

## Generalized Transduction by Phage P22 in *Salmonella typhimurium*

### I. Molecular Origin of Transducing DNA

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P22, a temperate phage which grows on *Salmonella typhimurium*, is capable of carrying out generalized transduction in this host. The generalized transducing particles are the same size as P22 phage particles but instead of phage DNA they contain bacterial DNA which was synthesized before phage infection. Further, DNA molecules isolated from the transducing particles have the same molecular weight as P22 DNA,  $27 \times 10^6$  daltons.

Examination of the formation of transducing particles under conditions in which the *S. typhimurium* recombination system (*rec*) and/or the P22 recombination system (*erf*) are defective indicates that neither of these general recombination systems is necessary for the formation of transducing particles.

### 1. Introduction

P22, a temperate phage which grows on *Salmonella typhimurium*, is capable of carrying out both generalized and specialized transduction in this host (Zinder & Lederberg, 1952; Smith-Keary, 1966; Wing, 1968). These two processes, both involving the transfer of genetic information from one bacterium to another by means of a phage vector, differ in several respects. Whereas specialized transduction is restricted to the region of the bacterial chromosome adjacent to the prophage attachment site, generalized transduction involves the transfer of any region of the bacterial chromosome.

Specialized transducing particles are formed only after induction of a lysogen (Morse, Lederberg & Lederberg, 1956; Wing, 1968), while generalized transducing particles seem to be formed in all lytic infections (Zinder, 1955). In addition, specialized transducing particles contain segments of both phage and bacterial DNA covalently joined to one another (Campbell, 1962; Smith, 1968), while the experiments of Ikeda & Tomizawa (1965) demonstrated that in the case of coliphage P1, generalized transducing particles contain bacterial DNA with little or no phage DNA.

We shall describe observations showing that the generalized transducing particles of phage P22 contain primarily bacterial DNA synthesized before infection, and little or no phage DNA. These transducing particles have the same sedimentation properties

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as phage P22 and contain DNA of the same molecular weight,  $27 \times 10^6$  daltons, as DNA isolated from vegetative phage.

Examination of the formation of transducing particles under conditions in which the bacterial recombination system (*rec*) and/or the phage recombination system (*erf*) are inactive indicates that neither of the known general recombination systems in *S. typhimurium* and P22 is necessary for the formation of transducing particles.

## 2. Materials and Methods

### (a) Bacterial strains

The following strains of *Salmonella typhimurium* LT2 were used: DB21, a prototrophic strain; DB25, a low thymine-requiring mutant of DB21; and DB47, a *rec*<sup>-</sup> prototroph which is nearly isogenic with DB21 (Botstein & Matz, 1970), and whose phenotype resembles that of *recA* mutants of *Escherichia coli* (Wing, Levine & Smith, 1968). PV78 is *hisD23metC30gal-50purC213* and was obtained from the culture collection of B. Magasanik; the lysogenic derivative, PV78 (P22 *sie*  $1m_3$ ), is described in Ebel-Tsipis & Botstein (1971).

### (b) Phage strains

The following P22 phage strains were used: P22*sie*<sup>+</sup> $m_3$ , called hereafter wild-type P22; P22*sie*  $1m_3$ , a derivative of a non-excluding mutant isolated by Rao (1968) which, when present as a prophage, allows transduction to occur with the same efficiency as in non-lysogenic cells instead of with the lower transducing efficiency found in lysogens of *sie*<sup>+</sup> phage (Ebel-Tsipis & Botstein, 1971); P22*m*<sub>3</sub>*c*<sub>2</sub>*h*<sub>21</sub>, a clear mutant carrying the morphological markers *m*<sub>3</sub> and *h*<sub>21</sub> (Levine & Curtiss, 1961); P22*c*<sub>1</sub>*ts* 19·1, a clear mutant which is temperature-sensitive for lysis; P22 *erf*-3, a recombination-deficient mutant of P22 which is unable to grow on a *rec*<sup>-</sup> host (Botstein & Matz, 1970).

