

Biochemical Changes in Lysogenic *Bacillus stearothermophilus* After Bacteriophage Induction¹

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ABSTRACT

WELKER, N. E. (University of Illinois, Urbana), AND L. LEON CAMPBELL. Biochemical changes in lysogenic *Bacillus stearothermophilus* after bacteriophage induction. *J. Bacteriol.* 90:1129-1137. 1965.—Cultures of *Bacillus stearothermophilus* 1503-4R (TP-1) continued to grow at an unaltered rate after induction with mitomycin C (MC). MC-induced cultures exhibited a 2.5-fold increase in cell number before lysis occurred. Prior to lysis, cells were observed to elongate and to contain areas of lesser density. Protein synthesis was slightly inhibited in MC- or ultraviolet light (UV)-induced cultures for a period of 5 to 10 min, and then proceeded at a rate identical to that in the noninduced culture. Ribonucleic acid (RNA) synthesis was not affected by MC induction. UV induction caused RNA synthesis to occur in two stages: in the first stage, the rate of RNA synthesis was one-third that observed in the noninduced culture and lasted for a period of 15 min; the second stage of RNA synthesis then proceeded at a rate identical to that in the noninduced culture. The synthesis of deoxyribonucleic acid (DNA) in an MC- or UV-induced culture occurred in two stages. In the first stage, DNA synthesis in induced cultures occurred at a rate of one-half (MC) and one-third (UV) of that observed in the noninduced culture. The first stage of DNA synthesis in MC- or UV-induced cultures lasted for 25 to 30 min and 15 to 20 min, respectively. In the second stage, the rate of DNA synthesis in MC- or UV-induced cultures occurred at a rate three times that of the noninduced culture. UV induction appeared to have a greater inhibitory effect than MC induction on protein, RNA, and DNA synthesis as well as phage yield. The differential rate (*K*) of inducible and constitutive α -amylase synthesis was inhibited by 75 and 100%, respectively, for a period of 20 min after MC induction. After 20 min, the *K* values for α -amylase synthesis were identical to those obtained in the absence of MC induction. The synthesis of TP-1 phage DNA occurred rapidly and was complete 25 min after MC induction, whereas bacterial DNA was degraded or its rate of synthesis was decreased. During the second stage of DNA synthesis, only bacterial DNA was synthesized, but at a rate greater than that found in the noninduced culture.

In a previous paper (Welker and Campbell, 1965), it was shown that an obligately thermophilic strain of *Bacillus stearothermophilus* carried a temperate bacteriophage (TP-1). The conditions for phage induction and assay, as well as some of the physical and chemical properties of the phage and the phage deoxyribonucleic acid (DNA) were reported. Growth of induced cultures

of *B. stearothermophilus* continued at an unaltered rate until lysis occurred. This paper describes some of the biochemical changes occurring in *B. stearothermophilus* after phage induction.

MATERIALS AND METHODS

Microscopic examination of induced and non-induced cultures. Cells from induced and non-induced cultures were prepared for microscopic examination as follows: glass microscope slides were coated with a thin film of agar by immersion in a beaker containing 2% melted agar (Difco). The slides were removed and placed upright in an empty beaker so that excess agar was drained off, leaving a thin film of hardened agar. One side of the slide was wiped clean, and a 0.01-ml sample of

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the culture was placed on the agar film and covered with a cover slip. The preparation was observed under phase contrast by use of a Zeiss photomicroscope. Photographs were recorded on Adox KB-14 film.

Measurement of cellular ribonucleic acid (RNA), DNA, and protein. Amounts of 5 ml of TYF-log cells of *B. stearothermophilus* 1503-4R (TP-1) were used to inoculate each of three 500 ml flasks containing 100 ml of TYF medium (Welker and Campbell, 1965). The flasks were shaken at 55 C on a rotary shaker. Samples (5 ml) were removed from the cultures induced with mitomycin C (MC; 0.05 $\mu\text{g}/\text{ml}$) or ultraviolet (UV) light (General Electric germicidal lamp at a distance of 50 cm for 5 min) and from noninduced cultures at the times indicated, and were placed in conical centrifuge tubes; the cells were removed by centrifugation. The pellet was suspended in 5 ml of cold (4 C) 5% trichloroacetic acid and placed in an ice bath for 4 hr. The precipitate was removed by centrifugation, washed once with 2 ml of 0.5 N HClO_4 , and suspended in 5 ml of 0.5 N HClO_4 . The tubes, containing the suspended cells, were heated at 90 C in a water bath for 20 min, cooled to ambient room temperature, and centrifuged at $3,000 \times g$ for 15 min. The supernatant fluids were decanted and used for the determination of RNA (Dische, 1955) and DNA (Burton, 1956). The pellets were dissolved in 0.1 ml of 1 N NaOH and brought to 10 ml with distilled water; protein was determined by the procedure of Lowry et al. (1951). Salmon sperm DNA, yeast RNA, and bovine serum albumin (Mann Research Laboratories, New York, N.Y.) were used as standards.

