

Development of Coliphage T5: Ultrastructural and Biochemical Studies¹

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Electron microscopic studies of *Escherichia coli* infected with bacteriophage T5⁺ have revealed that host nuclear material disappeared before 9 min after infection. This disappearance seemed to correspond to the breakdown of host deoxyribonucleic acid (DNA) into acid-soluble fragments. Little or no host DNA thymidine was reincorporated into phage DNA, except in the presence of 5-fluorodeoxyuridine (FUdR). Progeny virus particles were observed in the cytoplasm 20 min postinfection. Most of these particles were in the form of hexagonal-shaped heads or capsids, which were filled with electron-dense material (presumably T5 DNA). A small percentage (3 to 4%) of the phage heads appeared empty. On rare occasions, crystalline arrays of empty heads were observed. Nalidixic acid, hydroxyurea, and FUdR substantially inhibited replication of T5 DNA. However, these agents did not prevent virus-induced degradation of *E. coli* DNA. Most of the phage-specified structures seen in T5⁺-infected cells treated with FUdR or with nalidixic were in the form of empty capsids. Infected cells treated with hydroxyurea did not contain empty capsids. When *E. coli* F was infected with the DO mutant T5 amH18a (restrictive conditions), there was a small amount of DNA synthesis. Such cells contained only empty capsids, but their numbers were few in comparison to those in cells infected under permissive conditions or infected with T5⁺. The cells also failed to lyse. These results confirm other reports which suggest that DNA replication is not required for the synthesis of late proteins. The data also indicate that DNA replication influences the quantity of viral structures being produced.

The developmental cycle of bacteriophage T5 has not been as extensively studied as that of T-even coliphages. Due largely to the work of Lanni and her associates (28, 37), it is known that, upon infection, only 8% of the viral deoxyribonucleic acid (DNA) is injected into the host. This segment of T5 DNA (first-step-transfer DNA) is transcribed immediately into a class of ribonucleic acid (RNA; class I RNA) (40, 60) from which class I proteins presumably are translated (36). Included in class I proteins are the species responsible for the degradation of host DNA (27), the inhibition of host and class I functions (26, 36, 40, 60), and the transfer of the remaining 92% of T5 DNA from the phage into the host (25). Injection of the phage DNA is completed about 2 to 3 min after adsorption. This is followed by the synthesis of class II RNA (40, 60) and class II proteins (36). Time

sequence studies indicate that class II proteins include the enzymes involved in the replication of viral DNA, such as DNA polymerase (9, 44), deoxyribonucleotide kinase (3), thymidylate synthetase (2), and dihydrofolate reductase (39). The synthesis of class III RNA and class III proteins begins between 8 and 14 min after infection and continues until the time of cell lysis (45 to 60 min after infection (36, 40, 60)). Class III proteins presumably are composed mainly of phage structural components (22, 36).

The present report deals with the normal developmental cycle of coliphage T5 in *Escherichia coli* F as seen with the electron microscope. Attempts to assess the role of DNA synthesis in the morphogenesis of this virus are also described.

MATERIALS AND METHODS

Strains. *E. coli* F (23) and bacteriophage T5⁺ were kindly supplied by Y. T. Lanni, University of Texas at Dallas. Coliphage T5amH18a, an amber mutant defective in gene D9, which is the structural gene for T5 DNA polymerase, was the gift of D. J. Mc-

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Corquodale, University of Texas at Dallas. *E. coli* Fcb (*pro⁻*, *su⁺*), a permissive host for T5amH18a, was donated by R. W. Moyer of Columbia University. This strain has been redesignated *E. coli* F *su β ⁺* (15). *E. coli* F was used as a restrictive host (*su⁻*) for the amber mutant. Phage stocks were derived from single plaques, and all media used for the propagation of T5 contained 10^{-3} M CaCl_2 (23). Concentrated viral preparations were kept suspended in M/15 phosphate buffer (pH 7.0) containing 2×10^{-3} M MgSO_4 (23).

Growth and infection of bacteria. Bacteria in medium MGM (24) were brought to the exponential growth phase (1.5×10^8 cells/ml), at which time they were infected with an average multiplicity of 8.

Viable bacteria were enumerated by plating serial dilutions (0.1 ml) of cell cultures on nutrient agar plates. Infectious phage was determined by the double agar-layer technique (1).

Metabolic techniques. The metabolic techniques used in this study have been described previously (50, 51, 54).

Cesium chloride density gradient centrifugation. Portions (3.0 ml) of radioactive lysates were mixed with 2.7 g of CsCl, and the specimens were spun for 65 hr at 30,000 rev/min in the SW50 rotor of a Spinco model L-2 ultracentrifuge. Three-drop fractions were collected from the bottom (11). The density of selected fractions was calculated from the refractive index (17). Portions (20 μ liter) were removed from each fraction and deposited onto filter paper discs, which were placed in 5% cold trichloroacetic acid, washed with ethanol and ether, dried, and placed in vials. The radioactivity retained by the discs was determined in 10 ml of omnifluor (4 g per liter of toluene).

Chemical analyses. Colorimetric procedures were used to determine DNA and proteins (6, 32). Standards of calf thymus DNA and bovine serum albumin were used.

Electron microscopy. Cells were fixed by mixing cultures (30 ml) with 10 ml of cold 4% glutaraldehyde (56) in 0.1 M cacodylate buffer, pH 6.8, containing 2×10^{-3} M CaCl_2 . The cells were sedimented, suspended gently in 1.5% glutaraldehyde (also in cacodylate buffer), and fixed for 2 hr at room temperature. They were washed twice in cacodylate buffer, dispersed in 1% OsO_4 in cacodylate buffer, and kept overnight at 4 C. The suspensions were then washed three times in 0.05 M maleate buffer, pH 5.1, containing 2×10^{-3} M CaCl_2 and embedded in agar (2% in maleate buffer). They were chilled, diced into small pieces, and stained with 2% uranyl acetate in 0.05 M maleate buffer, pH 6.0 (18). After standing for 2 hr at room temperature, the specimens were washed once in maleate buffer, pH 5.1, dehydrated in ethanol, and embedded in Epon 812 (33). Thin sections were mounted on uncoated grids and doubly stained with uranyl acetate and lead citrate (48).

Negative staining with 1% phosphotungstate solution adjusted to pH 6.4 with 1 N KOH was carried out on the supernatant fluids of cultures at the time of lysis. To promote lysis, a drop of 1% OsO_4 was

added to 10 ml of culture (20). Specimens were supported on carbon-coated, Formvar-covered grids.

Electron micrographs were taken with an RCA EMU-3G electron microscope.

Materials. ^3H -labeled compounds were obtained from Schwarz BioResearch, Inc., Orangeburg, N.Y., except for ^3H -thymidine, which was purchased from International Chemical and Nuclear Corp., City of Industry, Calif. ^{14}C -thymidine was purchased from New England Nuclear Corp., Boston, Mass.; nucleosides and nucleotides from Schwarz BioResearch, Inc.; calf thymus DNA from Nutritional Biochemicals Corp., Cleveland, Ohio; and hydroxyurea either from Nutritional Biochemicals Corp., or from Sigma Chemical Co., St. Louis, Mo.

5-Fluorodeoxyuridine (FUdR) was the gift of J. J. Fox, Sloan-Kettering Institute for Cancer Research, New York, N. Y.; nalidixic acid was donated by Sterling-Winthrop Institute, Rensselaer, N.Y.

A stock solution of nalidixic acid (1 mg/ml) was prepared in 0.01 N NaOH as described by Winshell (Ph.D. thesis, Columbia University, New York, N.Y., 1967) and stored at -20 C. Only freshly prepared solutions of hydroxyurea were used.

RESULTS

Developmental cycle of bacteriophage T5⁺. Figure 1 illustrates uninfected *E. coli* F from the exponential phase of growth. The cytoplasm contains characteristically dense ribosomes; the nuclear material consists of fine fibrils. The result of T5⁺-induced degradation of host DNA can be seen in cells fixed 9 min postinfection (Fig. 2). The nuclear material has entirely disappeared, a phenomenon previously observed both by light (35, 41) and electron microscopy (19). By 20 min after infection (Fig. 3), fine fibrillar material has reappeared and probably represents newly synthesized viral DNA. A few phage heads are already visible. The maximum number of phage heads observed at this stage was about 10 per cell. Almost all of the phage heads are filled with electron-dense material, presumably T5 DNA. Just before lysis (60 min postinfection, Fig. 4, 5), as many as 100 or more virus particles are seen in the cytoplasm. Although the majority of head structures are filled, a small percentage (3 to 4%) appear to be empty or partially filled. On rare occasions, crystalline arrays of empty heads were seen (Fig. 6). Similar crystals composed of heads devoid of electron-dense material have been observed in phage-infected streptococci (7) and lactobacilli (10). Electron-dense heads were never seen in crystalline arrays during T5⁺ development. Crystalline aggregates of full heads or complete phage particles have been described in *E. coli* infected with phages f2 (59), T2 (19, 21), and N4 (58).