### (c) Media

LB broth,  $\lambda$  agar, soft top agar, Ozeki minimal agar, and minimal top agar are described in Ebel-Tsipis & Botstein (1971), as are buffered saline and dilution fluid.

Minimal medium supplemented with casein hydrolysate (LCG20) or without casein hydrolysate (LG20) is described by Botstein (1968). This was supplemented with 1  $\mu$ g thiamin·HCl/ml., 20  $\mu$ g adenine/ml. and 20  $\mu$ g amino acids/ml. or 10  $\mu$ g thymine/ml. when necessary.

Density-labeling minimal medium (also called heavy minimal medium) used to prepare purified transducing particles contains: 0·1 M-Tris (pH 7·4), 0·011 M-<sup>15</sup>NH<sub>4</sub>Cl,  $2\cdot5 \times 10^{-3}$  M-MgSO<sub>4</sub>, 0·012 M-NaCl, 0·2% (w/v) glucose, 20  $\mu$ g phosphorus (as phosphate)/ml., and 10  $\mu$ g thymine/ml. in D<sub>2</sub>O. Two drops of a trace-metals solution were added per 100 ml.

### (d) Enzymes

Deoxyribonuclease I (DNase), 1  $\times$  crystallized powder, code D, was obtained from Worthington Biochemical Corporation, Freehold, New Jersey. Egg white lysozyme, 3  $\times$  crystallized, grade 1, was obtained from Sigma Chemical Co., St. Louis, Missouri. Ribonuclease, bovine pancreatic, code RASE (Worthington), was diluted to a concentration of 1 mg/ml. in buffered saline and was heated at 80°C for 10 min before use. Pronase (B grade) was obtained from Calbiochem, Los Angeles, California. Solutions, prepared at a concentration of 20 mg/ml. in buffered saline, were incubated at 80°C for 10 min before use.

### (e) Chemicals

D<sub>2</sub>O (99·1 to 99·8% pure) and <sup>15</sup>NH<sub>4</sub>Cl (99% pure) were obtained from BioRad, Richmond, California. Sarkosyl NL30 was obtained from the Geigy Chemical Corp. CsCl, 99·95% pure, was obtained from Varlacoid Chemical Co., Elizabeth, New Jersey. Angio-Conray (sodium iothalamate, 80% (w/v) solution) was purchased from Mallinckrodt Chemical Works. Acid-hydrolyzed casein (vitamin and salt free; 10% solution) was obtained from Nutritional Biochemical Co., Cleveland, Ohio. EDTA (ethylene diamine

tetra-acetic acid) was obtained from Eastman, Rochester, New York, and prepared as described in Botstein (1968).

[2-<sup>14</sup>C]thymine (49 mCi/m-mole), [methyl-<sup>3</sup>H]thymine (53.5 Ci/m-mole) and [<sup>3</sup>H]toluene ( $1.62 \times 10^6$  disint./min/ml.) were obtained from the New England Nuclear Corp., Boston, Mass. [Methyl-<sup>3</sup>H]thymine (11.2 Ci/m-mole) was obtained from Schwarz/Mann, Orangeburg, New York.

(f) *Growth of bacteria in heavy minimal medium*

*S. typhimurium* growth in heavy minimal medium requires gradual adaptation. Fresh cultures of DB25 in LB broth or LCG20 were diluted into LG20 and allowed to grow up. A portion of this culture was then diluted 1 : 20 into medium containing equal volumes of LG20 and heavy minimal medium. When this culture had grown to saturation the cells were diluted 1 : 20 into medium containing 80% deuterium oxide. Cells were transferred to increasingly heavy medium (1 : 20 dilutions into 90%, 95% and finally 99.7% D<sub>2</sub>O) until the cells could grow in heavy medium. This process can take as long as two weeks since the cells grow slowly. A culture adapted in this way can be kept at 4°C for up to 2 months without substantial loss of viability. The substitution of either <sup>15</sup>NH<sub>4</sub>Cl for <sup>14</sup>NH<sub>4</sub>Cl or [<sup>13</sup>C]glucose for [<sup>12</sup>C]glucose had no effect on the growth rate of the cells.