Induction and assay of α -amylase. The preparation of pure maltose, induction of α -amylase in cultures growing in MCHF medium, and the assay of α -amylase activity were described by Welker and Campbell (1963a, b). α -Amylase formation was quantitated by measuring the differential rate of synthesis (K) of enzyme where the increase in enzyme (E) is directly proportional to the increase in cell mass ($A = \text{absorbancy}$). The rate is expressed as follows: $\Delta E/\Delta A = K$.

*Isolation of DNA from MC-induced and non-induced cultures of *B. stearothermophilus*.* One 500-ml flask containing 100 ml of TYF medium was inoculated with cells from an overnight Trypticase agar plate and placed on a rotary shaker for 2.5 hr at 55 C. Amounts of 5 ml of this culture were used to inoculate each of ten 500-ml flasks containing 100 ml of TYF medium plus 40 μC of thymidine- 2-C^{14} (New England Nuclear Corp., Boston, Mass.). The flasks were placed on a rotary shaker for 1 hr at 55 C, and then MC (0.05 $\mu\text{g}/\text{ml}$) was added to five of the flasks. At the indicated times, an induced and a noninduced culture were removed from the shaker, cells were removed by centrifugation, and the pellets were frozen (-20 C) until used.

DNA was isolated from the cells by use of a modification of the procedure of Marmur (1961). The cells were thawed, suspended in 10 ml of

saline-EDTA (0.15 M NaCl plus 0.1 M ethylenediaminetetraacetate, pH 8), and incubated at 37 C with 10 $\mu\text{g}/\text{ml}$ of lysozyme (Mann Research Laboratories) until lysis was complete. Duponol (0.67 ml of a 25% solution) was added, and the mixture was heated at 60 C for 10 min. The tube, containing the nucleic acids, was cooled to ambient room temperature, and was shaken on a wrist-action shaker for 30 min at 4 C with an equal volume of water-saturated phenol (adjusted to pH 7.5 with 10% NaOH). The layers were separated by centrifugation and the aqueous layer, containing the nucleic acids, was removed and shaken with an equal volume of anhydrous ethyl ether to remove the residual phenol. The aqueous layer was bubbled with N_2 to remove the ether and was incubated with 10 $\mu\text{g}/\text{ml}$ of ribonuclease (Mann Research Laboratories) for 30 min at 37 C. The DNA solution was shaken with an equal volume of chloroform-isoamyl alcohol and centrifuged, and the aqueous layer was dialyzed at 4 C against several changes of SSC (0.15 M NaCl-0.015 M trisodium citrate, pH 7). DNA isolated by this procedure still contains some RNA and protein and will be referred to as partially purified DNA. Purified bacterial and phage DNA were prepared as described by Welker and Campbell (1965).

Chromatographic fractionation of partially purified and purified DNA. Methylated-albumin-Kieselguhr (MAK) columns were prepared according to Mandell and Hershey (1960) by use of well-methylated albumin (Hayashi, Hayashi, and Spiegelman, 1963). The DNA samples were fractionated on single-layer MAK columns (10 ml of MAK in a 2.5-cm diameter column fitted with a sintered-glass plate) by use of a four-step elution procedure with increasing molarity of NaCl (0.4, 0.6, 0.65, and 1.25 M) in 0.05 M sodium phosphate buffer (pH 6.7). The fractionation was carried out at ambient room temperature. The effluent was monitored with an Ico, model UA, recording ultraviolet analyzer and was collected in 5-ml fractions. The absorbance at 260 $\mu\mu$ and the radioactivity of each fraction were measured with a Gilford spectrophotometer and a Nuclear-Chicago end-window gas-flow Gieger counter (model D-47), respectively.

