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Bacteriophage-induced Inhibition of Host Functions[1]

I. Degradation of *Escherichia coli* Deoxyribonucleic Acid After T4 Infection

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The kinetics of degradation of bacterial deoxyribonucleic acid (DNA) after infection of *Escherichia coli* with T4D, ultraviolet-irradiated T4D, and two amber mutants, N122 and N94, was studied by zone sedimentation through linear glycerol gradients. Within 5 min after infection with any of the bacteriophages, breakdown of host genome was evident. The first product was a high-molecular-weight material (50*S* to 70*S*) and further degradation appeared to occur in discrete steps. Rapid and extensive breakdown of bacterial DNA was seen after infection with *am* N122 and T4D. Infection with ultraviolet-irradiated phage or with *am* N94 resulted in an accumulation of high-molecular-weight material. These results suggest that the observed degradation of host DNA begins early and requires sequential action of several phage-induced endo- as well as exodeoxyribonucleases.

The synthesis of bacterial protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) ceases rapidly after infection of *Escherichia coli* with T-even bacteriophages (3, 6, 7, 14). Phage infection also inhibits the induction of β-galactosidase (1), and it has been suggested (9) that infection prevents the initiation of transcription of bacterial messenger RNA (mRNA). In infected cells pretreated with streptomycin or chloramphenicol (CM), there is an inhibition of synthesis of bacterial DNA and mRNA, the degree of inhibition depending on multiplicity of infection (16). Destruction of bacterial DNA normally occurs after infection (7, 10, 12, 13). Bacteria pretreated with CM, however, appear to have intact chromosomes (10, 15). Sedimentation analyses of bacterial DNA isolated from T4-infected *E. coli* led Nomura et al. (15) to postulate that the kinetics of degradation was too slow to account for the cessation of host functions.

The degradation of host DNA appears to be due to action of deoxyribonucleases induced by phage infection. The level of deoxyribonuclease activity increases several-fold after infection, and the increase requires protein synthesis (20). Wiberg (21) reported that mutations in genes 46 and 47 of the T4 chromosome lead to inability of breakdown of the host DNA to acid-soluble material. Two oligonucleotidases which preferentially hydrolyze partially degraded DNA (17, 18), and an endonuclease which preferentially cleaves denatured *E. coli* DNA (Bose and Nossal, Federation Proc. **23**:272, 1964), have been isolated from T2-infected cells.

In this report we present results on the gradual degradation of host DNA, as revealed by zone sedimentation studies, after infection of *E. coli* with T4, ultraviolet-irradiated T4 ($T4^{uv}$), and amber mutants N122 [no DNA synthesis (DO), hydroxymethylase-defective, gene 42 (5, 22)] and N94 [DNA arrest, defective in the breakdown of host DNA, gene 46 (21)]. In addition, conversion of bacterial DNA into acid-soluble material after infection was investigated. Our results indicate that the bacterial chromosome is converted to a slower sedimenting but still high-molecular-weight material within 5 min after infection. Further degradation of this material follows infection with T4 and *am* N122 but not with $T4^{uv}$ or *am* N94. It is likely that degradation of host DNA is brought about by sequential action of one or more endonucleases followed by exonucleolytic digestion by one or more oligonucleotidases.

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MATERIALS AND METHODS

Chemicals. Reagent grade chemicals, and Difco agar, tryptone, and peptone were used. Vitamin- and salt-free casein hydrolysate (acid-hydrolyzed) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Amino acids, nucleosides, and nucleotides were purchased from Calbiochem (Los Angeles, Calif.), Schwarz Bio Research (Orangeburg, N.Y.), and Sigma Chemical Co. (St. Louis, Mo.). Thymidine-methyl-^3H and thymine-2-^{14}C were purchased from Schwarz Bio Research, and ^{32}P-sodium phosphate from Nuclear Consultants, St. Louis, Mo. Sodium lauroyl sarcosinate was purchased from Geigy Industries, Inc.

