring *lexA*<sup>-</sup> strains were starved for thymidine, the kinetics of survival were similar to those of a nearly isogenic *lexA*<sup>+</sup> strain. The size distribution of cells in the *lexA*<sup>-</sup> and *lexA*<sup>+</sup> cultures were, however, quite different. Whereas most of the cells in the starved *lexA*<sup>+</sup> cultures grew into long filamentous forms (longer than 4.0  $\mu\text{m}$ ), many of the *lexA*<sup>-</sup> cells were found to have a normal rod shape (4.0  $\mu\text{m}$  or shorter). It was shown that *lexA*<sup>-</sup> cells undergo more divisions during thymidine starvation than *lexA*<sup>+</sup> cells. Furthermore, using an autoradiographic method to analyze deoxyribonucleic acid (DNA) distribution in the starved cells, we demonstrated that cells without DNA are produced in both normal and starved *lexA*<sup>-</sup> cultures at a much higher frequency than in *lexA*<sup>+</sup> cultures. Some of these cells may be produced by breakdown of DNA, but we favor the hypothesis that they result from an abnormal cell division process. Since *lexA* mutations are dominant, we conclude that a diffusible product decreases the synthesis or activity of an inhibitor of cell division in *lexA*<sup>-</sup> strains when DNA synthesis is blocked by thymidine starvation.

*lexA*<sup>-</sup> cells are sensitive to ultraviolet (UV) light and a variety of agents that damage deoxyribonucleic acid (DNA), but are recombination proficient. The dominance of *lexA*<sup>-</sup> over *lexA*<sup>+</sup> indicates that the *LexA*<sup>-</sup> phenotype is due to the synthesis of a diffusible product (18). When UV-resistant derivatives of *lexA*<sup>-</sup> strains are selected, about 50% appear to be defective in cell division and form filaments at 42 C. These derivatives are thought to carry a mutation that affects the same product as the original *lexA* mutation. This product appears to be necessary for DNA repair and cell division (19).

Inouye has reported that cultures of thymidine (TdR)-requiring *recA*<sup>-</sup> strains divide and produce cells without DNA (*N*<sup>-</sup> cells) during TdR starvation (15). The production of *N*<sup>-</sup> cells by *recA*<sup>-</sup> strains has also been reported by Capaldo and Barbour in normal growing cultures (3, 4). Many of the mutant properties observed in *lexA*<sup>-</sup> strains are also observed in *recA*<sup>-</sup> mutants of *Escherichia coli* K-12. However, *recA*<sup>-</sup> cells are recombination deficient and are more sensitive to UV than *lexA*<sup>-</sup> cells (17). These studies have indicated that *recA*, like the *lexA* gene, influences cell division in *E. coli* K-12.

We have looked for further evidence of the

involvement of the *lexA* gene product in the control of cell division by examining cell division in cultures of TdR-requiring *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cells with or without TdR. These studies have suggested a regulatory control of cell division by the *lexA* gene.

## MATERIALS AND METHODS

**Medium.** The media employed were described previously (10, 17, 18). TdR was added to the medium at a concentration of 2  $\mu\text{g}/\text{ml}$  unless otherwise noted. Complete Davis minimal medium (CDM) consists of Davis minimal salts (10), 0.1  $\mu\text{g}$  of thiamine per ml, 0.2% glucose, and 2  $\mu\text{g}$  of TdR per ml.

**Bacterial strains.** The properties of the strains and their sources are given in Table 1. The *lex* locus at 80.9 min on the *E. coli* K-12 linkage map (23) is designated here *lexA*.

**Transduction procedures.** The transduction procedures described by Willetts et al. (24) were used.

**UV survivals.** The UV survival technique described by Willetts and Mount (25) and Mount et al. (18) was used.

**Measuring survival during thymidine starvation.** Cells to be starved for TdR were grown in CDM broth to log phase ( $2 \times 10^8$  cells/ml). The cells were centrifuged and the pellet was washed once at 3 C and resuspended to the original volume in Davis minimal salts. The culture was then diluted 1,000-fold into CDM broth lacking TdR. Samples (0.1 to 1.0 ml)

TABLE 1. *Properties of bacterial strains*

Strain no.	Genotype <sup>a</sup>	Source or reference
DM49	F <sup>-</sup> , <i>lexA3</i> , <i>thr-1</i> , <i>leu-6</i> , <i>proA2</i> , <i>thi-1</i> , <i>argE3</i> , <i>lacY1</i> , <i>galK2</i> , <i>ara-14</i> , <i>xyl-5</i> , <i>tsx-33</i> , <i>strA31</i> , <i>sup-37</i> , λ <sup>-</sup>	18
DM837	F <sup>-</sup> , <i>malB32</i> , <i>lacY1</i> or <i>Z4</i> , <i>thi-1</i> , <i>xyl-5</i> or <i>-7</i> , <i>galK2</i> , <i>tsx-6</i> , λ <sup>-</sup>	18
DM992 <sup>b</sup>	As DM837, also <i>thyA</i> (?) <sup>c</sup> , <i>drm</i> (?) <sup>c</sup>	This paper
DM952 <sup>d</sup>	As DM992, also <i>malB</i> <sup>+</sup>	This paper
DM953 <sup>d</sup>	As DM992, also <i>malB</i> <sup>+</sup> , <i>lexA3</i>	This paper

<sup>a</sup> The nomenclature is that of Demerec et al. (11) and Taylor and Trotter (23). All markers are wild type except those listed.

