Fractionation of Deoxyribonucleic Acid from Phage-Infected Bacteria

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1. DNA has been isolated in 90% yield from T5-infected cultures of *Escherichia coli* 'pulse'-labelled with [^3H]thymidine. It had a buoyant density in caesium chloride solution identical with the DNA of mature T5 phage, and no components of unusual buoyant density were detected. 2. The DNA preparation was resolved into two major components of differing specific activity on a column of kieselguhr coated with methylated serum albumin. The DNA of high specific activity could be eluted from the column only with 2*N*-ammonia, and the firm binding did not appear to be due to an artifact of preparation. 3. A similar fractionation into two DNA components of differing specific activity was observed when the 'pulse'-labelled culture was lysed with sodium dodecyl sulphate and the lysate rocked with phenol. The DNA of high specific activity was found in the interface precipitate between the phenol and aqueous layers. 4. The amounts of DNA in the two fractions were measured at different times after infection and the radioactivity content of each was determined at various times after a short 'pulse' of [^3H]thymidine. The interface fraction contained the replicating phage DNA, and the DNA from mature phage particles appeared in the aqueous fraction. 5. Analogous results were obtained with T2-infected *E. coli*. In the presence of chloramphenicol the DNA in the interface fraction was not converted into DNA extractable into the aqueous layer. Since chloramphenicol prevents the condensation of DNA into phage heads, it is suggested that any DNA in extended configuration is trapped inside the rigid-layer framework of the cell wall. 6. Treatment with lysozyme released much of the DNA from the interface precipitate. This DNA was firmly bound by the chromatographic column and had the same buoyant density in caesium chloride solution as normal T5-phage DNA. Sucrose-gradient sedimentation studies showed that it was heterogeneous and that as much as 60% sedimented faster than T5-phage DNA.

Although many facts are known about the replication of DNA in biological systems, the only example where the structure of the replicating molecule is known is the chromosome of *Escherichia coli* (Cairns, 1963). We decided to investigate the structure of replicating DNA from phage-infected bacteria because phage DNA is smaller than unbroken bacterial DNA and therefore the replicating DNA may be easier to extract in an unbroken state from the bacteria. As well, phage-infected bacteria are capable of rapid and extensive DNA synthesis.

At any given time after infection with phage, bacteria are likely to contain bacterial DNA, replicating phage DNA, and DNA in incomplete and completed intracellular phage particles (Hershey, 1953; Hershey & Melechen, 1957; Koch &

Hershey, 1959; Kellenberger, 1959). The problem of contamination of DNA preparations with bacterial DNA can be largely surmounted by using T5-infected *E. coli*, since in this system the bacterial DNA is rapidly and extensively degraded after infection (Pfefferkorn & Amos, 1958; Crawford, 1959). The experiments in the present paper describe the fractionation of DNA from T5-infected *E. coli* as a first stage in studying the molecular size and configuration of the replicating DNA.

MATERIALS AND METHODS

Bacteriophage. Phages T2r⁺ and T5 were prepared from stocks by lysis of bacteria in liquid media as described by Lunt, Siebke & Burton (1964) and Stone & Burton (1962)

respectively. The preparations were assayed by the double-layer technique (Adams, 1950) by using *E. coli* B with phage T2 and *E. coli* B/2 with phage T5. The latter strain, like strain F (Lanni, 1958), gives a higher yield of T5 plaques than does strain B. The purity of each stock was checked by plating the phage on resistant bacteria (B/2 and B/5). The ^3H -labelled T5 phage was prepared as above by adding [^3H]thymidine (final concn. $2\mu\text{C}/\text{ml.}$) to the culture at intervals from 25 min. after infection. The ^{32}P -labelled T5 phage was prepared by using a tri β -glucose synthetic medium (Hershey, 1955) for growth of the bacteria, and immediately after infection [^{32}P]phosphate (final concn. $33\mu\text{C}/\text{ml.}$) was added. After lysis the bacteriophage was purified as described by Burgi (1963).

Bacteria. *E. coli* strains B, B/2 and B/5 were kept at 3° on nutrient agar slopes. Cultures were grown at 37° with aeration in a liquid synthetic medium with mannitol as the carbon source (Stone & Burton, 1962). A 15 hr. culture was added to 10 vol. of fresh medium and aerated. When the cell density reached 4×10^8 – $5 \times 10^8/\text{ml.}$, the bacteria were infected by adding 10–15 T5 particles/bacterium or five T2 particles/bacterium. In experiments with T5 phage, *E. coli* B/2 was used and immediately before infection the culture was made 1 mM with respect to CaCl_2 . It was important for reproducibility that aeration of T5-infected bacterial cultures was never interrupted.

DNA preparations. DNA was prepared from a suspension of purified T5 phage ($2 \times 10^{12}/\text{ml.}$) by a modification (Davison & Freifelder, 1962) of the phenol method of Mandell & Hershey (1960). DNA was prepared from *Micrococcus lysodeikticus* according to the method of Marmur (1961) and was a gift from Dr G. B. Petersen.

Radioactive materials. [^3H]Thymidine nominally labelled in the 6 position and of specific activity 8.6 c/m-mole was obtained from The Radiochemical Centre, Amersham, Bucks. Carrier-free [^{32}P]phosphate was obtained from the Oak Ridge National Laboratory, Tenn., U.S.A. Before use the solution was heated at 100° for 10 min. and neutralized to convert the pyrophosphate form.

Enzymes. Ribonuclease was purchased from the Sigma Chemical Co. (St Louis, Mo., U.S.A.) as a $5 \times$ crystallized material from bovine pancreas. Crystalline deoxyribonuclease was obtained from the Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). Lysozyme and papain were obtained from the British Drug Houses Ltd. (Poole, Dorset) and from the Sigma Chemical Co.

Buffers. Tris buffer solutions were prepared by mixing N-HCl and m-tris to obtain the desired pH; phosphate buffers were prepared from $0.2\text{M-KH}_2\text{PO}_4$ and $0.2\text{M-Na}_2\text{HPO}_4$ and by mixing $0.2\text{M-Na}_2\text{HPO}_4$ with N-NaOH . All pH measurements were made at room temperature with a glass electrode.