Effect of FUdR and nalidixic acid. When T5⁺-infected *E. coli* were treated with either FUdR

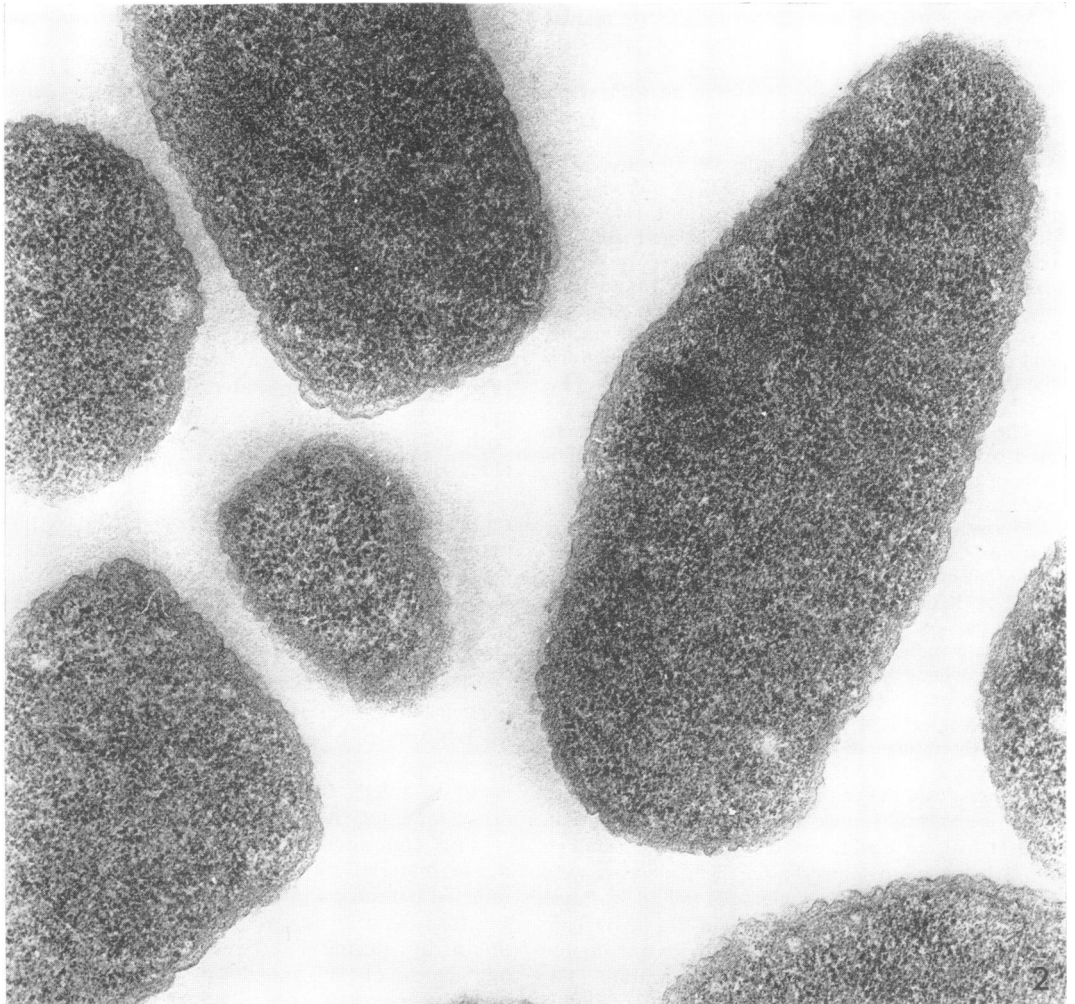
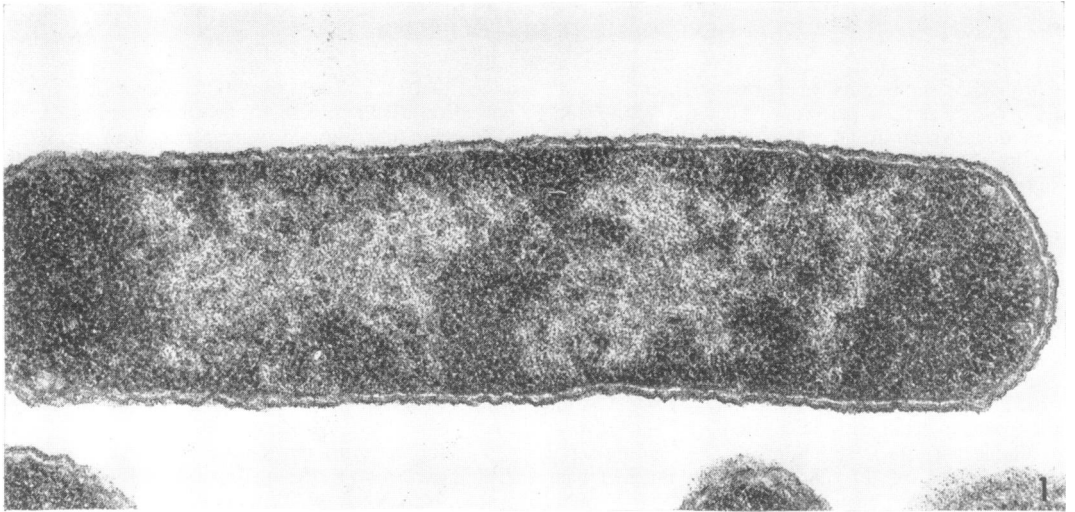


FIG. 1. Uninfected *E. coli* F fixed sequentially in glutaraldehyde, osmium tetroxide, and uranyl acetate. The bacterial wall is slightly separated from the underlying cytoplasmic membrane. The cytoplasm contains densely packed ribosomes and nuclear material consisting of the fine fibrils. 57,000 \times

FIG. 2. Bacteria 9 min after $T5^+$ infection. The cytoplasm of the cells contains ribosomes but is devoid of nuclear material. 57,000 \times

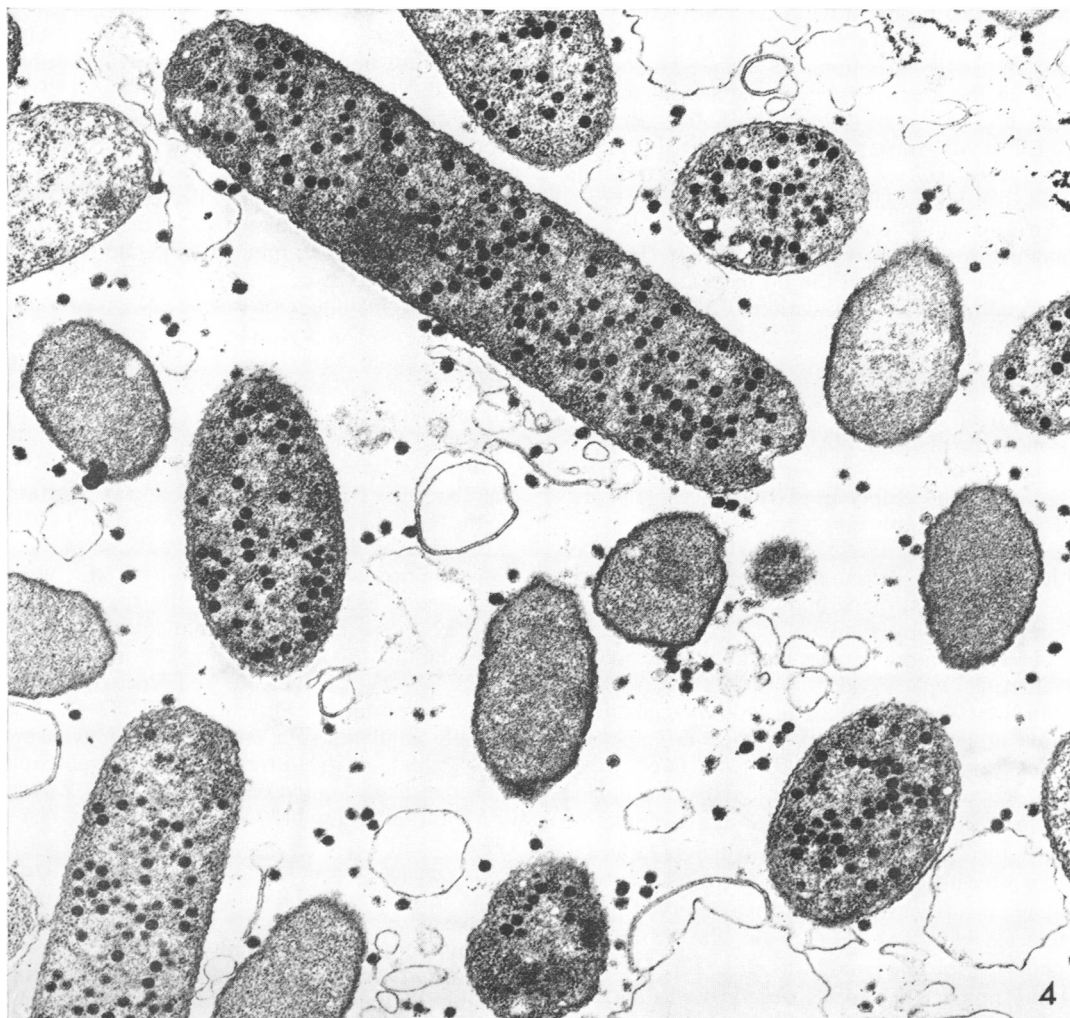
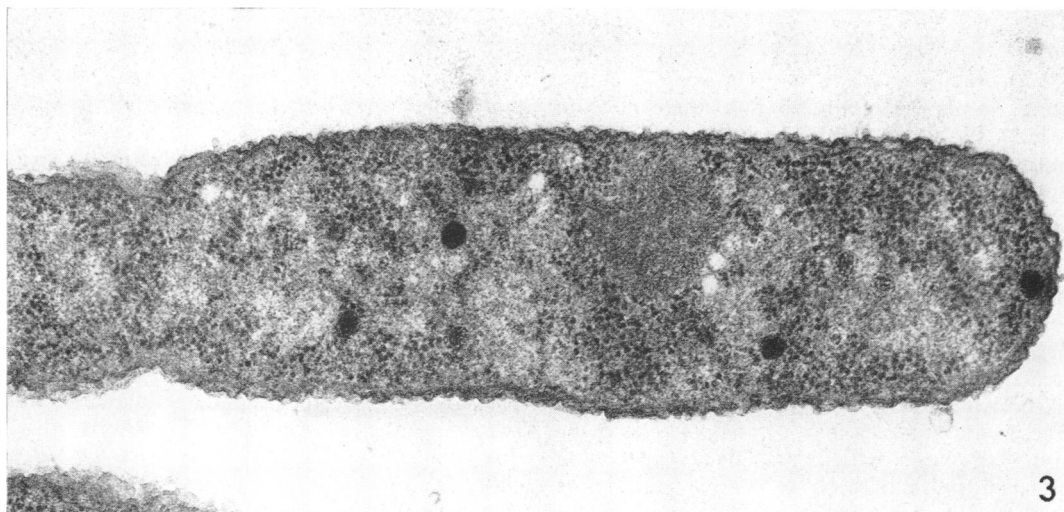


FIG. 3. *E. coli* F 20 min after infection. Fibrillar material has replaced some of the ribosomes in certain areas of the cytoplasm. A few viral heads have been assembled. 57,000 \times

FIG. 4. T5⁺-infected *E. coli* F at the stage of cell lysis (60 min after infection). Phage at various stages of development are seen. Remnants of membranes and walls are lodged between unlysed cells. 31,000 \times

(50 $\mu\text{g/ml}$) or nalidixic acid (10 $\mu\text{g/ml}$), empty phage heads were seen 60 min after infection (Fig. 7, 8). These unfilled heads never appeared in crystalline arrays as they sometimes did in untreated cells. A few full heads were also encountered, suggesting that some synthesis of phage DNA occurred in cells exposed to these agents. No nuclear material was seen in these cells, suggesting that degradation of cellular DNA had occurred. It will be noted in the negatively stained preparation of the FudR-treated cell illustrated in Fig. 8 that most of the particles released are empty heads lacking tails. These viral particles may be compared with the complete phage particles (i.e., full capsids with attached tails) released by untreated cultures (Fig. 9). When infected *E. coli* were exposed to a high concentration of nalidixic acid (100 $\mu\text{g/ml}$), no phage structures were seen in the cytoplasm 60 min after infection (*unpublished data*), although the absence of nuclear material revealed that breakdown of cellular DNA had occurred.

