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The Transformation of *Escherichia coli* with Deoxyribonucleic Acid isolated from Bacteriophage *λdg*†

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Deoxyribonucleic acid of *λdg*, isolated by phenol extraction, will transform galactose-negative strains of *Escherichia coli* K12. That the transforming agent is indeed DNA is shown by (1) its sensitivity to pancreatic DNase, (2) its resistance to anti-*λ* antibody, (3) its resistance to heat up to the characteristic melting temperature of *λdg* DNA, and (4) its buoyant density of 1·71 g cm^{-3}.

Transformation is found to occur only if the bacteria exposed to *λdg* DNA are simultaneously infected with ordinary *λ*.

The transforming agent appears to be the entire *λdg* chromosome since the phage genes *c* and *mi* are also present in the galactose-positive transformants.

1. Introduction

This paper describes a system for the genetic transformation of *Escherichia coli* K12. Recipient strains which are unable to metabolize galactose because they lack the enzymes galactokinase or galactose-1-phosphate uridyl transferase are transformed into galactose-metabolizing strains. The transforming agent is DNA isolated from *λdg*, a variant of coliphage *λ*.

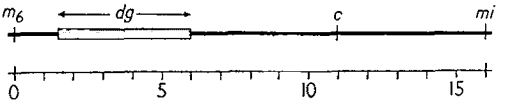

FIG. 1. This linkage map shows the position of the "*dg* region" relative to the phage genes m_6, *c*, and *mi*. The *dg* region is defined as that part of the *λ* chromosome which is not recoverable in active phage when *λdg* is crossed to normal *λ*. Data for the size and position of *dg* were taken from the experiments of Arber (1958). The distances are measured in recombination units whereby a cross involving two markers separated by one recombination unit gives 1% recombinants.

Lambda is a temperate phage whose locus on the chromosome of *E. coli* K12 is closely linked to the genes controlling several enzymes of galactose metabolism (Lederberg & Lederberg, 1953). Ultraviolet irradiation of bacteria lysogenic for *λ* initiates synthesis and release of phage particles. Most of the new phage particles are ordinary *λ*. However, approximately one in 10^6 of the new particles is *λdg*.

Lambda *dg* transduces galactose genes from the bacterium in which it was produced to the bacterium it infects (Morse, Lederberg & Lederberg, 1956). The transduced galactose genes control at least the enzymes galactokinase and galactose-1-phosphate uridyl transferase (Kalckar, Kurahashi & Jordan, 1959). The acquisition of galactose

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genes by *λdg* is accompanied by the loss of a part of the genetic material present in ordinary λ. The region which is inactive or absent in *λdg*, i.e., which is defective, is indicated on a linkage map of λ given in Fig. 1.

The defectiveness of *λdg* is expressed in the following way. Of the bacteria infected with ordinary λ under standard conditions, 20% become lysogenic. Under the same conditions only 1% of the bacteria infected with *λdg* become lysogenic. However, if bacteria are infected simultaneously with *λdg* and λ, then 20% become lysogenic for both *λdg* and λ (Arber, 1958). Thus ordinary λ acts as a "helper" for the lysogenization of *λdg*, presumably by supplying a normal *dg* region.

The defectiveness of *λdg* is expressed in another way. Bacteria lysogenic for *λdg*, induced with ultraviolet light (u.v.), lyse but do not produce any phage (Arber, 1958). However, doubly lysogenic bacteria, carrying both *λdg* and λ, induced with u.v., lyse and produce both *λdg* and λ. Lambda *dg* can be obtained substantially free of ordinary λ by density gradient centrifugation because *λdg* differs slightly in buoyant density from λ (Weigle, Meselson & Paigen, 1960).

Lambda *dg* would seem to be a good potential source of genetically active DNA. The DNA complement of one *λdg* particle is about 1/100 that of an *E. coli* cell, and since both carry the same galactose genes, the fraction of the DNA represented by the genes for galactokinase and galactose phosphate uridyl transferase is about one hundred-fold higher in *λdg* DNA than in *E. coli* DNA. Moreover, during the purification of *λdg*, the protein coat protects its charge of DNA from destruction by nucleases released during cell lysis. Finally, the knowledge of the genetic structure of λ (Jacob & Wollman, 1954; Kaiser, 1955) and *E. coli* K12 (Lederberg, Lederberg, Zinder & Lively, 1951; Wollman, Jacob & Hayes, 1956) provides a basis for the interpretation of experiments with isolated DNA.

DNA isolated from *λdg* is shown in this paper to be active in transformation. Phenol extraction of *λdg* denatures its protein and releases its DNA into aqueous solution. The *λdg* DNA, so prepared, will transform strains of *E. coli* K12 which lack either galactokinase or galactose transferase. Transformation is found to occur, however, only if the bacteria exposed to *λdg* DNA are simultaneously infected with "helper" phage (e.g. ordinary λ). Analysis of the transformed bacteria shows that the active DNA carries phage genes as well as galactose genes, and, in fact, appears to be the entire chromosome of *λdg*.

2. Materials and Methods

(a) *Media*

The growth media used include: Difco bacto-tryptone broth with 0·5% NaCl (TB medium) and its agar derivatives, TB soft agar (0·7% agar) and TB plate agar (1% agar); EMB-galactose agar as described by Lederberg (1950) except that 10 g/l. Difco bacto-tryptone is substituted for 8 g/l. of casein digest; H medium consisting of 0·1 M-potassium phosphate buffer, pH 7·0, 0·015 M-$(NH_4)_2SO_4$, 0·001 M-$MgSO_4$ and $1{\cdot}8 \times 10^{-6}$ M-$FeSO_4$; and P medium which is identical to H except that the phosphate concentration is 0·02 M.

Unless otherwise specified, bacteriophage stocks were kept in and diluted into a solution (termed λ-dil.) of 0·01 M-potassium phosphate buffer, pH 7·0, 0·01 M-$MgSO_4$, and 10 μg/ml. of bovine plasma albumin.

(b) *Special reagents*

The trypsin and pancreatic DNase were crystalline products of Worthington Biochemical Corp. Thymus DNA was prepared according to the procedure of Kay, Simmons & Dounce (1952). CsCl was obtained from American Potash and Chemical Corp.; its analysis

in per cent by weight was: CsCl—95·01; RbCl—4·11; KCl—0·39; NaCl—0·06; $NaHCO_3$—0·02; and moisture—0·03. The CsCl concentrations referred to in the text are not corrected for the presence of these impurities. For experiments involving the centrifugal banding of DNA in a CsCl gradient, ultraviolet light-absorbing impurities in the CsCl were reduced by heating the CsCl at 500°C for about 20 hr and then passing a 60% solution of the heated CsCl through a Norite column. The resulting solutions had absorbancies of less than 0·05 at 260 mμ. All other chemicals were C.P. grade.

(c) *Bacterial strains*

The galactose-negative mutants of *E. coli* K12 were isolated by Dr. E. Lederberg who generously made them available to us. Mutant W3102 (Gal_2^-) is defective in the synthesis of galactokinase, mutants W3101 (Gal_1^-) and W3104 (Gal_4^-) are defective in the synthesis of galactose-1-phosphate uridyl transferase. All three strains are nonlysogenic. The three were derived from W3092, W3091, and W3094, respectively, whose enzyme defects have been analyzed (Kalckar *et al.*, 1959). The double mutant $Gal_1^-Gal_4^-$ was isolated by Dr. M. L. Morse (stock no. 550) who kindly permitted us to use it. It is also methionine-requiring, lactose-negative, T1-resistant, streptomycin-resistant, and F^-.

Strain C600 is a nonlysogenic, galactose-positive derivative of K12 (Appleyard, 1954).

Lysogenic derivatives of these strains were isolated from the survivors of phage infection. The symbol for a lysogenic strain, e.g. C600(λ), is read: C600 lysogenic for λ.

(d) *Bacteriophages*

Wild type λ, implied whenever the symbol λ is written without qualification, forms turbid plaques about 3 mm in diameter. Its origin has been described (Kaiser, 1957).

The plaques formed by λ *co mi* are clear (*co*) and minute, with well defined halos (*mi*). The *co* mutation, which is very closely linked to *c*, gives 5% recombination with *mi* (Fig. 1) (Kaiser, 1957).

λi^{434}, which has also been called 434 *hy*, is identical with λ except that it has the immunity specificity gene of 434. The i^{434} and i^{λ} genes are two different alleles at the *c* locus (Kaiser & Jacob, 1957).

Isolation of λdg. A stock containing 10^9 λ and 10^2 λdg/ml. was obtained by u.v. irradiation of the galactose-positive strain C600(λ). It is known that several types of λdg, differing in buoyant density, occur in such a stock (Weigle *et al.*, 1960). All of the experiments reported here involve the same λdg, which was isolated as follows. K12 Gal_4^- was exposed to the aforementioned stock and a single galactose-positive colony isolated. Cultures grown from this colony, when induced with ultraviolet light, produce both λ and λdg. It is, therefore, lysogenic both for λ and λdg, symbolized Gal_4^- (λ, λdg). To facilitate the preparation of u.v.-induced lysates from Gal_4^- (λ, λdg), a mutant unable to adsorb λ was selected from it: Gal_4^- (λ, λdg)/λ. This strain served as the source of λdg.

Concentrated stocks of λ, λi^{434}, and λ *co mi* to be used as helper were prepared by u.v. induction of Gal_4^- (λ), Gal_4^-(λi^{434}), and Gal_4^- (λ *co mi*), respectively, and then purified by the procedure described in a succeeding section for λdg.