Bacteria. E. coli B3 and CR34 were obtained from Norman Melechen and Ronald Rolfe, respectively, of this department. Both are thymine-requiring auxotrophs, and CR34 has additional requirements for thiamine, threonine, leucine, and uracil. Strains R2, a variant of *E. coli* B, resistant to premature lysis, and CR63 were acquired from G. R. Greenberg. CR34 and CR63 are permissive, and B3 and R2 are nonpermissive, hosts for the suppressible amber mutants.

Bacteriophages. T4D and the amber mutants N122 and N94 were supplied by R. S. Edgar. They were propagated on R2 and CR63, respectively, and purified by differential centrifugation. Ultraviolet-irradiated T4 was prepared as described before (2*a*).

Growth medium. The composition of the glycerol-Casamino Acids medium (FJ) employed for this investigation has been described (2). In the low-phosphate medium (TFJ) used to prepare ^{32}P-labeled bacteriophage, the phosphate buffer in FJ was replaced with 0.1 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.4, and KH_2PO_4 (817 mg/liter).

Labeled cell DNA. The DNA of *E. coli* was labeled by growing the thymine auxotrophs B3 and CR34 at 37 C in media supplemented with thymine (5 μg/ml) and thymidine-methyl-^3H (amount indicated in the legends to figures). The cells were grown to a density of about 2×10^8/ml, washed twice with thymine-supplemented nonradioactive medium, suspended in one-half volume of nonradioactive medium, and aerated at 37 C for 20 min. After infecting with the appropriate phage in the presence of L-tryptophan (50 μg/ml), aeration was continued; 0.5-ml samples of infected cells were pipetted at intervals into tubes immersed in an ethyl alcohol-dry ice bath. The cells were stored overnight at −20 C and then prepared for sedimentation analyses.

Preparation of lysates. The procedure of Smith and Levine (19) was adopted with the following modifications. To 0.5 ml of frozen infected cells, the following were added: ethylelenediaminetetraacetate (*p*H 8.0), 5 mM; NaCl, 150 mM; Tris (*p*H 7.4), 10 mM; and sodium lauroyl sarcosine to a final concentration of 3%. After incubation at 65 C for 10 min, more NaCl was added to 1.2 M. After further incubation for 10 min, 1 mM Tris (*p*H 7.4) containing 30 mM NaCl was added to a final volume of 2.6 ml. The lysate was allowed to stand overnight at room temperature and was warmed to 47 C for 2 min prior to sedimentation analysis.

Labeled phage DNA. ^{32}P- or ^{14}C-labeled T4 DNA was utilized as a sedimentation marker in each centrifuge tube. To prepare ^{32}P-labeled phage, *E. coli* R2 cells, grown in isotope-containing TFJ, were infected after they reached a density of about 2×10^8/ml. To prepare ^{14}C-labeled phage, 40 μc of thymine-2-^{14}C and phage were added to 20 ml of cells at a density of 2×10^8/ml, in the presence of 5-fluorodeoxyuridine (5 μg/ml). After 5 hr of aeration at 37 C, the infected cells were lysed by adding chloroform. The lysates were left overnight at room temperature, and the phage was purified by three cycles of differential centrifugation. To prepare T4 DNA, the purified phage, at a density of approximately 10^{11} plaque-forming units per ml, was lysed by the technique utilized for the infected bacteria.

Sedimentation analysis. Linear 40 to 60% (v/v) gradients (4.8 ml) of glycerol in 10 mM Tris (*p*H 7.4) were prepared at room temperature and left at 4 C overnight. A 200-μliter sample, containing ^{3}H-labeled bacterial DNA and ^{14}C- or ^{32}P-labeled T4 DNA marker, was layered on the top of the gradient. Centrifugation was performed in a Spinco SW39 rotor at 112,000 × *g* for 7 hr at 24 C. Serial fractions were collected directly into scintillation vials from the top by pumping 80% glycerol (v/v) through a needle puncturing the bottom of the centrifuge tube. After adding 5 ml of scintillation fluid (690 ml of toluene, 310 ml of ethyl alcohol, 80 g of naphthalene, 5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene), radioactivity was counted in a Nuclear-Chicago Mark I liquid scintillation spectrometer. Recovery of input radioactivity was >90%. The position of reference T4 DNA, present in each gradient, is indicated by an arrow. The thin vertical line drawn across the figures indicates the position of DNA in uninfected *E. coli*.

