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Isolation of the Nonviable Cells Produced During Normal Growth of Recombination-Deficient Strains of *Escherichia coli* K-12

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During normal growth, cultures of recombination-deficient (Rec$^-$) strains contain a population of cells that do not form colonies. Such cells are not present in a culture of an isogenic Rec$^+$ strain. We present a procedure for isolating and studying this defective population of cells. Exposure of a growing Rec$^-$ or Rec$^+$ culture to low levels of penicillin causes the dividing cells to elongate. The size of the nonviable cells present in the Rec$^-$ cultures is unaffected. The nonviable cells are then separated from the elongated cells by velocity sedimentation. This isolation technique provides a convenient way of analyzing the composition, biosynthetic capacity, and enzymatic function of the nonviable cells before isolation. In this paper we present data showing that before fractionation the nonviable cells in the Rec$^-$ culture are defective in their ability to synthesize β-galactosidase, whereas the Rec$^-$ viable cells behave like the Rec$^+$ cells in this regard. This observation confirms the existence of at least two classes of cells in liquid cultures of Rec$^-$ strains grown under normal conditions. That class of cells which is unable to synthesize β-galactosidase is the same class that cannot form colonies when plated on solid medium.

The known recombination-deficient mutants of *Escherichia coli* K-12 fall into two distinct classes on the basis of physiological characteristics (5, 6, 8, 11, 22, 23). One class, comprised of mutants with lesions in the *recA* gene, is characterized by high ultraviolet (UV) sensitivity, rapid deoxyribonucleic acid (DNA) breakdown after UV irradiation, low spontaneous production of λ phage from lysogens, no detectable recombination proficiency, alterations in cell division processes, reduced growth rate, and 50 to 60% viability (colony-forming ability) compared with an isogenic *rec*$^+$ strain (4, 9, 10, 12, 14, 21). The other class, comprised of mutants with lesions in the *recB* and *recC* genes, is characterized by moderate UV sensitivity, reduced DNA breakdown after UV irradiation, normal spontaneous production of λ phage from lysogens, reduced recombination proficiency, absence of an adenosine triphosphate-dependent deoxyribonuclease (exonuclease V), reduced growth rate, and 20 to 30% viability (1, 2, 4, 9, 17, 20, 21). A strain carrying both *recA* and *recB* mutations is 15 to 20% viable (F. Capaldo, unpublished data); however, strains carrying either a *recA* or a *recB* mutation together with a *polA* mutation (polymerase I-deficient) are completely inviable (15).

The products of the three *rec* genes are clearly important (they are essential in the absence of functional DNA polymerase I) for the maintenance of normal growth rate and viability. One approach to determining the roles played by these gene products in cell growth and viability is to study the nonviable cells (those cells which have been most affected by the lack of a functional *rec* gene product), especially with regard to the presence and the nature of macromolecular species and to the ability of the cells to synthesize these macromolecules.

In this paper we present a technique for isolating the nonviable cells from cultures of Rec$^-$ mutants. The culture is treated with a low concentration of penicillin which causes the dividing cells to elongate while not altering the size of the nonviable cells. The two size classes of cells are then separated by velocity sedimentation. This technique is useful to study the nonviable cells *before isolation*. These cells are shown to be defective in their ability to synthesize β-galactosidase.

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Thus, during normal growth, Rec⁻ cultures contain a population of non-colony-forming cells that are defective in enzyme synthesis and which can be separated from viable cells by our technique.

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MATERIALS AND METHODS

Bacterial strains. All bacterial strains used were *E. coli* K-12. Their *rec* genotypes and other phenotypic properties are listed in Table 1. Nomenclature conforms to that of Demerec et al. (7).

Media. M9 salts (4) and Luria medium (22) have been described. EM9 glycerol medium consisted of M9 salts (1 liter), Casamino Acids (Difco) (2.5 g), glycerol (4 g), and thiamine (0.1 mg). NaCl (0.85% saline) was used as a diluent.

Growth of strains. Each strain was grown overnight (37 C on a rotating wheel) at least two successive times in 5 ml of EM9 glycerol medium. The culture was stored in the refrigerator and used as the stock for experiments with that particular strain. The night before an experiment, a 1:25 (Rec⁻) or 1:50 (Rec⁺) dilution of the stock was made into 5 ml of fresh EM9 glycerol medium and incubated overnight at 37 C on a rotating wheel. On the morning of the experiment, the overnight culture was diluted (between 1:40 and 1:75 depending on the strain) into fresh EM9 glycerol medium (25 or 30 ml in a 125-ml flask) containing ^{14}C-leucine-UL (1 μCi/ml) (Schwarz-Mann, lot XR-2033, 310 mCi/mmol) for uniform labeling. The flasks were incubated in a shaking 37 C water bath long enough to allow each strain to undergo four to five doublings (pregrown cells). The growth rate was determined by making periodic optical density measurements at 650 nm in a spectrophotometer. Since the nonviable cells are formed from viable cells and since, after four generations of growth, greater than 90% of cells present in the culture will have been formed during the period of exposure to ^{14}C-leucine, we expect the specific activity of ^{14}C-leucine in the viable and in the nonviable cells to be identical. Pregrown cells (5 ml) were harvested at 6,000 rpm for 10 min in the SS-34 rotor of the Sorvall RC2-B refrigerated centrifuge. Before centrifugation, unlabeled L-leucine was added to a final concentration of 100 μg/ml. The cells were washed twice with 1-ml volumes of M9 salts containing unlabeled L-leucine (100 μg/ml). The cells were resuspended in 1 ml of M9 salts + leucine and used immediately for the penicillin treatment. For measurement of the rate of induction of β-galactosidase in whole cultures, 20 ml of pregrown cells was diluted with an equal volume of fresh EM9 glycerol medium in a 125-ml flask and incubated an additional 45 min as before. β-galactosidase was induced with isopropyl-β-D-thiogalactoside (Sigma Chemical Co.) at a final concentration of 10^{-3} M. Samples were removed at intervals during the subsequent 30 min to measure the absorbance at 650 nm and the units of β-galactosidase present.