Buoyant density (ρ) measurements. The four DNA fractions eluted from the MAK column were placed separately in dialysis tubing, and surrounded with Carbowax 4000 (Union Carbide Chemical Co., Charleston, W. Va.) in a glass cylinder. The cylinder was placed at 4 C until all but 4 to 5 ml of the liquid was removed. The contents of the dialysis tubing were dialyzed against several changes of SSC at 4 C. Portions of the dialyzed DNA samples were centrifuged to equilibrium in a Spinco model E analytical ultracentrifuge at 44,770 rev/min at 25 C for 18 to 24 hr in approximately 5.7 M CsCl-0.02 M tris (hydroxymethyl) aminomethane (Tris) buffer (pH 8.5) with N^{15} -labeled *Pseudomonas aeruginosa* DNA (ρ , 1.742 g/cc) as a reference standard. The banded DNA was photographed by use of ultraviolet-ab-

sorption optics. Photographs were traced with a Joyce-Loebl double-beam recording microdensitometer. Buoyant densities were calculated according to the equation of Sueoka (1961).

RESULTS AND DISCUSSION

Microscopic examination of mitomycin C-induced and non-induced cultures. Samples from induced and noninduced cultures were removed as indicated in Fig. 1 (A to H). Photomicrographs of these samples are shown in Fig. 2. Induced cultures continued to grow at an unaltered rate (Fig. 1), resulting in a 2.5-fold increase in cell number. The continued growth of induced lysogenic cultures was termed "residual growth" by Lwoff, Siminovitch, and Kjeldgaard (1950). Residual growth occurs in other lysogenic species of bacteria (see review by Lwoff, 1953), but at growth rates lower than those observed in non-induced cultures. In *B. megaterium* 899 (1), residual growth is a result of an increase in cell mass by elongation and not cell division (Delaporte and Siminovitch, 1952). Microscopic examination of a noninduced culture of *B. stearothermophilus* in the early logarithmic phase of growth (Fig. 2A) and in the early stationary phase of growth (Fig. 2H) shows cells in active cell division and of relatively uniform density. Cells from induced cultures were observed to elongate and contain areas of lesser density (Fig. 2C to E) prior to lysis. Cell lysis (Fig. 2F and G) is caused by the removal of the cell wall by a phage lytic enzyme, a study of which will be reported separately. In many instances, lysis occurred after the cells were immobilized on the agar film (Fig. 2F and G). Cells from UV-induced cultures also undergo these same changes before lysis.

Effect of phage induction on cellular protein, DNA, and RNA synthesis. The effect of phage induction on cellular protein, RNA, and DNA synthesis is shown in Fig. 3, 4, and 5, respectively. A comparison of the rates of protein, RNA, and DNA synthesis in MC- and UV-induced and noninduced cultures is shown in Table 1. A slight inhibition of protein synthesis occurs after induction with MC or UV, lasts for 5 to 10 min, and then proceeds at a rate identical to that in the noninduced culture (Fig. 3). Inhibition of cellular protein synthesis was not observed when cell mass was determined by the turbidimetric method (Fig. 1). RNA synthesis was not affected by induction with MC (Fig. 4). These results are similar to those reported by Siminovitch and Rapkine (1952) where RNA synthesis in an induced lysogenic culture of *Bacillus megaterium* paralleled the rate of growth. RNA synthesis in a UV-induced culture of *B. stearothermophilus* occurs in two stages. The rate of RNA synthesis

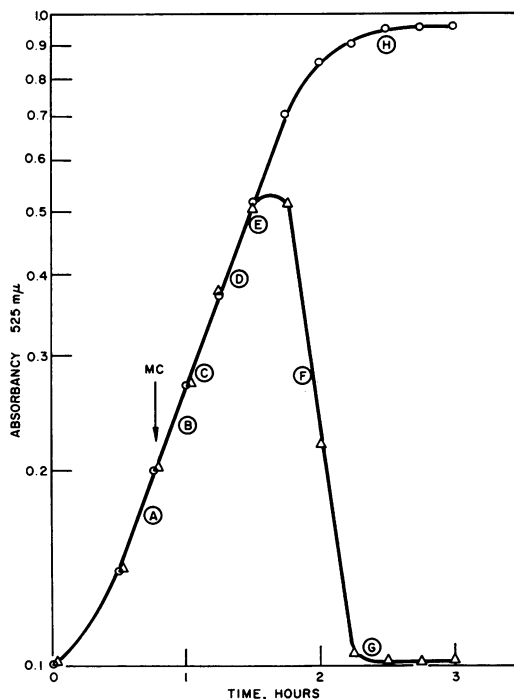


FIG. 1. Growth of mitomycin C-induced and non-induced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Cultures were grown in a TYF medium at 55 C. Samples (0.01 ml) were removed as indicated by the letters (A to H) and observed under phase contrast. Mitomycin C (0.05 $\mu\text{g}/\text{ml}$)-induced culture, Δ ; noninduced culture, \circ .