(g) *Density-labeling of phage particles*

Three types of lysates of P22<sub>m<sub>3</sub>c<sub>2</sub>h<sub>21</sub></sub> were prepared: (1) a Light-Light lysate in which cells are grown in light medium before infection and maintained in light medium following infection; (2) a Heavy-Heavy lysate in which cells are both grown and infected in heavy minimal medium; (3) a Heavy-Light lysate in which cells are grown before infection in heavy minimal medium but shifted to light medium immediately after phage adsorption. Input phage used in the preparation of these lysates were grown before use on PV78. Since PV78 (P22<sub>sie-1m<sub>3</sub></sub>) is used as the recipient for all transduction assays, the input phage in these lysates contained no detectable transducing activity. The procedures for the preparation of these lysates are as follows.

(i) *Light-Light lysates*

Light-Light phage stocks were generally grown on DB21. Cells were grown in LCG20 or LB, with shaking at 39°C, to a density of 2 to 4 × 10<sup>8</sup> cells/ml. Phage were then added at a multiplicity of 5 phage/cell (unless otherwise specified) and incubation continued until lysis occurred. The phage were then purified and concentrated by differential centrifugation and resuspended in buffered saline.

If the bacteria were labeled with [methyl-<sup>3</sup>H]thymine before phage infection in order to make radioactive transducing particles, then the following modifications were made in the procedure. The bacterial strain used was DB25 and the cells were grown in LCG20 medium containing 5 μg thymine/ml. and [methyl-<sup>3</sup>H]thymine at a specific activity of 10 μCi/μg thymine. 15 min before phage infection, a large excess of cold thymine (200 μg/ml.) was added to the culture. The phage were then added, and, after 2 min, NaCN (2.5 × 10<sup>-3</sup> M) was added and adsorption continued for an additional 8 min. The infected cells were then deposited on Millipore filters (GSWPO4700), washed well with dilution fluid, resuspended in 3 vol. warmed LCG20 medium supplemented with 200 μg thymine/ml., and incubated. After lysis, the bacterial debris was removed by low-speed centrifugation (9000 rev./min for 5 min in a Sorvall SS34 rotor). The lysate was treated with DNase (10 μg/ml.) for 60 min at 37°C in order to degrade bacterial DNA in the lysate. The phage and transducing particles were then pelleted (17,000 rev./min for 90 min in a Sorvall SS34 rotor), resuspended in a small volume of buffered saline and incubated for 1 hr with a purified P22 base plate preparation (Israel, Anderson & Levine, 1967) containing 1 × 10<sup>4</sup> plaque-forming units per ml.

(ii) *Heavy-Heavy lysates*

Heavy-Heavy lysates in which both plaque-forming units and transducing particles are uniformly density-labeled were prepared by infecting DB25 (grown in heavy minimal medium to a density of 5 × 10<sup>8</sup> cells/ml.) with phage at a multiplicity of 5 phage/cell. The

infected cells were incubated at 39°C for 3.5 hr and then lysed with  $\text{CHCl}_3$ . Phage were purified and concentrated by differential centrifugation; again the suspension was treated with purified P22 base plate parts before analysis.

(iii) *Heavy-Light lysates*

A Heavy-Light lysate was prepared on DB25 donor bacteria which had been grown to a cell density of  $5 \times 10^8$ /ml. in radioactive heavy medium ( $5 \mu\text{g}$  thymine/ml.;  $10 \mu\text{Ci}$  [*methyl*- $^3\text{H}$ ]thymine/ $\mu\text{g}$  thymine). 15 min before phage infection a large excess of cold thymine ( $200 \mu\text{g}/\text{ml.}$ ) in heavy medium was added to the culture. The cells were then infected and treated as described for the preparation of light radioactive transducing particles; the cells were incubated for 150 min before phage growth was terminated by the addition of  $\text{CHCl}_3$ . The particles were purified, concentrated and treated with purified base plate parts.