For measurement of synthesis by nonviable cells, the inducer was added to the pregrown cells 20 min before harvesting.

Penicillin treatment. A sample (0.5 ml) of washed, resuspended, pregrown cells was inoculated into 50 ml of EM9 glycerol medium (no ^{14}C-leucine) containing 35 U of penicillin (buffered, potassium salt; Eli Lilly) per ml in a 250-ml flask. A portion (10 ml) was immediately sampled for a control (penicillin untreated) gradient. The flask was incubated for 105 min in a shaking 37 C water bath. At the end of the incubation, 10 ml was removed for the penicillin-treated gradient. During the penicillin treatment, periodic optical density measurements at 650 nm were made. Under these conditions, lysis begins at about 135 min for all three strains.

Velocity sedimentation. Each 10-ml sample was harvested as described for pregrown cells. The cells were washed twice with 1-ml volumes of 0.05 M tris(hydroxymethyl)aminomethane (Tris; Sigma)-hydrochloride buffer (pH 7.4) containing unlabeled L-leucine (100 μg/ml) and were resuspended in 0.25 ml of buffer. The cell suspension was kept refrigerated until layered on the gradient.

Linear, preformed, 5 to 20% (wt/vol) sucrose gradients (4.6 ml) were prepared by using 2.3 ml each of 5 and 20% filter sterilized sucrose (Baker, A.S.C. grade) solutions made up in 0.05 M Tris-hydrochloride buffer (pH 7.4). A cell suspension (0.5 ml) was layered on each gradient with a 0.1-ml pipette. The gradients were centrifuged in the SW-39 rotor of the Spinco model L-2 centrifuge for 8 min at an average speed of 3,000 rpm.

Gradients were collected by puncturing the bottom of the tube with a needle and collecting 12-drop fractions (approximately 0.2 ml each, 23 fractions per gradient) into sterile test tubes. Each fraction was diluted five-fold with saline. All assays were done with the diluted fractions. After collecting all fractions, the centrifuge tube was washed with 1 ml of 20% sucrose: saline (1:4). The wash was poured into a test tube and treated identically with the fraction tubes in all assays. To preserve maximum viability, all manipulations of the cells after they came in contact with sucrose were performed in the cold, and chilled reagents were used in all cases.

Viable cell measurements. We have previously defined the number of viable cells per milliliter of culture as the number of cells in a liquid culture which, when diluted and plated as described, give rise to visible (without magnification) colonies on solid medium (4). To assay the number of viable cells in each fraction, a 0.1-ml sample of each fraction was

TABLE 1. *Bacterial strains*[a]

Strain no.	*rec* Genotype	Reference
JC4583	+	2
JC4584	B21 C22	4
JC4588	A56	2

[a] All strains are F⁻, His⁻, Gal⁻, Thi⁻, Str^S, and EndA⁻.

diluted serially through 0.9 ml of saline, and duplicate 0.1-ml samples were plated on Luria agar. For fractions plated without dilution, 0.1 ml of saline was put on the plate and spread with the sample. The fraction tubes and dilution tubes were kept on ice during these manipulations. The plating itself was carried out at room temperature. The plates were incubated at 37 C for 1 to 2 days and the colonies were counted.

Total cell measurements. The total number of cells per milliliter of culture or in each fraction was determined directly by counting samples in a Levy-Hauser counting chamber at a magnification of 400×. Where necessary, samples were diluted through saline. The smallest division on a Levy-Hauser chamber is 50 μm long. Under the growth conditions described above, 95% or more of the cells in cultures of each of the three strains in the absence of penicillin are less than or equal to one-fifth the length of this division, or 10 μm. After 105 min of penicillin treatment as described above, the sizes of the long cells range approximately from 3 to 10 times the length of normal size cells for cultures of all three strains.

Radioactivity measurements. The radioactivity present in each fraction was determined by adding a 0.1-ml sample from each fraction to glass vials containing 10 ml of scintillation fluid [naphthalene (1, 125 g), 2,5-diphenyloxazole (67.5 g), 1,4-bis-2-(5-phenyloxazolyl)-benzene (6.57 g), *p*-dioxane (8,739 ml), and methanol (600 ml)] plus 0.4 ml of distilled water, and counted in a Packard Tri-Carb liquid scintillation spectrometer. Under these conditions ^{14}C is counted with 80% efficiency. All the radioactivity was in 5% trichloroacetic acid-precipitable form.