Effect of hydroxyurea. Cells treated with hydroxyurea (0.2 M) at the time of infection contained no intracellular phage particles, either 60 min after infection (*unpublished data*) or when incubation was extended to 120 min (Fig. 10). Since the cells lacked the fibrils characteristic of nuclear material, degradation of the DNA presumably had taken place. In this respect, the cells resembled those exposed to 100 μg of nalidixic acid per ml. When hydroxyurea was added 20 min after infection [at which time the cells synthesize phage DNA and structural proteins (Fig. 3)] and the cells were fixed 40 min later, both full and empty heads were seen (Fig. 11). There were more empty capsids in these cells than in untreated cells fixed 20 min after infection (Fig. 3). Apparently some unfilled heads were assembled or perhaps synthesized after the addition of hydroxyurea.

Ultrastructural studies with amber mutant. When *E. coli* F $\text{su}\beta^+$ was infected with T5 amH18a (permissive condition), the cells at the time of lysis (55 min after infection) appeared (Fig. 12) very much like *E. coli* F infected with T5⁺ (Fig. 5). On the other hand, *E. coli* F infected with T5 amH18a (restrictive condition) contained only empty heads when examined 60 min after infection (Fig. 13). The total number of phage heads was less than that observed in cells infected with T5⁺ (Fig. 5) or with T5 amH18a under permissive conditions (Fig. 12). The maximum number of capsids observed was about 20 per cell. Many cells contained no particles at all, but nuclear material was absent, which suggested that degradation of host DNA had taken place.

Effect of inhibitors on virus production. Although the burst size of T5⁺ growing on *E. coli* F was about 300 (38 min after infection, Fig. 14), there was no net production of virus in hydroxyurea- or FudR-treated cells; cells exposed to nalidixic acid (10 $\mu\text{g/ml}$) gave a small burst of about 7 plaque-forming units (PFU) per infected cell. Disrupting the cultures with chloroform 120 min after infection did not increase the number of PFU, indicating the absence of infectious intracellular particles. A control experiment showed that exposure of cell-free T5⁺ to inhibitors for as long as 120 min did not change the infectious titer. The effects of hydroxyurea on phage production were reversible (Fig. 15). Thus a 1:100 dilution of infected cells into drug-free medium 25 min after infection resulted in a normal burst (250 PFU per cell).

Virus production in cells infected with DO mutant. Infection of *E. coli* F with the amber mutant did not result in lysis of the culture (Fig. 16) or in production of progeny phage (Fig. 17). On the other hand, lysis did occur when *E. coli* F $\text{su}\beta^+$ was the host. This was accompanied by a burst of about 50 PFU per infected cell 38 min after infection. Treatment of T5-infected *E. coli* F with chloroform 60 min after infection did not reveal the presence of intracellular phage.

Effect of inhibitors on viral DNA synthesis. By using ³H-deoxycytidine as a precursor of DNA, it could be shown (Fig. 18) that phage DNA synthesis began about 10 min after infection and continued until lysis (50 min after infection). This confirmed the pattern of net DNA synthesis as measured colorimetrically (8, 27, 45). Nalidixic acid (10 $\mu\text{g/ml}$) inhibited incorporation of the radioactive label by 92%, and FudR (50 $\mu\text{g/ml}$) by 98%. Inhibition in excess of 99% was obtained with hydroxyurea (0.2 M). When these agents were added after the beginning of DNA synthesis (i.e., 21 to 22 min after infection), they inhibited further DNA replication (Fig. 19), thus indicating that they blocked processes necessary for continued synthesis of T5 DNA. Essentially similar results were obtained by using ³H-thymidine or measuring net DNA production colorimetrically (*unpublished data*; M. Zweig, Ph.D. thesis, Columbia Univ., New York, N.Y., 1970).

T5⁺-induced breakdown of *E. coli* DNA. Degradation of *E. coli* DNA induced by T5⁺ was examined by prelabeling cellular DNA with ¹⁴C-thymidine and determining the amount of radioactivity remaining acid-insoluble after infection. In untreated, T5⁺-infected cells, about 90% of the cellular DNA was rendered acid-soluble during the first 6 min of infection (Fig. 20). After degradation of host DNA, the level of

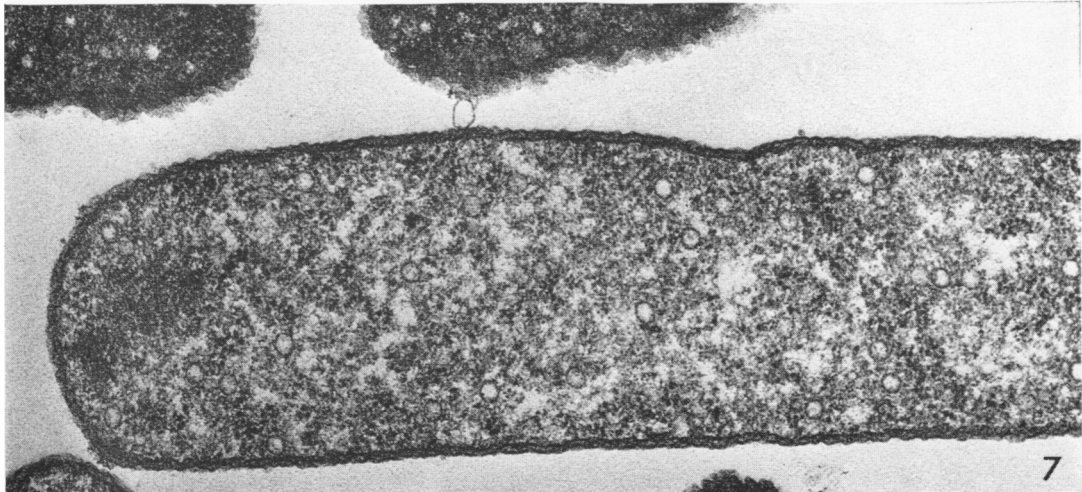
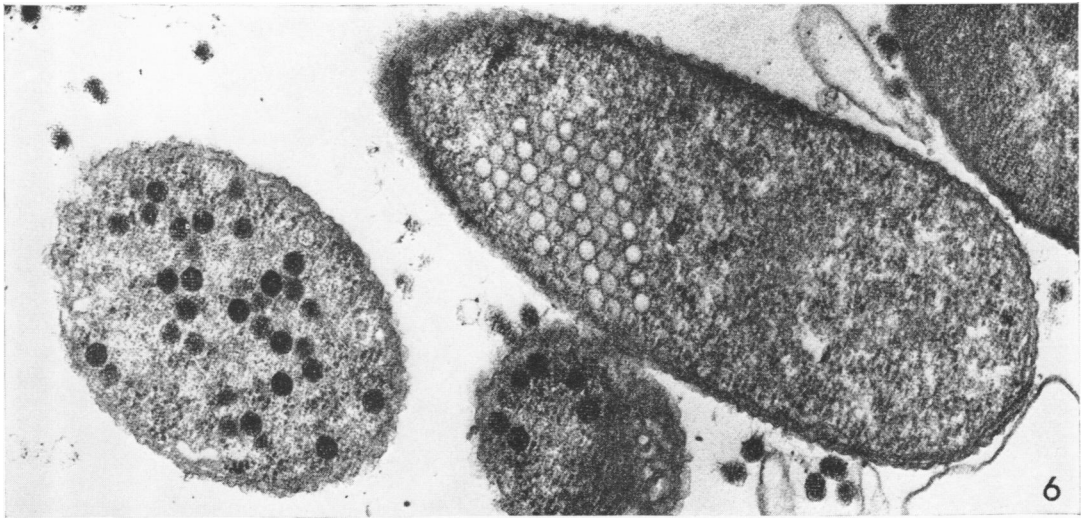
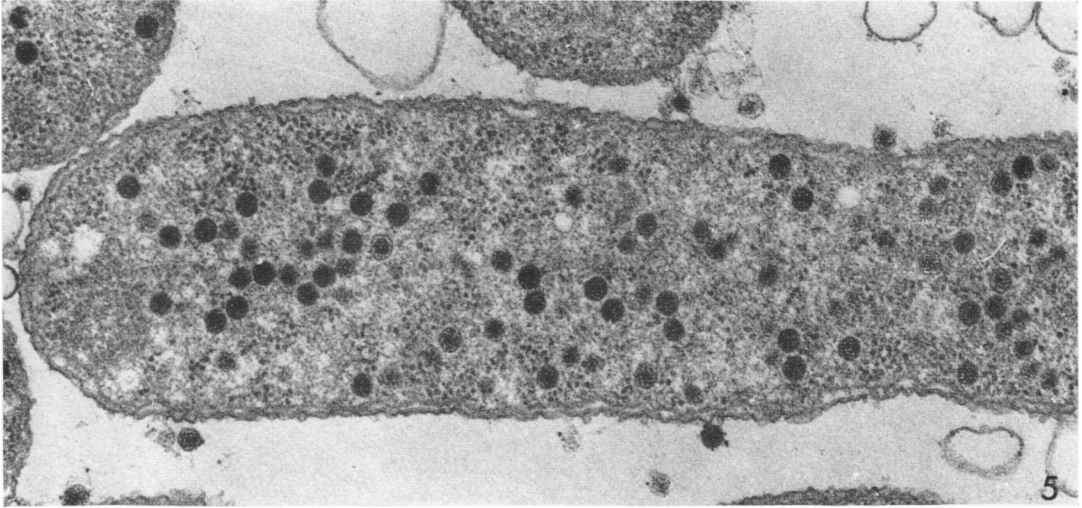


FIG. 5. *E. coli* F 60 min after $T5^+$ infection. Numerous phage heads are visible. Most contain electron-dense material, but occasional empty heads are seen as well. 57,000 \times

FIG. 6. A crystalline array of empty heads at 60 min after infection with $T5^+$. 57,000 \times

FIG. 7. Cells 60 min after infection in the presence of FUdR. The cytoplasm contains empty head forms. The ribosomes are smaller and less densely packed than in untreated cells. 57,000 \times