Acid-soluble nucleotide measurements. For total DNA measurements, duplicate samples taken at various times after infection were placed in 1 ml of 10% trichloroacetic acid and hydrolyzed for 10 min at 95 C. This was taken as 100% radioactivity in all calculations. Additional samples were coprecipitated at 0 C with approximately 7×10^8 nonradioactive cells by adding one-fifth volume of 50% trichloroacetic acid (final concentration, 10%). After standing for 10 min at 0 C, the acid-insoluble material was sedimented at 6,000 × *g* for 15 min. A sample of the acid-soluble nucleotides was removed from the supernatant fluid and counted.

Estimation of S values. Sedimentation coefficients assigned are estimates based on the position of the radioactive material in the gradient relative to T4 DNA reference. The sedimentation coefficient of T4 DNA as determined by analytical centrifugation was 62.5*S*.

RESULTS

Breakdown by T4 infection. Zone sedimentation through 40 to 60% glycerol gradients showed that the DNA material of *E. coli* B3 was de-

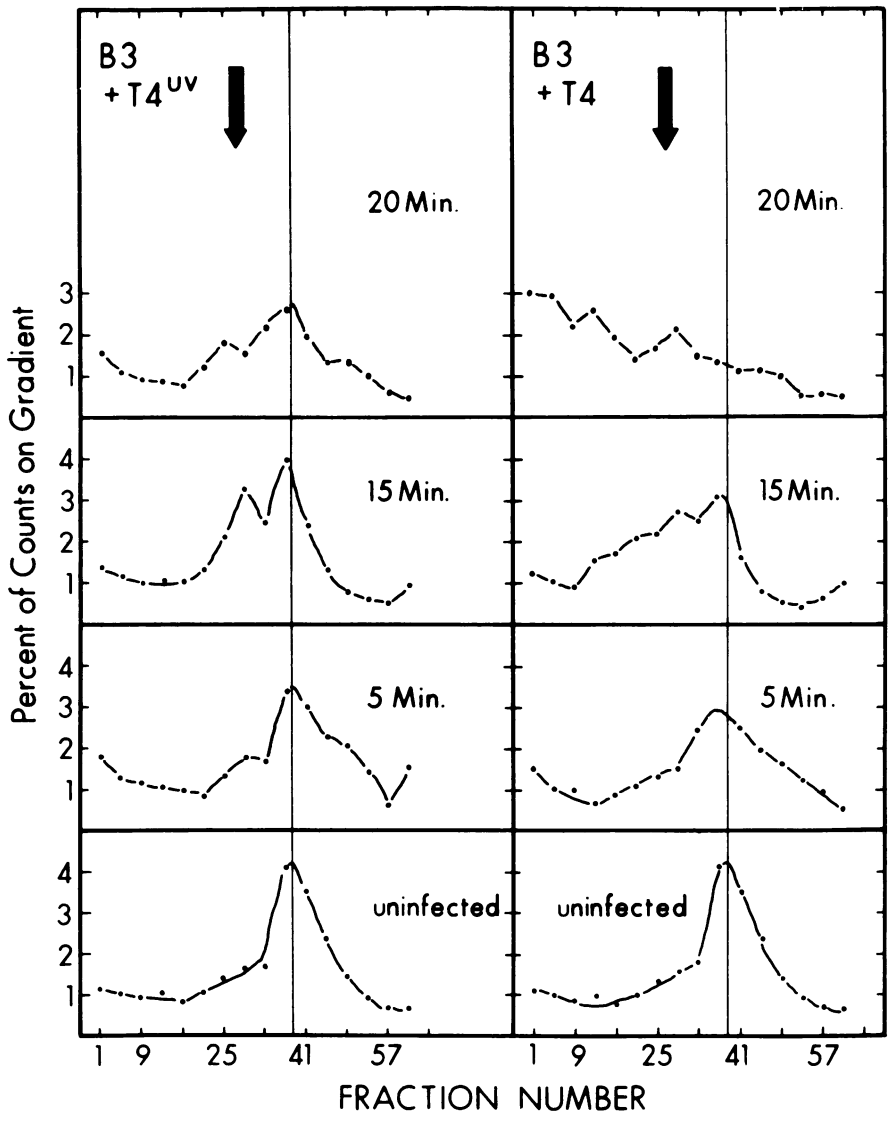

FIG. 1. *Fate of Escherichia coli B3 DNA after infection with T4 or T4^{uv}. E. coli B3 was grown to a density of 2 × 10^8 cells/ml in the presence of thymine (5 μg/ml) and thymidine-methyl-^{3}H (100 μc/ml; 15 c/mmole). One-half of the washed cells were infected with T4, the other with T4^{uv}, both at a multiplicity of 1.4 (which killed 90% of the cells). Samples of the infected cells were removed at the indicated times; lysates were prepared and centrifuged through linear glycerol gradients. Input per gradient, 2 × 10^4 to 2.5 × 10^4 counts/min. The position of the ^{32}P-phage DNA marker in this and other figures is indicated by the arrow. Direction of sedimentation in this and all similar figures is to the right.*