β-galactosidase assay. Samples (0.1 ml) of either the induced culture or the diluted fractions were added directly to tubes containing 0.9 ml of M9 salts plus 1 drop each of toluene and 5% sodium lauroyl sarcosinate. β-galactosidase was assayed according to the procedure of Pardee et al. (18). No correction was necessary for turbidity. For experiments using unfractionated cultures, the units of β-galactosidase in each sample are expressed per unit of absorbance at 650 nm in the culture at the time each sample was taken. For experiments using fractionated cultures, the units of β-galactosidase in each sample are expressed per ^{14}C counts per minute in that sample.

RESULTS

Effects of penicillin treatment. When cultures of either the *rec*$^+$, *recB*$^-$ *recC*$^-$, or *recA*$^-$ strains are treated with penicillin as described above, the culture continues to increase in optical density for about 150 min, undergoing 1.5 to 2 doublings in mass. However, direct microscope observation of the cultures reveals that this mass increase is largely the result of cell elongation rather than cell division. The total number of particles per milliliter of culture remains approximately constant for about 135 min, at which time cell lysis becomes evident. The number of viable cells per milliliter of culture remains nearly unchanged for the first hour (even though cells are visibly elongated), then decreases rapidly to 1 to 2% of the initial value at the end of the second hour (Fig. 1). We have chosen 105 min of penicillin treatment as being an optimal time because at this point (i) there is no evidence for cell lysis (the mass of the culture is still increasing at a nearly exponential rate and the doubling time for all three cultures is only increased approximately 10% over that of an untreated culture, the total number of particles is unchanged, and no "ghost" particles are visible in the culture); (ii) there is relatively little penicillin-induced lethal damage among the initially viable cells (all three cultures retain approximately 10% of their initial viability); and (iii) the long cells are significantly larger than the normal size cells and readily separable from them on a sucrose gradient.

The efficiency of penicillin in inducing cell elongation in cultures of each of the three strains is presented in Fig. 2. The percentage of cells longer than 10 μm in normal, untreated cultures of each of the three strains is approximately 10%. After 105 min of penicillin treatment, more than 95% of the cells in the *rec*$^+$ culture have elongated. However, in the *recB*$^-$ *recC*$^-$ culture only 55% of the particles are long cells, and in the *recA*$^-$ culture 75 to 80% are long cells after identical penicillin treatment.

Velocity sedimentation. Untreated cultures of each of the three strains behave identically in a neutral sucrose velocity gradient under the conditions used (Fig. 3). In all cases the radioactivity, total particles, and viable cells cosediment to the same position in the gradient (fractions 12–19), and in all cases 70% or more of the total material (radioactivity, total particles, viable cells) recovered from the gradient is found in this region. Approximately 5% of the total recovered material is found in the tube wash. More than 70% of the radioactivity and total particles layered on the gradient are recovered.

After penicillin treatment, the *rec*$^+$ culture sediments much more rapidly than before and has a considerably broader band, reflecting the heterogeneity in the length of the long cells (Fig. 4A). More than 50% of the total radioactivity recovered from the gradient sediments in this broad band (fractions 4–11). Less than 20% of the total recovered radioactivity appears in fractions 12 through 19 where normal size cells are found. The ratio of radioactivity to total particles is constant across the gradient. The ratio of viable cells to total particles varies and is greatest in fractions 11 through 18. The average ratios of radioactivity to total particles and viable cells to total particles for peak regions are given in Table 2.