<sup>b</sup> A Thy<sup>-</sup> derivative of DM837 using trimethoprim selection (22).

<sup>c</sup> An allele number has not yet been assigned.

<sup>d</sup> A Mal<sup>+</sup> transductants of DM992 using P1 *vira* (24) growth on DM49.

were taken, inoculated to CDM plates (using Davis minimal overlays plus 3 μg of TdR per ml), and incubated at 37 C for about 24 h.

**Optical density measurements.** Cells were grown to log phase as stated before and then centrifuged, and the pellet was resuspended in CDM broth without TdR. The centrifugation and resuspension process was repeated twice and then the cells were starved for TdR. Optical density (OD) measurements were taken on a Klett-Summerson photoelectric colorimeter.

**Cell number counts.** Cultures were treated in the same manner as in the OD experiments. Samples (0.9 ml) were taken and 0.1 ml of 20% Formalin was added to each. When necessary, the samples were diluted into Davis minimal salts containing 2% Formalin. Portions were placed on a Petroff-Hausser counter and photographed. Four sets of pictures were taken of each sample; each set was taken at two focal planes in the sample in order to score all cells. Kodak Tri-X film was used for the photography. The film was developed and printed on paper (8 by 10 inch; about 20 by 24 cm) (#3) for cell counting.

**Autoradiographs.** The cells were grown and starved in the same way as in the OD experiments except that the DNA was labeled by adding [<sup>3</sup>H]TdR (specific activity 20 μCi/μg, New England Nuclear Corp.) to cultures prior to starvation (minimum of 10 generations). Samples for the autoradiographs (5, 6) were taken before and after 4 and 8 h of TdR starvation. These were diluted into an equal volume of 4% Formalin. Samples were filtered (45 μm pore size, Millipore Corp., 13 mm) with a syringe, washed with distilled water, resuspended in distilled water, spread on subbed slides, and dried. Subbed slides were prepared by cleaning microscope slides in 95% ethanol, drying them, dipping them in a solution of

0.5% gelatine and 0.01% chromium potassium sulfate, and drying them in a vertical position. Slides with the dried cultures were dipped in cold 5% trichloroacetic acid for 10 min and distilled water twice for 2 min. These slides were then dipped in emulsion (Ilford L-4 at 40 to 45 C) in the dark. Developing of the slides was done after 1 to 8 days in the dark by dipping them into D19 developer (5 min), distilled water (1 min), F24 fixer (240 g of sodium thiosulfate, 25 g of sodium bisulfite, and 10 g of sodium sulfite in 1,000 ml of distilled water, 5 min), and then five consecutive baths of distilled water (2 min each). The autoradiographs were analyzed on a phase contrast microscope. The cells were classified as N<sup>+</sup> if they had grains and N<sup>-</sup> if they did not (14). A normal cell was defined as a cell with a length of 4.0 μm or less and a filament was defined as a cell longer than 4.0 μm. This definition was based on our observation that 90% of the cells in *lexA*<sup>+</sup> cultures growing in CDM medium were found to have lengths in the range of ≈0.7 to 4.0 μm, the average length being ≈2.0 μm. A length of 4.0 μm for the normal cell was selected as the longest cell which, after completing the process of cell division, would yield progeny of lengths near the average.

**Measuring DNA degradation.** Preparation of the cultures was similar to the autoradiographs except [<sup>3</sup>H]TdR specific activity was 10 μCi/μg, and the cell concentration was 2 × 10<sup>6</sup>/ml. Samples (10 ml) were taken, and then 5 ml of 15% cold trichloroacetic acid was added to each sample. Samples were kept cold for 105 min, filtered (0.45 μm pore size, Millipore Corp., white 25 mm), dried at 60 C for 30 min, placed in scintillation fluid (7.55 g of 2,5-diphenyloxazole and 0.189 g of 1,4-bis-[2]-(5-phenyloxazoly)-benzene per 1,900 ml of toluene), and counted on a Nuclear-Chicago Mark I or Beckman LS-250 liquid scintillation counter. Sample counts were in the range of 10<sup>4</sup> to 10<sup>6</sup> counts/min with background at 200 counts/min or less. To obtain the data points for Fig. 8B, the points in Fig. 3 were used in combination with those in Fig. 8A. Hour points 1 through 8 (*lexA*<sup>+</sup>, Fig. 3) were averaged and used to normalize the *lexA*<sup>+</sup> curve in Fig. 8A to obtain the *lexA*<sup>+</sup> curve in Fig. 8B. The 2-h point (*lexA*<sup>-</sup>, Fig. 3) was used to normalize the 2-h point in Fig. 8A for Fig. 8B; the hour points 3 through 8 (*lexA*<sup>-</sup>, Fig. 3) were averaged to normalize the same *lexA*<sup>-</sup> points in Fig. 8A for construction of Fig. 8B.