graded in apparently discrete steps. In samples taken 5 min after infection, the first indication of degradation was a slower sedimenting shoulder and broadening of the peak of labeled material (Fig. 1). Considerable heterogeneity in the distribution of radioactivity was seen 15 min after infection, and degradation of bacterial DNA continued, ultimately showing material sedimenting below 10*S*. The gradients used in the present study did not permit resolution of smaller breakdown products, which tended to pile up at the interface between the lysate and the top of the gradient.

Breakdown by T4^{uv} infection. There was a

more clearly resolved decrease in the sedimentation rate of bacterial DNA after infection with T4^{uv}. Within 5 min after infection, a 70*S* shoulder was discernible (Fig. 1). By 15 min, about half of the host DNA was found in the 70*S* peak. The smallest DNA material in the 20-min sample appeared to be a 58*S* product. We saw only partial degradation of the host genome in samples taken after 40 min of infection. In addition to the rather large degradation products, infection with T4^{uv} allowed considerable amounts of nearly intact (78*S*) bacterial DNA material to remain, even after 20 min. No viral DNA synthesis occurs after infection with UV-irradiated T-even bacteriophages (4), although it has been suggested that synthesis of bacterial DNA continues (11). Determination of acid-soluble product from T4^{uv}-infected B3 and CR34 corroborate the incomplete degradation revealed by zone sedimentation (Fig. 2 and 3). More than 90% of the bacteria were rendered nonviable after infection with T4^{uv}.

Breakdown by infection with amber mutants. The DNA of permissive as well as nonpermissive hosts was degraded after infection with amber