Other materials. Phenol was AnalaR grade from British Drug Houses Ltd. and was redistilled before use. Liquefied phenol for chromatography (Fisher Scientific Co., Fairlawn, N.J., U.S.A.) was also used without further purification. Ether was 'anaesthetic' grade from Harrington Brothers (London) and was not usually redistilled before use. The product of the J. T. Baker Chemical Co. (Philadelphia, Pa., U.S.A.) was also used, but after redistillation. Caesium chloride was a product of British Drug Houses Ltd. and was designated 'for ultracentrifuge work'. The product of the Kaweck Chemical Co. (New York, N.Y., U.S.A.) was also used. Sodium dodecyl sulphate was obtained from

L. Light and Co. Ltd. (Colnbrook, Bucks.), and also from Bios Laboratories (New York, N.Y., U.S.A.). Chloramphenicol was a product of Parke, Davis and Co. Ltd. (Hounslow, Middlesex). Thymidine was obtained from the California Corp. for Biochemical Research (Los Angeles, Calif., U.S.A.).

'Pulse'-labelling of cultures. Cultures of infected bacteria were 'pulse'-labelled by the addition of [^3H]thymidine (final concn. 0.1 – $0.2\mu\text{C}/\text{ml.}$, except when DNA was required for zone centrifugation, when $7\mu\text{C}/\text{ml.}$ was used) and allowing aeration to continue for 30 sec. (a 1 min. 'pulse' was used in the preparation for zone centrifugation) before either pouring the culture on to ice or adding a several thousand-fold excess of unlabelled thymidine.

Preparation of lysates. Cultures of infected bacteria were poured on to 0.5 vol. of crushed frozen 0.1M-NaCl . The mixture was stirred and centrifuged at $2500g$ for 10 min. and the pellet was washed by suspending in ice-cold 0.1M-NaCl and centrifuging. The resultant pellet was suspended evenly in 0.08M-tris buffer, pH 8.0, containing EDTA (0.05M), to make a bacterial concentration of 5×10^8 – 8×10^9 cells/ml. Lysates were prepared by either of the following methods.

(a) Lysozyme was added to the suspension to make a final concentration of $300\mu\text{g}/\text{ml.}$ The mixture was incubated at 37° for 5 min. and the resultant viscous lysate frozen and thawed three times with the use of an ethanol-solid CO_2 bath. Sodium dodecyl sulphate was then added (final concn. 0.75% , w/v) and the mixture incubated for a further 5 min. at 37° . The resultant solution was usually clear.

(b) Sodium dodecyl sulphate was added to the washed suspension of infected bacteria to make a final concentration of 0.75% (w/v) and the mixture was incubated at 37° for 5 min. The resultant lysate was very viscous but still turbid. In the experiments with phage T2r $^+$, samples of the culture were added directly to a mixture of KCN and sodium dodecyl sulphate (final concn. 10mM-KCN and 0.06% dodecyl sulphate). The mixture was incubated at 37° for 5 min. and cooled in ice. This modification of the phage-T5 procedure was necessary because T2-infected bacteria often lysed spontaneously on collection by centrifuging.

Phenol treatment of the lysate. This was performed by using a water-saturated solution of redistilled phenol. About 7 ml. of $0.2\text{M-Na}_2\text{HPO}_4$ was added to 100 ml. of phenol solution so that the pH of the aqueous phase was 7.4. Usually an equal volume of cold phenol solution was added to the lysate (contained in a glass-stoppered tube) and the mixture was rocked back and forth by hand at a rate just sufficient to cause complete emulsification of the mixture in about 10 min. The mixture was kept cold (in ice-water) and rocked for about 30 min. before centrifuging (2°) at $2500g$ in a swing-out rotor for 20 min. The upper aqueous phase was removed with a wide-bore Pasteur pipette, except when the DNA was required for physical measurements, when the phenol phase was removed and the solution containing DNA left in the tube. When there was an interface precipitate the aqueous layer was removed and the interface material obtained either by using a Pasteur pipette or by carefully removing the phenol layer so that the precipitate adhered to one side of the glass tube.

With lysates of T2r $^+$ -infected bacteria 0.3 vol. of aqueous phenol was used, and after centrifuging the emulsion at $2500g$ as above the phenol layer was removed and the

mixture of interface material and aqueous phase centrifuged (2°) at 5000*g* for 30 min. in a toughened glass tube. The clear supernatant was decanted from the precipitate.

The interface material was suspended evenly in 0.1 M-tris-HCl buffer, pH 8.0, containing EDTA (0.05 M). Residual phenol was removed from this suspension and from the aqueous phase by gently rocking with an equal volume of ice-cold ether with at least three changes. Residual ether was removed by rotary evaporation.

Removal of RNA from DNA preparations. RNA in the DNA preparations was degraded by incubation with ribonuclease (20 μ g./ml.) at 37° for 30 min. The ribonuclease solution was previously heated at 80° for 20 min. to inactivate any possible deoxyribonuclease activity. The products of the ribonuclease digestion were often removed as completely as possible by dialysis against 0.5 M-NaCl in 0.05 M-phosphate (Na^+ and K^+ salts) buffer, pH 6.7.

Isolation of DNA from the interface precipitate. The interface suspension prepared as described above was incubated with lysozyme (300 μ g./ml.) for 30 min. at 37° . Usually ribonuclease (10–20 μ g./ml.) was included with the lysozyme for 15 min. A further incubation with 0.7% (w/v) sodium dodecyl sulphate for 5 min. at 37° usually improved the yield of DNA. The clear viscous solution was gently rocked with phenol for 30 min. as described, and the aqueous phase obtained by centrifuging was dialysed for 2 days with at least four changes against 1 l. of 0.04 M-NaCl–0.002 M-EDTA, pH 8.0. The dialysed solution was centrifuged at 18000*g* for 20 min. to remove possible particulate material that had not been removed by the original centrifuging. Usually the faint precipitate contained only traces of DNA and radioactivity. Occasionally the procedure released a very small percentage of the interface DNA (10–20% instead of 40–90%). The reason for this failure of the procedure is unknown. The interface DNA preparation obtained by the above procedure contained only traces of protein but did contain fragments of RNA produced by the ribonuclease digestion. These contaminants did not interfere with the analytical procedures employed.