## RESULTS

**Survival and the change in OD during thymidine starvation.** Survival curves of *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures are shown in Fig. 1. The strains (Table 1) carry the same *thyA* and *drm* mutations (20) and are isogenic except for the *malB-lexA* region of the *E. coli* K-12 genetic map (23). The results shown are the average of three experiments each of which gave similar results. (All experiments to be described have been repeated at least two times and show the same general trends, except when noted.) The kinetics of survival are similar for the two

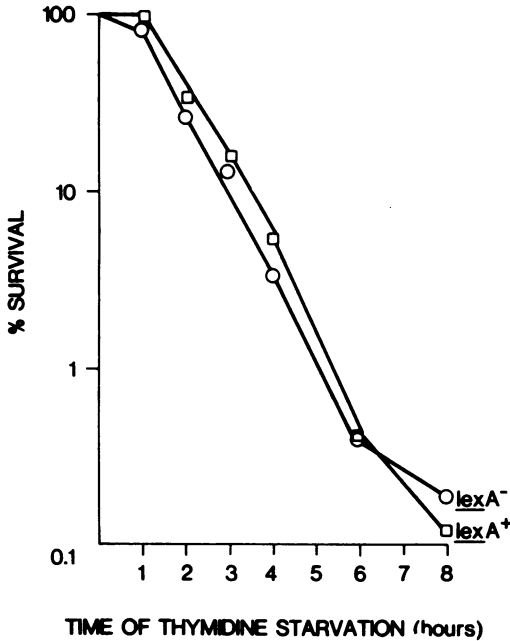


FIG. 1. Loss of viability of *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation.

strains, indicating that the *lexA* gene product does not appear to affect the repair of DNA lesions arising from TdR starvation. Similar observations were made by Anderson and Barbour (1) with *recA* cells, but not by Inouye (15) who found a different *recA*<sup>-</sup> strain to be more resistant to starvation of TdR. Similar kinetics of survival (9) were also observed in *exrA* mutants (thought to be identical to *lexA* mutants of *E. coli* K-12 [13] of *E. coli* B and a related strain *E. coli* B/r). However, since these strains differ by at least one mutation other than *exrA* (*sul*[16]) that affects cell division, the interpretation of this result is difficult.

The OD changes seen in Fig. 2 are slightly greater for *lexA*<sup>-</sup> cells than *lexA*<sup>+</sup> cells during TdR starvation. This infers that the increase in total mass during TdR deprivation is approximately the same and may be slightly greater in *lexA*<sup>-</sup> cultures.

**Petroff-Hausser cell count.** The total number of cells in TdR-starved *lexA*<sup>-</sup> cultures (Fig. 3) increases over an 8-h interval whereas the increase in *lexA*<sup>+</sup> cells is very slight. This indicates that there is much more cell division during TdR starvation in *lexA*<sup>-</sup> cultures than in *lexA*<sup>+</sup> cultures. In *lexA*<sup>+</sup> cultures the number of normal-sized cells (4.0 μm or less) decreases (Fig. 4), while there is an increase in filaments (cells that are longer than 4.0 μm) (Fig. 5). This result suggests that most of the short rods in

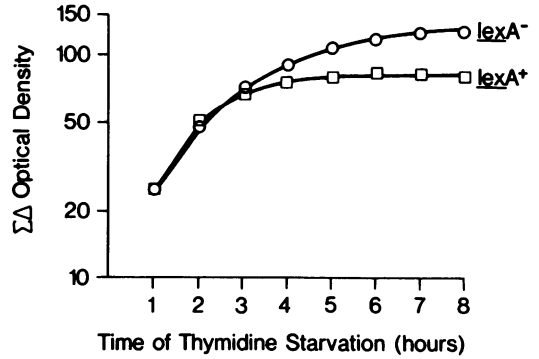


FIG. 2. Changes in optical density of *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation.

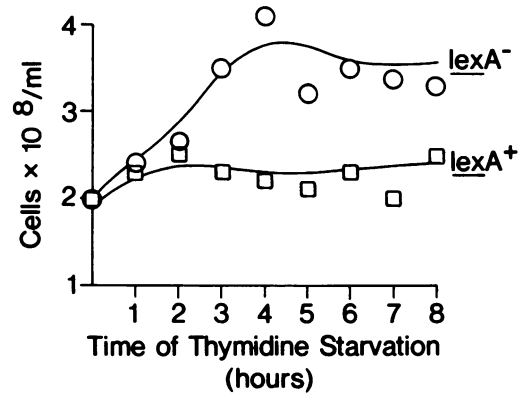


FIG. 3. Total cell counts of *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation (Petroff-Hausser count).