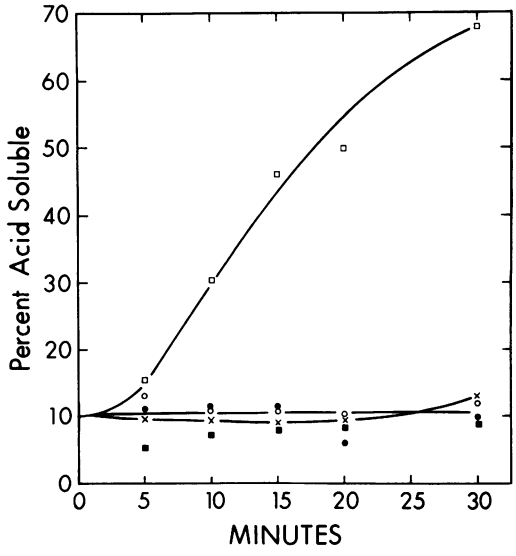

FIG. 2. *Formation of acid-soluble nucleotides in phage-infected B3. Samples of Escherichia coli B3, grown and infected as described in the legend to Fig. 1, were coprecipitated with 7 × 10^8 cells in 10% trichloroacetic acid at 0 C. The percentage acid-soluble determination is based on the number of counts in the supernatant fluid after cold acid precipitation of the infected cells, relative to the number of counts after hydrolysis of the infected cells in 10% trichloroacetic acid for 10 min. (●) T4; (×) T4^{uv}; (□) am N122; (○) am N122^{uv}; (■) am N94.*

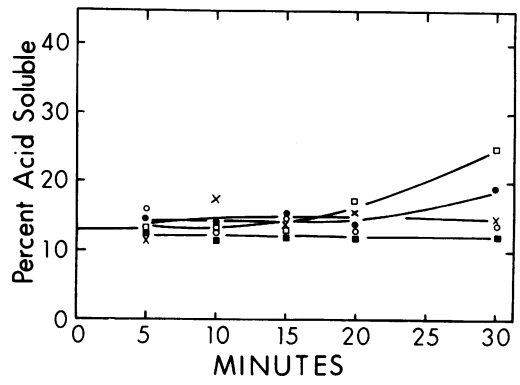

FIG. 3. *Formation of acid-soluble nucleotides in phage-infected CR34. Samples of Escherichia coli CR34, grown and infected as described in the legend to Fig. 1, were coprecipitated with 7 × 10^8 cells in 10% trichloroacetic acid at 0 C. Symbols as in the legend to Fig. 2.*

mutants N122 and N94. With *am* N122, extensive breakdown of host DNA was observed. Within 5 min, a sharp peak, at 57*S* in the case of B3 (*su*$^-$) and 63*S* after infection of CR34 (*su*$^+$), was resolved (Fig. 4). The rapid conversion of bacterial DNA to the 57*S*–63*S* material was reproducible after infection with *am* N122, and the speed of degradation was reflected in the appearance of acid-soluble product after infection of B3 with *am* N122 (Fig. 2). There was no synthesis of viral DNA in this system (*am* N122 is a DO amber mutant) and thus no reincorporation of bacteria DNA degradation product. Infection with ultraviolet-irradiated N122 of B3 or CR34 resulted in the absence of production of acid-soluble material (Fig. 2 and 3).

Infection with *am* N94 does not induce breakdown of DNA of a nonpermissive host into acid-soluble material (21), and the present study confirms this observation (Fig. 2 and 3). However, degradation of the host DNA to high-molecular-weight material, a possibility considered earlier (21), was clearly seen by zone sedimentation (Fig. 5). It is of interest to compare the pattern of degradation induced by infection of B3 with *am* N94 to that induced with T4^{uv} (Fig. 1). In *am* N94-infected B3, 5 min after infection an 83*S* material was seen which was degraded to a 58*S* product by 15 min and to 40*S* in 30 min (Fig. 5). Smaller products were not seen in these gradients. Infection of an *su*$^-$ host with a gene 46 amber mutant results in the synthesis of a limited amount of viral DNA before further synthesis is arrested (5). It remains to be determined whether the incomplete degradation of host chromosome plays a role in the arrest of synthesis of viral