Centrifuging in a caesium chloride density gradient. This was performed in a Spinco model E analytical ultracentrifuge and in an MSE Speed 40 or a Spinco model L ultracentrifuge. Solutions for the analytical ultracentrifuge were prepared in 0.02 M-tris buffer, pH 8.0, and contained up to 4 μ g. of each DNA/ml. and CsCl to make a density of 1.715 at 25° . The density of the solution was adjusted by using a refractometer and the cells were filled without special precaution to prevent shear degradation of the DNA. A 12 mm. standard cell and a 12 mm. cell with a negative wedge window were centrifuged together at 44770 rev./min. for 24 hr. at 25° in a rotor with a reference hole. An aperture mask was used so that photographs of each cell could be obtained, and the photographs were analysed in an Analytrol integrating densitometer (Beckman Instruments Inc., Palo Alto, Calif., U.S.A.). The buoyant density of each band was calculated (Sueoka, 1961) with reference to the position in the cell of the band of reference DNA (*M. lysodeikticus* DNA, $\rho = 1.731$). Some experiments were performed at pH 11.0 with 0.05 M-phosphate buffer (Vinograd, Morris, Davidson & Dove, 1963).

For preparative density-gradient centrifuging the solutions contained DNA and CsCl to make a density of 1.70 ± 0.01 at 2° . Density was measured by using a 200 μ l. micropipette as a pycnometer. Three tubes were centri-

fuged at 110000*g* for 40 hr. at $2-4^\circ$. At the end of the run, fractions were collected from the tube by piercing the bottom with a needle and collecting fractions of 5 drops directly into polyethylene counting bottles.

Sedimentation of DNA in the Spinco model E ultracentrifuge. This was performed at 31410 rev./min. and 20.0° . The solutions contained about 20 μ g. of DNA/ml. in 0.7 M-NaCl–0.05 M-phosphate buffer, pH 6.7 (Burgi & Hershey, 1961), and were very slowly introduced into a partly assembled 12 mm. standard cell by using a syringe with a wide-bore hole (no needle). Photographs were taken at 2 min. intervals and analysed by using a densitometer. All calculations were made by using the mid-point of the sedimenting boundary and the values obtained were corrected to that in water. On some occasions insufficient material was available to make a solution of concentration 20 μ g. of DNA/ml. Because the sedimentation coefficient of DNA is dependent on concentration, the values obtained in these cases were corrected to the value at 20 μ g./ml., by using as a basis the concentration-dependence of T5-phage DNA.

Zone centrifuging. This was performed by layering 0.5 μ g. of labelled DNA on a 5–20% (w/v) linear gradient of sucrose in 2 M-NaCl and centrifuging in the SW 39 head of a Spinco model L ultracentrifuge for 3 hr. at 30000 rev./min. See Burgi & Hershey (1963) for details.

Chromatography of DNA. This was performed on columns of kieselguhr coated with methylated serum albumin as described by Mandell & Hershey (1960) and Lacks (1962). Linear gradients of increasing NaCl concentrations were obtained by using two reservoirs one of which acted as a mixing chamber. Usually about 1 mg. of DNA was applied to the column and 5 ml. fractions were collected. All operations were performed at room temperature, and air pressure of about 3 lb./in.² was applied to increase the flow rate to about 30 ml./hr. Methylated serum albumin of decreased basicity was made as described by Mandell & Hershey (1960) except that methylation was stopped after 24 hr. instead of 72 hr. Only a slight precipitate had formed but good yields were obtained by precipitation of the remainder of the protein with ether.

Increase of extinction of DNA at 260 m μ on heating. This was measured in a Unicam SP.500 spectrophotometer fitted with a water-jacketed cell holder. Solutions for analysis contained 0.15 M-NaCl–0.015 M-sodium citrate and DNA (about 20 μ g./ml.), and were first briefly evacuated to remove air bubbles.

Tritium content of samples of DNA. This was measured by mixing with the sample about 1 mg. of bovine plasma albumin, cooling the mixture in ice and adding cold HClO_4 to give a final concentration of 0.25 N. After a few minutes in ice the solution was centrifuged (2°) at 2500*g* for 15 min. and the precipitate washed once or twice with cold 0.2 N- HClO_4 . The DNA was hydrolysed in 0.5 N- HClO_4 at 70° for 25 min. and the solution centrifuged. A portion (0.05 ml.) of the supernatant was added to 1.2 ml. of scintillation fluid and the samples were counted three times for 1000 sec. in a tritium coincidence scintillation counter (Isotope Developments Ltd., Beenharn, Reading, Berks.). The standard error of the results was usually not more than 2%. The scintillation fluid contained 100 ml. of sulphur-free toluene, 100 ml. of dioxan, 60 ml. of methanol, 21 g. of naphthalene, 1.2 g. of 2,5-diphenyloxazole and 26 mg. of 1,4-bis-(5-phenyloxazol-2-yl)benzene. In some experiments

herring-sperm DNA was added as a co-precipitant instead of albumin, and fractions from the chromatographic columns containing NH_3 were first neutralized. From time to time internal standards were used to check possible quenching of the scintillators. The presence of HClO_4 in the mixture decreased the count rate of standards by about 50%, but apart from this no quenching was observed with samples of different origin. The efficiency of counting ^3H in the presence of the HClO_4 was about 10%.

Mechanical disruption of bacteria. This was performed with a bacterial press (Hughes, 1951) at -25° .

Chemical estimations. DNA was measured by the method of Burton (1956), with deoxyribose as standard. Only the purine-bound deoxyribose reacts in this method and so the values were doubled to obtain the amounts of DNA deoxyribose that are given in the paper. RNA was measured by the orcinol reaction under the conditions used by Ogur & Rosen (1950), with ribose as the standard. Again, the colour represents purine-bound ribose and so they were likewise doubled to give the results that are presented. Protein was measured by using the Folin-Ciocalteu phenol reaction (Lowry, Rosebrough, Farr & Randall, 1951), with bovine serum albumin (Armour Laboratories, Eastbourne, Sussex) as standard.

RESULTS

Analysis of the total DNA of pulse-labelled T5-infected E. coli

Cultures of T5-infected bacteria were pulse-labelled with [^3H]thymidine as described in the Materials and Methods section, and DNA was extracted in good yields (cf. Table 5) by incubating the cells with lysozyme, freezing and thawing three times, warming the lysate with 0.75% sodium dodecyl sulphate and subsequently deproteinizing the clear solution with water-saturated phenol. The solution was treated with ribonuclease and the properties of the DNA were investigated by using caesium chloride density gradients and chromatography on methylated albumin columns.

Fig. 1. Density-gradient centrifuging of DNA from T5-infected *E. coli*. DNA was isolated as described in the text from a culture 37 min. after infection, and 4 μg . of this DNA was centrifuged together with 1 μg . of *M. lysodeikticus* DNA ($\rho=1.731$) in CsCl solution (see the Materials and Methods section) in the ultracentrifuge. Photographs were analysed in a densitometer.