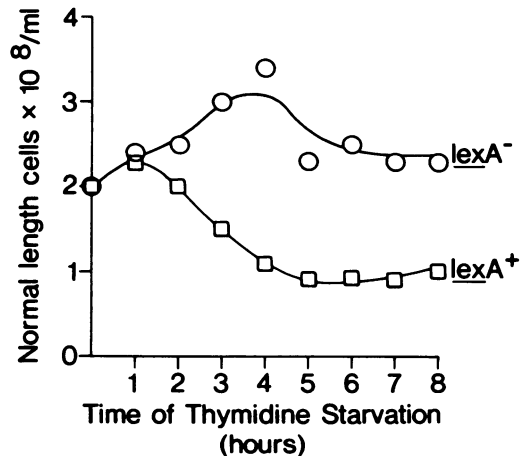


FIG. 4. Number of normal-sized cells in *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation (Petroff-Hausser count).

*lexA*<sup>+</sup> cultures grow into filaments during TdR deprivation. The increase in the number of normal-sized cells (Fig. 4) and the total number of cells in *lexA*<sup>-</sup> cultures (Fig. 3) are very

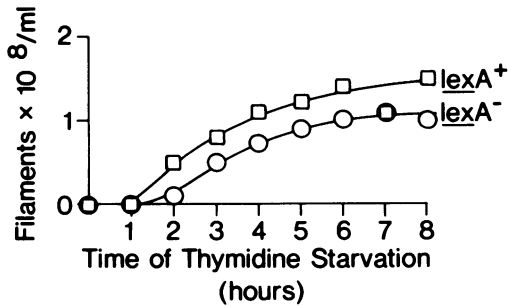


FIG. 5. Number of filaments counted in *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation (Petroff-Hausser count).

similar. This suggests that the short rods are produced in *lexA*<sup>-</sup> cultures during TdR starvation and that these probably do not elongate into filaments. Similar results were obtained with *recA*<sup>-</sup> cells during TdR starvation by Inouye (15).

**Autoradiographs.** Typical examples of autoradiographs of *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures that have been starved for TdR for 4 h are shown in Fig. 6 and 7, respectively. The following tables have been constructed from four different autoradiographic experiments. The distribution of cell sizes of all cells counted (sum of N<sup>+</sup> and N<sup>-</sup>) is shown in Table 2. *lexA*<sup>-</sup> cultures have many more normal-sized cells (75%) than filaments (25%) during TdR starvation when compared to *lexA*<sup>+</sup> cultures (75% filaments). This infers that the average cell in *lexA*<sup>-</sup> cultures is much smaller than those of *lexA*<sup>+</sup> cultures. Since the increase in OD in these experiments is slightly larger in *lexA*<sup>-</sup> versus *lexA*<sup>+</sup> cultures, then the *lexA*<sup>-</sup> cultures must contain more cells than *lexA*<sup>+</sup> cultures, in agreement with the data in Fig. 3.

The classification of cells in the above experiment into N<sup>+</sup> and N<sup>-</sup> types is shown in Tables 3 and 4, respectively. There are substantially more N<sup>+</sup> normal-sized rods in *lexA*<sup>-</sup> (50%) than in *lexA*<sup>+</sup> cultures (25%) (Table 3). The question as to why the *lexA*<sup>-</sup> cultures have many more normal-sized N<sup>+</sup> cells is under further investigation.