(h) *Transduction procedure*

Transduction was carried out as described by Ebel-Tsipis & Botstein (1971) using PV78 (P22*sie 1m*<sub>3</sub>) as the recipient in all cases. Under these conditions, transduction is linear with number of phage up to a multiplicity of 10 plaque-forming units per cell.

(i) *Reference phage and DNA*

Phage P22 labeled with [ $^{14}\text{C}$ ]thymine was prepared by the method of Botstein (1968) The lysate contained  $1.5 \times 10^{11}$  plaque-forming units/ml. at a specific activity of  $10^{-5}$  cts/min/phage.

$^{14}\text{C}$ -labeled *S. typhimurium* DNA was isolated from two bacterial cultures: DNA was isolated from DB25 which had grown for many generations in LG20 medium containing [ $^{14}\text{C}$ ]thymine at a specific activity of  $0.25 \mu\text{Ci}/\mu\text{g}$  thymine and from DB25 grown in heavy minimal medium containing [ $^{14}\text{C}$ ]thymine at a specific activity of  $0.3 \mu\text{Ci}/\mu\text{g}$  thymine.

The densities of these reference DNA's are given in Table 1.

(j) *DNA isolation procedures*

(i) *Phage P22 DNA*

DNA was isolated from P22 by lysing the phage with Sarkosyl at 65°C, as described by Botstein (1968). The sedimentation rate and density of DNA isolated in this manner is indistinguishable from P22 DNA isolated by phenol extraction.

(ii) *Bacterial DNA*

Bacteria grown in either LCG20 or heavy minimal medium were centrifuged twice and resuspended in 1 ml. of 0.1 M-Tris, 0.1 M-EDTA, 0.15 M-NaCl, pH 8.0. Lysozyme was added to 1 mg/ml. and the solution incubated at 37°C for 10 min. RNase was added to 0.1 mg/ml. and incubation continued for an additional 5 min. The cell suspension was then heated to 70°C for 3 min; lysis is effected by the addition of Sarkosyl to a concentration of 2%. Heating at 70°C was continued for 20 min. The lysis mixture was then shifted to 37°C and incubated for 1 hr before the addition of pronase. 0.1 vol. of pronase (to give 2 mg/ml.) was added and incubated for 2 to 4 hr. An additional 0.1 vol. of pronase was added just before dialysis; the DNA was dialyzed twice against 1000 vol. of 0.01 M-Tris, 0.01 M-EDTA, 0.15 M-NaCl (pH 8), at 37°C.

All pipetting of the DNA was carried out using Falcon 1-ml. disposable pipets (no. 7506, Falcon Plastics, Oxnard, California) with a Clay-Adams pipetting device. DNA isolated by this method is of high molecular weight (70 to  $100 \times 10^6$  daltons).

(k) *CsCl density-gradient centrifugation*

Phage were centrifuged to equilibrium in a CsCl density gradient prepared by adding 3.2 g solid CsCl to 4 ml. phage in buffered saline. The density was adjusted to approximately  $1.51 \text{ g}/\text{cm}^3$  and the tubes overlaid with mineral oil. The phage were centrifuged in a Spinco SW39 rotor for 24 to 36 hr at 22,000 rev./min, 20°C.

CsCl gradients for DNA were prepared by adding 5 g solid CsCl to 4 ml. DNA in 0.01 M-Tris, 0.01 M-EDTA, pH 8. The density of the final solution was adjusted to about  $1.71 \text{ g}/\text{cm}^3$

and the tubes overlaid with mineral oil. DNA gradients were centrifuged for 48 to 60 hr either in a Spinco SW39 rotor at 35,000 rev./min or in a Spinco 50 fixed-angle rotor at 37,000 rev./min, 20°C. Densities were calculated from refractive index measurements (Weigle, Meselson & Paigen, 1959).