Density-gradient centrifuging. In a density gradient of caesium chloride in the analytical ultracentrifuge only one major component was detected in DNA obtained from T5-infected *E. coli* (Fig. 1). This component had a buoyant density of 1.702, which is identical with the reported value for DNA of purified T5 phage (Schildkraut, Marmur & Doty, 1962). It is evident that by 37 min. after infection most of the *E. coli* DNA ($\rho=1.710$) has been degraded, although significant amounts were found when the infected culture was harvested before 30 min. after infection.

Similar results to Fig. 1 were obtained in another experiment, performed in caesium chloride buffered at pH 11.0 with 0.05 M-phosphate buffer. Under these conditions single-stranded DNA has a considerably increased buoyant density (Vinograd *et al.* 1963).

Chromatography of pulse-labelled DNA from T5-infected E. coli. Chromatography of DNA from pulse-labelled T5-infected *E. coli* on columns of kieselguhr coated with methylated serum albumin resolved the DNA preparation into components of differing specific activity (counts/sec./ $E_{260\text{m}\mu}$) (Fig. 2). About 50% of the applied DNA was eluted as a heterogeneous peak with 0.7 M-sodium chloride (peak 1) and a second smaller peak (peak 2) was obtained by increasing the salt concentration and pH of the eluting fluid. The remainder of the DNA

Fig. 2. Chromatography of DNA from pulse-labelled T5-infected *E. coli*. DNA was isolated as described in the text from a culture pulse-labelled at 33 min. and subsequently treated with ribonuclease. Then 1-mg. of this DNA in 50 ml. of 0.65 M-NaCl in 0.05 M-phosphate buffer, pH 6.7, was adsorbed on to a 20 cm. $\times$ 2 cm. diam. column and eluted with: (A) a linear gradient of 0.65–0.8 M-NaCl in 0.05 M-phosphate buffer, pH 6.7; (B) 1.5 M-NaCl in 0.1 M-tris buffer, pH 8.0; (C) 1.5 M-NaCl in 2 N- NH_3 . Fractions of volume 5 ml. were collected. $\circ$, $E_{260\text{m}\mu}$; $\blacktriangle$, radioactivity.

could be eluted only with 2*N*-ammonia-1.5*M*-sodium chloride (peak 3). Occasionally a fourth peak was obtained when changing from the buffered salt to the ammoniacal solution, but this was not usually observed and its significance is unknown. The ^3H content of the peaks differed markedly; peaks 2 and 3 had a similar specific activity and contained about 80% of the total ^3H . Peak 1 was heterogeneous; the leading edge consistently contained DNA of higher specific activity than the remainder of the peak, the specific activity of which was less than 10% that of peaks 2 and 3. Recovery of DNA from the column measured as 260*mμ*-absorbing material usually accounted for up to 90% of the applied material, and only traces of RNA were observed in the peaks.

The fractions comprising the peaks were pooled, neutralized where necessary, concentrated and dialysed. Measurements of radioactivity and deoxyribose content (Table 1) substantiated the differences observed in Fig. 2. Since the DNA of peak 1 was eluted at the salt concentration that would be required to elute T5-phage DNA, the sedimentation coefficients of the DNA of selected fractions were determined and are listed in Table 2. The DNA from the leading edge of peak 1 had a relatively low sedimentation coefficient, whereas the remainder of peak 1 had a sedimentation coefficient similar to that of DNA from T5 phage. The shape of the sedimenting boundaries of the DNA from the leading edge of peak 1 and from peak 2 suggested that these fractions were much more polydisperse than the DNA of the centre fractions of peak 1. The boundary of this material was of similar shape to that obtained with native T5-phage DNA.

The labelling pattern observed in Fig. 2 suggested that peak 3 may contain DNA that is a precursor of the DNA in peak 1. To test this idea a culture

of T5-infected *E. coli* was pulse-labelled with [^3H]-thymidine 27 min. after infection, by stopping the incorporation of ^3H after 30 sec. with a large excess of unlabelled thymidine. The culture was aerated for a further 10 min., the bacteria were collected and the DNA was isolated from a lysozyme lysate. A sample of this DNA preparation was incubated

Table 2. *Sedimentation coefficients of DNA samples from the column*

Fraction no.	Concn. of DNA ($\mu\text{g./ml.}$)	$S_{20,w}$ (observed) (s)	$S_{20,w}$ (corrected) (s)
60-68	11.5	33	31
70-73	21.0	45	45
74-80	18.0	45	44.5
122-125	13.5	42	40
T5-phage DNA	20.0	44	44

Fig. 3. Chromatography of DNA from a culture of T5-infected *E. coli* pulse-labelled 10 min. before collection of the bacteria. The culture was pulse-labelled as described in the text and the DNA isolated. Then 1.1 mg. of the DNA was adsorbed on to a column and eluted as described in Fig. 2 except that buffered 0.55*M*-NaCl was used for adsorption and a linear gradient of 0.55-0.77*M*-NaCl in buffer was used. ○, $E_{260m\mu}$; ▲, radioactivity.

Table 1. *Specific activity of fractions from the column*

Fractions from the column described in Fig. 2 were combined as indicated, neutralized, concentrated by rotary evaporation and dialysed against 0.7*M*-NaCl in 0.05*M*-phosphate buffer, pH 6.7, and the DNA was precipitated in 0.2*N*-HClO₄. Deoxyribose and radioactivity were measured in each fraction.

Fraction no.	Total deoxyribose (μmole)	Total radioactivity (counts/sec.)	Specific activity (counts/sec./ μmole of deoxyribose)
60-68	0.053	46.5	886
70-73	0.551	147.5	273
74-80	0.365	95.0	260
122-125	0.120	225.0	1875
129-134	0.114	31.5	276
136-143	0.900	2710.0	3010

with ribonuclease and applied to a chromatographic column and eluted as before (Fig. 3). The DNA of peak 3 was of slightly lower specific activity than that of peak 1, which is consistent with the idea that the former is a precursor of the latter. We were not able to recover the DNA of peak 3 from the column without the use of 2*N*-ammonia. With the described columns the use of saturated sodium chloride, saturated ammonium sulphate and 1.5*M*-sodium chloride buffered at pH 9.6 was unsuccessful, as was an attempt with a column with methylated serum albumin that was less extensively methylated than that described by Mandell & Hershey (1960).