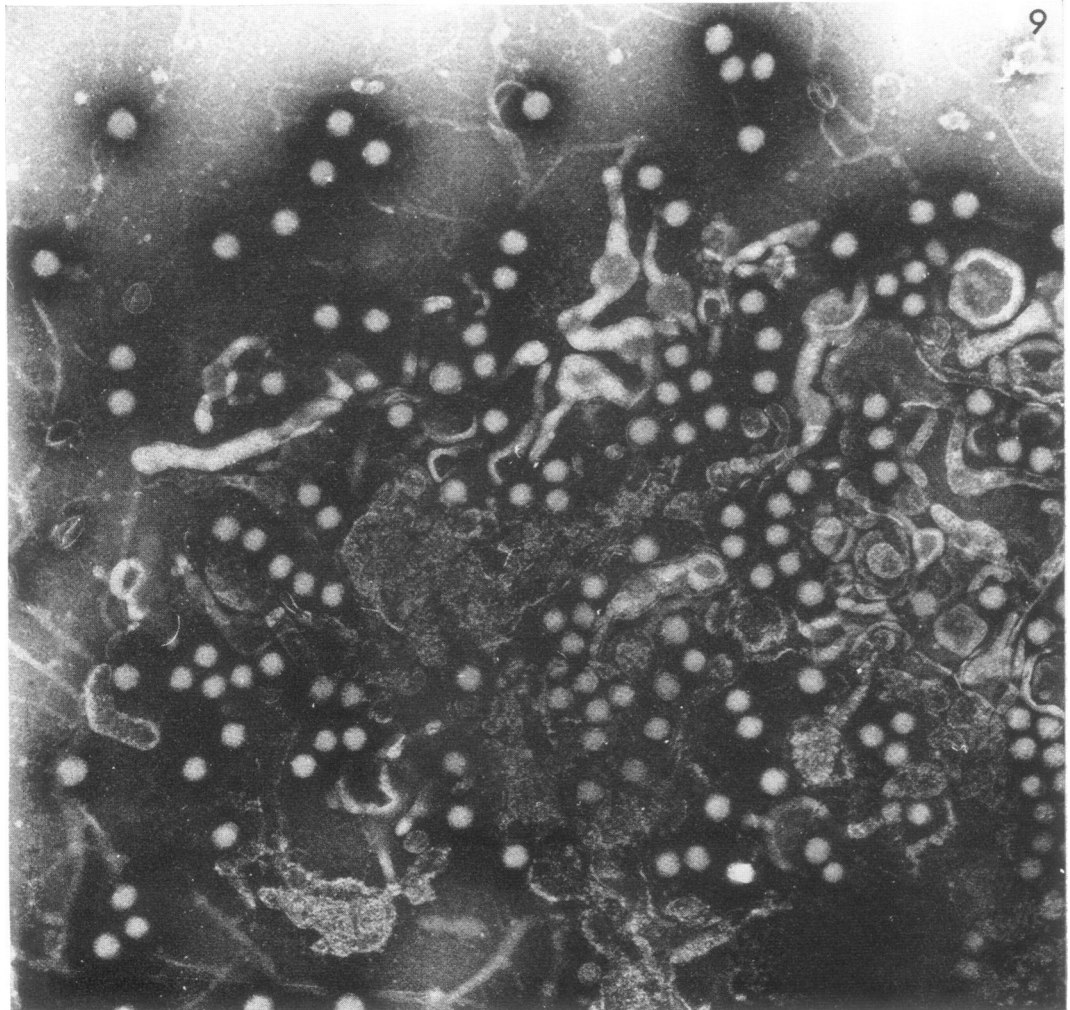
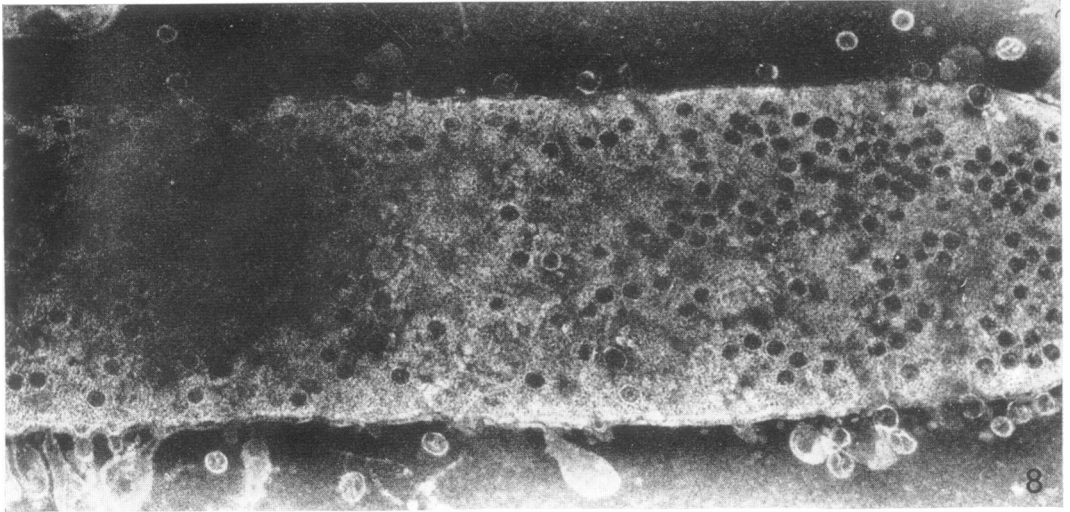


FIG. 8. A negatively stained cell which has been infected in the presence of FUdR. There are numerous, mostly empty heads devoid of tails. 57,000 \times

FIG. 9. A negatively stained cell at the moment of lysis. The phage particles ($T5^+$) in process of release consist of full heads and attached tails. 57,000 \times

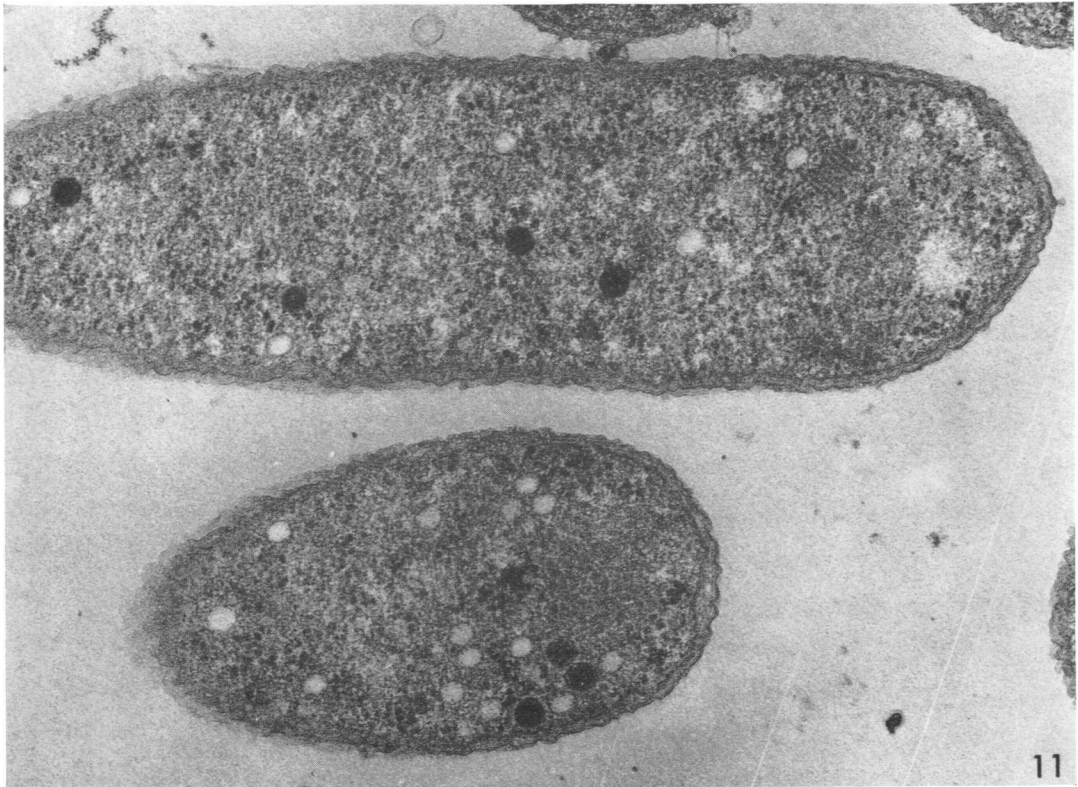
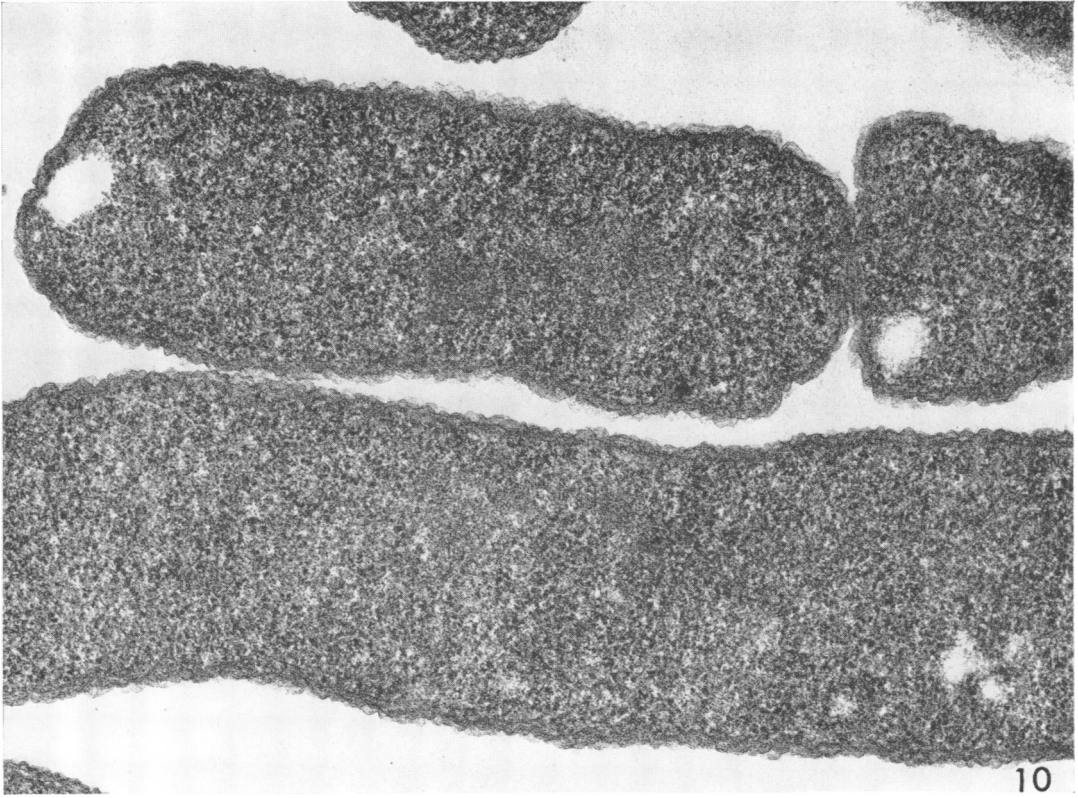