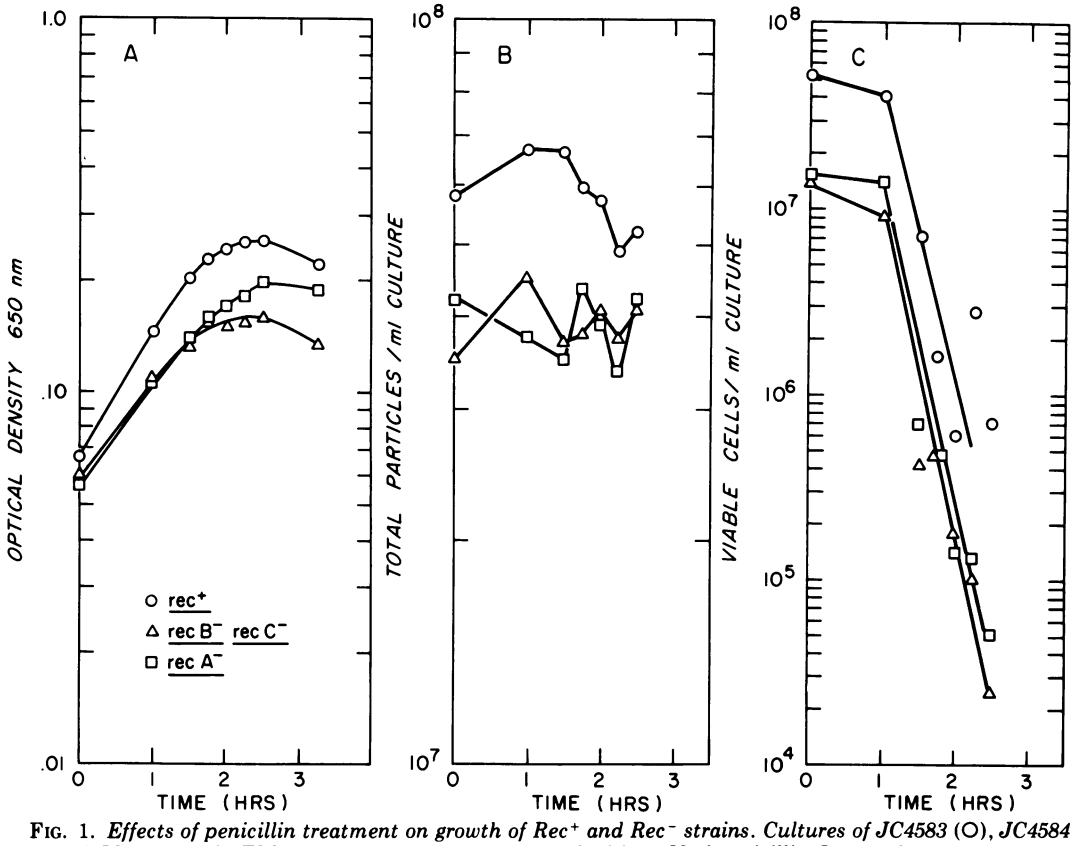

FIG. 1. *Effects of penicillin treatment on growth of Rec⁺ and Rec⁻ strains. Cultures of JC4583* (○), *JC4584* (△), *and JC4588* (□) *in EM9 glycerol medium were treated with 35 U of penicillin G per ml.*

Sedimentation of the *recB⁻ recC⁻* strain after penicillin treatment reveals a clearly bimodal distribution of cell sizes (Fig. 4B). The radioactivity is distributed in two regions. The first band (fractions 1-11) sediments in the same position as penicillin treated *rec⁺* cells, whereas the second band (fractions 12-19) sediments in approximately the same position as untreated cells. Each band contains approximately 35% of the total radioactivity recovered from the gradient. Microscope observation of the particles in each fraction reveals that the faster-moving band is composed almost exclusively of long cells, whereas the slower-moving band contains normal size cells as well as a trailing of longer cells. The ratio of radioactivity in ^{14}C-leucine to total particles is constant throughout the two bands, indicating that this ratio is the same for long and short cells. This result is consistent with out earlier prediction that at the end of the uniform labeling period the specific activity of ^{14}C-leucine is the same in both viable and nonviable cells. Because the penicillin treatment is carried out in the absence of ^{14}C-leucine, any new protein synthesized during cell elongation would be unlabeled and would not alter the ratio of counts in ^{14}C-leucine per particle. The distribution of viable cells throughout the gradient coincides with the distribution of long cells, indicating that the short cells are nonviable (Table 2).

Sedimentation of the *recA⁻* strain after penicillin treatment results in a distribution of particles, radioactivity, and viability similar to that observed for the *recB⁻ recC⁻* strain (Fig. 4C). The band of short cells is much smaller in the *recA⁻* gradient than in the *recB⁻ recC⁻* gradient, an observation that is consistent with the smaller fraction of nonviable cells present in the *recA⁻* culture initially (Table 2).

Induction of β-galactosidase. Figure 5 shows the rates of induction of β-galactosidase by the *rec⁺*, *recB⁻ recC⁻*, and *recA⁻* strains. In panel A the units of β-galactosidase are plotted as a function of time after addition of the inducer. Both Rec⁻ strains show significantly reduced rates of formation of the enzyme compared with the Rec⁺ strain. However, correction of the time scale for the slower growth rate of the Rec⁻ cultures eliminates the differences in rates (panel B). That is, the reduction in the rate of appearance of enzyme in Rec⁻ strains