The chromatographic results described suggested that the pulse-labelled DNA was of an unusual structure, but no unusual properties of this DNA were detected. All the DNA samples of fractions from the column denatured on heating at the same temperature, and the magnitude of the hyperchromic effect on heating was similar in all cases except that for the DNA of peak 3, which showed a rather smaller increase (25% instead of 30–33% for the other samples), but these fractions were contaminated with protein from the column. No differences in buoyant density in caesium chloride were detected; all the DNA samples had a buoyant density of 1.702 even when centrifuged at pH 11.0.

Since it is known that DNA damaged by various procedures can be firmly bound to these chromatographic columns (Hershey, Goldberg, Burgi & Ingraham, 1963), it was important to establish whether or not the observed retention of the highly labelled DNA was an artifact of the procedures employed for the characterization and isolation of replicating DNA. To test the idea that the retention was a result of damage caused by the decay of ³H in the DNA, a sample of DNA was prepared from purified T5 phage labelled with [³H]thymidine. The resultant DNA preparation had a specific activity of 1130 counts/sec./μmole of deoxyribose. It was subjected to chromatography 3 days after preparation and again 2 months later. In both cases about 90% of the applied DNA could be eluted with sodium chloride solutions, pH 6.7, alone (Table 3). This recovery is similar to that obtained with unlabelled phage DNA and therefore ³H decay cannot be responsible for the aforementioned chromatographic effects.

Another possible explanation for the retention is that replicating DNA is susceptible to nuclease action during preparation of the lysates, whereas DNA contained inside phage heads would be refractory. The following experiments were designed to examine this possibility. A sample of ³H-labelled T5-phage DNA was added to a suspension of unlabelled T5-infected *E. coli* and the bacteria were lysed with sodium dodecyl sulphate (see the

Table 3. *Retention of DNA on chromatographic columns*

Samples (1mg.) of each of the following DNA preparations (see the text for details) were applied to chromatographic columns and the DNA was eluted as described in Fig. 2. The percentage of the labelled DNA that could be eluted only with 2*N*-NH₃ was measured.

DNA sample applied	Labelled DNA retained (% of total)
1. Tritium-labelled T5-phage DNA 3 days after preparation	11.5
2. Tritium-labelled T5-phage DNA 2 months after preparation	11.5
3. Tritium-labelled T5-phage DNA added to a suspension of T5-infected <i>E. coli</i> before lysing with sodium dodecyl sulphate	11.7
4. Tritium-labelled T5-phage DNA (500 μg.) and unlabelled interface DNA (400 μg.)	20.6
5. Tritium-labelled T5-phage DNA added to a lysozyme lysate of T5-infected <i>E. coli</i> and incubated at 37° for 5 min.	24

Materials and Methods section). The lysate was treated with water-saturated phenol as described and the aqueous-phase fraction was collected. The solution was dialysed to remove phenol and then digested with ribonuclease. After further dialysis against 0.5*M*-sodium chloride a sample of the DNA was subjected to chromatography and the amount of labelled DNA that could be eluted only with 2*N*-ammonia was measured. This proportion (sample 3, Table 3) was similar to that observed with labelled T5-phage DNA alone. A similar experiment was performed by adding labelled T5-phage DNA to a lysozyme lysate of T5-infected bacteria and incubating at 37° for 5 min. before adding sodium dodecyl sulphate (0.75%) and isolating the DNA in the usual manner. In this case rather more labelled DNA was firmly bound to the column (sample 5, Table 3). However, we do not consider that this behaviour was due to nuclease action on the labelled DNA during the incubation with the lysozyme lysate, because of the behaviour of sample 4 (Table 3). In this case, the labelled T5-phage DNA was mixed with unlabelled interface DNA that was obtained from a sodium dodecyl sulphate lysate as described in the next section. The interface DNA is firmly bound to the column and at the same time increases the proportion of labelled T5-phage DNA that is also bound in this way.

Fractionation of DNA from phage-infected E. coli by phenol extraction of sodium dodecyl sulphate lysates

When a suspension of T5-infected *E. coli* was lysed at 37° with 0.75% sodium dodecyl sulphate and the viscous lysate rocked with water-saturated

phenol and centrifuged, a copious white precipitate appeared at the interface between the aqueous and phenol layers. This interface precipitate contained an appreciable amount of DNA. When T5-infected bacteria were pulse-labelled with [^3H]thymidine for 30 sec. at 35 min. after infection, the interface material contained about half the DNA and as much as 90% of the radioactivity that was incorporated into DNA (Table 4).

This fractionation of the phage DNA was very reproducible and was unaffected by the rate and time of rocking of the lysate with phenol. The amount of sodium dodecyl sulphate used for lysis did not affect the subsequent fractionation, although some DNA was extracted from bacteria that had

not been lysed with detergent. If the infected bacteria were first lysed with lysozyme before treatment with sodium dodecyl sulphate and phenol, very little interface material was found; almost all the DNA was found in soluble form in the aqueous layer, and the specific activity of this DNA in the interface fraction was close to that in the aqueous layer (Table 5). Similarly, the fractionation was not obtained if the infected bacteria were broken in a bacterial press (Table 5).

Conversion of interface DNA into aqueous-phase DNA during multiplication of phage. To test the hypothesis that the interface DNA was a precursor of the aqueous-phase DNA, a culture of T5-infected *E. coli* was supplied with [^3H]thymidine 29 min. after infection, and a large excess of unlabelled thymidine was added 30 sec. later. At intervals thereafter samples of the culture were removed, the bacteria were collected and lysed with sodium dodecyl sulphate, and the lysates were rocked with phenol. The changes in ^3H and DNA content of the aqueous-phase and interface fractions obtained are shown by Fig. 4.

For the period between 29 and 40 min. after infection the interface DNA content remained constant, whereas the DNA content of the aqueous-phase fraction increased linearly. Initially, most of the radioactivity of the pulse was found in the interface fraction, but this was subsequently transferred to the aqueous-phase fraction. Approx. 50% of the peak radioactivity was transferred in 6 min.

A similar experiment, pulse-labelled at 18 min. after infection, showed essentially the same pattern of ^3H transfer.

Changes in DNA content of the aqueous and interface pools of DNA after infection with T5 phage.

Table 4. *Fractionation of the DNA from a sodium dodecyl sulphate lysate of pulse-labelled T5-infected E. coli*

A sodium dodecyl sulphate lysate (see the text for details) of pulse-labelled infected *E. coli* was rocked with an equal volume of water-saturated phenol (see the Materials and Methods section) for 1 hr. The emulsion was centrifuged and the aqueous phase and the interface precipitate were separated. The precipitate was suspended in 0.1 M-NaCl and the acid-insoluble deoxyribose and radioactivity were measured in both fractions after removal of phenol by ether extraction.