during the first stage is decreased by a factor of three immediately after induction and lasts for a period of 15 min. The second stage of RNA synthesis is rapidly attained and then proceeds at a rate identical to that in the noninduced culture. The synthesis of DNA in MC- or UV-induced cultures occurs in two stages (Fig. 5). The first stage of DNA synthesis in the MC-induced culture proceeds at a rate of a little over one-half that observed in the noninduced culture, and lasts for a period of 25 to 30 min. In the second stage, DNA synthesis commences at a rate approximately three times that observed in the noninduced culture. The first stage of DNA synthesis in a UV-induced culture lasts for 15 to 20 min and at a rate one-third that observed in the noninduced culture. In the second stage, DNA synthesis commences at a rate of almost three times that observed in the noninduced culture. These results are similar to those reported by Siminovitch and Rapkine (1952), who showed that DNA synthesis in an induced lysogenic culture of *B. megaterium* occurs in two stages. Dur-

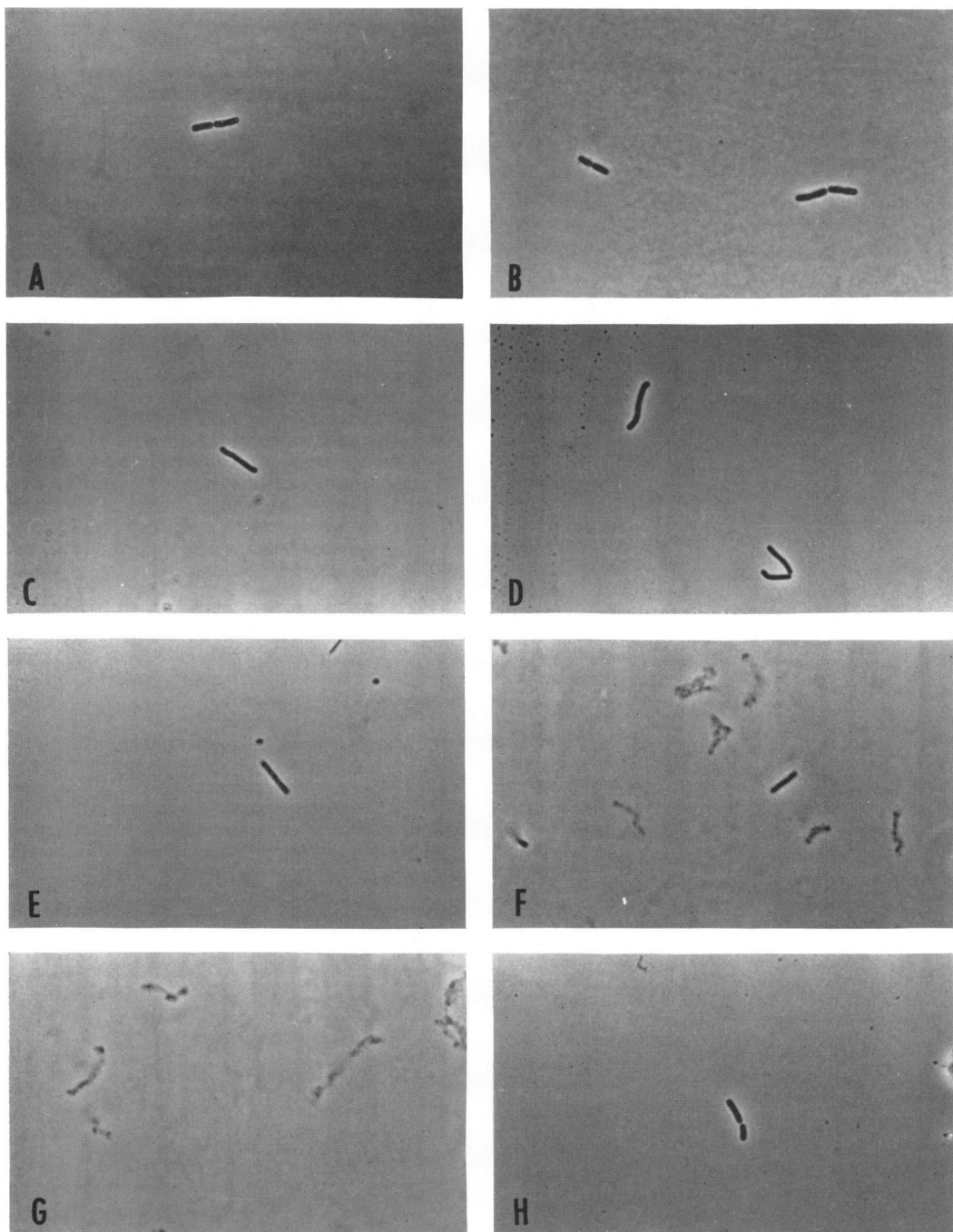


FIG. 2. Photomicrographs of cells from mitomycin C-induced and noninduced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Experimental procedure the same as described under Fig. 1. Samples (0.01 ml) were placed on glass microscope slides which were coated with a thin film of 2% agar, covered with a cover slip, and the slides were observed under phase contrast by use of a Zeiss photomicroscope. Photographs were recorded on Adox KB-14 film. Cells from mitomycin C-induced culture, B to G; cells from noninduced culture, A and H.

TABLE 1. Comparison of the rates of protein, deoxyribonucleic acid, and ribonucleic acid synthesis in mitomycin C and ultraviolet light-induced and noninduced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1)*

Culture	Protein doublings/hr†	RNA doublings/hr	DNA doublings/hr
Noninduced	1.57	1.71	1.66
Induced			
Mitomycin C	1.57	1.71, 1.71‡	0.92, 4.44