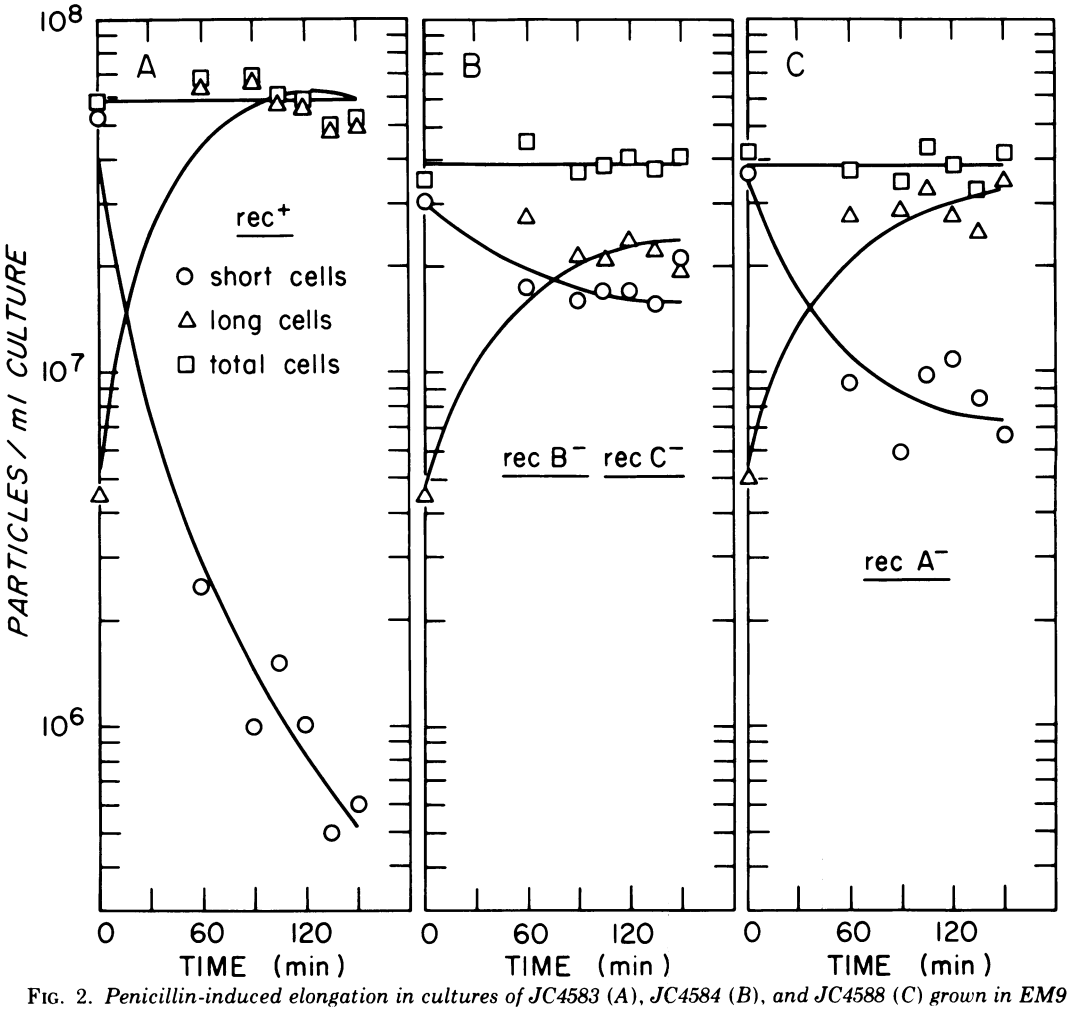

FIG. 2. *Penicillin-induced elongation in cultures of JC4583 (A), JC4584 (B), and JC4588 (C) grown in EM9 glycerol medium. Symbols: ○, short cells; △, long cells; □, total cells.*

compared with the Rec$^+$ strain is concomitant with the reduction in growth rate of Rec$^-$ strains.

Two simple alternative models may be proposed to explain these results. According to the first model, all of the cells in the Rec$^-$ cultures are able to synthesize β-galactosidase, but they do so at a slower rate than do Rec$^+$ cells. According to the second model, only the dividing cells are able to synthesize β-galactosidase, but they do so at the same rate as do Rec$^+$ cells. The rate of synthesis of β-galactosidase seen in whole cultures is therefore an average for synthesizing and nonsynthesizing cells. These models predict clearly distinguishable results if cells which have been induced to synthesize β-galactosidase are treated with penicillin and subjected to velocity sedimentation to separate the nondividing from the dividing cells.

According to the first model, treatment of both Rec$^+$ and Rec$^-$ cultures in this manner would result in identical specific activities of β-galactosidase in all fractions of a gradient, but the specific activities of the enzyme would be lower in the Rec$^-$ gradients than in the Rec$^+$ gradient.

According to the second model, for Rec$^-$ cultures the specific activity of β-galactosidase should vary through the gradient, being highest (and equal to the Rec$^+$ specific activity) near the bottom, where the dividing cells band, and lowest across the region where the nondividing cells band. The following experiment tests these alternatives.

Fractionated cultures. Figure 6 shows the results of an experiment in which Rec$^+$ and Rec$^-$ cultures, prelabeled with ^{14}C-leucine, were induced for β-galactosidase, treated with peni-

cillin, and sedimented through a preformed sucrose gradient. Before penicillin treatment all three strains behaved identically, with cells and enzyme cosedimenting in a sharp band in the upper half of the gradient (panel A). Throughout this band the ratio of enzyme units to radioactivity is nearly constant for each strain, but is significantly lower for the Rec⁻ strains compared with the Rec⁺ strain. After penicillin treatment (panel B), the Rec⁺ cells sediment in a faster-moving and broader band, but the ratio of enzyme units to radioactivity remains about the same across the entire gradient. On the other hand, the *recB⁻ recC⁻* cells (panel C)

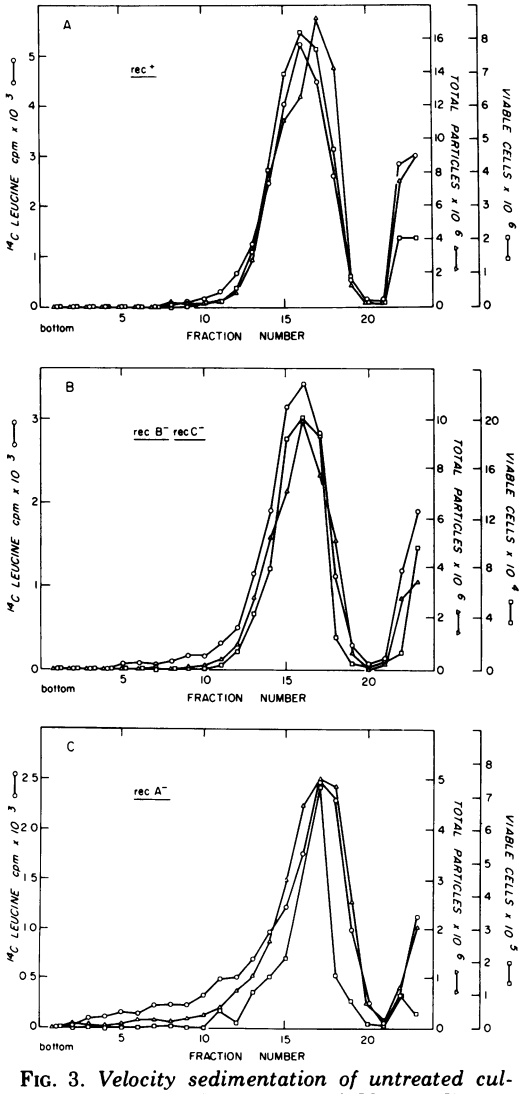

FIG. 3. *Velocity sedimentation of untreated cultures. JC4583 (A), JC4584 (B), and JC4588 (C) were grown in EM9 glycerol medium. Symbols: O, radioactivity; Δ, total particles (all short); □, viable cells.*