(e) *Assays*

λ.—The number of active λ in a phage suspension was determined by counting the number of plaques produced by an appropriately diluted sample of the suspension using the agar layer technique described by Adams (1959). The indicator bacteria were strain W3104.

λdg.—The number of functional λdg particles in a phage suspension was determined from the number of galactose-positive colonies produced by an appropriately diluted sample in a standard transduction assay. The assay was carried out as follows: an 0·1 ml. portion of the λdg to be assayed was mixed with 0·2 ml. of exponentially growing cells of W3104 in TB medium (2×10^9 cells/ml.) and 0·1 ml. of helper phage λ (8×10^9 λ/ml.). The mixture was incubated at 37°C for 20 min, then 2 ml. of soft TB agar was added and the entire mixture poured onto an EMB-galactose plate. After incubation for 40 to 48 hr at 37°C, the plates were scored for Gal^+ colonies.

Since 20% of the λdg lysogenize under these conditions, the number of λdg particles is obtained by multiplying the number of Gal^+ colonies by 5. The validity of this procedure

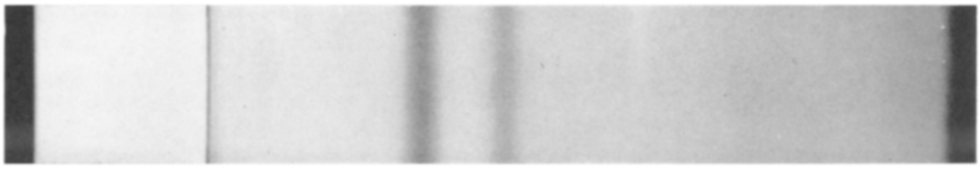

PLATE I. The separation of *λdg* and *λ* phage in a cesium chloride gradient after 8 hr centrifugation at 34,410 rev/min, 5°C. This phage suspension was similar to fraction CsCl-1 except that $\rho_4 = 1{\cdot}51$. The more concentrated band of ultraviolet-absorbing material nearest the meniscus is *λdg* and has a buoyant density of 1·49. The other band is *λ* with a density of 1·50. These buoyant densities are mean values determined by direct density measurements of the fractions obtained during the preparation of fraction CsCl-3 ("Materials and Methods").

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is confirmed by the observation that in purified preparations of λ the ratio of active λ to absorbancy at 260 mμ is 5 times larger than the ratio of *Gal*$^+$ to absorbancy at 260 mμ for purified *λdg*.

(f) *Chemical and physical determinations*

Orthophosphate was estimated by the method of Fiske & SubbaRow (1925) and total phosphorus was measured as orthophosphate after digestion in concentrated H_2SO_4 to which H_2O_2 was added. The diphenylamine reaction of Dische (1955) was used to determine deoxypentose. Pentose was determined by the Mejbaum (1939) procedure, using a 40-min heating period and adenosine 5′-phosphate as a standard. Protein was determined by the phenol method of Lowry, Rosebrough, Farr & Randall (1951) using crystalline bovine plasma albumin as a standard. Absorbancy at a single wavelength was determined in a Beckman model DU spectrophotometer using a 1·00 cm light path, whereas absorption spectra were measured in a Cary model 14 recording spectrophotometer. The viscosity of DNA solutions was determined with a capillary viscometer (Schachman, 1957) at concentrations of about 25 μg/ml. Densities of cesium chloride solutions were determined with a 0·2 ml. pycnometer.

(g) *Purification of λdg*

A culture of Gal_4^- (λ, *λdg*)/λ growing exponentially in H medium containing 1% galactose was induced by irradiation with u.v. after it had achieved a concentration of 2×10^9 cells/ml. and had been cooled to 0°C. After irradiation of 50 liters of culture, 12 liters of 10% Difco bacto-tryptone broth were added, the mixture brought to 37°C, and incubated with aeration until maximum lysis occurred (measured by the absorbancy of the culture at 600 mμ). This lysate was immediately cooled to 0°C and centrifuged in a Sharples centrifuge to clear it of bacterial cells and debris. The supernatant fraction is termed the *crude lysate*. All further purification steps were performed in the cold room at 4 ± 1°C.

The crude lysate was concentrated by adding 250 g of $(NH_4)_2SO_4$/liter of lysate and allowing a precipitate to form and settle to the bottom of the container by letting the mixture stand overnight. After siphoning off the clear supernatant fluid, the precipitate-containing portion was centrifuged to yield a pellet containing the phage. This pellet was taken up in λ-dil. to a final volume one-fortieth of that of the crude lysate and, after dialysis against 0·01 M-$MgSO_4$ in 0·01 M-potassium phosphate buffer, pH 7·0, was termed the *$(NH_4)_2SO_4$ precipitate fraction*.

This fraction was centrifuged for 10 min at 14,000 ***g***, the supernatant fluid decanted and centrifuged for 3 hr at 21,000 ***g***. The resulting pellet, containing the phage, was taken up in λ-dil. to a final volume that was 40% of that of the $(NH_4)_2SO_4$ precipitate fraction and after dialysis as above was termed the *21G fraction*.

Sufficient CsCl was added to the 21G fraction to make the phage suspension 41·5% CsCl (w/w), having a density at 4°C (ρ_4) of 1·46 g cm^{-3}. This solution was centrifuged 1 hr at 21,000 ***g*** and the clear liquid separating a small pellet and a floating gel removed and termed the *CsCl-1 fraction*.

The CsCl-1 fraction was centrifuged 42 hr at 27,000 rev/min in a Spinco model 30 rotor (maximum centrifugal force = 86,000 ***g***). Since λ and *λdg* have different buoyant densities in CsCl solutions (Weigle *et al.*, 1960), they form bands at different positions in the CsCl density gradient established in the centrifuge tube. This banding, as observed with the ultraviolet absorption optics of the Spinco model E centrifuge, is shown in Plate I. If a small hole is pierced in the bottom of the nitrocellulose centrifuge tube with an insect pin (size 00 or 0 pin attached to the tip of a soldering gun and heated prior to piercing), fractions can be collected, drop by drop, without appreciable disturbance of the CsCl and phage distributions. Fractions containing 80 to 90% of the active *λdg* phage were combined and dialyzed against 0·01 M-$MgSO_4$ in 0·01 M-potassium phosphate or 2-amino-2-hydroxymethylpropane-1:3-diol (tris-) buffers, pH 7·0, to yield *fraction CsCl-2*.

In some cases the *λdg* phage from fraction CsCl-2 were not dialyzed but were diluted in 41·5% CsCl solution and centrifuged 67 hr in a Spinco model SW25·1 swinging bucket rotor at 22,000 rev/min (maximum centrifugal force = 70,000 ***g***). Fractions were collected and dialyzed as described above to yield *fraction CsCl-3*.

A summary of the data obtained from the purification procedure is given in Table 1. Its effectiveness is indicated by the fact that the $(NH_4)_2SO_4$ precipitate fraction contains 60 times more 260 mμ-absorbing material and 400 times more protein per active λdg than does the purified CsCl-2 fraction. The 21,000 *g* centrifugation, addition of CsCl, and the preliminary 1 hr centrifugation, together, inactivate about one third of the phage. Since some, if not all, of the inactive phage fractionate with the active phage (e.g. in the CsCl gradient step), these purification factors are, therefore, minimal relative to total phage particles. Because of the uncertainty caused by this inactivation, a better criterion of the purity of the phage preparations in the latter stages of purification is given by the ratio of the absorbancy at 260 mμ to the protein concentration.

TABLE 1

The purification of λdg

Fraction	$\frac{\lambda dg}{\text{ml.}} \times 10^{-12}$	$\frac{\lambda dg}{\lambda}$	$\frac{\lambda dg/\text{ml.}}{As_{260}} \times 10^{-10}$	$\frac{As_{260}}{\text{mg prot./ml.}}$	% yield
Crude lysate	0·06	6·0	—	—	100
$(NH_4)_2SO_4$ precipitate	1·3	5·5	0·55	2·5	53
21G	2·4	5·5	2·8	4·0	42
CsCl-1	2·0	5·0	30	4·4	33
CsCl-2	14	12	42	13	26
CsCl-3	60	70	35	16	18

The method of preparation and assay of the fractions is given in "Materials and Methods." The reproducibility of the assay for λdg and λ is about 15% and that for the protein about 5%. As_{260} refers to the absorbancy at 260 mμ over a 1·00 cm light path after correcting for light scattered by the phage particles. The method of correction is given in the legend of Table 2. No values for the crude lysate are given in columns 4 and 5 because of the large contribution of the Difco bacto-tryptone to the protein and absorbancy values.

If one assumes that all inactive phage fractionate with active phage during centrifugation, then an approximate value of 5×10^7 for the gram-molecular weight of the DNA per λdg particle can be calculated from the data in Table 1, using a 260 mμ molar absorbancy index relative to phosphorus (or deoxyribose) of $6{\cdot}8 \times 10^3$ M^{-1} cm^{-1} (see Table 2). If, on the other hand, all of the λdg particles in the CsCl-2 fraction are assumed to be active, a value of 7×10^7 is found. These values are in agreement with the approximate gram-molecular weight of the DNA per λ particle of 5×10^7 which can be calculated from the data of Stent & Fuerst (1955) concerning the number of phosphorus atoms per λ that have a lethal effect when subject to ^{32}P decay if one assumes that the fraction of total phosphorus atoms exhibiting this effect is 0·1, the value found for phages T1, T2, T3, T5, and T7 (Stent & Fuerst, 1955). Since DNA constitutes 50% of the λdg mass (see "Experimental," Section (a)), the molecular weight of the λdg particle is twice the above values, i.e., about 1×10^8.