(1) *Measurement of radioactivity*

Radioactive samples were deposited on 21-mm filter paper discs (no. 895E from Carl Schleicher and Schuell Co.) and dried with a heat lamp. The filter papers were then washed twice with iced 5% trichloroacetic acid, 15 to 30 min each time, and once with cold acetone or 95% ethanol. The dried filter discs were placed in low-potassium glass vials (Wheaton Glass Co., Milville, New Jersey), containing 5 ml. of scintillation fluid, prepared by adding 160 ml. Liquifluor (New England Nuclear, Boston, Mass.) to 1 gallon toluene. The counting efficiency of  $^3\text{H}$  under these conditions was determined to be 0.20.

### 3. Results

(a) *Physical studies on density-labeled transducing particles and their DNA*

In order to determine the molecular origin of transducing DNA in P22 particles, three types of lysates were examined: Light-Light, Heavy-Light and Heavy-Heavy. In all cases substantial numbers of both infectious phage and transducing particles were made although the frequency of transducing particles in the lysates was not the same in all cases. A summary of the properties of these lysates is given in Tables 1 and 2.

TABLE 1  
*Buoyant density determinations*

	L-L† Lysate	Density (g/cm <sup>3</sup> ) H-L† Lysate	H-H† Lysate
Phage particles			
Plaque-forming units	1.506	1.506	1.546
<i>his</i> <sup>+</sup> transducing particles	1.507	1.523	1.545
<i>gal</i> <sup>+</sup> transducing particles	1.510	1.526, 1.518	1.548
<i>pur</i> <sup>+</sup> transducing particles	1.511	1.527	—
Total $^3\text{H}$ -labeled transducing particles	1.509	1.525	—
P22 DNA	1.691	—	—
<i>S. typhimurium</i> DNA			
$^{14}\text{C}$ -labeled reference DNA	1.697	—	1.729
$^3\text{H}$ -labeled bacterial DNA	1.697	1.728	—
Transducing particle DNA	—	1.726	—

The densities of plaque-forming units, *his*<sup>+</sup> transducing particles, *gal*<sup>+</sup> transducing particles and  $^3\text{H}$ -labeled particles were determined by CsCl density-gradient analysis of a Light-Light phage lysate (Fig. 1), a Heavy-Heavy phage lysate (Fig. 3), and a Heavy-Light phage lysate (Fig. 4). The densities were calculated from refractive index measurements.

The densities of P22 and *S. typhimurium* DNA were determined by CsCl density gradient analysis in a Spinco SW39 rotor; the absolute densities were again determined from refractive index measurements. Transducing DNA was isolated from purified transducing particles obtained from the Heavy-Light lysate (Fig. 5).

† L-L, H-L, H-H lysates are Light-Light, Heavy-Light and Heavy-Heavy lysates, respectively.

TABLE 2  
*Phage and transducing particle titers†*

Lysate	p.f.u./ml.¶	<i>his</i> <sup>+</sup> /ml.	<i>pur</i> <sup>+</sup> /ml.	<i>gal</i> <sup>+</sup> /ml.	<sup>3</sup> H cts/min/ml.‡	Total transducing particles/ml.§
Light-Light	1.1 × 10 <sup>12</sup>	1.2 × 10 <sup>7</sup>	4.8 × 10 <sup>5</sup>	—	3.7 × 10 <sup>5</sup>	2.2 × 10 <sup>10</sup>
Heavy-Heavy	1.0 × 10 <sup>10</sup>	2.7 × 10 <sup>4</sup>	—	1.0 × 10 <sup>4</sup>	—	—
Heavy-Light	3.2 × 10 <sup>12</sup>	2.7 × 10 <sup>7</sup>	2.2 × 10 <sup>6</sup>	8.3 × 10 <sup>6</sup>	7.1 × 10 <sup>5</sup>	4.2 × 10 <sup>10</sup>

† All numbers were calculated from lysates which were analyzed on CsCl density gradients (Figs 1, 3 and 4).