Fraction	Total deoxyribose (μmoles)	Total radioactivity (counts/sec.)	Specific activity (counts/sec./ μmole of deoxyribose)
Aqueous phase	1.20	1560	1300
Interface	0.92	12200	13250

Table 5. *Effect of prior lysozyme treatment and mechanical breakage of T5-infected cells on the phenol fractionation*

Expt. A. A suspension of T5-infected *E. coli* (pulse-labelled at 33 min.) was divided into two samples. One was lysed with sodium dodecyl sulphate as described in Table 4; the other was incubated at 37° with lysozyme (300 $\mu\text{g.}/\text{ml.}$) and frozen and thawed three times before treatment with sodium dodecyl sulphate. Expt. B. Two unequal portions of a similar suspension were obtained and treated as in Expt. A, except that instead of lysing one portion with lysozyme the cells were broken in a bacterial press at -25°. In both experiments the lysates were fractionated with phenol and assayed as described in Table 4.

Method of disruption	Fraction	Total radioactivity (counts/sec.)	Total deoxyribose (μmoles)	Specific activity (counts/sec./ μmole of deoxyribose)
A. Sodium dodecyl sulphate alone	Aqueous	310	1.06	290
	Interface	1140	0.51	2240
Lysozyme and sodium dodecyl sulphate	Aqueous	1230	1.50	820
	Interface	50	0.06	835
B. Sodium dodecyl sulphate	Aqueous	113	0.76	148
	Interface	607	0.92	660
Mechanical disruption	Aqueous	1350	2.84	476
	Interface	18	< 0.03	—

Since the preceding results indicated that the interface DNA fraction contained the replicating DNA, it was decided to measure the amount of DNA in the interface and aqueous-phase fractions at various times after infection of the bacteria with

T5 phage. Samples of a culture of T5-infected *E. coli* were removed at intervals from 18 min. after infection, and the distribution of DNA was measured as before. The results of this experiment are shown by Fig. 5. The DNA content of the interface fraction increased in the period between 18 and 30 min. after infection, reaching a constant value of about 55 T5-phage DNA molecules/bacterium. DNA began to accumulate in the aqueous phase from about 23 min. after infection, and by 30 min. the increase in size of this pool accounted for most of the DNA synthesis of the culture. At 18 and 23 min. after infection 70% and 40% respectively of the aqueous-phase DNA was *E. coli* DNA as determined by caesium chloride density-gradient centrifuging.

Before 15 min. after infection the aqueous-phase fraction and the interface fraction had a similar DNA content, most of which would be *E. coli* DNA.

Fractionation of the DNA by phenol is probably due to condensation of the DNA. Published studies by electron microscopy of developing phage T2 (Kellenberger, 1961) prompted the idea that the

Fig. 4. Changes in (a) DNA and (b) ^3H content of the aqueous-phase and interface fractions after a pulse of ^3H -thymidine. Experimental details are described in the text. ●, Total DNA; ▲, interface DNA; ■, aqueous-phase DNA.

Fig. 5. Changes in the distribution of DNA between the aqueous-phase and interface fractions after infection with T5 phage. Experimental details are given in the text. ●, Total DNA; ▲, interface DNA; ■, aqueous-phase DNA.

Fig. 6. Changes in (a) DNA and (b) ^3H content of the aqueous-phase and interface fractions from T2-infected *E. coli* after a pulse of ^3H thymidine. Experimental details were similar to those described for Fig. 4. ●, Total DNA; ▲, interface DNA; ■, aqueous-phase DNA.

DNA in the aqueous-phase pool might be derived from that phage DNA which had condensed to form phage heads. If this were so, inhibition of DNA condensation should alter the normal distribution of DNA between the aqueous phase and interface of phenol-treated lysates of infected bacteria.

Since chloramphenicol inhibits DNA synthesis in T5-infected bacteria (Pfefferkorn & Amos, 1958; Crawford, 1959), T2-infected cultures were used for the following experiments. Chloramphenicol is known to inhibit the condensation process in this system (Kellenberger, Séchaud & Ryter, 1959).

In the absence of chloramphenicol (Fig. 6) the distribution of DNA and radioactivity between the aqueous and interface fractions was very similar to that obtained with the T5-phage system described above. The DNA content of the interface fraction remained constant, whereas that of the aqueous-phase fraction increased at the rate of overall DNA synthesis. Further, ^3H initially entered the interface fraction and was subsequently transferred to the aqueous-phase fraction. When chloramphenicol (50 $\mu\text{g./ml.}$) was added 11 min. after infection, a completely different situation was found (Fig. 7). The increase in total DNA could be completely

accounted for by the increase in DNA content of the interface fraction, whereas that of the aqueous phase remained constant. Also, most of the radioactivity incorporated from the pulse of [^3H]thymidine entered the interface and stayed there throughout the experiment. This finding is consistent with the idea that the interface fraction of DNA consists of the fibrillar DNA that has not been condensed to form phage heads.

Release of DNA from the interface fraction

The interface fraction obtained from T5-infected bacteria by phenol fractionation contained appreciable amounts of RNA and protein as well as DNA (e.g. 1.5 μmoles of RNA ribose and 4.2 mg. of protein/ μmole of DNA deoxyribose). The DNA was not extracted from interface suspensions by various combinations of heating at 55°, 2M-sodium chloride and pH 10.6. The suspension was partly clarified by treatment with papain but the DNA was still associated with the insoluble material. On centrifuging in a caesium chloride density gradient, virtually all of the radioactivity was associated with the insoluble material on the surface. After fixation with osmic acid, examination in an electron microscope showed that the interface fraction contained many particles of similar shape and size to bacteria. This suggested that the DNA was not extracted by the above procedures because it was contained by the structures of the bacterial cell wall, which are resistant to the detergent and phenol.

The interface precipitate was found to be susceptible to lysozyme and this enzyme released 40–75% of the DNA in a soluble form. The specific activity of the released DNA was similar to that of the residual DNA that was not released from the interface material (Table 6). As much as 90% of the DNA has been released in some of the experiments in which sodium dodecyl sulphate was added to 0.3% (w/v) concentration after the lysozyme incubation and incubation continued at 37° for 5 min.