Ultraviolet light	1.57	0.58, 1.71	0.53, 4.00

* Data calculated from Fig. 3, 4, and 5.

† Rates reported as the number of doublings in quantity per hour.

‡ Numbers represent first and second stages.

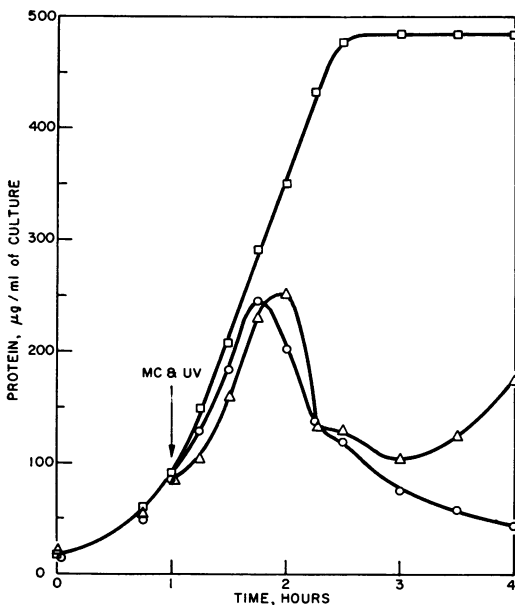


FIG. 3. Cellular protein synthesis in mitomycin C and ultraviolet light-induced and noninduced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Cultures were grown in a TYF medium at 55 C. Induction with mitomycin C (0.05 µg/ml) or ultraviolet light (General Electric germicidal lamp at a distance of 50 cm for 6 min) was carried out as indicated by the arrow. Samples (5 ml) were removed at the indicated times, and the cells were removed by centrifugation. The pellets were suspended in 5 ml of cold 5% trichloroacetic acid and left in an ice bath for 4 hr. The precipitate was removed by centrifugation, washed once with 2 ml of 0.5 N HClO₄, and suspended in 5 ml of 0.5 N HClO₄. The tubes, containing the suspended cells, were heated at 90 C in a water bath for 20 min, cooled to ambient room temperature, and centrifuged. The supernatant fluids were decanted and used for the determination of ribonucleic acid and deoxyribonucleic acid, and the pellets were assayed for protein. The results are reported as micrograms of protein per milliliter of culture. Noninduced culture, □; mitomycin C-induced culture, ○; ultraviolet light-induced culture, △.

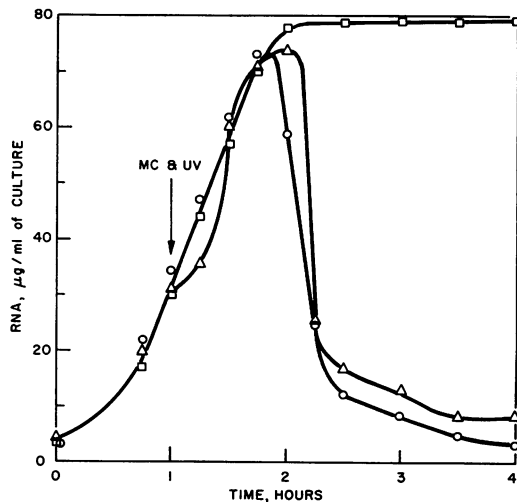


FIG. 4. Ribonucleic acid synthesis in mitomycin C- and ultraviolet light-induced and noninduced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Experimental conditions the same as described in Fig. 3. The results are reported as micrograms of RNA per milliliter of culture. Noninduced culture, □; mitomycin C-induced culture, ○; ultraviolet light-induced culture, △.

ing the first stage, net DNA synthesis was completely inhibited for a period of 30 min, followed by the second stage where DNA synthesis proceeded at a rate greater (1.2 to 1.4 times) than that observed in the noninduced culture.