‡ Includes only those <sup>3</sup>H counts associated with the peak of transducing activity.

§ Calculated from the specific activity of the bacterial DNA at the time of infection (4.2 × 10<sup>5</sup> cts/min/μg DNA).

¶ Abbreviation used: p.f.u., plaque-forming unit.

#### (i) *Light-Light phage lysates*

A lysate of P22 containing radioactively-labeled transducing particles was prepared, concentrated and analyzed in a CsCl equilibrium density-gradient. Fractions were collected and assayed for infectivity, transducing activity and radioactivity. Phage and transducing particles have similar, though not identical, buoyant densities (Fig. 1(a)). Plaque-forming particles form a well-defined peak at a density of 1.506 g/cm<sup>3</sup>, whereas transducing particles carrying different regions of the bacterial chromosome have different mean densities: *his*<sup>+</sup>|| transducing particles have a buoyant density of 1.507 g/cm<sup>3</sup> and *pur*<sup>+</sup> transducing particles form a peak at a density of 1.511 g/cm<sup>3</sup>. *gal*<sup>+</sup> transducing particles, assayed in another experiment, are found at a density of 1.510 g/cm<sup>3</sup>.

The density differences among transducing particles carrying different markers appear to reflect small density differences which exist in different regions of the bacterial chromosome rather than small differences in the amount of DNA encapsulated in the transducing particles. This conclusion is supported by the findings of Roth & Hartman (1965) which demonstrate that particles assayed for carrying the *ile*<sup>+</sup> region alone are more dense than particles scored for the joint transduction of both *ile*<sup>+</sup> and *hisR*.

The radioactivity profile is shown in Figure 1(b). The <sup>3</sup>H-labeled particles form a peak at a density of 1.509 g/cm<sup>3</sup>. It is reasonable to assume that most of the [<sup>3</sup>H]DNA found in these labeled particles represents bacterial DNA which had been synthesized before infection since the radioactive label was present in the medium only before infection and since P22 infection does not cause any significant degradation of the bacterial chromosome into acid-soluble fragments (Schmieger, 1971). Therefore the average density of transducing particles formed under these conditions is 1.509 g/cm<sup>3</sup>.

The DNA extracted from transducing particles has a density very close to that of reference *S. typhimurium* DNA; in contrast, phage DNA has a density substantially less than that of the bacterial DNA (Fig. 2).

|| Genetic markers used are: *his*, requirement for histidine; *pur*, requirement for adenine or guanine; *gal*, inability to utilize galactose.

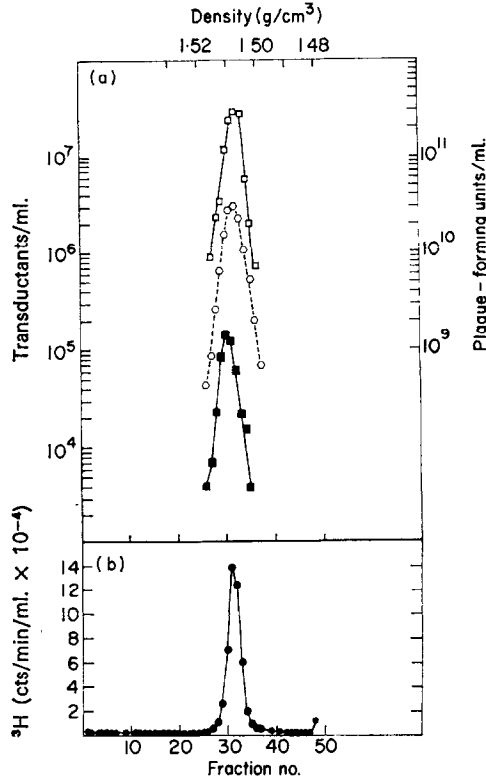


FIG. 1. CsCl equilibrium density-gradient analysis of a Light-Light phage lysate containing radioactive transducing particles. Three-drop fractions were collected from the bottom into 0.3 ml. buffered saline. (a) Each fraction was assayed for plaque-forming units, *his*<sup>+</sup> transducing activity and *pur*<sup>+</sup> transducing activity. (b) A 50- $\mu$ l. sample from each fraction was counted for radioactivity. —□—□—, Plaque-forming units; —■—■—, *pur*<sup>+</sup> transducing activity; —○—○—, *his*<sup>+</sup> transducing activity; —●—●—, <sup>3</sup>H radioactivity.