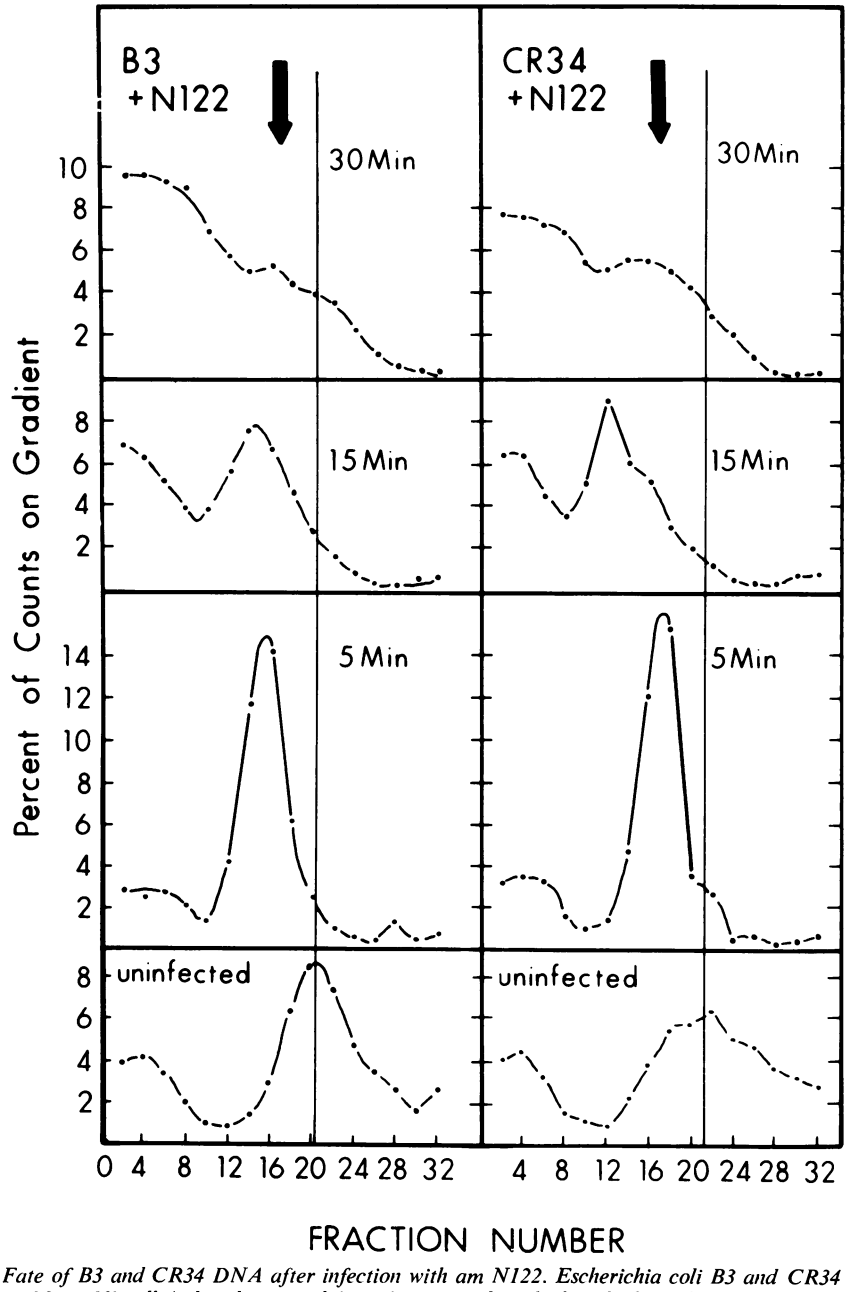

FIG. 4. *Fate of B3 and CR34 DNA after infection with am N122. Escherichia coli B3 and CR34 were grown to a density of 2 × 10⁸ cells/ml and prepared for infection as described in the legend to Fig. 1, except that thymidine-methyl-³H (20 μc/ml) was used. One-half of the washed-cell suspension was infected with am N122 at a multiplicity of 5, killing 99% of the cells. The remainder of the experiment was as described in the legend to Fig. 1. Input per gradient, 1.8 × 10⁵ to 2 × 10⁵ counts/min. The position of the ³²P-phage DNA marker is indicated by the arrow.*

DNA. Evidence for breakdown of host DNA after infection with gene 46 amber mutants into acid-insoluble material has also been obtained by zone sedimentation through sucrose gradients (E. Kutter and J. S. Wiberg, *personal communication*).