UV induction appears to have a greater inhibitory effect on cellular protein, RNA, and DNA synthesis as well as on phage yield [MC induction, 6×10^4 plaque-forming units (PFU)/ml; UV induction, 4×10^4 PFU/ml] than does MC induction. In all subsequent studies, MC was used as the inducing agent.

Effect of phage induction on the synthesis of an extracellular α -amylase. Welker and Campbell (1963a) showed that this strain of *B. stearothermophilus* produces an extracellular α -amylase during the logarithmic phase of growth, and that it is

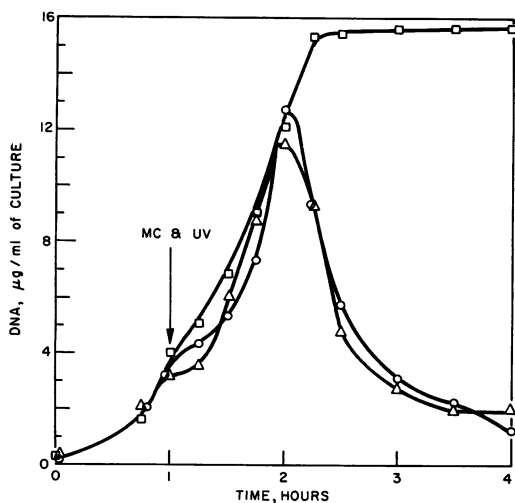


FIG. 5. Deoxyribonucleic acid synthesis in mitomycin C- and ultraviolet light-induced and non-induced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Experimental conditions the same as described in Fig. 3. Results are reported as micrograms of DNA per milliliter of culture. Noninduced culture, \square ; mitomycin C-induced culture, \circ ; ultraviolet light-induced culture, Δ .

partially constitutive with respect to α -amylase synthesis (Welker and Campbell, 1963b). The effect of MC on the constitutive and inducible differential rate (K) of α -amylase synthesis is shown in Fig. 6. The inducible and constitutive K values are inhibited by 75 and 100%, respectively, for a period of 20 min after MC treatment, followed by K values identical to those in cultures not treated with MC. Table 2 shows that the decreased synthesis of inducible and constitutive α -amylase lasts for a period of 20 min. This time period corresponds to that observed for phage DNA synthesis and the inhibition of host DNA synthesis (see Fig. 5 and 8). If pure maltose is added at the same time the culture is treated with MC, the inducible K value is not attained until after a 20-min period of 100% inhibition in α -amylase synthesis. If pure maltose is added 25 min after MC treatment, the inducible K value is immediately attained. The constitutive K value is inhibited (100%) for a period of 20 min, irrespective of the time of MC addition. These results show that α -amylase synthesis is inhibited for a period of 20 min after MC addition.

Chromatographic fractionation of DNA on MAK columns. The difference in guanine plus cytosine (G + C) composition between *B. stearothermophilus* DNA (50%) and TP-1 phage DNA

(42%) shown previously (Welker and Campbell, 1965) makes it possible to separate these two DNA preparations on MAK columns. Figure 7 shows an elution diagram from a MAK column of a partially purified DNA sample obtained from a culture induced with mitomycin C (MC) for 40 min. Buoyant density measurements on the DNA fractions eluted from the column were determined, and the results are shown in Table 3 along with buoyant densities of purified bacterial and phage DNA fractionated on MAK columns. Bacterial and phage DNA elute from the MAK column with 0.6 and 0.65 M NaCl, respectively. The material eluting with 0.4 M NaCl contained RNA, protein, and DNA of low molecular weight. This finding is similar to that reported by Mandell and Hershey (1960) for a mixture of *Escherichia coli* and phage T2 DNA where the initial effluent contained DNA of low molecular weight and soluble RNA. DNA eluting with 1.25 M NaCl is made up of denatured bacterial and phage DNA having buoyant densities of 0.008 and 0.007 g/cc,