(h) *The preparation of λ and λdg DNA*

The DNA of λ or λdg phage was separated from the protein by a modification of the phenol method of Gierer & Schramm (1956). All operations were carried out at cold room temperatures of about 4°C. Fraction CsCl-2 was diluted in λ-dil. to a 260 mμ absorbancy of 10 (about 4×10^{12} λdg per ml.). Equal volumes of this phage suspension and freshly distilled phenol saturated with water at 4°C were mixed and agitated by hand for 1 min. The two phases were separated by centrifugation and the aqueous phase recovered. This process was repeated two more times, fresh water-saturated phenol being mixed with the recovered aqueous phase in each case. The three phenolic phase residues were extracted

serially with a volume of λ-dil. equal to one-fifth the volume of the phage suspension. The resulting two aqueous fractions were combined and dialyzed against 0·15 M-NaCl in 0·01 M-potassium phosphate buffer, pH 7·0, until all of the phenol was removed. The resulting clear, viscous solution is termed *λdg* DNA. It was stored, after freezing quickly in an ethanol-dry ice bath, at − 15°C and showed no loss of biological activity over the period of one year.

The DNA from helper phage λ, termed λ DNA, was also prepared according to the above procedure.

(i) *The nucleotide analysis of λdg DNA*

Fraction CsCl-3 was used as a source of *λdg* DNA for the determination of its nucleotide composition. Prior to isolating the DNA by the above phenol method, the phage suspension was dialyzed against 0·001 M-$MgSO_4$, 0·07 M-NaCl and 0·006 M-tris buffer, pH 7·5, and then treated with pancreatic DNase at a concentration of 1·0 μg per ml. for 1 hr at 37°C in order to hydrolyze any contaminating DNA. The phage were washed twice in the above tris medium by 3-hr centrifugations at 24,000 ***g*** and then treated with phenol. The phenol was removed by dialysis against 0·15 M-NaCl in 0·01 M-tris buffer, pH 7·5.

This DNA solution, containing 14 μmoles of phosphorus, was hydrolyzed to mononucleotides by the successive action of pancreatic DNase and venom phosphodiesterase (Koerner & Sinsheimer, 1957) according to the procedure of Lehman, Bessman, Simms & Kornberg (1958) with minor modifications in the concentrations of DNA and enzymes. The resulting digest contained 98 to 100% of the total phosphorus in the form of 5′-mononucleotides as indicated by the fact that this amount of phosphorus was released as orthophosphate after treatment of a portion of the digest with purified semen monoesterase (gift of Dr. L. Heppel) under conditions in which the contaminating phosphodiesterase activity of this enzyme preparation was not significant.

The 5′-mononucleotides were separated on a column of Dowex 1 (10×) acetate at 4°C using ammonium acetate buffers at pH 4·3 according to the method of Sinsheimer & Koerner (1951) except that 0·05, 0·25, 0·50, and 1·0 M buffer solutions were used to elute the 5′-monophosphates of deoxycytidine, deoxythymidine, deoxyadenosine, and deoxyguanine, respectively. The mononucleotides were identified by their absorption spectra and by their position in the elution diagram when compared to that of known nucleotides.

3. Experimental

(a) *The preparation and chemical analysis of λdg DNA*

Extraction of *λdg* with cold, water-saturated phenol releases the phage nucleic acid from its protein coat into aqueous solution. The chemical analysis of this material is given in this section. Its biological activity will be described in the following section.

The *λdg* phage that are used as the source of DNA in the phenol extraction ("Materials and Methods") are 50 ± 3% by weight DNA, as calculated from their phosphorus and deoxyribose content (Table 2). The remaining material in the phage is assumed to be protein, although protein analysis by the method of Lowry *et al.* (1951) yields the value of 62%. The inconsistency of this high protein percentage is most easily resolved by assuming that the bovine plasma albumin, used as a standard, is less reactive in this protein assay than is the average *λdg* phage protein.

The aqueous solution obtained after phenol extraction of the phage is termed *λdg* DNA and contains approximately 90% of the 260 mμ-absorbing material present in the phage. Since both the molar absorbancy index relative to phosphorus (α_p) at 260 mμ and the molar ratio of deoxyribose to phosphorus of the *λdg* DNA and of the phage are not significantly different (Table 2), the yield of DNA is also about 90%. On the other hand, less than 2% of the phage protein remains in the aqueous phase after phenol extraction. This is a maximum value determined by the significance level of the protein assay for the *λdg* DNA. The actual protein content of *λdg* DNA is

probably very much lower than this 2% value. Thus Dr. Simmons, in our laboratory, has recently found that in the phenol extraction of λ*dg* labeled with ^{35}S only 0·1% of the phage sulfur appears in the aqueous phase.

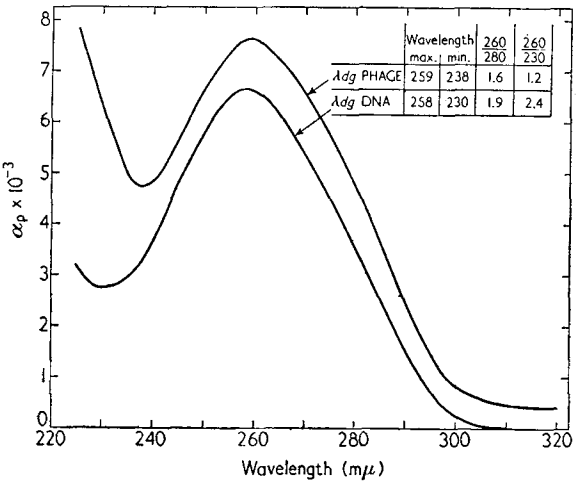

FIG. 2. The ultraviolet absorption spectra of λ*dg* phage (fraction CsCl-3) and of λ*dg* DNA at pH 7. The phage were suspended in 0·01 M-$MgSO_4$ and 0·01 M-potassium phosphate buffer and no correction was made for scattered light. The DNA was dissolved in 0·15 M-NaCl and 0·01 M-potassium phosphate buffer. α_p = molar absorbancy index relative to phosphorus.

TABLE 2

The chemical analysis of λdg phage and λdg DNA

Material	Percent phosphorus by weight	Deoxyribose to phosphorus molar ratio	α_p (260 mμ)	Molar ratio of deoxynucleotides
λ*dg* phage	4·7	0·98	$6{\cdot}8 \times 10^3$	—
λ*dg* DNA	9·0	1·00	$6{\cdot}6 \times 10^3$	A-1·00
				T-1·00
				G-0·95
				C-0·94

The λ*dg* phage (fraction CsCl-2) and λ*dg* DNA were analyzed for phosphorus and deoxyribose after dialysis against water and freeze-drying, followed by drying over P_2O_5 *in vacuo* at room temperature. The λ*dg* DNA was prepared by phenol extraction of phage fraction CsCl-2, except that for the analysis of deoxynucleotides fraction CsCl-3 was used (see "Materials and Methods"). The α_p at 260 mμ for λ*dg* phage and λ*dg* DNA was determined under conditions given in Fig. 2, except that a correction for the light scattered by the λ*dg* phage has been made. The amount of scattered light was determined by the extrapolation to lower wavelength of a λ^{-3} curve fitted to the measured optical densities between 315 mμ and 400 mμ.

The ultraviolet absorption spectrum of λ*dg* DNA (Fig. 2) is that expected of solutions of native DNA (Beaven, Holiday & Johnson, 1955) and exhibits a typical hyperchromic effect when treated with pancreatic DNase, the α_p at 260 mμ increasing 1·35-fold at pH 7 in 0·15 M-NaCl. Treatment of λ*dg* DNA with pancreatic DNase also causes a decrease in the reduced viscosity from 65 dl. g^{-1} to less than 1 dl. g^{-1} when measured at 37 °C in 0·14 M-NaCl, 0·015 M-sodium citrate at pH 7·6.

Hydrolysis of *λdg* DNA to mononucleotides can be accomplished by the successive catalytic action of pancreatic DNase and venom phosphodiesterase ("Materials and Methods"). Chromatography on Dowex 1 of the resulting digest ("Materials and Methods") showed that 98% of the 260 mμ-absorbing material and 97% of the phosphorus in the digest could be accounted for by the deoxynucleotides of adenine, thymine, guanine, and cytosine. No 5-hydroxymethyl-deoxycytidine-5′-monophosphate nor its *o*-mono- or diglucosylated derivatives was detected, although these nucleotides are separable by this technique (Lehman, personal communication) and would have been detectable if any one of them constituted greater than 0·3% of the mononucleotides in the digest.

TABLE 3

Components required for transformation

	Number of *Gal*$^+$
Complete system	2×10^4†
omit *λ*	0
omit *λdg* DNA	6
replace *λdg* DNA with *λ* DNA	10
replace *λdg* DNA with DNase-treated *λdg* DNA	6
omit *λdg* DNA and *λ*	0
omit bacteria	0

† A 10^{-2} dilution of the incubated mixture showed 204 *Gal*$^+$ colonies. The plates obtained from the undiluted mixture contained more than 5000 *Gal*$^+$ colonies and were therefore not countable.

The complete system contained 190 μg *λdg* DNA and $1{\cdot}5 \times 10^8$ λ-infected K12 $Gal^-{}_1Gal_4^-$ bacteria, 2 μmoles tris, pH 7·1, 2 μmoles $CaCl_2$, and 2 μmoles $MgSO_4$ in a total volume of 0·2 ml. The mixture was incubated 60 min at 37°C and then plated.