DISCUSSION

Conversion of host chromosome into viral DNA normally obscures degradation of the former. In the absence of synthesis of bacteriophage DNA, such as after infection of a non-

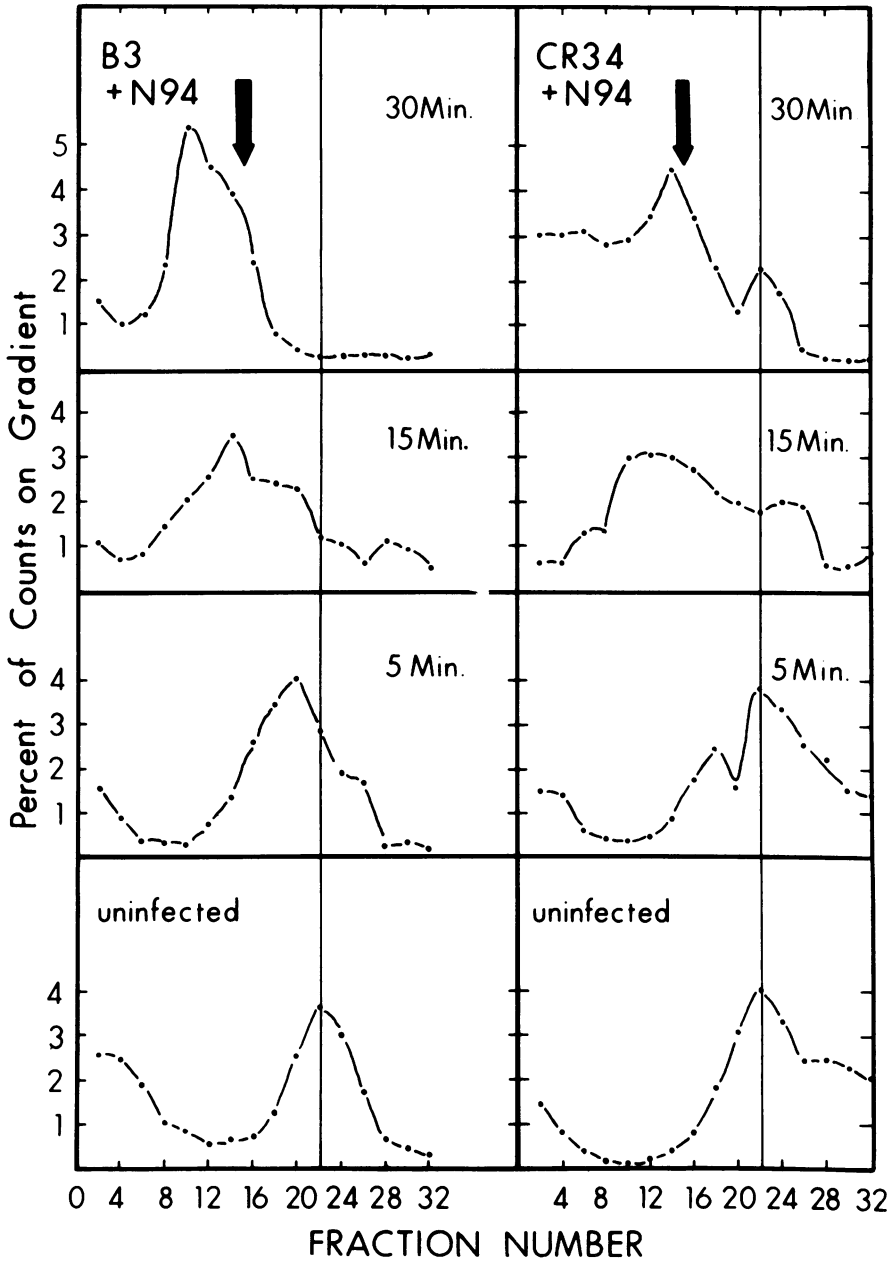

FIG. 5. *Fate of B3 and CR34 DNA after infection with am N94. The remainder of the washed B3 and CR34 cells described in the legend to Fig. 4, which had been washed and divided, were infected with N94 at a multiplicity of 5; 99% of the cells were killed. The remainder of the experiment was as described in the legend to Fig. 4.*