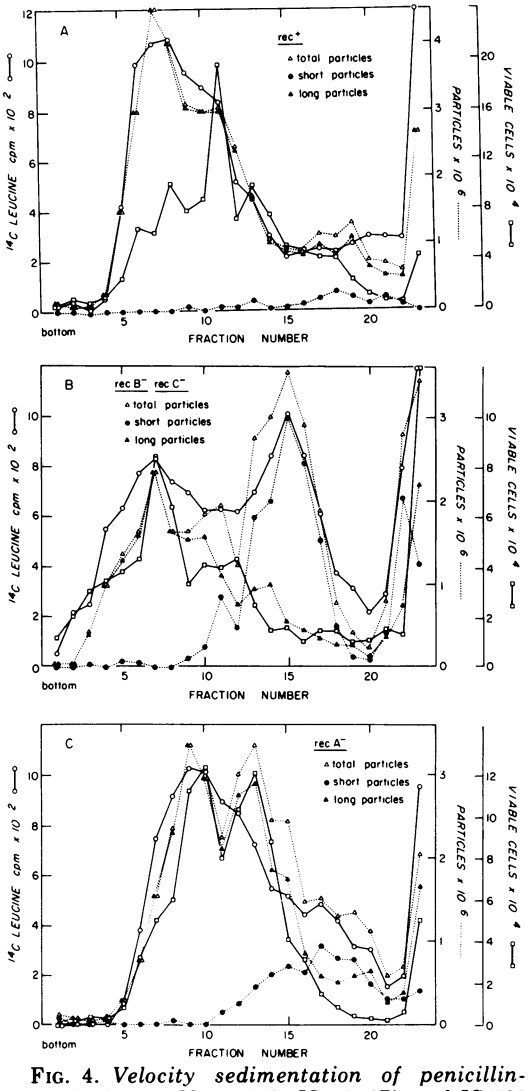

FIG. 4. *Velocity sedimentation of penicillin-treated cultures. JC4583 (A), JC4584 (B), and JC4588 (C) were grown in EM9 glycerol medium and treated with 35 U of penicillin G per ml for 105 min. Symbols: O, radioactivity; Δ, total particles (long and short); □, viable cells; ●, short particles; ▲, long particles.*

sediment in a bimodal pattern, but the enzyme activity is distinctly skewed toward the lower end of the gradient. The ratio of enzyme units to radioactivity is two- to threefold lower in the region of the gradient where nondividing cells band than in the region where dividing cells band. Similarly, for the *recA⁻* strain the ratio of enzyme units to radioactivity is twofold lower in the upper region of the gradient than in the lower part (panel D). The ratio of enzyme units to radioactivity for different regions of the gradients are given in Table 3. These results

TABLE 2. *Specific activity and viability of penicillin-treated particles isolated from different regions of the gradient after velocity sedimentation*

Strain	Fraction or pooled fractions	Specific activity[a]	Viability[b]
JC4583 (*rec*$^+$)	7	2.4	1.4
	6-11	2.8	2.9
	15	1.8	4.3
	13-18	2.3	5.0
JC4584 (*recB*$^-$ *recC*$^-$)	7	3.5	3.5
	4-10	4.3	3.0
	15	2.9	0.45
	13-18	3.0	0.64
JC4588 (*recA*$^-$)	10	3.4	4.2
	6-14	3.2	3.5
	17	3.1	1.0
	15-21	2.7	1.1

[a] Expressed as counts per minute of ^{14}C-leucine per 10^{-4} particles.

[b] Expressed as viable cells per 10^2 particles.

indicate that the nondividing Rec$^-$ cells present in a liquid culture *before* penicillin treatment are unable to be normally induced to synthesize β-galactosidase, thus supporting the second model.

DISCUSSION

It has previously been shown that Rec$^-$ strains are significantly less viable than Rec$^+$ strains (5, 10, 11) and that Rec$^-$ strains show an increased frequency of spontaneous lethal sectoring compared with Rec$^+$ strains (10). These two observations suggest that cultures of Rec$^-$ strains contain a population of cells that are unable to divide. The existence in Rec$^-$ cultures of a population of cells that (i) do not elongate in the presence of penicillin, (ii) are less viable than those cells that elongate in the presence of penicillin, and (iii) are absent in cultures of an identically treated, isogenic Rec$^+$ strain is consistent with this hypothesis. The percentage of cells that fail to elongate in the presence of penicillin appears to be correlated with the viability of each strain under normal growth conditions. We believe that the population of normal size cells isolated from the *recB*$^-$ *recC*$^-$ culture by velocity sedimentation after penicillin treatment is, in fact, the population of nondividing (hence nonviable) cells present in the culture at the start of the penicillin treatment. It is important to emphasize that after sedimentation of penicillin-treated Rec$^-$ cultures, the region of the gradient showing the lowest viability is that region where the cells showing the least visible effects of penicillin treatment band. Conversely, those cells which respond to penicillin most like wild-type cells, i.e., by elongating, also are most viable. Moreover, the nonviable cells isolated by this procedure are, during normal growth before isolation, unable to synthesize β-galactosidase, whereas the viable cells behave like Rec$^+$ cells in this regard.