λ-infected bacteria were prepared by exposing $1{\cdot}5 \times 10^9$ bacteria to $1{\cdot}5 \times 10^{10}$ λ in P medium (made up to 0·01 M in $MgSO_4$) at 37°C for 15 min. At the end of the incubation the bacteria were centrifuged and resuspended in the diluent indicated above. Uninfected bacteria (for the line "omit λ") were similarly treated except that no phage was added.

DNase-treated *λdg* DNA was prepared by preincubating 380 μg *λdg* DNA with 0·2 μg pancreatic DNase at 22°C for 40 min in 0·2 ml. The inorganic components of this preincubation were the same as those in the complete system.

The amount of *λ* DNA or DNase-treated *λdg* DNA employed was also 190 μg.

The relative molar concentrations of the four mononucleotides present in the digest are given in Table 2. The expected equalities of adenine and thymine and of guanine and cytosine (Chargaff, 1955; Watson & Crick, 1953) occur. The fraction of bases which are guanine and cytosine in *λdg* DNA is 0·49. This quantity is a characteristic of the biological origin of a DNA preparation, varying from 0·3 to 0·7 for different microorganisms (Chargaff, 1955; Lee, Wahl & Barbu, 1956). The value for *λdg* DNA is equivalent, within experimental error, to that found for the DNA of a virulent mutant of *λ* (Lwoff, 1953) and of the *λ* host cell, *E. coli* K12 (Gandelman, Zamenhof & Chargaff, 1952).

The foregoing data show that the aqueous phase resulting from the phenol extraction of a *λdg* phage suspension is a solution of DNA. Less than 2% of the phage material in this solution is protein and, on the basis of its phosphorus and deoxynucleotide content, at least 95% is DNA.

(b) *Description of the transformation system and its requirements*

λdg DNA will transform galactose-negative (*Gal*$^-$) bacteria only if the bacteria have been exposed to ordinary λ, either simultaneously with or before the addition of the DNA. The requirement for added "helper phage," as it will be called in subsequent discussion, is shown in Table 3. Experiments which explore the role of helper will be presented in the next section; here, the point to be emphasized is that the level of transformation is at least 10^4-fold higher in the presence of helper than in its absence.

For these experiments the helper phage was propagated on *Gal*$_4^-$ bacteria so that it could not mediate a transduction of the *Gal*$_4^-$ recipient bacteria to *Gal*$^+$. The *Gal*$^+$ bacteria which arise in the absence of DNA, Table 3, are most likely due to reversions of the *Gal*$^-$ recipient bacteria which occur when it is growing on the assay plate.†

The transformation of *Gal* genes is a specific property of *λdg* DNA in the sense that neither λ DNA nor DNase-treated *λdg* DNA can replace *λdg* DNA. The results of both experiments are shown in Table 3.

The transforming activity of *λdg* DNA for *Gal*$^-$ mutants defective in the synthesis of galactokinase or in the synthesis of galactose phosphate uridyl transferase is similar. Thus 2·5 μg of *λdg* DNA produced 205 *Gal*$^+$ colonies from a *Gal*$_4^-$, transferase-less mutant and 130 *Gal*$^+$ colonies from a *Gal*$_2^-$, kinaseless mutant. The conditions of the experiment were those of the standard transformation assay described at the end of the next section. Therefore, *λdg* DNA, like *λdg*, carries both the gene for galactose phosphate uridyl transferase and the gene for galactokinase.

(c) *The quantitative determination of transforming activity*

The aim of the experiments described in this section was to develop an assay for the transforming activity of *λdg* DNA. These experiments also give some insight into the mechanism of the reaction between DNA and recipient cell and the role of helper.

(i) *Choice of helper phage*

Although wild type λ acts as helper, the variant *λi*^{434} acts more efficiently. At the same multiplicity of infection, 10 adsorbed phage per bacterium, 3·5 times more *Gal*$^+$ bacteria arose when *λi*^{434} was used as helper than when wild type λ was used. Phage *λi*^{434} was therefore adopted as helper for the standard transformation assay.

To avoid lysis of the recipient bacteria due to multiplication of helper phage and subsequent reinfection of the survivors during the assay, a *Gal*$^-$ lysogenic for *λi*^{434} was used as recipient.

(ii) *Time course of the reaction between λdg DNA and helper-infected bacteria*

If *λdg* DNA, helper phage, and recipient bacteria are mixed and DNase is added at various times later, the number of *Gal*$^+$ bacteria increases slowly to reach a plateau at 120 min, having attained one-half the maximum value at 40 min. The results of this experiment are represented in Fig. 3.

(iii) *Dependence on DNA concentration*

The number of *Gal*$^+$ bacteria obtained is proportional to the concentration of *λdg* DNA, as is shown in Fig. 4. This is true not only for an incubation of 120 min, a

† The data of Table 3 indicate that more revertants arise among the helper-infected bacteria than in the uninfected. This may be due to selection imposed by lysis and the products of lysis acting on the assay plates.

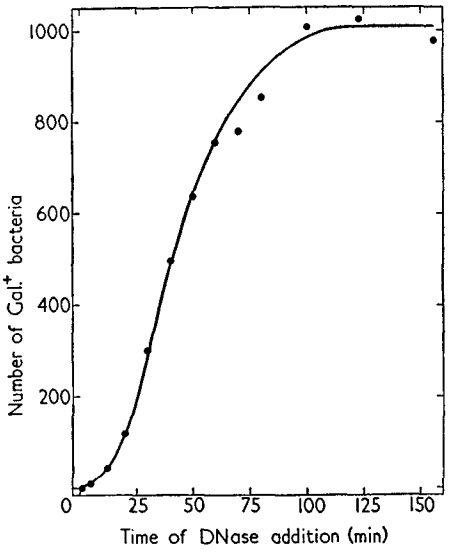

FIG. 3. Time course of the reaction between λdg DNA and helper-infected bacteria. DNA, helper, and bacteria were incubated together at 37°C in the following concentrations: λdg DNA—31 μg per ml., λi^{434} helper—$2{\cdot}6\times10^{9}$ per ml., and K12 Gal_4^- (λi^{434})—$4{\cdot}3\times10^{8}$ per ml. Media and diluents were the same as those described in the standard transformation assay. At the times indicated on the abscissa duplicate 0·3 ml. samples were removed from the incubation mixture and added to 0·1 ml. of 20 μg per ml. pancreatic DNase, incubated 5 min at 37°C and then plated. The number of Gal^+ bacteria given on the ordinate is the sum of that for two duplicate incubation mixtures.

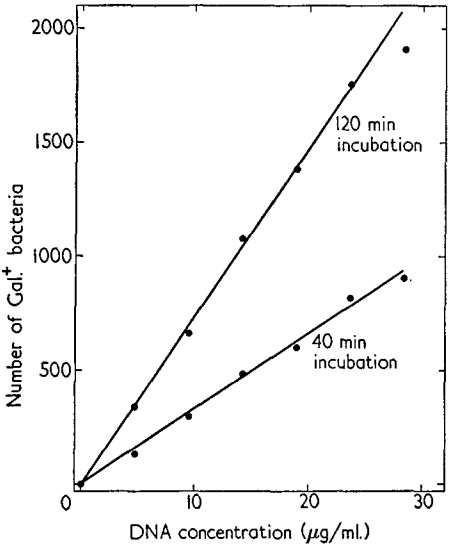

FIG. 4. Dependence on the concentration of DNA. Duplicate samples of λdg DNA at the concentrations indicated on the abscissa were incubated with K12 Gal_4^- (λi^{434}) at $5{\cdot}6\times10^{8}$ per ml. and λi^{434} helper at $2{\cdot}6\times10^{9}$ per ml. in a total volume of 0·3 ml. After 40 or 120 min at 37°C, 0·1 ml. pancreatic DNase at 30 μg per ml. was added, the mixtures incubated 5 min at 37°C and then plated. The number of Gal^+ recorded on the ordinate is the average of that for two duplicate incubation mixtures.

time which falls on the plateau of the time curve (Fig. 3), but also for an incubation of 40 min, the time at which uptake is one-half the maximum plateau value.

The specific transforming activity measured in this experiment after 120 min incubation is 75 *Gal*$^+$ per μg DNA. More recently specific activities of 8×10^5 *Gal*$^+$ per μg DNA have been obtained with the same DNA preparation but with different conditions of helper infection and DNA uptake.† The specific activity of whole λdg phage as measured in a transduction assay (see Table 1) is 2×10^9 *Gal*$^+$/μg DNA. Therefore, 4×10^{-4} of the activity present in λdg has been accounted for in terms of transformation by λdg DNA.

(iv) *Dependence on the concentration of helper-infected bacteria*

For a given amount of λdg DNA, the number of *Gal*$^+$ bacteria produced during a two-hour exposure is a linear function of the number of helper-infected bacteria,

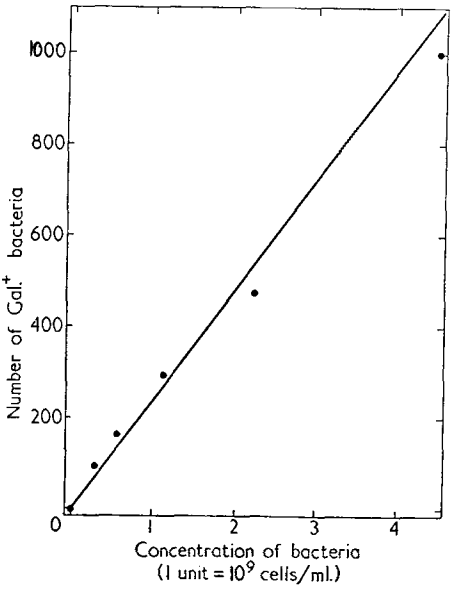

FIG. 5. Dependence on the concentration of helper-infected recipient bacteria. A number of 0·3 ml. incubation mixtures were made, each containing 8 μg per ml. λdg DNA and variable amounts of K12 Gal_4^- (λi^{434}) ranging from $5{\cdot}7\times10^8$ to $4{\cdot}7\times10^9$ per ml. as indicated on the abscissa. The mixtures were completed by the addition of variable amounts of helper so as to keep a constant ratio of 9 phage per bacterium. After 120 min incubation at 37°C, 0·1 ml. pancreatic DNase at 20 μg per ml. was added, incubated 5 more min at 37°C, and then plated. The ordinate indicates the total number of *Gal*$^+$ bacteria on plates from two duplicate incubation mixtures.

as can be shown by (1) varying the concentration of bacteria, keeping the multiplicity of helper infection constant (Fig. 5), or (2) varying the multiplicity of helper infection for a given bacterial concentration.