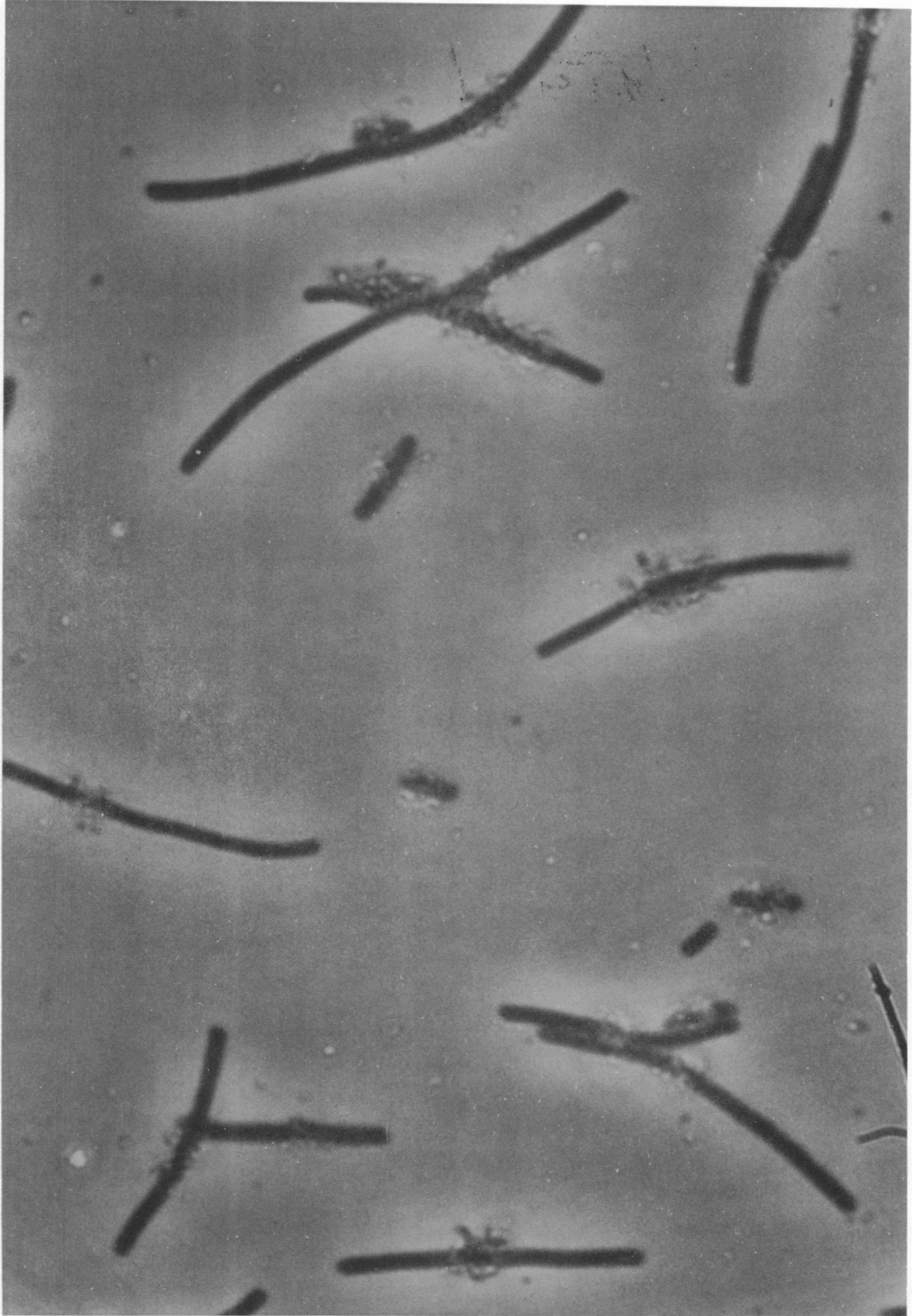
The number of N<sup>-</sup> cells in *lexA*<sup>+</sup> cultures is found to be very small (0.1%) (Table 4). This low frequency could represent the lower limit of resolution of the autoradiographic technique for the detection of N<sup>-</sup> cells. This percentage increases to 1.4% at 4 h and 1.9% at 8 h of TdR deprivation. This indicates that a small number of N<sup>-</sup> cells are produced in *lexA*<sup>+</sup> cultures. Unstarved *lexA*<sup>-</sup> cultures have 2.1% N<sup>-</sup> cells. This is considerably more than *lexA*<sup>+</sup> cultures

(0.1%), indicating that lethal sectoring is present in *lexA*<sup>-</sup> cells as it is in *recA*<sup>-</sup> cells (3, 4). At 4 and 8 h of TdR starvation, the N<sup>-</sup> cells increase to 15.8% and 18.4% in *lexA*<sup>-</sup> cultures, respectively. This result shows that there is a substantial number of N<sup>-</sup> cells produced in *lexA*<sup>-</sup> cultures during TdR deprivation. This amount (20%) is 10-fold higher than that of *lexA*<sup>+</sup> at the same time of starvation (2%) and that of *lexA*<sup>-</sup> cultures during normal growth (2%). In summary, approximately 10 times more N<sup>-</sup> cells are found in both unstarved and starved *lexA*<sup>-</sup> cultures than are present in similarly treated *lexA*<sup>+</sup> cultures.

**DNA degradation.** N<sup>-</sup> cells could be formed by an abnormal cell division process (defined here as the sum total of the steps needed for the division of the cell and not just the step of septum formation) or by DNA degradation during TdR starvation. It has been reported that there is 15% DNA breakdown during thymidine starvation (2) and that this breakdown is probably at the replicative fork(s) (21). The problem with measuring DNA degradation during TdR starvation is to stop the reutilization of labeled DNA precursors. To reduce such reutilization, DNA degradation experiments were performed at a low cell concentration (10<sup>6</sup> cells/ml). The rate of loss of trichloroacetic acid-insoluble counts in *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures (Fig. 8A) is approximately the same within a reasonable margin of experimental error. These DNA breakdown data assume that the cell number in each sample remains constant. This is probably not true because we have shown (Fig. 3) that *lexA*<sup>-</sup> cells divide during TdR deprivation whereas *lexA*<sup>+</sup> cells do not. If the DNA breakdown data is normalized to the total cell number (Fig. 3), the DNA degradation curves (Fig. 8B) are obtained. Approximately 10% more breakdown per cell is calculated to occur in *lexA*<sup>-</sup> cultures by this analysis. The implications of these results are discussed below.