FIG.10. *Hydroxyurea*-treated *E. coli* F 120 min after $T5^+$ infection. Phage particles are not seen in the cytoplasm. 57,000 \times

FIG. 11. *E. coli* F 60 min after infection with $T5^+$; *hydroxyurea* was added 20 min postinfection. Cells contain a mixture of full and empty heads. 60,000 \times

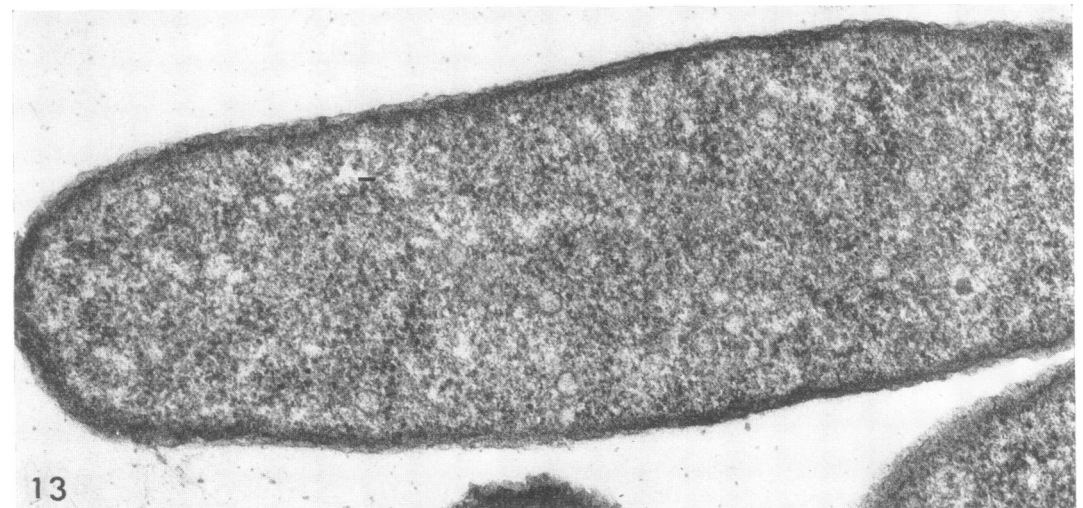
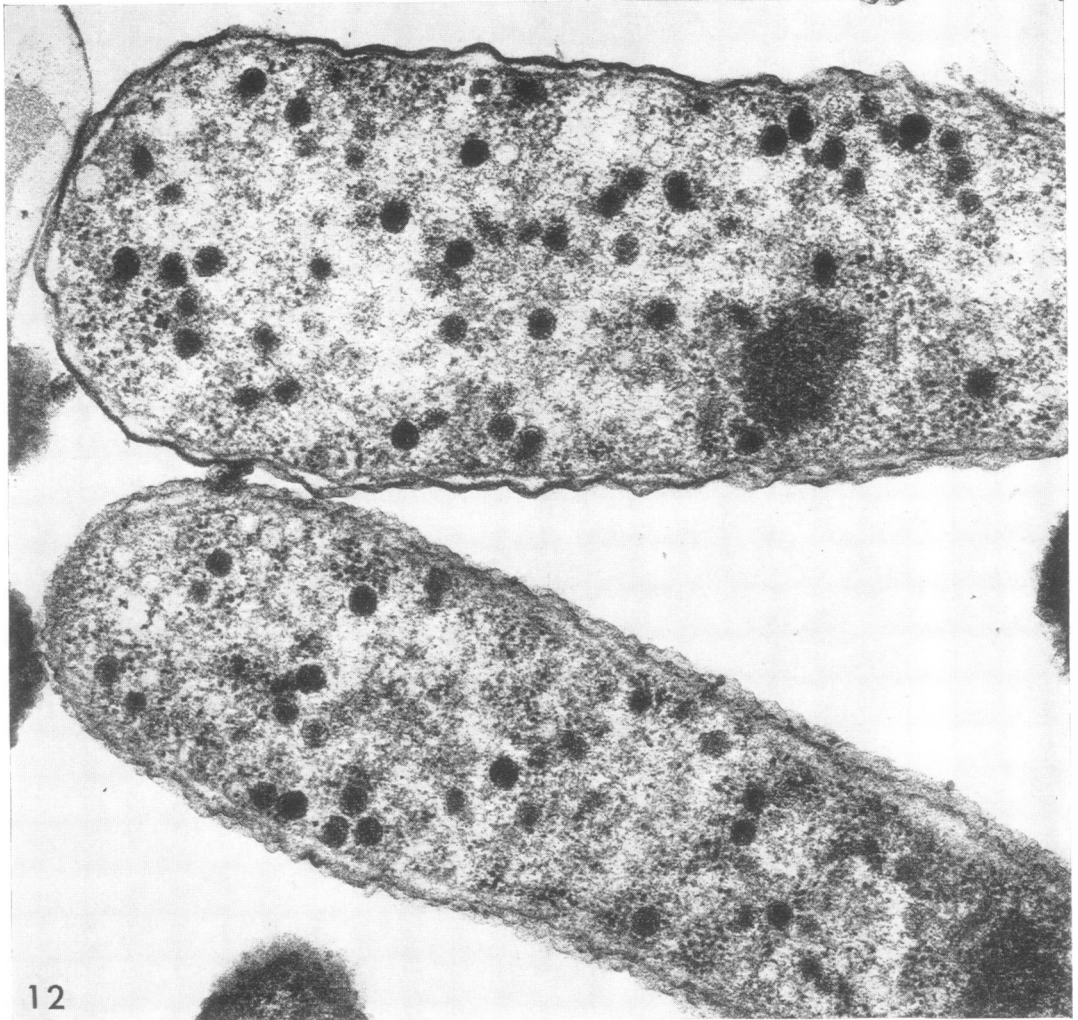


FIG. 12. *E. coli* $F_{su\beta}^+$ 55 min after infection (time of lysis) with T5 amH18a. These cells contain phage particles which are mostly in the form of full capsid structures. The separation of the cell wall from the underlying cell membrane indicates that the cell is near lysis. 57,000 \times

FIG. 13. *E. coli* F 60 min after infection with T5 amH18a. A few unfilled capsids are seen in the cytoplasm. The total number of phage structures in this cell is considerably less than that seen in T5 amH18a infection under permissive conditions (Fig. 12) or T5 $^+$ infection (Fig. 4, 5). 57,000 \times

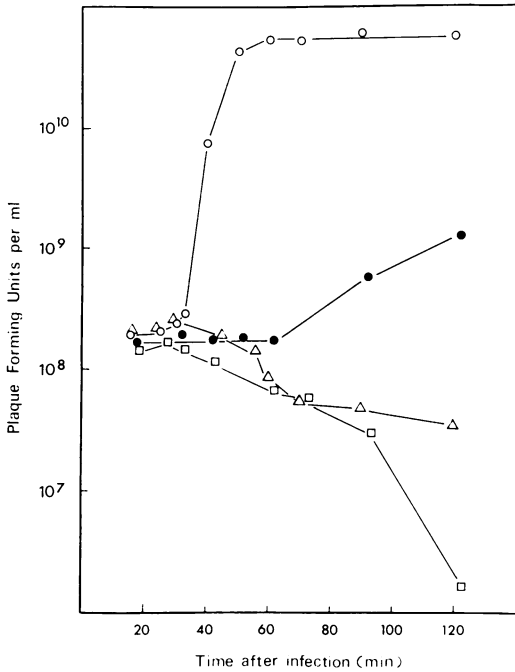


FIG. 14. Effect of inhibitors of DNA synthesis on $T5^+$ multiplication. Cells were infected at a multiplicity of 8. Five minutes after infection, portions of infected cultures were diluted 100-fold into flasks containing inhibitors. Further dilutions were made to insure that the number of plaques was between 25 and 250 (1). Symbols: \circ , control; \bullet , nalidixic acid (10 $\mu\text{g/ml}$); Δ , FUDR (50 $\mu\text{g/ml}$); \square , hydroxyurea (0.2 M).

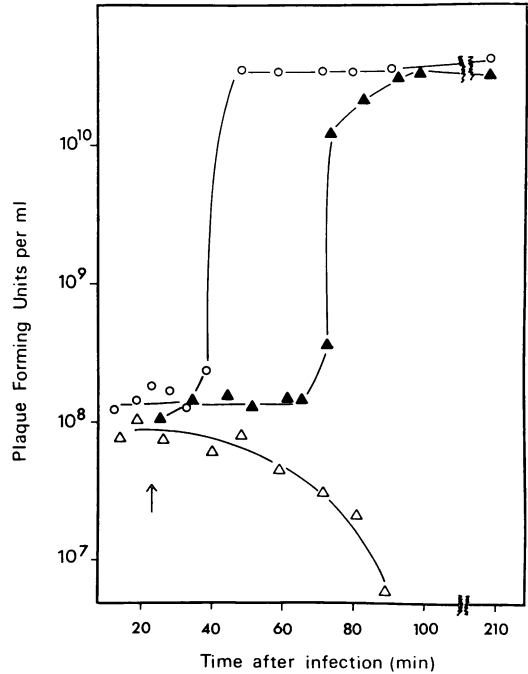


FIG. 15. Reversible effect of hydroxyurea on $T5^+$ multiplication. Hydroxyurea (0.2 M) was added 5 min after infection. Twenty-five minutes after infection, part of the hydroxyurea-treated cells were diluted 1:100 in medium free of hydroxyurea. Symbols: \circ , control; Δ , hydroxyurea-treated cells; \blacktriangle , hydroxyurea removed 25 min after infection.

acid-insoluble ^{14}C -thymidine did not rise, even though intense synthesis of phage DNA was taking place (Fig. 18). This suggested that little or none of the preformed thymidine derived from host DNA could be reutilized for the synthesis of T5 DNA, even though infected cells were capable of using exogenously supplied radioactive thymidine (unpublished data; M. Zweig, Ph.D. thesis, Columbia Univ., New York, N.Y., 1970) and deoxycytidine (Fig. 18).