### (ii) Heavy-Heavy phage lysates

In order to show that the density pattern found in the Light-Light lysate is not affected by heavy medium, uniformly density-labeled phage and transducing particles, grown in the presence of <sup>2</sup>H and <sup>15</sup>N, were prepared. The phage were analyzed on a CsCl equilibrium density-gradient and the results are presented in Figure 3. The plaque-forming units have an average density of 1.546 g/cm<sup>3</sup>, the *his*<sup>+</sup> transducing particles have a density of 1.545 g/cm<sup>3</sup> and the *gal*<sup>+</sup> transducing particles form a peak at a density of 1.549 g/cm<sup>3</sup>. Density-labeling of the particles in this way increases the densities of all particles examined by about 0.04 g/cm<sup>3</sup>, leaving the relative positions of the transducing particles and the plaque-forming units unaltered.

### (iii) Heavy-Light phage lysates

Differentially-labeled transducing particles and phage were prepared, using bacteria which had been grown before infection in heavy minimal medium containing [*methyl*-<sup>3</sup>H]thymine. The infected cells were then incubated until lysis in light medium without radioactive label. The purified, concentrated phage were analyzed by CsCl equilibrium density-gradient centrifugation.

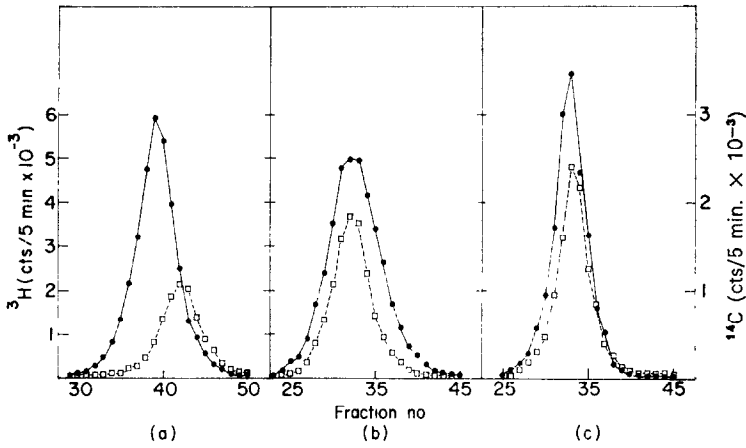


FIG. 2. CsCl equilibrium density-gradient analysis of  $^3\text{H}$ -labeled *S. typhimurium* DNA and  $^3\text{H}$ -labeled transducing DNA from a Light-Light lysate in a Spinco 50 rotor. (a)  $^3\text{H}$ -labeled *S. typhimurium* DNA and  $^{14}\text{C}$ -labeled P22 DNA. The total number of fractions was 83. (b)  $^3\text{H}$ -labeled transducing DNA, extracted from particles in the radioactive peak of the Light-Light phage lysate (fraction 31 in Fig. 1) and  $^{14}\text{C}$ -labeled *S. typhimurium* DNA. The total number of fractions was 82. (c)  $^3\text{H}$ -labeled *S. typhimurium* DNA and  $^{14}\text{C}$ -labeled *S. typhimurium* DNA. The total number of fractions was 85. —●—●—,  $^3\text{H}$ -radioactivity; —□—□—,  $^{14}\text{C}$ -radioactivity.