The isolation of this population of cells is an important step in determining the role of the *rec* gene products in normal cell growth and viability, because analysis of the nonviable cells may identify the specific lethal damage incurred by cells in the absence of functional *rec* gene products. At least two possibilities consistent with our findings can be suggested for the role of the *rec* genes in normal growth.

(i) The products of the *rec* genes might be involved in the repair of normal metabolically produced DNA lesions. It is reasonable to assume that the formation and repair of single-strand breaks in the DNA duplex is a normal and essential part of cell metabolism. Such breaks might be necessary to allow transcription (19) and replication (3, 16) to occur. The products of the *rec* genes have been shown to be involved in the repair of X ray-induced single-strand breaks (13). In the absence of functional *rec* gene products, it is possible that some DNA breaks are not efficiently repaired. If, as a result of chromosome segregation during cell division, a daughter cell were to receive only damaged chromosomes, then it would be unable to replicate its DNA and undergo cell division (i.e., it would be a nonviable cell) and might also be unable to transcribe its DNA.

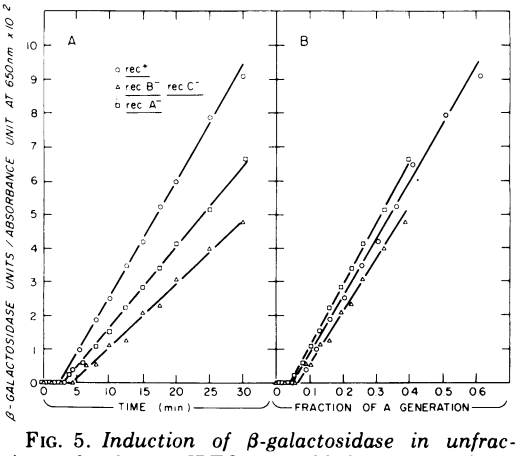

FIG. 5. *Induction of β-galactosidase in unfractionated cultures. IPTG was added at 0 min. Symbols:* ○, *JC4583;* △, *JC4584;* □, *JC4588.*

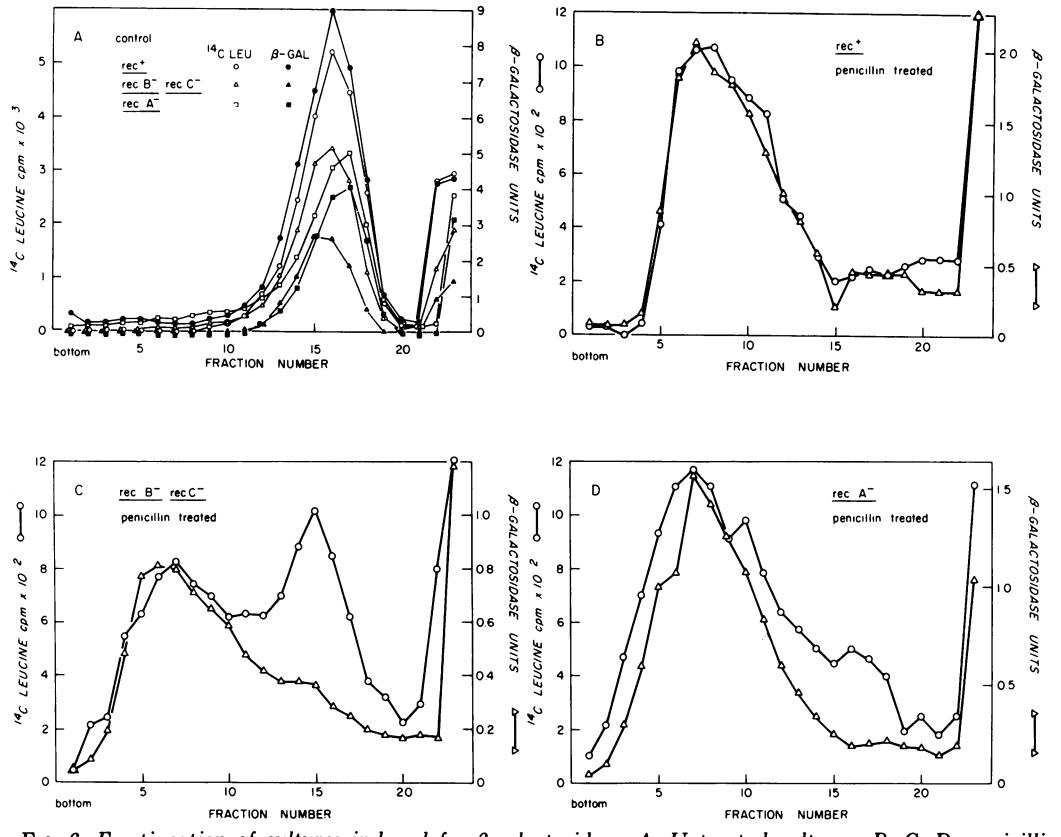

FIG. 6. *Fractionation of cultures induced for β-galactosidase. A, Untreated cultures; B, C, D, penicillin-treated cultures. Symbols for A: ○, ●, JC4583; △, ▲, JC4584; □, ■, JC4588. Open symbols represent radioactivity, closed symbols represent units of β-galactosidase. Symbols for B (JC4583), C (JC4584), and D (JC4588): ○, radioactivity; △, units of β-galactosidase.*