Since breakdown of host DNA occurred rapidly after infection, the effects of inhibitors of DNA synthesis were studied by adding them to the cultures just before infection, to insure that the entire process of phage-induced degradation of *E. coli* DNA took place in their presence. It was found that none of the agents tested [hydroxyurea (0.2 M), nalidixic acid (10 $\mu\text{g/ml}$), and FUDR (50 $\mu\text{g/ml}$)] interfered with $T5^+$ -induced breakdown of *E. coli* DNA (Fig. 20), thus indicating that neither phage adsorption nor injection and expression of the first-step-transfer fragment of T5 DNA was prevented. In the presence of FUDR (50 $\mu\text{g/ml}$), about 20% of the original acid-insoluble ^{14}C -thymidine was re-

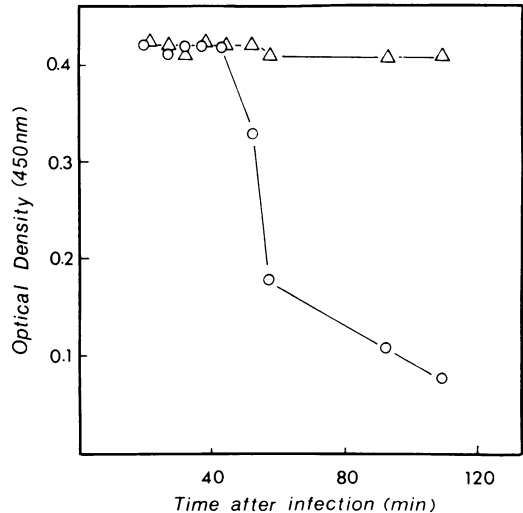


FIG. 16. Cell lysis in *E. coli* infected with $T5\text{ amH18a}$. Cultures of *E. coli* F (nonpermissive host) and *E. coli* F $su\beta^+$ (permissive host) were grown to a density of 1.1×10^8 per ml, whereupon each culture was infected with $T5\text{ amH18a}$ at a multiplicity of 8. At intervals, the turbidity of the cultures was measured. Symbols: \circ and Δ , *E. coli* F $su\beta^+$ and *E. coli* F, respectively, infected with $T5\text{ amH18a}$.

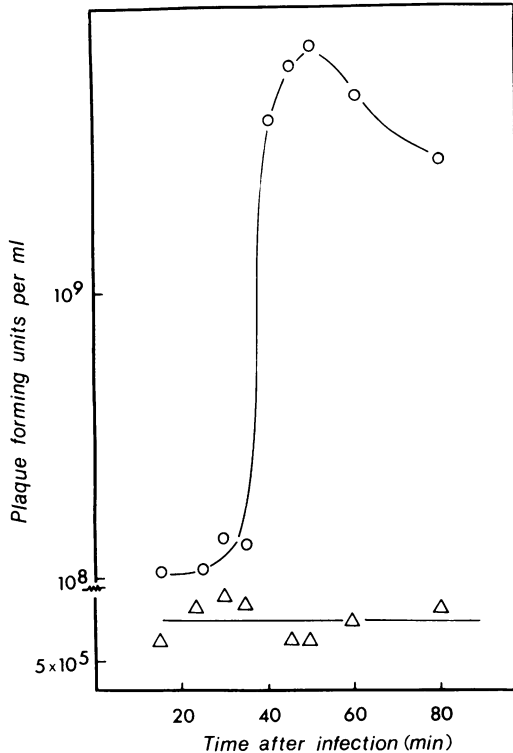


FIG. 17. Multiplication of *T5 amH18a*. Cultures of *E. coli F* and *E. coli F suβ⁺* were grown to a density of 1.5×10^8 per ml. Each was infected with *T5 amH18a* at a multiplicity of 8. Five minutes after infection, the cells were diluted 100-fold to prevent further phage adsorption. Appropriate additional dilutions were made. Symbols: ○, *E. coli F suβ⁺*; △, *E. coli F*.

utilized between 12 and 40 min after infection (Fig. 20). It appears, then, that only in FUDR-treated cells did host nuclear material supply significant amounts of thymidine for viral DNA synthesis during *T5⁺* infection. In cells exposed to hydroxyurea or nalidixic acid, the level of acid-insoluble ^{14}C -thymidine remaining after degradation was about half that detected in untreated cells. Degradation was slightly delayed in the presence of hydroxyurea.

Hydroxyurea (0.2 M) completely inhibited FUDR-induced reutilization of host DNA thymidine (Fig. 21). This suggests that one mechanism whereby hydroxyurea blocks DNA replication is the prevention of transfer of deoxynucleosides to *T5* DNA.

It could be shown that a portion of the "FUDR-induced" viral DNA was actually packaged into phage particles. Cellular DNA was prelabeled with ^{14}C -thymidine, and the cells were infected in the presence of FUDR. When the resulting lysate was centrifuged to equilibrium in a gradient

of CsCl (Fig. 22), a radioactive band appeared at the density of *T5⁺* (1.54 g/ml) (29). The radioactivity in this band represented 42% of the total amount applied to the gradient and 9% of that incorporated into host DNA prior to infection, thus indicating that a significant amount of host DNA thymidine was incorporated into phage particles in the presence of FUDR [although the particles were noninfectious (Fig. 14)].

Metabolism of cells infected with DO mutant. Incorporation of ^3H -deoxythymidine into the DNA of *E. coli F* infected with *T5 amH18a* was 5% of that observed in cells infected with *T5⁺* (Fig. 23). This small incorporation was further inhibited (Fig. 23) by treatment with hydroxyurea (0.2 M), thus suggesting that cells infected with *T5 amH18a* may have synthesized a very small amount of phage DNA that was sensitive to inhibition by hydroxyurea. This small amount of DNA was not packaged, however, into phage particles (Fig. 24) and therefore may be host *E. coli* DNA.

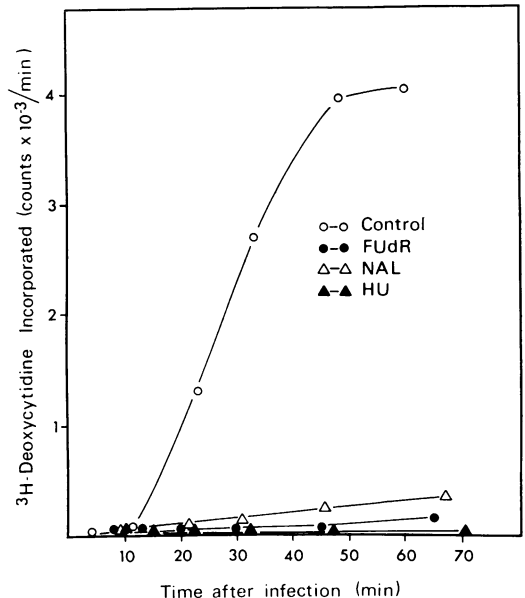


FIG. 18. Effect of inhibitors of DNA synthesis on incorporation of ^3H -deoxythymidine into DNA. ^3H -deoxythymidine (final concentration 3.2 $\mu\text{g/ml}$, 0.05 $\mu\text{Ci/ml}$) was added 2 min after infection with *T5⁺*, and, between 4 and 5 min after infection, portions of infected culture were distributed into prewarmed flasks containing premeasured amount of inhibitors. At intervals, portions from each culture were removed, precipitated with trichloroacetic acid, digested with alkali, and reprecipitated with acid (54). Insoluble residues were collected on filter discs and washed with cold acid (54), and radioactivity retained by the discs was determined. Symbols: ○, control; ●, FUDR (50 $\mu\text{g/ml}$); △, nalidixic acid (10 $\mu\text{g/ml}$); ▲, hydroxyurea (0.2 M).

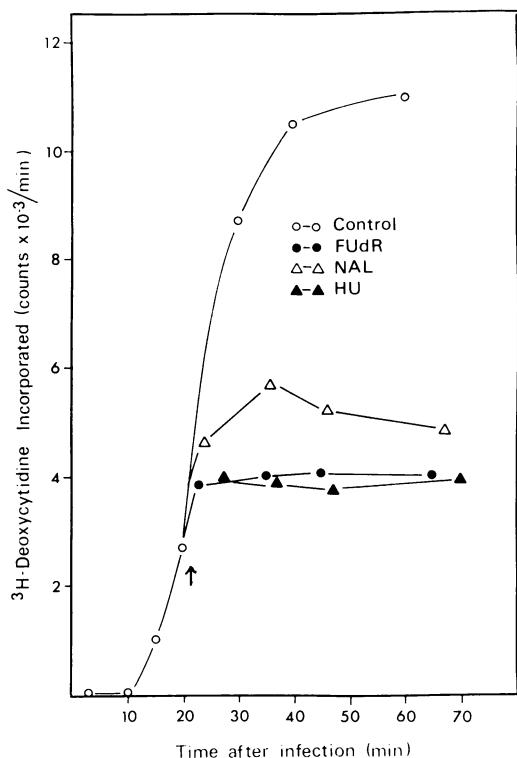


FIG. 19. Effect of delayed addition of inhibitors of DNA synthesis on incorporation of ^3H -deoxycytidine into DNA in $T5^+$ -infected *E. coli* F. ^3H -deoxycytidine (final concentration $3.2 \mu\text{g/ml}$) was added 2 min after infection; 21 to 22 min after infection, portions of the culture were treated with inhibitors. Symbols: \circ , control; \bullet , FUdR ($50 \mu\text{g/ml}$); \triangle , nalidixic acid ($10 \mu\text{g/ml}$); \blacktriangle , hydroxyurea ($0.2 M$).

The breakdown of host DNA after $T5$ amH18a infection under restrictive conditions resembled that following infection with $T5^+$ (Fig. 25), although the level of acid-insoluble ^{14}C -thymidine remaining was about half that observed during $T5^+$ infection. In striking contrast to $T5^+$, however, the reutilization of host thymidine was not induced by FUdR, suggesting that $T5$ specific DNA polymerase is required for reincorporation to occur.

The rates of protein synthesis in *E. coli* F infected with $T5^+$ and $T5$ amH18a were determined to assess the influence of a mutational block in replication of phage DNA (Fig. 26). These rates were similar for the first 15 min after infection, but thereafter the rate in cells infected with the latter agent was comparatively lower.