permissive strain of *E. coli* with amber mutants of T4, defective in early functions, production of acid-soluble material from host DNA is clearly observed (22). Epstein et al. (5) reported that infection by a number of amber mutants, including those in gene 42 or 46, induced nuclear breakdown as revealed by phase-contrast microscopy. However, no information was available about the kinetics of breakdown of the bacterial chromosome other than that performed by zone sedimentation of a purified DNA prepared from T4-infected *E. coli* (15). However, the DNA, even

from uninfected bacteria, had a sedimentation coefficient of 31*S*, and further degradation of this material was not seen until 10 min after infection. The apparent delay in the breakdown of this bacterial DNA was taken to suggest that the phage-induced inhibition of host-specific biosynthesis of macromolecules was unrelated to breakdown of bacterial genome.

Infection of *E. coli* with T4 bacteriophage results in the appearance of several apparently new deoxyribonucleases (Y-C. Yeh and G. R. Greenberg, *personal communication*) and at least three of them have been described (17, 18; Bose and Nossal, Federation Proc. **23**:272, 1964). Increase in ribonuclease-stimulated deoxyribonuclease activity, with denatured DNA as substrate, has been seen in endonuclease I-deficient strains infected with T4, $T4^{uv}$, and *am* N122 within 5 min after infection (S. K. Bose, *unpublished data*). In view of this early increase in deoxyribonuclease activity, it appeared reasonable that degradation of host DNA could be an early function. The present studies show that the sedimentation rate of bacterial DNA decreases within 5 min after infection, and this slower sedimenting material is of high molecular weight (50*S* to 70*S*). It is therefore likely that the first step in degradation is an endonucleolytic cleavage. Work is now in progress to determine whether a single-strand scission precedes this cleavage and is responsible for the inhibition of host functions. The enzymes responsible for the production of 50*S* to 70*S* material from bacterial DNA would be induced after infection with T4, $T4^{uv}$, *am* N122, and *am* N94. Further degradation appears to involve at least another endonuclease and one or more exonucleases which act on partially degraded DNA, such as the ones described by Koerner and his coworkers (8, 17, 18). The second endonuclease would be lacking after infection with $T4^{uv}$ and *am* N94. The relationship of the deoxyribonucleases described and those postulated remain to be determined. The problem is complicated by the obviously high degree of specificity regarding at least the size of the substrate, insofar as devising in vitro assay systems.

In the present study, no attempt was made to purify further the labeled bacterial DNA which sedimented at 90*S* to 100*S* in glycerol gradients. The possibility of a DNA-protein complex is therefore not ruled out, although incubation of the lysate (containing sodium lauroyl sarcosine) with Pronase (100 μg/ml) for 16 hr at room temperature had no effect on sedimentation behavior of the thymidine-labeled material. Similarly, one cannot reject the possibility that the observed decrease in sedimentation rate of bacterial DNA material after infection with T4 could be due to alterations in the conformation of the host chromosome, and possible conversion of a nuclease-resistant material to either a more shear- or deoxyribonuclease-sensitive state.

The foregoing data and other lines of evidence, such as the "freezing" of degradation of host DNA by adding CM or puromycin at various times after infection and the use of deoxyribonuclease-deficient hosts (R. J. Warren and S. K. Bose, *in preparation*), suggest strongly that the degradation of *E. coli* DNA after infection with T4 bacteriophage is an early function with sequential participation of several endo- as well as exonucleases whose synthesis appears to require de novo protein synthesis.

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