## DISCUSSION

*lexA*<sup>-</sup> cultures are sensitive to UV (18), but not to TdR starvation. As they are starved for TdR, the *lexA*<sup>-</sup> cells divide, and many of the normal-sized cells do not appear to elongate into filaments. This is in contrast to the *lexA*<sup>+</sup> cultures where there are fewer cell divisions and most of the cells grow into filaments. Unstarved *lexA*<sup>+</sup> cultures have very few N<sup>-</sup> cells (0.1%), whereas similar *lexA*<sup>-</sup> cultures have a considerable number of N<sup>-</sup> cells (2%). There is an increase in N<sup>-</sup> cells in both *lexA*<sup>+</sup> (2%) and



**FIG. 6.** Autoradiograph of TdR-starved *lexA*<sup>+</sup> culture. Culture contains mostly filaments with a few normal-sized cells of which one is *N*<sup>-</sup>. The length of one of the small normal-sized cells is approximately 2  $\mu$ m.

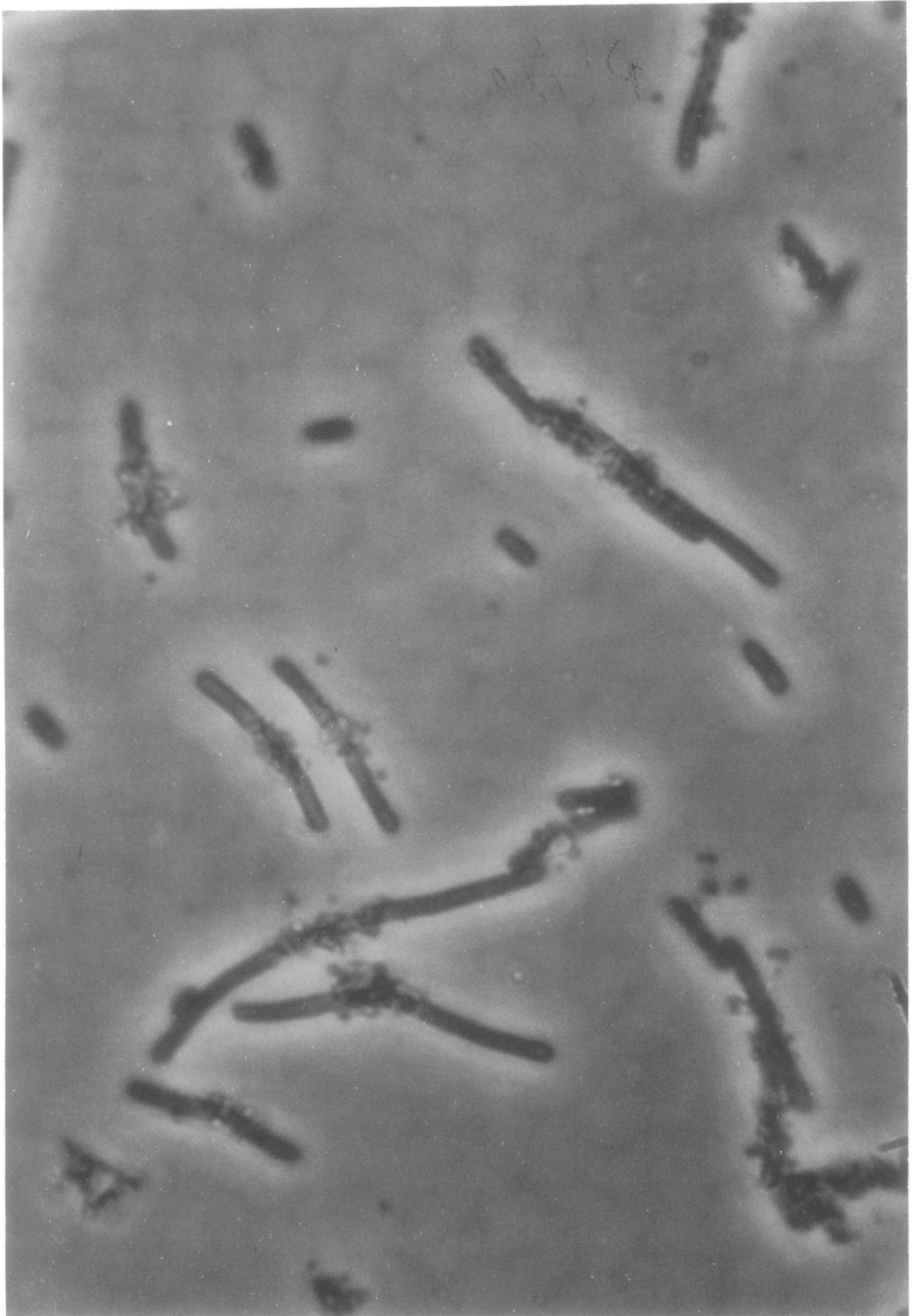


FIG. 7. Autoradiograph of *TdR*-starved *lexA*<sup>-</sup> culture. Culture contains some filaments and some normal-sized rods of which 5 are *N*<sup>-</sup>. The length of one of the smaller normal-sized cells is approximately 2  $\mu$ m.