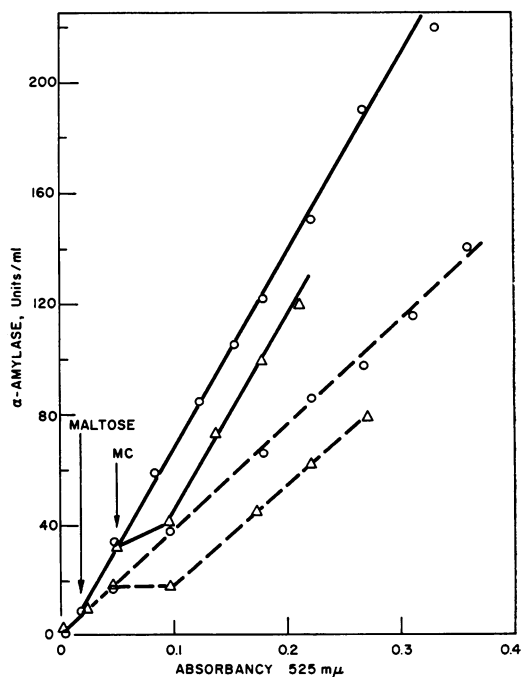


FIG. 6. Effect of mitomycin C on the constitutive and inducible differential rate of α -amylase synthesis in cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Cultures were grown in a MCHF medium at 55 C. Pure maltose (α -amylase induction) and mitomycin C (phage induction) were added as indicated by the arrows. Solid lines, pure maltose (10^{-3} M); dashed lines, no maltose. Mitomycin C (0.05 μ g/ml), Δ ; no mitomycin C, \circ .

respectively, heavier than the native DNA's. Sueoka and Cheng (1962) showed that denatured *E. coli* DNA elutes at a higher NaCl concentration than does native DNA. When the DNA fractions were rechromatographed on MAK columns, they eluted at the position corresponding to that from which they were taken. The recovery of partially purified DNA (radioactivity, counts per

TABLE 2. Effect of mitomycin C on inducible and constitutive synthesis of α -amylase in cultures of *Bacillus stearothermophilus* 1503-4R (TP-1)

Time	Inducible α -amylase		Constitutive α -amylase	
	Mitomycin C*	No mitomycin C	Mitomycin C	No mitomycin C
min	units/ml	units/ml	units/ml	units/ml
0	38.7	39.0	18.0	18.8
15	45.1	59.3	18.5	38.3
20	53.5	75.8	18.7	50.5
30	75.0	108.9	40.5	63.8
40	87.0	120.8	58.0	80.5
50	120.3	145.6	73.7	93.6
60	157.0	187.5	83.4	112.7

* Mitomycin C (0.05 μ g/ml) added after the zero-time sample.

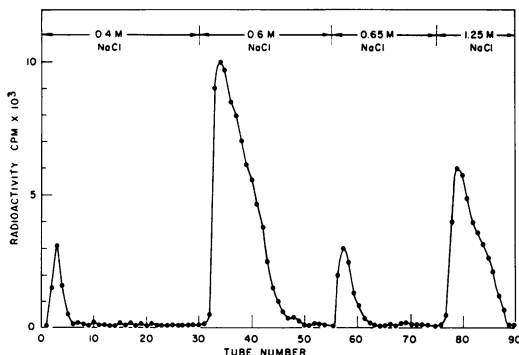


FIG. 7. Chromatographic fractionation of partially purified deoxyribonucleic acid obtained from a mitomycin C-induced culture of *Bacillus stearothermophilus* 1503-4R (TP-1). Partially purified DNA was prepared, as described in the text, from cultures grown in a TYF medium at 55 C and induced with mitomycin C for 40 min. The MAK column was loaded with 0.5 mg of partially purified DNA. Fractionation was accomplished by use of a four-step elution procedure with increasing molarities of NaCl (0.4, 0.6, 0.65, and 1.25 M) in 0.05 M sodium phosphate buffer (pH 6.7). The fractionation was carried out at ambient room temperature. The effluent was collected in 5-ml fractions, and the radioactivity of each fraction was measured with a Nuclear-Chicago end-window gas-flow Gieger counter.

TABLE 3. Buoyant densities of DNA fractions eluted from methylated-albumin-Kieselguhr columns

DNA	NaCl concn in elution buffer	Buoyant density
	M	g/cc
Partially purified DNA*	Culture of <i>Bacillus stearothermophilus</i> 1503-4R	—
	(TP-1) induced with MC for 40 min	1.713
		1.706
		1.713, 1.721
Purified DNA†	<i>B. stearothermophilus</i> 1503-4R (TP-1)	1.713
		1.721
	TP-1 phage	1.706
		1.713

* The partially purified DNA sample was prepared and fractionated on a MAK column as described under Fig. 7.