A sample of interface DNA that was released by detergent as described above was treated with ribonuclease and chromatographed on a column of kieselguhr coated with methylated serum albumin. This DNA, like part of the DNA from a lysozyme lysate of T5-infected bacteria, was firmly bound by the chromatographic column and could be eluted only with ammonia (Fig. 8). By contrast, DNA from the aqueous-phase fraction could be eluted with sodium chloride solutions at pH 6.7.

The ^3H -labelled DNA isolated from the interface fraction was mixed with DNA isolated from ^{32}P -labelled T5 phage and centrifuged in a density gradient of caesium chloride. Fig. 9 shows that the distributions of ^{32}P and ^3H coincide and that there

Fig. 7. Effect of chloramphenicol on the distribution of (a) DNA and (b) ^3H between the interface and aqueous-phase fractions of T2-infected *E. coli*. Experimental details were similar to those described for Fig. 6 except that chloramphenicol (final concn. 50 $\mu\text{g./ml.}$) was added 11 min. after infection. ●, Total DNA; ▲, interface DNA; ■, aqueous-phase DNA.

Table 6. Release of DNA from the interface precipitate by lysozyme

Interface material was prepared from pulse-labelled T5-infected *E. coli* as described in Table 4, and evenly suspended in 0.1 M-tris buffer, pH 8.0, containing EDTA (0.06 M). Residual phenol was removed by three ether extractions and final traces of ether were removed by evacuation. Samples of this suspension were incubated at 37° for 30 min. in the presence and absence of lysozyme (final concn. 300 µg./ml.) and then subjected to a further phenol extraction. The ³H and deoxyribose contents of the resultant fractions were measured.

Treatment	Fraction	Total deoxyribose (µmole)	Total radioactivity (counts/sec.)	Specific activity (counts/sec./µmole of deoxyribose)
Control	Aqueous	0.068	72	1060
	Interface	0.314	1030	3280
Lysozyme	Aqueous	0.319	956	3000
	Interface	0.104	308	2960

Fig. 8. Chromatography of interface DNA and DNA from T5 phage. DNA was isolated from labelled interface as described in Table 6. Then 300 µg. of this DNA and 400 µg. of unlabelled DNA from T5 phage were applied to a chromatographic column (as described in Fig. 2) with (A) a linear gradient of 0.65–0.95 M-NaCl in 0.05 M-phosphate buffer, pH 6.7; (B) 2 M-NaCl in 0.1 M-tris buffer, pH 8.0; (C) 2 M-NaCl in 2 N-NH₃. O, *E*_{280mμ}; ▲, radioactivity.

Fig. 9. Density-gradient centrifuging of ³H-labelled interface DNA and ³²P-labelled T5-phage DNA. The CsCl solution (1.5 ml.) contained 0.15 µg. of T5-phage DNA and 2.6 µg. of interface DNA and was centrifuged for 46 hr. (see the Materials and Methods section). Fractions were collected directly into counting vials and the ³H and ³²P contents were measured. —, ³H; ----, ³²P. The graph shows, for each isotope, the percentage of the total radioactivity that was found in each fraction.

is no tritium at the top or the bottom of the density gradient. This result shows that the DNA released from the interface fraction is not attached to measurable amounts of protein.

To obtain an indication of the size of the interface DNA, a ³H-labelled sample was centrifuged together with a sample of ³²P-labelled T5-phage DNA in a 5–20% (w/v) gradient of sucrose in 2.0 M-sodium chloride. Fig. 10 shows that approx. 50% of the ³H-labelled DNA sedimented as a broad band much faster than the sharp band of the ³²P-labelled marker DNA. This sedimentation pattern was unaffected by the sodium chloride concentration in the sucrose and the amount of DNA applied (in the range 0.2–1.5 µg.) and cannot be due to non-specific aggregation since the T5-phage DNA sedimented normally. The sedimentation behaviour is not due to any association with a large bulk of

protein or other non-DNA material because essentially the same sedimentation results were obtained with DNA that had been recovered from an equilibrium centrifugation in caesium chloride.

DISCUSSION

Although the chromatographic experiments and the phenol fractionation indicated that an appreciable amount of the total DNA of T5-infected *E. coli* is replicating, caesium chloride density-gradient centrifuging with the total DNA of lysozyme lysates and with the purified interface DNA revealed

Fig. 10. Zone sedimentation of ^3H -labelled interface DNA and ^{32}P -labelled T5-phage DNA. A mixture of $0.4\text{ }\mu\text{g.}$ of ^3H -labelled interface DNA and $0.1\text{ }\mu\text{g.}$ of ^{32}P -labelled T5-phage DNA was layered on a 5–20% (w/v) sucrose gradient and centrifuged as described in the Materials and Methods section. Fractions were collected by piercing the tube and collecting drops directly into counting vials. The graph shows, for each isotope, the percentage of the total radioactivity that was found in each fraction. —, ^3H ; ----, ^{32}P .

no components having an unusual buoyant density. The same conclusion was reached with experiments performed at pH 11.0, at which pH single-stranded DNA has a much greater density than at neutral pH (Vinograd *et al.* 1963).

Pulse-labelled DNA of T5-infected bacteria therefore differs from that of non-infected *E. coli*, where components of unusual buoyant density have been detected (Rolfe, 1963; Rosenberg & Cavalieri, 1964). In these cases the unusual components were detected only after special treatments and it is conceivable that the techniques employed in the present study were inadequate. In any case, if single-stranded regions are present in molecules of replicating T5-phage DNA these regions must be very short compared with the length of the molecule. This conclusion is in accord with present evidence on DNA replication in *E. coli*, which suggests that only a minute portion of the chromosome is involved with replication at any specified time (Cairns, 1963).

Chromatography of the total DNA of phage-infected bacteria on columns of kieselguhr coated with methylated serum albumin resolved the DNA into two major components of different specific activity. The leading edge of peak 1 (Fig. 2) apparently contained fragments of DNA molecules, whereas the DNA of the remainder of the peak was probably derived from intracellular phage particles. The highly labelled DNA, on the other hand,

was firmly bound to the column and could be eluted in good yield only by 2N-ammonia. Hershey *et al.* (1963) have shown that DNA will bind to these columns in a similar manner if it is damaged by any of several methods, which include decay of incorporated radioactive atoms, intense u.v. light and localized denaturation in internal regions of the molecule caused by shear forces. In the present study ^3H decay cannot be responsible for the retention because a sample of ^3H -labelled T5-phage DNA was eluted normally and in the pulse-chase experiment the DNA of peak 1 had a rather higher specific activity than the DNA of peak 3.