TABLE 2. Analysis of autoradiographs for the normal length cells and filaments during thymidine starvation

Time of starvation (h)	<i>lexA</i> <sup>+</sup>			<i>lexA</i> <sup>-</sup>		
	No. of filaments	No. of normal length cells	Total no.	No. of filaments	No. of normal length cells	Total no.
0						
Count of total cells <sup>a</sup>	428	5,000	5,428	249	5,475	5,724
% of total cells	7.9	92.1	100	4.4	95.6	100
4						
Count of total cells	3,881	1,073	4,954	1,382	3,382	4,764
% of total cells	78.3	21.7	100	29.0	71.0	100
8						
Count of total cells	3,796	1,292	5,088	1,301	4,010	5,311
% of total cells	74.6	25.4	100	24.5	75.5	100

<sup>a</sup>Total cells = N<sup>+</sup> + N<sup>-</sup> cells.

TABLE 3. Analysis of autoradiograph for N<sup>+</sup> cells during thymidine starvation

Time of starvation (h)	<i>lexA</i> <sup>+</sup>			<i>lexA</i> <sup>-</sup>		
	No. of N <sup>+</sup> filaments	No. of N <sup>+</sup> normal cells	Total no. of N <sup>+</sup>	No. of N <sup>+</sup> filaments	No. of N <sup>+</sup> normal cells	Total no. of N <sup>+</sup>
0						
Count of N <sup>+</sup> cells	428	4,993	5,421/5,428 <sup>a</sup>	247	5,356	5,603/5,724
% of N <sup>+</sup> cells	7.9	92.0	99.9	4.3	93.6	97.9
4						
Count of N <sup>+</sup> cells	3,879	1,006	4,885/4,954	1,377	2,635	4,012/4,764
% of N <sup>+</sup> cells	78.3	20.3	98.6	28.9	55.3	84.2
8						
Count of N <sup>+</sup> cells	3,784	1,206	4,990/5,088	1,287	3,047	4,334/5,311
% of N <sup>+</sup> cells	74.4	23.7	98.1	24.2	57.4	81.6

<sup>a</sup>Total number of cells counted = N<sup>+</sup> + N<sup>-</sup> cells.

TABLE 4. Analysis of autoradiographs for N<sup>-</sup> cells during thymidine starvation

Time of starvation (h)	<i>lexA</i> <sup>+</sup>			<i>lexA</i> <sup>-</sup>		
	No. of N <sup>-</sup> filaments	No. of N <sup>-</sup> normal length cells	Total no. of N <sup>-</sup>	No. of N <sup>-</sup> filaments	No. of N <sup>-</sup> normal length cells	Total no. of N <sup>-</sup>
0						
Count of N <sup>-</sup> cells	0	7	7/5,428 <sup>a</sup>	2	119	121/5,724
% of N <sup>-</sup> cells	0	0.1	0.1	<0.1	2.1	2.1
4						
Count of N <sup>-</sup> cells	2	67	69/4,954	5	747	752/4,764
% of N <sup>-</sup> cells	<0.1	1.4	1.4	0.1	15.7	15.8
8						
Count of N <sup>-</sup> cells	12	86	98/5,088	14	963	977/5,311
% of N <sup>-</sup> cells	0.2	1.7	1.9	0.3	18.1	18.4

<sup>a</sup>Total number of cells counted = N<sup>+</sup> + N<sup>-</sup> cells.

*lexA*<sup>-</sup> (20%) cultures during starvation, indicating that N<sup>-</sup> cells are produced in both strains during TdR deprivation, but the production of N<sup>-</sup> cells in *lexA*<sup>-</sup> cultures is 10-fold more frequent than in *lexA*<sup>+</sup> cultures.