In an experiment of the latter type, the number of *Gal*$^+$ bacteria is directly proportional to the multiplicity of infection for multiplicities below 0·1. Above 0·1 the

† The conditions were as follows. Gal_4^- (λi^{434}) were infected with λi^{434} in P medium with $MgSO_4$ added to 0·01 M. The multiplicity of infection was 10. After 15 min incubation at 37°C the infected bacteria were centrifuged and resuspended in a solution containing 0·01 M-tris, pH 7·1, 0·01 M-$MgSO_4$, 0·01 M-$CaCl_2$. λdg DNA diluted in the same solution was added. Infected bacteria and DNA were incubated for 60 min at 30°C; then pancreatic DNase, 10 μg/ml. final concentration, was added and the mixture plated.

number of *Gal*$^+$ increases slowly with increasing multiplicity, reaching a maximum at a multiplicity of 4. The maximum is broad, extending from 4 to 14. The growth of the recipient bacteria is inhibited at multiplicities higher than 14.

(v) *Procedure for a standard transformation assay*

The following procedure was used to assay transforming activity in all subsequent experiments unless otherwise indicated. DNA, helper phage, and recipient bacteria were prepared as three separate reagents. The DNA was diluted to an appropriate concentration in 0·15 M-NaCl, 0·01 M-potassium phosphate buffer at pH 7·0. Helper λi^{434} was diluted to 10^{10} phage per ml. in λ-dil. The recipient bacteria Gal_4^- (λi^{434}) were grown in P medium containing 1·25 mg /ml. glucose to $1{\cdot}6\times10^9$ viable cells/ml., chilled, and then $MgSO_4$ was added to a final concentration of 0·01 M.

The assay was begun by mixing 0·1 ml. each of the diluted DNA, helper, and recipient bacteria. After 120 min at 37°C, 0·1 ml. of a 20 μg per ml. solution of pancreatic DNase was added, and the 37°C incubation was continued for 5 min more. Finally, 2 ml. of TB soft agar was added and the contents poured onto an EMB-galactose plate. The number of *Gal*$^+$ colonies was counted after 40 to 48 hr at 37°C.

Under the conditions of this assay, λ*dg* DNA had a specific transforming activity of 75 *Gal*$^+$ per μg DNA. The mean deviation between duplicate assays with the same culture of bacteria was 15%, although the activities measured for the same DNA preparation using different cultures of recipient bacteria might differ by several-fold.

(d) *The nature of the active material*

The chemical and physical properties of λ*dg* DNA are those of deoxyribonucleic acid. However, since only 4×10^{-4} of the activity present in whole λ*dg* has been accounted for in terms of transformation by λ*dg* DNA, it is possible that only 4×10^{-4} of the λ*dg* complements of DNA, or even 8×10^{-5} since no more than 20% of the λ*dg* phage particles register in a transduction assay, are still biologically active. If this were true, and if the loss in activity were due to a change in composition, the bulk chemical properties of λ*dg* DNA might not represent those of the active material. It is necessary, therefore, to use other techniques to identify the active material.

(i) *The effect of anti-λ serum*

Since the active material is obtained from phage particles, it is pertinent to compare its properties with those of whole phage.

Neutralizing antibody reacts with the proteins of the phage tail to block some early step in the infection process, either adsorption or injection (Nagano & Oda, 1955). To see whether the active material in λ*dg* DNA contains those structures of whole phage which react with neutralizing antibody, λ*dg* DNA was treated with rabbit anti-λ serum.

The antiserum was first heated to 70°C for 45 min to denature serum nucleases. After heating, the serum contained less nuclease activity/ml. than would be equivalent to a solution containing 10^{-5} μg per ml. of crystalline pancreatic DNase, measured viscometrically with calf thymus DNA as substrate.

Results of this experiment are presented in Fig. 6. Whereas more than 95% of the transducing activity of λ*dg* was neutralized in 160 min, no neutralization of the infectivity of λ*dg* DNA was detected. The 10% decrease in infectious activity of λ*dg* DNA which occurred in the presence of anti-λ serum occurred also in its absence.

To test the hypothesis that DNA might inhibit the neutralization reaction so that a small amount of whole *λdg* could escape detection in a large amount of *λdg* DNA, λ DNA was added to *λdg* and the course of neutralization of the mixture followed. The result, also presented in Fig. 6, was identical to the neutralization of *λdg* in the absence of added DNA.

The conclusion is that those structures of whole phage responsible for its reaction with neutralizing antibody are not necessary for the activity of *λdg* DNA.

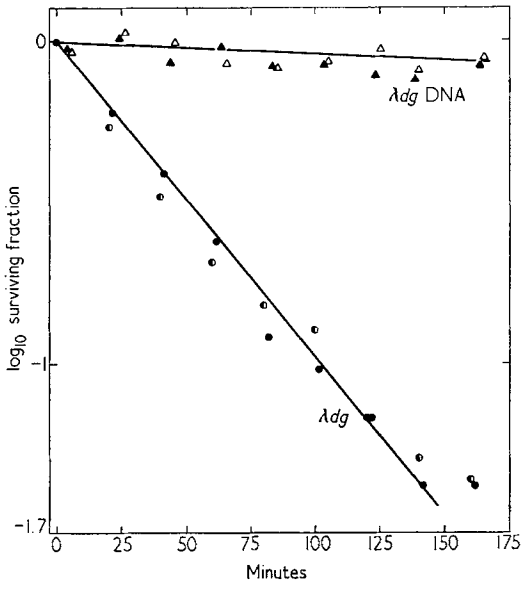

FIG. 6. The effect of anti-λ serum on the transducing activity of *λdg* and the transforming activity of *λdg* DNA.

1. (▲) 112 μg *λdg* DNA in 2 ml. anti-λ serum. The anti-λ serum had been diluted 1 to 33 in 0·15 M-NaCl, 0·01 M-potassium phosphate buffer, pH 7·0, heated 45 min at 70°C and further diluted 1500-fold in the same diluent.
2. (△) 112 μg *λgd* DNA in 2 ml. 0·15 M-NaCl, 0·01 M-potassium phosphate buffer, pH 7·0.
3. (●) $1{\cdot}6\times10^5$ *λdg* in 2 ml. anti-λ serum which had been treated as in 1.
4. (◐) $1{\cdot}6\times10^5$ *λdg* and 125 μg λ DNA in 2 ml. anti-λ serum which had been treated as in 1.

The four mixtures were incubated at 37°C and at the times indicated duplicate 0·1 ml. samples withdrawn and added to the standard transformation assay system as described in the text. A low concentration of antiserum was employed in the experimental mixtures so that neutralization of helper phage during the 2-hr assay would be negligible.

Initial values in the incubation mixtures were $7{\cdot}8\times10^3$ *Gal*$^+$ per ml. for 1 and 2; $1{\cdot}6\times10^4$ *Gal*$^+$ per ml. for 3 and 4.

(ii) *The effect of pancreatic DNase*

Whole phage is resistant to DNase because a shell of protein completely encases the nucleic acid. By contrast the transforming activity of *λdg* DNA is sensitive to this enzyme.

Sensitivity to pancreatic DNase was measured as a function of enzyme concentration from 10^1 to 10^{-4} μg per ml. For purposes of comparison whole *λdg* was treated with the same concentrations of enzyme. Results of this experiment, presented in Table 4, show that 10^{-4} μg per ml. of enzyme was sufficient to destroy 94% of the transforming activity in 30 min. It is unlikely, therefore, that inactivation is due to other enzymes contaminating the preparation of DNase.

The kinetics of inactivation of transforming activity and of decrease in viscosity were measured during exposure to 8×10^{-5} μg per ml. pancreatic DNase at 37°C. The results are plotted in Fig. 7. Transforming activity fell within 35 min to the limit of detectability which was for this experiment 0·4% of the initial activity. The viscosity, however, decreased more slowly; 70% remained after 50 min of enzyme treatment.

TABLE 4

Effect of DNase on the transducing activity of λdg and on the transforming activity of λdg DNA

DNase concentration (in mixture)	*Gal*+	
	λdg DNA	Whole *λdg*
μg/ml.		
10	0	245
1	0	229
10^{-1}	0	261
10^{-2}	0	197
10^{-3}	1	221
10^{-4}	16	220
0	264	246

Whole *λdg* or 4·7 μg *λdg* DNA and the indicated concentration of pancreatic DNase (in 0·01 M-$MgSO_4$, 0·01 M-potassium phosphate buffer, pH 7, 12 μg per ml. serum albumin) were incubated at 28°C in a total volume of 0·4 ml. After 30 min incubation 0·1 ml. of a λ suspension containing $4{\cdot}4 \times 10^{10}$ phage per ml. and 0·1 ml. of a TB culture of K12 Gal_4^- containing $7{\cdot}5 \times 10^9$ cells/ml. were added. The mixtures were incubated 20 min more at 28°C; then 0·2 ml. was withdrawn and plated.