† Purified bacterial and phage DNA were prepared as described by Welker and Campbell (1965) and fractionated on MAK columns as described in the text.

minute) from MAK columns in all experiments was 65 to 76%, with 5 to 10% and 25 to 36% eluting with 0.4 and 1.25 M NaCl, respectively. The per cent recovery of DNA was not affected by the amount of material placed on the column as long as the capacity of the column (0.8 mg of DNA per 10 ml of MAK) was not exceeded.

The amount of bacterial and phage DNA in the 1.25 M NaCl eluate was estimated from the area within the relevant band of the microdensitometer tracing made from the UV-absorption photograph. The quantity of denatured bacterial and phage DNA was found to vary in proportion to the total amount of each type of DNA in the sample.

Purified bacterial and phage DNA, when fractionated on MAK columns, elute with 0.6 and 0.65 M NaCl, respectively, with 100% recovery of the starting material. Of the total bacterial and phage DNA, 10 and 45%, respectively, elute with 1.25 M NaCl. Purified bacterial DNA, when prepared by the procedure described for the preparation of purified phage DNA (phenol extraction), contains a 40% fraction that elutes with 1.25 M NaCl. These results indicate that denaturation of DNA is a result of the use of phenol in the extraction procedure.

The input DNA which could not be recovered from the MAK columns (24 to 35%) is assumed to consist of both denatured bacterial and phage DNA in ratios identical to those observed in the 1.25 M NaCl eluate.

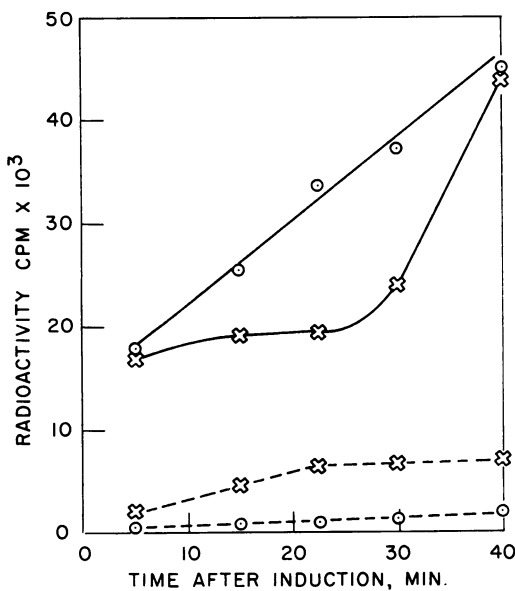


FIG. 8. Synthesis of bacterial and phage deoxyribonucleic acid in mitomycin C-induced and non-induced cultures of *Bacillus stearothermophilus* 1503-4R (TP-1). Partially purified DNA samples were prepared from mitomycin C-induced and non-induced cultures and fractionated on MAK columns as described in the text. The total bacterial and phage DNA in each sample was determined from the radioactivity in the 0.6 and 0.65 M NaCl eluates. Solid lines, bacterial DNA; dashed lines, phage DNA. Noninduced culture, O; mitomycin C (0.05 μ g/ml)-induced culture, X.

The synthesis of bacterial and phage DNA was followed after MC induction, and the incorporation of radioactivity into each type of DNA is shown in Fig. 8. The synthesis of phage DNA occurs rapidly during the first stage and is complete within 25 min after MC induction, whereas bacterial DNA is either degraded or its rate of synthesis is decreased. In the second stage, only bacterial DNA is synthesized. In support of this is the finding that the synthesis of mature phage is complete 30 min after induction. In the non-induced culture, the synthesis of phage DNA (1 phage per 2.8×10^6 cells) occurs at a low rate and has no effect on the synthesis of bacterial DNA.

The induction of phage TP-1 results in the inhibition of net synthesis of host DNA. Bacterial DNA synthesis does not resume until phage DNA synthesis is complete (25 min after MC induction). Siminovitch and Rapkine (1952) implied that during the first stage of DNA synthesis (no

net increase in DNA) in an induced lysogenic culture of *B. megaterium*, the host DNA is being continuously degraded and that the synthesis of phage DNA compensates for this loss. In the second stage of DNA synthesis, however, these authors suggest that the observed increase in DNA is a result of bacteriophage multiplication. In induced cultures of *B. stearothermophilus*, the increase in the rate of synthesis of DNA during the second stage is due to bacterial DNA synthesis and not to phage DNA synthesis. We are unable to say at this time why bacterial DNA synthesis resumes at a rate greater than that observed in the noninduced culture.

ACKNOWLEDGMENTS

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