The major question that arises from these observations is: How are the N<sup>-</sup> cells which are

present in *lexA*<sup>-</sup> cultures during TdR starvation produced? N<sup>-</sup> cells could be produced by the cell division process being abnormal or by DNA degradation in N<sup>+</sup> cells. Cell division and DNA breakdown do occur in *lexA*<sup>-</sup> cultures during TdR starvation and so both explanations are tenable. In the autoradiograph experiments,



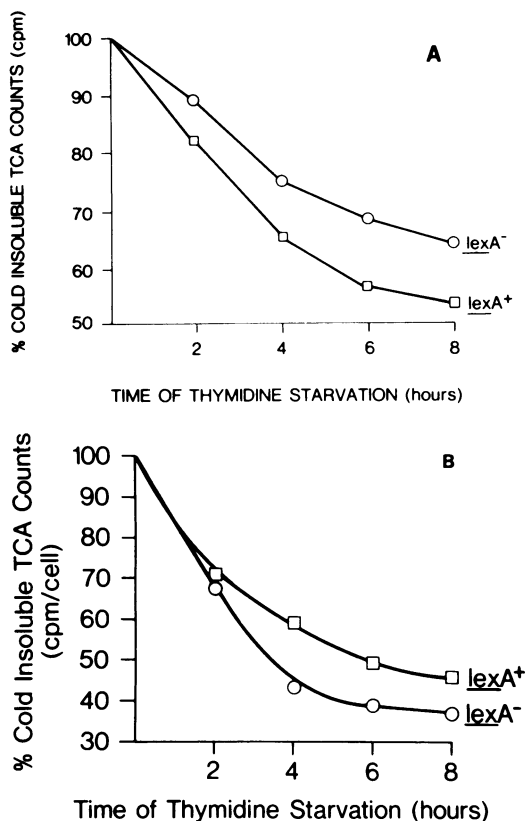


FIG. 8. (A) Percent cold insoluble trichloroacetic acid count in *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation. (B) Percent cold insoluble trichloroacetic acid count per cell in *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures during TdR starvation.

cells could be seen that have the septum positioned such that there are grains on one side but not on the other. This observation supports the first hypothesis of cell division. Furthermore, 20% more DNA breakdown in the *lexA*<sup>-</sup> cultures would be necessary to account for the high yield of N<sup>-</sup> cells, assuming that the pattern of degradation in individual cells is the same in both *lexA*<sup>+</sup> and *lexA*<sup>-</sup> cultures, and this is not observed (Fig. 8A and B). We conclude that some of the N<sup>-</sup> cells could arise by DNA breakdown, but that the most reasonable interpretation of our data is that cell division is perpetuated in *lexA*<sup>-</sup> cultures under conditions that block division in wild-type *E. coli* K-12 to produce N<sup>-</sup> cells.

*recA*<sup>-</sup> cells may react to TdR starvation in the same manner as *lexA*<sup>-</sup> cells, showing increased residual divisions and the production of N<sup>-</sup> cells. Inouye has also shown that *recA*<sup>-</sup> strains have higher levels of survival than *recA*<sup>+</sup>

cultures during TdR deprivation. He did not find N<sup>-</sup> cells in unstarved *recA*<sup>-</sup> or control *recA*<sup>+</sup> cultures (15). On the other hand, Anderson and Barbour (1), working with a different *recA*<sup>-</sup> strain, reported no difference in survivals of starved *recA*<sup>-</sup> and *recA*<sup>+</sup> cultures. Furthermore, Capaldo and Barbour (3, 4) have demonstrated that untreated *recA*<sup>-</sup> cultures contain 10% N<sup>-</sup> cells. Since there is DNA breakdown during normal growth in *recA*<sup>-</sup> cells (8), these N<sup>-</sup> cells could arise through DNA degradation. Clearly these discrepancies indicate that more work should be done with *recA*<sup>-</sup> strains. Inouye's *recA*<sup>+</sup> *lexA*<sup>+</sup> DNA degradation results are not in agreement with ours, because he found no DNA breakdown (15) and we show 50% DNA degradation. His procedure probably did not inhibit the reutilization of DNA precursors. His conclusion that N<sup>-</sup> cells are produced by cell division in *recA*<sup>-</sup> cultures during TdR starvation, based on his result of no breakdown, now appears to be open to question.

That the presence of a *lexA* mutation leads to the production of N<sup>-</sup> cells may or may not be related to the increase in cell number and normal-sized cells during TdR starvation. These effects show that the *lexA* gene product influences the process of cell division. *tsl*<sup>-</sup>, a UV-resistant derivative of a *lexA* mutant, forms filaments at 42 C (19). *lexA* mutations (or *exrA*) suppress the filamentation of *tif*<sup>-</sup> ( $\lambda$ <sup>-</sup>) cells at 42 C on minimal medium (7) and of *lon*<sup>-</sup> cells after UV irradiation on complete medium (12, 13).

These reports provide further evidence that the *lexA* gene product affects the cell division process. Witkin (26) previously proposed that UV irradiation induces the synthesis of an inhibitor of cell division. Possibly TdR starvation has the same effect. *lexA* mutations are dominant and so produce a diffusible product (18). We propose that this diffusible product acts in some manner to decrease the synthesis or activity of an inhibitor of cell division during TdR starvation. This product may also influence cell division during normal growth because *lexA*<sup>-</sup> cultures have a significant number of N<sup>-</sup> cells and there are fewer long filamentous cells than in *lexA*<sup>+</sup> cultures (Table 2).

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