The rate of inactivation of transforming activity is very close to that observed for *Hemophilus* transforming DNA (Zamenhof, Alexander & Leidy, 1953). Moreover, just as was observed for *Hemophilus* DNA, the decay of transforming activity is faster than the decay of viscosity.

(iii) *The effect of trypsin*

The active material in *λdg* DNA can be compared to the urea-treated T2 phage (Pi particles) which are capable of infecting protoplasts of *E. coli* but not the normal cells (Spizizen, 1957; Mahler & Frazer, 1959). The Pi particles are clearly differentiable from T2 phage but apparently still retain a structure consisting of other components in addition to the DNA.

The Pi particles exhibit a high and unique sensitivity to the action of trypsin. Whereas T2 phage are insensitive to 30 min exposure at 37°C to concentrations of trypsin as high as 100 μg per ml., Pi particles are over 90% inactivated at a trypsin concentration of 5×10^{-4} μg per ml. (Mahler & Frazer, 1959). When *λdg* DNA was treated with trypsin, no significant change in its activity could be demonstrated. Thus *λdg* DNA at a concentration of 130 μg per ml. was mixed at 37°C with from 1 to 90 μg per ml. of trypsin in either 0·05 M-potassium phosphate or tris buffer, pH 8·0. Samples of the reaction mixture taken at zero and at 60 min after the addition of the trypsin were diluted tenfold in 0·14 M-NaCl, 0·01 M-potassium phosphate buffer, pH 7·0, at 0°C and

then assayed for their activity. No significant difference (± 15%) in activity was observed between the zero and 60-min samples, the results being the same for control mixtures in which trypsin was absent.

While this result does not exclude the presence of some protein in the active *λdg* DNA molecules, it does indicate that trypsin-sensitive proteins, such as those present in Pi, are not necessary for activity.

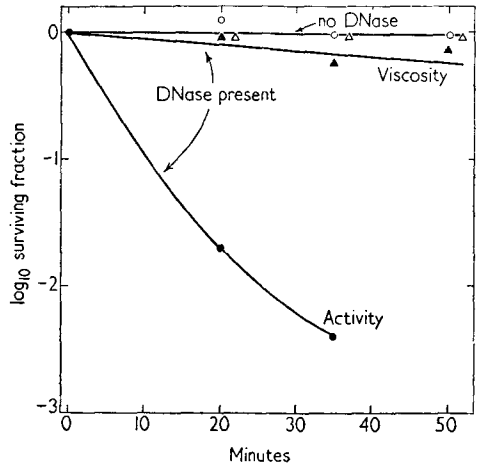

FIG. 7. Time course of the inactivation of *λdg* DNA by pancreatic DNase. Two incubation mixtures were prepared, each containing 210 μg *λdg* DNA, 20 μmoles $MgSO_4$, 10 μmoles potassium phosphate buffer, pH 7·0, and 20 μg serum albumin in a total volume of 1 ml. One mixture contained in addition 8×10^{-5} μg pancreatic DNase. After incubation for the times indicated at 37°C, 0·1 ml. samples were removed and diluted 1/20 in 0·15 M-NaCl, 0·01 M-potassium phosphate buffer, pH 7·2. Duplicate 0·1 ml. samples of the diluted mixture were then assayed by the standard transformation assay. Reconstruction controls carried out at the beginning and at the end of the experiment showed that the carry-over of 4×10^{-7} μg of DNase into the assay had no effect. As soon as the samples for the transformation assay were removed the remainder of the 20-fold dilution was used to measure the relative viscosity at 37°C.

(●) transforming activity, DNase present.
(○) transforming activity, DNase absent.
(▲) relative viscosity, DNase present.
(△) relative viscosity, DNase absent.

The initial values were 5×10^4 *Gal*$^+$ per ml. in the incubation mixture and a η_{sp} of 0·037 for the twentyfold dilution.

(iv) *The heat denaturation of λdg DNA*

A further criterion for defining the molecular nature of the infectious agent in the *λdg* DNA preparation is its stability to heat. As the temperature is raised, the DNA in an aqueous salt solution exhibits a transition from the relatively ordered structure of a double helix to a disordered coil (Rice & Doty, 1957; Doty, Boedtker, Fresco, Haselkorn & Litt, 1959). In the case of DNA from *Hemophilus influenzae* and *Diplococcus pneumoniae*, this transition results in the inactivation of that DNA with respect to genetic transformation (Zamenhof *et al.*, 1953; Marmur & Lane, 1960). The temperature at the midpoint of this transition, T_m, is a linear function of the guanine-cytosine content of a given DNA preparation (i.e. the fraction of bases which are guanine and cytosine) and lies between 84° and 96°C for the solutions of DNA from natural sources that have been examined (Marmur & Doty, 1959). With a guanine-cytosine content of 0·49, *λdg* DNA should have a T_m of about 89°C, the exact value depending upon the method used for measuring the transition.

Bacteriophage, on the other hand, are inactivated at considerably lower temperatures since the mechanism of their attachment and DNA injection into the host cell is dependent upon phage proteins which are more thermolabile than is the DNA. The characterization of the heat stability of the infectious activity of the *λdg* DNA preparation should therefore determine whether the DNA or some phage protein is the most thermolabile substance necessary for activity of the infectious agent.

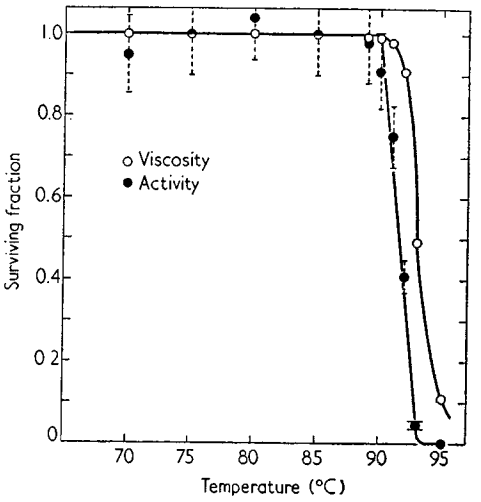

FIG. 8. The heat denaturation of *λdg* DNA with respect to transformation activity (●) and viscosity (○). The surviving fraction is the ratio of activity, or of viscosity, after heating for 60 min at the indicated temperature to that before heating. All activities and viscosities were determined at 37°C after cooling the heated material quickly in an ice-water bath. The dashed vertical lines associated with the activity values indicate the significance level ($\pm \sigma = \pm 10\%$) for this series of assays. Before heating, the 25 μg per ml. solution of DNA in 0·15 M-NaCl and 0·015 M-sodium citrate, pH 7·7, had an activity of 2×10^3 transformation units (*Gal*$^+$) per ml. and a reduced viscosity of 65 dl. g^{-1}.

The data presented in Fig. 8 indicate that the loss of transforming activity closely parallels the transition of the DNA structure as it is measured by the drop in viscosity attendant upon the loss of the double helical configuration. Thus, as the temperature is raised, the first significant loss in infectious activity and in viscosity occurs after one hour's exposure to a temperature between 90 and 91°C, the T_m values being 92°C for the activity and 93°C for the viscosity. In contrast, when complete *λdg* phage are heated under the conditions stated in Fig. 8, the surviving fraction of transducing activity is only 4×10^{-2} and 2×10^{-7} at 50° and 70°C, respectively.

It follows from these results that DNA is the most thermolabile substance in the active material of the *λdg* DNA preparation and further confirmation is given to the hypothesis that the proteins necessary for adsorption and DNA injection of *λdg* phage are not operative in this agent.

(v) *The buoyant density of the transforming agent in cesium chloride solutions*

Rolfe & Meselson (1959) and Sueoka, Marmur & Doty (1959) have shown that the buoyant density of DNA in cesium chloride solutions is related to its guanine-cytosine content (GC) by the following empirical equation:

$$\rho = 1{\cdot}66 + 0{\cdot}10\ (\mathrm{GC})\ \mathrm{g\ cm^{-3}}.$$

Since λdg DNA has a guanine-cytosine content of 0·49, it would be expected to exhibit a density of 1·71 g cm^{-3}. The λdg phage used in these experiments has a density of 1·49 g cm^{-3}, a value consistent with its 50% DNA content if one assigns a density of 1·3 g cm^{-3} to the phage protein. Thus one would expect that any product of λdg phage degradation would exhibit a density determined by its protein to DNA mass ratio. Provided the active material in the λdg DNA preparation is large enough to form a band in a cesium chloride gradient, the density at the band position should yield a measure of the protein to DNA mass ratio of the active unit.