TABLE 3. *Distribution of β-galactosidase in different regions of the gradient after velocity sedimentation of penicillin-treated cultures*

Strain	Fraction or pooled fractions	Amt of β-galactosidase[a]
JC4583 (*rec⁺*)	7	19.2
	6–11	17.8
	16	19.3
	13–18	17.6
JC4584 (*recB⁻ recC⁻*)	6	10.6
	4–10	10.0
	15	3.6
	13–18	4.3
JC4588 (*recA⁻*)	7	13.5
	4–12	10.1
	16	3.7
	14–19	5.6

[a] Expressed as β-galactosidase units per 10^4 counts per minute of ^{14}C leucine.

(ii) Alternatively, it is possible that during cell division in Rec⁻ strains, cells which lack DNA are segregated. Such cells would also be unable to divide or to be induced to synthesize an enzyme. With the method described in this paper we are testing these possibilities. We are determining whether nonviable cells synthesize DNA, contain DNA, and, if so, whether the DNA contains more single-strand breaks than does viable cell DNA.

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LITERATURE CITED

1. Barbour, S. D. 1972. Biochemical and genetic studies of recombination proficiency in *Escherichia coli* K-12. IV. Analysis of recombinants formed by a recombination deficient (*recB21 recC22*) strain. Mol. Gen. Genet. **117**:303-309.
2. Barbour, S. D., and A. J. Clark. 1970. Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. I. Enzymatic activity associated with $recB^+$ and $recC^+$ genes. Proc. Nat. Acad. Sci. U.S.A. **65**:955-961.
3. Cairns, J. 1963. The bacterial chromosome and its manner of replication as seen by autoradiography. J. Mol. Biol. **6**:208-213.
4. Capaldo-Kimball, F., and S. D. Barbour. 1971. Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. J. Bacteriol. **106**:204-212.
5. Clark, A. J. 1967. The beginning of a genetic analysis of recombination proficiency. J. Cell Physiol. **70** Suppl. 1:165-180.
6. Clark, A. J., and A. D. Margulies. 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K-12. Proc. Nat. Acad. Sci. U.S.A. **53**:451-459.
7. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61-76.
8. Emmerson, P. T. 1968. Recombination deficient mutants of *Escherichia coli* K-12 that map between *thyA* and *argA*. Genetics **60**:19-30.
9. Haefner, K. 1968. Spontaneous lethal sectoring, a further feature of *Escherichia coli* strains deficient in the function of *rec* and *uvr* genes. J. Bacteriol. **96**:652-659.
10. Hertman, I. 1969. Survival, DNA breakdown and induction of prophage in *Escherichia coli* K12 *recA uvrB* double mutant. Genet. Res. **14**:291-307.
11. Howard-Flanders, P., and L. Theriot. 1966. Mutants of *Escherichia coli* K12 defective in DNA repair and in genetic recombination. Genetics **53**:1137-1150.
12. Inouye, M. 1971. Pleiotropic effect of the *recA* gene of *Escherichia coli*: uncoupling of cell division from deoxyribonucleic acid replication. J. Bacteriol. **106**:539-542.
13. Kapp, D. S., and K. C. Smith. 1970. Repair of radiation-induced damage in *Escherichia coli*. II. Effect of *rec* and *uvr* mutations on radiosensitivity, and repair of X-ray-induced single-strand breaks in deoxyribonucleic acid. J. Bacteriol. **103**:49-54.
14. Low, B. 1968. Formation of merodiploids in matings with a class of Rec^- recipient strains of *Escherichia coli* K-12. Proc. Nat. Acad. Sci. U.S.A. **60**:160-167.
15. Monk, M., and J. Kinross. 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **109**:971-978.
16. Ogawa, T., J. Tomizawa, and M. Fuke. 1968. Replication of bacteriophage DNA. II. Structure of replicating DNA of phage lambda. Proc. Nat. Acad. Sci. U.S.A. **60**:861-865.
17. Oishi, M. 1969. An ATP-dependent deoxyribonuclease from *Escherichia coli* with a possible role in genetic recombination. Proc. Nat. Acad. Sci. U.S.A. **64**:1292-1299.
18. Pardee, A. B., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**:165-178.
19. Pauling, C., and P. Hanawalt. 1965. Nonconservative DNA replication in bacteria after thymine starvation. Proc. Nat. Acad. Sci. U.S.A. **54**:1728-1735.
20. Tomizawa, J., and H. Ogawa. 1972. Structural genes of ATP-dependent deoxyribonuclease of *Escherichia coli*. Nature N. Biol. **239**:14-16.
21. Willetts, N. S., and A. J. Clark. 1969. Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. J. Bacteriol. **100**:231-239.
22. Willetts, N. S., A. J. Clark, and B. Low. 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97**:244-249.
23. Willetts, N. S., and D. W. Mount. 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thyA*. J. Bacteriol. **100**:923-934.