DISCUSSION

Development of $T5^+$. Within the first 9 min, one of the most prominent morphologic changes

observed in *E. coli* F after $T5^+$ infection is disappearance of the nucleoplasm (Fig. 2). This presumably reflects the known breakdown of host DNA into acid-soluble components (Fig. 20). In contrast, $T4$ -infected cells, in which DNA breakdown occurs simultaneously with synthesis of phage DNA, do not lose their nucleoplasm, which instead migrates to the periphery and then back toward the center of the cell (21, 35, 38).

Twenty minutes after $T5^+$ infection, fine, nuclearlike filaments appear close to the center of the cells (Fig. 3). This material probably represents pools of $T5$ DNA. Between 20 min after infection and the time of cell lysis, this DNA is packaged into viral heads, which accumulate in the cytoplasm. Tails are not visible in cells, probably because of their small diameter (10 nm) and because they are obscured by surrounding cytoplasmic material. However, it is reasonable to assume that a substantial number of $T5$ capsids seen just before lysis have tails at-

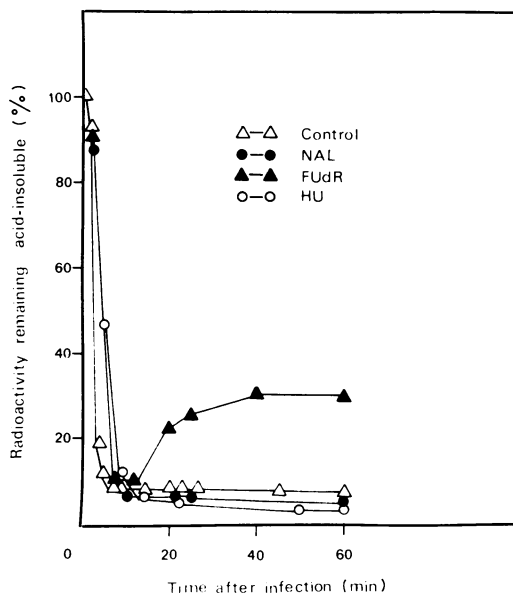


FIG. 20. Effect of inhibitors of DNA synthesis on $T5^+$ -induced breakdown of *E. coli* DNA labeled with ^{14}C -thymidine. Bacteria were grown for several generations in a medium supplemented with ^{14}C -thymidine and "cold" uridine (5). When the cells reached a density of 7×10^7 per ml, they were washed with MGM buffer, resuspended in fresh MGM, and allowed to grow to a concentration of 1.5×10^8 per ml. Two minutes before infection, portions of labeled culture were distributed into prewarmed flasks containing premeasured amounts of inhibitors. Samples were withdrawn 1 min before infection and at intervals thereafter, and radioactivity incorporated into acid-insoluble form was determined (54). Symbols: \triangle , control; \circ , hydroxyurea ($0.2 M$); \blacktriangle , FUdR ($50 \mu\text{g/ml}$); \bullet , nalidixic acid ($10 \mu\text{g/ml}$).

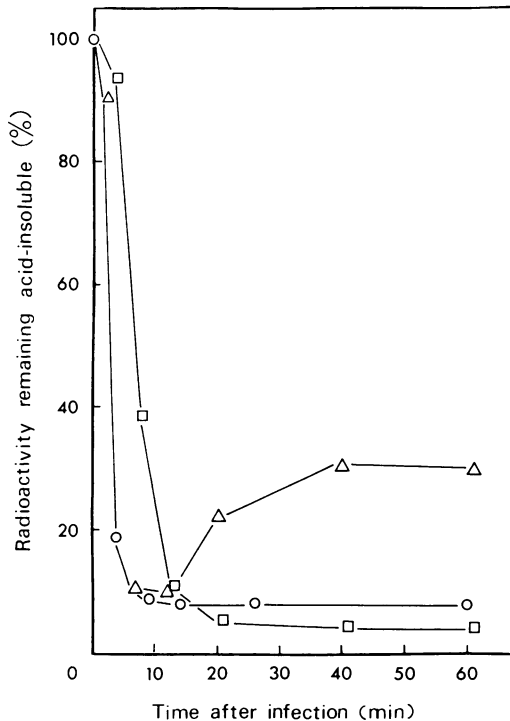


FIG. 21. Effects of FUdR and hydroxyurea on T5⁺-induced breakdown of *E. coli* DNA labeled with ¹⁴C-thymidine. Two minutes before infection, portions of labeled culture were distributed into flasks containing (i) MGM (control), (ii) FUdR (50 μg/ml), (iii) a mixture of FUdR (50 μg/ml) and hydroxyurea (0.2 M). Samples were withdrawn immediately before infection and at intervals thereafter. Symbols: ○, control; △, FUdR; □, FUdR-hydroxyurea mixture.

tached to them, since Lunt and Kay (34) have shown that tails attach to T5 heads *in vivo* immediately after head assembly. Although the majority of phage heads are filled with electron-dense material, a small but significant number of heads appear empty. The presence of empty heads was not described in an earlier electron microscopic study of T5 development (19). Empty heads have been seen in the course of development of T4 (38), of the marine bacterial virus NCMB 385 (62), and of φ25, a phage of *Bacillus subtilis* (31).

Degradation of *E. coli* DNA induced by T5⁺. During normal T5⁺ infection, about 90% of *E. coli* DNA labeled with ¹⁴C-thymidine became acid-soluble within 5 min (Fig. 20). As cited above, the extent of the degradation was reflected by the absence of nuclear material 9 min after infection (Fig. 2). In previous studies, in which T5-induced degradation was monitored by the diphenylamine reaction (8, 26, 27, 45),

the extent of DNA breakdown was reported to be slightly less than of the present study, but the rate of degradation appeared similar.

The technique used here for examining DNA breakdown had the additional advantage of permitting the determination of whether thymidylic acid residues of host DNA were used as precursors for the synthesis of T5 DNA. After degradation of host DNA, when infected cells were synthesizing T5 DNA rapidly, the level of acid-insoluble ¹⁴C-thymidine did not rise significantly, thereby indicating that little or no thymidine derived from host DNA was incorporated into T5 DNA. A small amount of reutilization was suggested by the fact that, when synthesis of phage DNA was prevented by nalidixic acid or by hydroxyurea (Fig. 20) or when nonpermissive cells were infected with a conditional lethal mutant blocked in its ability to induce synthesis of T5 DNA polymerase (Fig. 25), the level of acid-insoluble radioactivity remaining after the breakdown of host DNA was about half that detected during normal development. It is also possible that a greater amount of degradation occurs when DNA synthesis is prevented.

Between 20 and 30% of host DNA thymidine was reutilized for DNA synthesis in cells treated with FUdR (Fig. 20, 21). Part of the reincorporated ¹⁴C-thymidine was packaged into phage particles, since a radioactive peak was observed in CsCl at the density of T5 (Fig. 22). This

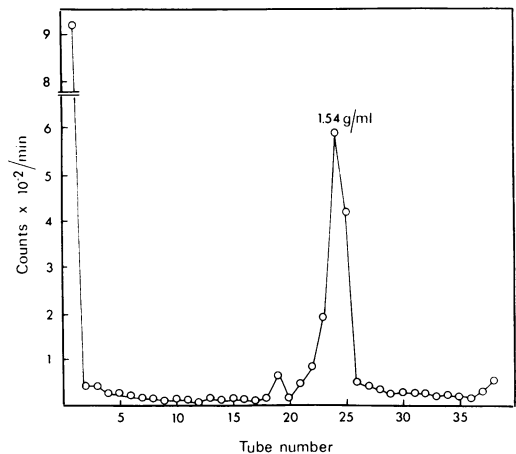


FIG. 22. Equilibrium centrifugation of a lysate obtained from cells infected with T5⁺ in the presence of FUdR (50 μg/ml). Bacteria were labeled with ¹⁴C-thymidine prior to infection; 60 min later, portions of the lysate (9×10^7 PFU) were spun in CsCl for 65 hr at 30,000 rev/min in the SW50 rotor of a Spinco model L-2 centrifuge. Samples were processed as described in the text.

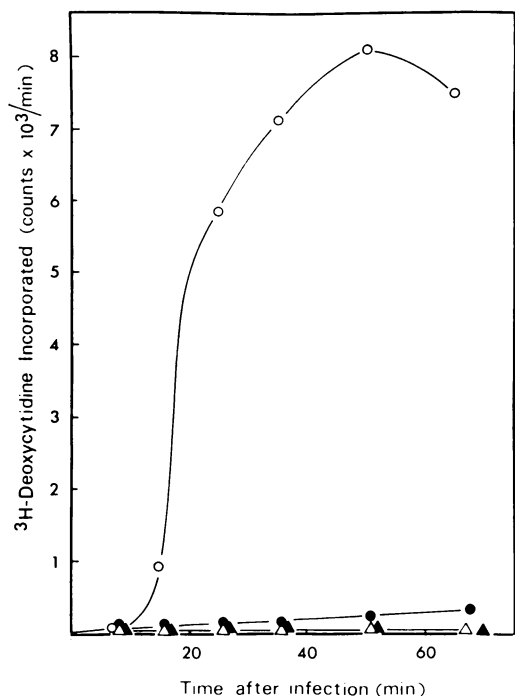


FIG. 23. DNA synthesis in cells infected with coliphage T5. Growing cells were infected with T5⁺ and T5 amH18a; 2 min later, the cultures were supplemented with ³H-deoxycytidine (0.25 μCi/ml, 3.5 μg/ml). Five minutes after infection, portions from each culture received hydroxyurea (0.2 M). At intervals, samples were withdrawn from each culture for the determination of radioactivity incorporated into DNA. Symbols: ○, cells infected with T5⁺; ●, cells infected with T5 amH18a; △, T5⁺-infected cells exposed to hydroxyurea; ▲, T5 amH18a-infected cells exposed to hydroxyurea.

reutilization and packaging theoretically could account for the production of about five to ten phages per infected cell, assuming of course that this preformed thymidine was incorporated into normal T5 DNA. However, no production of infectious phage occurred in the presence of FUdR (Fig. 14). The reason for this discrepancy is unknown.