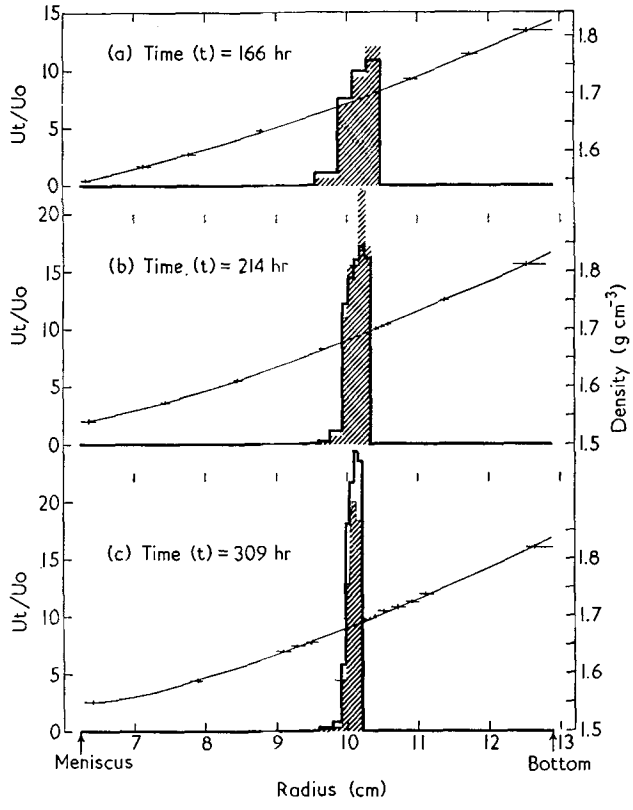

FIG. 9. Centrifugation of λdg DNA in a cesium chloride gradient.

(1) Left-hand ordinate. The ratio of 260 mμ absorbancy (heavy solid line) or of transformation activity (shaded) at time (t) to that at the beginning of centrifugation ($t = 0$).

(2) Right-hand ordinate. The density at 4°C of fractions whose size is indicated by the short horizontal lines, the short vertical line being at the mean radius of each fraction. Density determinations are accurate to $\pm$ 0·0015 g cm^{-3}.

(3) Conditions: At $t = 0$ hours, CsCl = 5·24 M; tris = 0·023 M; pH = 8·6; $\rho_4 = 1{\cdot}661$; 260 mμ absorbancy = 0·77; activity = 8×10^3 transformation units per ml.; and temperature = 4°C. The solution was centrifuged in a Spinco SW 25·1 swinging bucket rotor at $22{\cdot}5 \times 10^3$ rev/min and the temperature at the end of each run was 5 $\pm$ 1°C.

(4) Percent recovery: 260 mμ absorbancy = (a) 101, (b) 103, and (c) 98; transformation activity = (a) 96, (b) 108, and (c) 86.

To this end, λdg DNA in cesium chloride solutions was centrifuged for various times under the conditions indicated in Fig 9. Fractions were collected from the centrifuge tubes by the technique employed in the cesium chloride gradient purification of λdg phage ("Materials and Methods"). Analysis of these fractions for their ultraviolet

absorption spectrum, their density at 4°C, and their transforming activity after dialysis against 0·15 M-NaCl containing 0·01 M-potassium phosphate buffer, pH 7·0, yielded the results given in Fig. 9 and Table 5.

Since the recovery of absorbancy at 260 mμ and of transforming activity after each centrifugation was complete within the experimental error of the techniques employed (± 15% for activity and ± 4% for the absorbancy), and since better than 98% of the recovered value was found in the visually obvious band regions of Fig. 9, it is sufficient to limit the analysis of the fractions to this band region.

TABLE 5

Comparison of the DNA and transforming activity distributions during centrifugation in a cesium chloride gradient

Time (hours)	Mean density (g cm^{-3})		Standard deviation (g cm^{-3})		Density gradient (g cm^{-4})
	DNA	activity	DNA	activity	
166	1·691	1·692	0·0084	0·0080	0·045
214	1·687	1·687	0·0061	0·0056	0·048
309	1·685	1·685	0·0042	0·0043	0·048

The distributions analyzed are those shown in Fig. 9. The DNA was measured by its absorbancy at 260 mμ.

The mean density and standard deviation of density for the activity and absorbancy distributions (Table 5) are not significantly different from each other at each centrifugation time. Furthermore, there are no significant differences in the transforming activity per unit absorbancy at 260 mμ (specific activity) among the various fractions and that of the input material, except for one to two per cent of total recovered activity occupying the lowest density region of the bands, in which case the specific activity was about 60% of that found in the other fractions. The discrepancy between the observed density of 1·685 g cm^{-3} and the value of 1·71 g cm^{-3} predicted above is due to the different conditions employed in this experiment from those used in determining the empirical equation relating density to guanine-cytosine content, since the DNA used in this experiment yielded a buoyant density of 1·708 ± 0·001 when measured under the latter conditions (Rolfe, personal communication).†

These results indicate that the active molecules have the same protein to DNA mass ratio as that of the bulk of the DNA. As indicated in Section 1 the value of this ratio is less than or equal to 0·02 on the basis of the protein assay of Lowry *et al.* (1951) and less than or equal to 0·001 on the basis of sulfur content. Furthermore, from the equality of the activity and DNA distributions at the various times of centrifugation, it is concluded that the size of the active molecules is of the same order of magnitude as that of the DNA molecules. Thus the conclusion derived from this experiment and

† The significant difference in the conditions is that the CsCl used in our experiments was impure, containing 4·11% rubidium chloride (see "Materials and Methods"), whereas that used by Rolfe & Meselson (1959) was 99·9% CsCl (Maywood Chemical Works). Thus we have recently obtained a mean buoyant density of 1·71 for λ*dg* DNA under the conditions stated in Fig. 9 when using the purer CsCl.

consistent with the previous results is that the active material consists of molecules of DNA in aqueous solution.

(e) *Cotransformation of Gal and phage genes*

The galactose-positive bacteria produced by exposure to *λdg* DNA have two characteristic properties. First, they are unstable. Isolated colonies, previously purified by at least two single colony isolations, contain one galactose-negative bacterium per several hundred galactose-positive bacteria. Secondly, they produce lysates after irradiation with u.v. light which are capable of transducing the gene for galactose-l-phosphate uridyl transferase at high frequency.

In both respects these strains behave like *Gal*$^-$ (*λdg*). When a *λdg* prophage is lost, which happens about one in 10^3 cell generations, the host becomes *Gal*$^-$ (Morse *et al.*, 1956; Campbell, 1957). U.v. irradiation of bacteria doubly lysogenic for *λdg* and *λ* induces the production of both *λ* and *λdg* in roughly equal amounts. The lysate produced will, therefore, transduce with high frequency.

A quantitative comparison of segregation and production of *λdg* for a series of galactose-positive strains produced by exposure to *λdg* DNA and those produced by transduction with *λdg* is presented in Table 6. The similarity of the results obtained

TABLE 6

Segregation of Gal$^-$ *and phage production by Gal*$^+$ *transformants*

Donor	Segregation of *Gal*$^-$ bacteria		Production of *λdg*			
				number having *λdg*/*λ* values in the range		
	number tested	number segregating	number tested	0·5–2·0	2·1–4·0	4·1–6·0
λdg DNA	56	52	21	10	9	2
λdg	56	51	18	9	6	3

Using the procedure of the standard DNA transformation assay, galactose-positive bacteria were isolated after exposure to *λdg* DNA or to *λdg*. From each of the two series a number of galactose-positive bacteria were picked and streaked out on EMB-galactose plates. To avoid bias in the selection of colonies, all galactose-positive bacteria within an arbitrary sector of each of two plates were picked. A single galactose-positive colony was picked from each streak and restreaked so as to obtain more than 500 isolated colonies. These streaks were examined for galactose-negative colonies. From streaks which showed at least one galactose-negative colony a single galactose-positive colony was picked and grown in H-glucose liquid medium and a lysate prepared by u.v. induction. The lysates were assayed for their plaque-forming and transducing activities using the assays for *λ* and *λdg* described in the section on "Materials and Methods." It should be noted that the helper phage for both series of experiments was λi^{434}.

with *λdg* DNA and *λdg* supports the hypothesis that bacteria transformed by *λdg* DNA become galactose-positive because they are lysogenic for *λdg*. In both experiments λi^{434} was helper. All of the lysates examined contained both i^{λ} and i^{434} phages; therefore, the *dg* prophage could be either i^{λ} or i^{434}. It is conceivable that only the segment bearing the galactose gene(s) came from *λdg* DNA and the rest of the *λdg*

genome necessary for the formation of a complete prophage was supplied by recombination with the helper phage. This possibility was examined by using *λdg* derived from wild type *λ* as the source of *λdg* DNA and helper phage marked in the *c* region and at the *mi* locus. The *c* region and *mi* are on the same side of the defective region; *c* is 5 map units and *mi* 10 map units from the nearest end of the defective region (see Fig. 1) (Arber, 1958). Genes present in *λdg* can be detected among the active *λ* produced by bacteria lysogenic for both *λdg* and *λ* because genetic recombination occurs between *λ* and *λdg* during their multiplication together in the same cell.

In the first experiment *λdg* DNA was employed with *λ* co_1 *mi* as helper and nonlysogenic K12 Gal_4^- as recipient. The co_1 marker is in the *c* region. Control transductions were performed in which *λdg* replaced the DNA extract, the experiments being otherwise identical. Galactose-positive bacteria were picked and purified by three single colony isolations in EMB-gal plates, grown in H-glucose medium, induced with u.v. light, and plated on sensitive bacteria. These plates were examined for the presence of co_1, co_1^+, *mi*, and mi^+. Approximately 200 plaques were examined from each lysate. The results are presented in Table 7.

TABLE 7

Cotransformation of Gal^+ and phage genes by λdg DNA

Agent	Number of Gal^+ containing:					Total
	co^+ and mi^+	co^+ only	mi^+ only	neither	i^λ	Gal^+ tested
λdg (1)	17	0	1	3		21
λdg DNA (1)	24	2	2	27		55
λdg DNA input to CsCl, Fig. 9 (2)					1746 (97%)	1808
Peak fraction, 214 hr, Fig. 9b (2)					1935 (> 99%)	1942

(1) $2{\cdot}5 \times 10^3$ *λdg* or 12 *μg λdg* DNA were added to 2×10^8 K12 Gal_4^- and 4×10^9 *λ co mi* and the mixtures plated. Isolated Gal^+ colonies were picked and restreaked twice. The purified strains were induced to produce phage by exposing them to u.v. light, and the active phage produced examined by plating on C600. *λ co mi* forms small clear plaques while co^+ causes turbid plaques and mi^+ large plaques. At least 500 plaques were examined from each lysate. When either co^+ or mi^+ was present, it was present in roughly 25% of the active phage.