Similarly, no evidence has been obtained to suggest that specific nuclease action on replicating DNA during lysis of the bacteria was responsible for the effect, although the experiments could not negate the possibility of nuclease action exclusively at the moment of lysis of the bacterial cell. It is more likely that the retention of the highly labelled DNA by the column is a consequence of the physical configuration of replicating DNA. Conceivably, either forked molecules or short single-stranded regions in the molecule where base-pairing of incoming nucleotides was about to occur could be responsible.

The fractionation of the DNA of phage-infected bacteria with phenol was reproducible and provided a convenient method for the selective isolation of pulse-labelled DNA. That this DNA fraction contained replicating DNA was demonstrated by the kinetics of ^3H transfer from the interface fraction to the aqueous-phase fraction. The kinetics differ somewhat from the ideal case (Zilversmit, Entenman & Fishler, 1943) in that the specific activity of the interface fraction decreased rather more slowly with time. This divergence is not unexpected in a complex system involving the synthesis of phage DNA and the assembly of phage particles, since each DNA molecule will need to remain in the replicating pool for an appreciable time and, further, the specific activity of the aqueous-phase DNA is not necessarily a measure of the specific activity of the immediate product of replication. Moreover, some of the completed DNA must remain in the replicating pool to act as templates for the synthesis of new material.

The ability to separate a fraction of DNA from phage-infected bacteria that is enriched in replicating DNA allowed some measure to be made of the pool size of replicating DNA at different times after infection. The results obtained with T5-infected bacteria are similar to those obtained with T2-infected bacteria by Hershey (1953) in that the pool of replicating (or precursor) DNA first increases in size and eventually reaches a constant value. In T5-infected bacteria this value was 55 T5-phage DNA molecules/bacterium (assuming a T5-phage

particle contains 1.8×10^{-16} g. of DNA; Sinsheimer, 1960), and in the T2-phage system it was 60 T2-phage DNA molecules/bacterium. The latter value is higher than previous estimates of 17 in a synthetic medium and 35 in nutrient broth (Koch & Hershey, 1959). However, comparison is difficult because of the different growth conditions employed, and also it is possible that the interface fraction contained bacterial DNA and/or phage DNA that was not involved in the process of replication.

If T5-phage DNA replicates with only one growing point/T5-phage DNA molecule, and if it is assumed that two-thirds of the interface DNA acts as a template for the synthesis of new DNA (since the average template strand will be associated with half a strand of new material), the constant size of the interface pool of 55 T5-phage DNA molecules/bacterium and the constant rate of increase of the aqueous-phase pool at 10 T5-phage DNA molecules/bacterium/min. provide an estimate of 3.7 min. for the maximum time of synthesis of a T5-phage DNA molecule under the described growth conditions. Similar considerations with the less reliable values from the T2-phage system give an estimate of 10 min., which is close to a previous estimate of 9 min. (Koch & Hershey, 1959).

The accumulation of DNA in the aqueous-phase fraction with time, and the observation that ^3H from a short pulse of [^3H]thymidine is transferred from the interface fraction, suggest that the DNA in the aqueous phase may be derived from completed phage particles. This conclusion was supported by the observation that in T2-infected cells chloramphenicol, which is known to inhibit the condensation of fibrillar replicating DNA to form phage heads (Kellenberger *et al.* 1959), also inhibited the production of aqueous-phase DNA. However, chloramphenicol may affect processes other than condensation, since unusually large DNA molecules have been found in chloramphenicol-treated T2-infected *E. coli* (Frankel, 1962). It is not known whether or not the DNA in the aqueous-phase fraction is derived solely from infective intracellular phage. It is perhaps reasonable to suppose that the phenol procedure cannot differentiate between mature infective particles and any immature particles in which the head portion has been completely assembled.

The phenol fractionation was not observed if the infected bacteria were lysed with lysozyme before treatment with sodium dodecyl sulphate and phenol, or if the cells were broken in a bacterial press (Table 5). These facts suggest that the integrity of some part of the bacterial cell wall is an important factor in the separation. Bolle & Kellenberger (1958) showed that *E. coli* lysed with sodium lauryl sulphate retain the characteristic rod-shaped morphology. It is also known that the rigid layer of

E. coli cell walls is unaffected by detergent or phenol treatment but is degraded by lysozyme (Weidel, Frank & Martin, 1960). In the present study the interface precipitate contained bacteria-like structures; and of several procedures tried (including digestion with papain) incubation with lysozyme was the only method that released the DNA in a soluble form.

It appears possible that the replicating DNA may be trapped within the rigid-layer framework of the bacterial cell wall. DNA that has been condensed into phage-head structures may conceivably pass through smaller holes than the replicating DNA, which has both a higher molecular weight and a more extended fibrillar structure. Replicating bacterial DNA may be attached to the bacterial cell wall (Jacob, Brenner & Cuzin, 1963) and it is conceivable that the replicating phage DNA may be attached in a similar manner. As yet, our experiments give no clear indication about this possibility.

The DNA isolated from the interface fraction, like part of the DNA from a lysozyme-sodium dodecyl sulphate lysate of T5-infected cells, was completely retained by the chromatographic column. Although the cause of this retention is not known, the experiment showed that the interface fraction is free of normal T5-phage DNA. By contrast, interface DNA had the same buoyant density in caesium chloride as T5-phage DNA. This fact means that within the limits of the experiment the interface DNA was not covalently linked to protein.

Sedimentation studies in sucrose density gradients revealed that only a small amount of the isolated DNA was smaller than normal T5-phage DNA, suggesting that extensive degradation of the DNA had not occurred during isolation. The most striking observation from the sedimentation studies was that more than 50% of the DNA sedimented as a broad band appreciably faster than T5-phage DNA. The sedimentation behaviour is not due to any association with a large bulk of protein or other non-DNA material. If the relationship between sedimentation rate and molecular weight derived by Burgi & Hershey (1963) is valid for this material, the molecular weight of the fastest-sedimenting components (3.3 cm. from the top) is about five times that of T5-phage DNA. It is also possible that the rapid sedimentation is a result of a change in configuration of the DNA associated with replication or recombination or both. This large DNA is reminiscent of that obtained by Frankel (1962) from T2-infected cells treated with chloramphenicol, but differs in that the T5-phage interface DNA is firmly bound by the chromatographic column. The question remains whether or not such DNA is itself part of an even larger structure that was degraded during isolation.

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