Effects of hydroxyurea. Hydroxyurea is a specific and reversible inhibitor of bacterial DNA synthesis (51–53). The exact mechanism of action of this drug is a matter of some controversy. Cells treated with hydroxyurea (0.2 M) did not synthesize DNA during T5⁺ infection (Fig. 18). These bacteria did not lyse (*unpublished data*), and electron microscope studies showed that they contained no phage structures either 60 (*unpublished data*) or 120 min after infection (Fig. 10). The absence of phage particles could be the result of a block in either the synthesis or the

assembly of structural proteins. Indeed, metabolic studies indicate that, in *E. coli* F, hydroxyurea affects protein synthesis by some unknown mechanism (*unpublished data*; M. Zweig, Ph.D. thesis, Columbia Univ., New York, N.Y., 1970). It is apparent therefore that, despite its usefulness in animal virus systems (4, 30, 42, 43, 47, 55), hydroxyurea is not an ideal tool for determining the influence of DNA synthesis on T5 development in *E. coli* F.

Effect of nalidixic acid. Nalidixic acid, like hydroxyurea, is an inhibitor of bacterial DNA synthesis (13, 14; Winshell, Ph.D. thesis, Columbia Univ., New York, N.Y., 1967). The mode of action of this drug is still largely unknown, but it appears to involve the structure(s) responsible for the synthesis of nascent DNA (63). Nalidixic acid (10 μg/ml) allowed a small amount of phage DNA to replicate (7% of the control) (Fig. 18). This finding agrees reasonably well with the fact that infected cultures treated with nalidixic acid yielded some infectious virus (3% of the control) (Fig. 14). Sixty minutes after infection, electron microscopic examination revealed relatively small numbers of viral particles in the form of empty heads, although full head

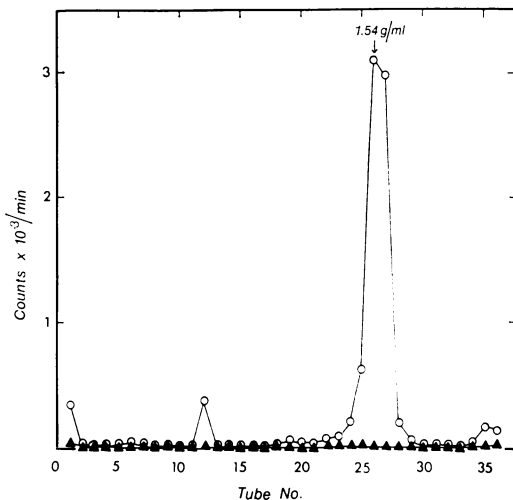


FIG. 24. Banding in cesium chloride of lysates derived from *E. coli* F infected with T5⁺ or T5 amH18a (multiplicity of infection, 7 and 8, respectively). Each culture was supplemented with ³H-thymidine (25 μCi). At the end of 60 min of aeration, each culture received a drop of chloroform. Cell debris was removed by centrifugation, and portions of the supernatant fluids were banded in gradients of cesium chloride. After equilibrium was reached, samples were collected and analyzed for content of radioactivity and refractive indexes were determined. Symbols: ○, lysate obtained from T5⁺-infected cells; ▲, lysate obtained from cells infected with T5 amH18a.

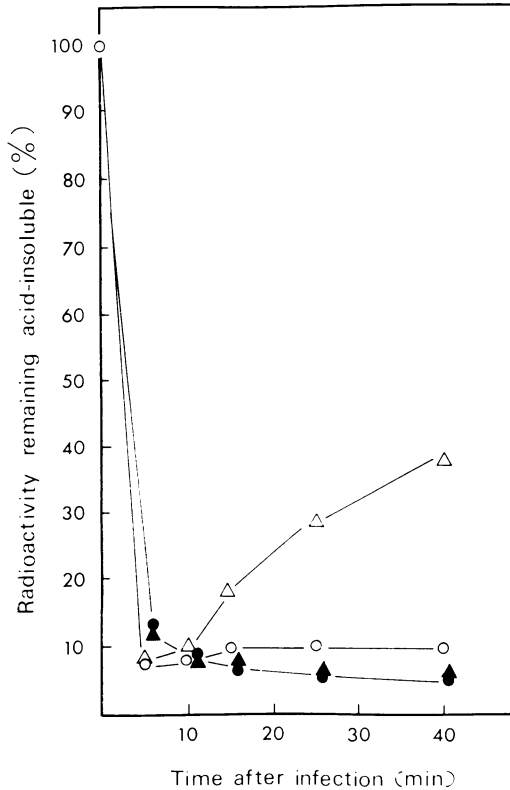


FIG. 25. Effect of FUDR on the virus-induced degradation of cellular DNA. Host DNA was prelabeled by growing *E. coli* F in the presence of ^{14}C -thymidine. One minute before infection and at intervals thereafter, samples were withdrawn, and radioactivity remaining acid-insoluble was determined (54). Symbols: ○ and ●, *E. coli* F infected with $T5^+$ and $T5$ amH18a, respectively; △ and ▲, cells infected with $T5^+$ and $T5$ amH18a, respectively, in the presence of FUDR (50 $\mu\text{g/ml}$).

structures were occasionally observed as well (unpublished data, Fig. 8). In addition to halting DNA production, nalidixic acid also reduced the rate of protein synthesis in infected cells (unpublished data), and this presumably explains the small number of viral particles seen 60 min after infection. In view of its inability to block DNA synthesis completely and because of its effect on protein synthesis in *E. coli* F, nalidixic acid, like hydroxyurea, is not a good agent for dissecting the role of DNA synthesis during the development of T5.

DO mutant. The study of phage mutants unable to induce DNA synthesis (DO mutants) throws light on the functions of DNA replication in the normal course of development. Such mutants have been used successfully in studies of T4 infection, on the assumption that in these

well characterized mutants no type of metabolism other than DNA synthesis was altered directly. Such specificity provides a distinct advantage over hydroxyurea and nalidixic acid, which affect protein synthesis as well in T5-infected *E. coli* F (see above; unpublished data).

Only a slight amount of DNA synthesis occurred in *E. coli* F after infection with DO mutant T5 amH18a (Fig. 23). It is of interest that the DNA was not incorporated into the viral particles (Fig. 24); hence it might be host rather than viral DNA. Studies to determine the nature of this DNA are in progress.

Sixty minutes after T5 amH18a infection, the cells contained empty capsids (Fig. 13), in contrast to cells infected with DO mutants of T4 wherein no capsids were observed (12). This suggests that DNA replication is not required for the synthesis of late proteins.

As previously indicated, there were fewer phage particles in T5 amH18a-infected cells (Fig. 13) than in cells infected with $T5^+$ (Fig. 5) or with T5 amH18a under permissive conditions (Fig. 12). The rate of protein synthesis in the former case was less than that seen in $T5^+$ infection about 15

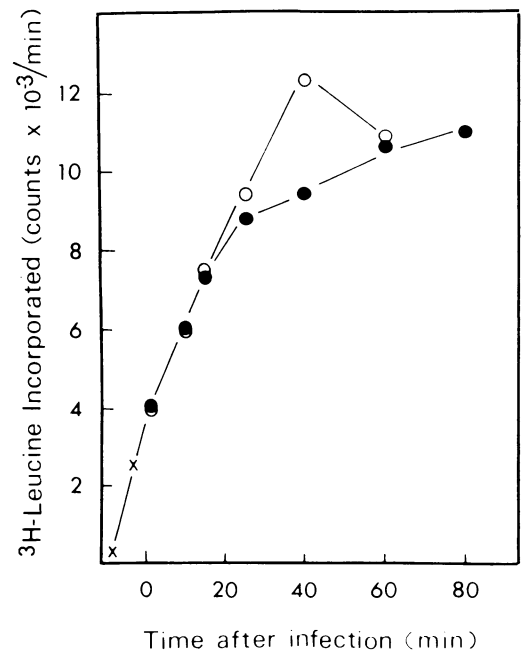


FIG. 26. Effect of T5 infection on protein synthesis. ^3H -leucine (final concentration 0.5 $\mu\text{Ci/ml}$, 15 $\mu\text{g/ml}$) was added to the cells 5 min before infection. The cells were divided, and one portion was infected with $T5^+$ (multiplicity of infection, 7) and the other with T5 amH18a (multiplicity of infection, 8). Symbols: ×, culture prior to infection; ○, $T5^+$ -infected cells; ●, T5 amH18a-infected cells.

min after infection [Fig. 26; approximately the time when synthesis of structural proteins begins (22, 36)]. Cells infected with the DO mutant did not lyse (Fig. 16). These observations indicate that DNA replication influences the quantity of late proteins (lysozyme and structural proteins) formed. These findings are in contrast to those of other investigators (16, 46). Thus Hendrickson and McCorquodale (16) suggested that replication of T5 DNA had little influence on the amount of late proteins produced. Although Pispá and his collaborators (46) found that viral DNA synthesis controlled the quantity of late RNA synthesized, they also observed the accumulation of almost normal amounts of T5-induced "lysozyme" (presumably a late protein) in cells infected under restrictive conditions with a DO mutant of T5. Three possible mechanisms may be proposed to account for this regulation:

(i) Phage particle synthesis is controlled by DNA replication, as suggested by Sauerbier and Brautigam (57) for T4 development. They proposed that a small amount of replicating phage DNA was necessary to initiate the synthesis of late messenger RNA and late proteins. However, DNA replication was not needed to sustain synthesis once it had started.

(ii) Continuous DNA replication is required for maintaining the normal rate of formation of viral structural components. Such a coupling has been postulated by Riva et al. (49) for normal T4 infection. A reduction in the normal rate of DNA synthesis or a total block in DNA synthesis after its initiation would cause a sharp decrease in the rate of transcription of late messenger RNA with resulting reduction in the rate of synthesis of late proteins.

(iii) Protein synthesis is governed by a gene dosage effect. This postulates that the rate of synthesis of structural components is a function of the quantity of DNA template available for transcription of late messenger RNA. DNA replication, by increasing the amount of template, enhances the rate of synthesis of late proteins. In the absence of DNA synthesis, the only template available is that of the parental virus. Such a mechanism has been suggested for the regulation of T7 development (61).

Hendrickson and McCorquodale (16) observed that T5 amH18a infection induced all three classes of viral proteins. This finding and the detection in this laboratory of empty heads in cells infected with T5 amH18a (Fig. 13) suggest that a block in DNA replication does not affect the types or kinds of viral-specific proteins being formed. Such a conclusion was also reached recently by Pispá and his collaborators (46) with respect to the classes of viral RNA. In this

report, it has not been possible, however (Fig. 23), to exclude entirely the possibility that a small quantity of phage DNA occurs in cells infected with T5 amH18a which may be responsible for the production of structural proteins. If the small amount of incorporation of specific DNA precursors really reflects viral DNA synthesis, then late protein synthesis may require at least some DNA replication. These findings suggest that, for the development of coliphage T5, continuous synthesis of DNA is required for the "normal" production of viral proteins.

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