(2) Galactose-positive bacteria which had arisen from a standard transformation assay and were growing on the EMB-galactose plate were scored for the presence of a halo of lysis. A halo would indicate that the bacteria were producing phage with the immunity specificity of *λ*, symbolized i^λ. See the text for a more complete explanation of the technique.

In the control series with *λdg*, 17 out of 21 or 81% of the galactose-positive bacteria isolated had both alleles of *λdg*, co_1^+, and mi^+. Among the Gal^+ arising from DNA-treated cells, 24 out of 55 or 44% had both co_1^+ and mi^+.

In another experiment the *c* gene was detected by making use of the fact that it controls the specificity of immunity. Transformation with *λdg* DNA was carried out with λi^{434} as helper and K12 Gal_4^- (λi^{434}) as recipient, plating finally on EMB-galactose agar. Normally the untransformed K12 Gal_4^- (λi^{434}) recipient bacteria grow to form a confluent, white background on the EMB plates. Because bacteria carrying a prophage with the immunity gene of *λ*, symbolized i^λ, will produce some active phage with the immunity specificity of *λ*, a halo of lysis of the background will

surround a galactose-positive colony if it has received the i^{λ} gene in addition to a galactose gene from the *λdg* DNA. The EMB plates are therefore scored for the total number of galactose-positive colonies and for the number of galactose-positive colonies with a halo of lysis.

The results of this experiment are presented in Table 7 also. The i^{λ} gene was present in at least 97% of the galactose-positive bacteria.

It should be pointed out that both experiments give minimum estimates of the frequency of cotransfer of *Gal* and phage genes because loss of prophage genes is known to occur during growth of polylysogenic bacteria (Appleyard, 1954; Campbell, 1957). The lower frequency of cotransfer in the first experiment might be explained by the greater number of bacterial divisions occurring between exposure to DNA and testing.

Thus bacteria which become galactose-positive because they have been transformed by *λdg* DNA have a high probability of receiving both the *c* and *mi* genes as well. The interval between *mi* and the farthest end of the defective region is about 90% of the known map of *λ* (see Fig. 1). If it is assumed that cotransformation means that *Gal*, *c*, and *mi* are on the same DNA molecule, this molecule must include essentially all of the *λdg* genome.

4. Discussion

A comparison of some of the chemical and biological properties of the *λdg* DNA system with those of the *D. pneumoniae* and *H. influenzae* transformations, the *λdg* transduction, and with urea-treated T2 phage (Pi particles) is given in Table 8. The active material in *λdg* DNA is similar to the *Pneumococcus*- and *Hemophilus*-transforming DNA in that their physical and chemical properties define them as molecules of DNA in aqueous solution. This is not the case for the Pi particles which evidently retain appreciable T2 protein as necessary components for infection.

The *λdg* DNA system is appreciably less efficient than the other systems listed in Table 8 in terms of the number of transformed cells per μg of DNA added. This difference is more marked relative to classical transformation if the measure of efficiency is the number of transformed cells per phage- or bacterial-equivalent of added DNA, this being the closest approximation that can presently be made to the number of cells transformed per donor genome. Because of the twenty-five- to sixty-fold greater amount of DNA per bacterial cell than per *λdg*, the *λdg* DNA system is, on this basis, 10^{-3} to 10^{-4} as efficient as classical transformation. The lower efficiency might be due either to the nature of the recipient cells, the DNA, or both.

The most important factor in determining the competence of recipient cells in the *λdg* DNA system is their infection with helper phage. If it is assumed that the active DNA molecule contains essentially all of the *λdg* genome, one of the roles played by helper phage in transformation should be the same as that which it performs in transduction: it should alleviate the *dg* defect in the reactions leading to lysogenization. However, the effect of helper phage in the transformation system is to increase the efficiency by a factor of at least 10^4 while having only a twenty-fold effect in the *λdg* transduction. Consequently it must be supposed that helper phage also supply a function present in the whole *λdg* phage, but absent in the *λdg* DNA. This function could involve the adsorption of the DNA to the cell surface, the penetration of that surface, or the several reactions by which the injected phage chromosome becomes prophage. One would expect that *λdg* DNA labeled with ^{32}P could be used to further explore these possibilities.

TABLE 8

A comparison of the properties of the D. pneumoniae *and* H. influenzae *transforming DNA, λdg DNA, λdg phage and Pi particles*

Property	Transforming DNA(1)	*λdg* DNA	*λdg* phage	Pi particles(2)
I. *Chemical*				
(1) effect of DNase	$< 10^{-4}$ μg/ml. inactivates	$< 10^{-4}$ μg/ml. inactivates	none	$> 10^{-2}$ μg/ml. partially inact.
(2) effect of trypsin	none	none	—	$< 10^{-4}$ μg/ml. inactivates
(3) effect of antiserum	—	none for anti-λ	anti-λ inactivates	anti-T2 inactivates(3)
(4) thermolability	same as DNA	same as DNA	more labile than DNA	more labile than DNA
(5) density, CsCl	—	same as DNA (1·71)	less than DNA (1·5)	CsCl inactivates
(6) protein content of active preparation	< 0·02%	< *ca.* 1%	50%	—
II. *Biological*				
(1) efficiency: no. of transformed cells per μg DNA added(4)	1×10^8	1×10^6	2×10^9	2×10^7
(2) DNA per donor bacterium or phage	$2\text{–}5 \times 10^{-9}$ μg per bact.(5)	8×10^{-11} μg per phage(6)	8×10^{-11} μg per phage(6)	2×10^{-10} μg per phage(7)
(3) Percent of genome transferred	*ca.* 1(8)	*ca.* 100	100	100

(1) Zamenhof (1957).

(2) Mahler & Frazer (1959).

(3) Whether anti-T2 or DNase in the serum caused the inactivation was not determined (Mahler & Frazer, 1959).

(4) This efficiency of transforming principle and of *λdg* DNA varies with recipient cell concentration. The figure for transforming principle is for *H. influenzae* at $1{\cdot}6 \times 10^9$ cells per ml. (Goodgal & Herriott, 1957) and that for *λdg* DNA is with 1×10^9 helper-infected cells per ml. under the most recent assay conditions (see "Experimental," Section 3c). The value for *λdg* transduction is taken from Table 1, fraction CsCl-2. Pi particles were assayed at 2×10^9 protoplasts per ml., and the efficiency was calculated assuming 2×10^{-16} g DNA per T2 particle and no loss of DNA during preparation of Pi particles (Mahler & Frazer, 1959). In this latter case, one plaque is taken as one "transformed" cell.

(5) *H. influenzae*: 2×10^{-15} g/cell (Zamenhof *et al.*, 1953).
D. pneumoniae: 2×10^{-15} g/cell (Fox, 1957).
D. pneumoniae: 5×10^{-15} g/cell (Lerman & Tolmach, 1957).

(6) See " Materials and Methods," Section 7.

(7) Herriott & Barlow (1952); Stent & Fuerst (1955).

(8) The value must be considerably less than 100% since most known transforming factors are transferred independently (Hotchkiss, 1954). An estimate of this value can be made from the molecular weight of $1{\cdot}5 \times 10^7$ calculated for the streptomycin-resistance factor of *H. influenzae* (Goodgal & Herriott, 1957) and the value of 2×10^{-15} g DNA, or 80 DNA molecules of molecular weight $1{\cdot}5 \times 10^7$ per cell. Assuming one genome contains these 80 molecules, genetic transfer by one molecule would amount to the transfer of about 1% of the genome.

The unique features of the *λdg* system which most easily distinguish it from transformation in *Pneumococcus* and *Hemophilus* are the small size of the donor genome and the knowledge of its structure from phage genetics. These characteristics are reflected in the fact that the *Gal*$^+$ transformants always have *c* and in half of the cases *mi* which come from the DNA. Since the linkage between *λdg* and *c* has been observed at DNA concentrations as low as 0·1 *λdg*-equivalents per recipient bacterium, and since the number of *Gal*$^+$ is a linear function of the DNA concentration, the simplest interpretation is that the active molecules carry *dg*, *c*, and *mi*. Assuming that the entire *dg* region, as mapped by Arber (1958), is present, and that the segments between *dg* and *c*, and between *c* and *mi* are also present, this represents 90% of the *λdg* map (Fig. 1). If the *λdg* genome contains all of the DNA in the phage, the active molecules should have a molecular weight of 5×10^7 ("Materials and Methods," Section (g)).

In *Pneumococcus* and *Hemophilus*, on the other hand, most of the known transforming factors are transferred independently. This is to be expected in view of the fact that a donor bacterium contains the equivalent of 25 to 60 DNA molecules of molecular weight 5×10^7 whereas the molecule of transforming DNA is certainly not more than 5×10^7 (see footnote 8, Table 8). Therefore, the *Pneumococcus*- and *Hemophilus*-transforming DNA's are heterogeneous in that different molecules represent different segments of the bacterial chromosome. *λdg* DNA does not exhibit this form of heterogeneity since the active molecules comprise the entire donor phage chromosome. The absence of this form of heterogeneity and the availability of a variety of markers, all linked, should make *λdg* DNA useful for an analysis of the chemical structure of the phage chromosome.

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