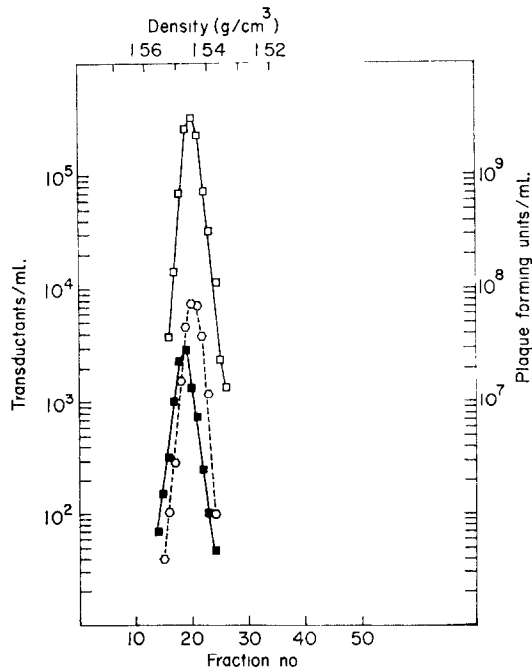


FIG. 3. CsCl equilibrium density-gradient analysis of a Heavy-Heavy phage lysate. The lysate was prepared as described in Materials and Methods and a portion of the lysate containing  $10^{10}$  plaque-forming units was analyzed. Two-drop fractions were collected into 1 ml. buffered saline and the fractions were assayed for plaque-forming activity, *his*<sup>+</sup> transducing activity and *gal*<sup>+</sup> transducing activity. The total number of fractions collected was 58. —□—□—, Plaque-forming units; —■—■—, *gal*<sup>+</sup> transducing activity; —○—○—, *his*<sup>+</sup> transducing activity.



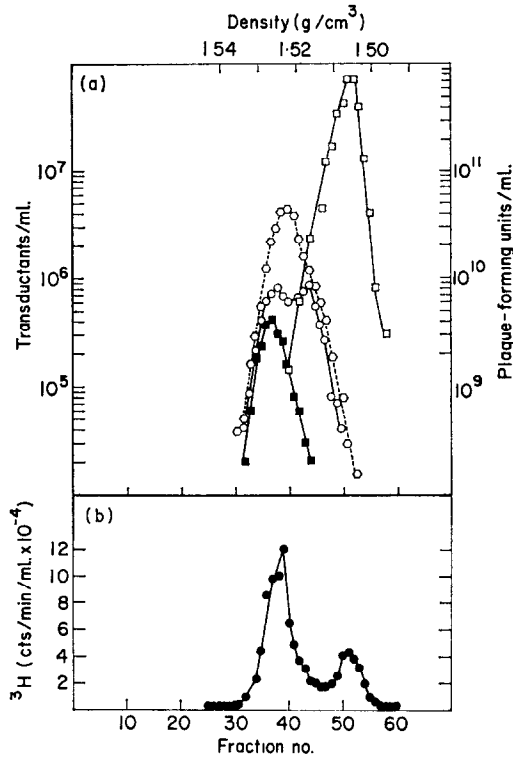


FIG. 4. CsCl equilibrium density-gradient analysis of a Heavy-Light phage lysate containing radioactive transducing particles. Two-drop fractions were collected into 0.4 ml. buffered saline. (a) Fractions were analyzed for plaque-forming units, *his*<sup>+</sup> transducing activity, *gal*<sup>+</sup> transducing activity and *pur*<sup>+</sup> transducing activity. (b) A 10- $\mu$ l. sample from each fraction was counted for radioactivity. The total number of fractions collected was 72. —□—□—, Plaque-forming units; —○—○—, *gal*<sup>+</sup> transducing activity; —◇—◇—, *his*<sup>+</sup> transducing activity; —■—■—, *pur*<sup>+</sup> transducing activity; —●—●—, <sup>3</sup>H-radioactivity.

The fractions collected from the gradient were analyzed for plaque-forming activity, transducing activity and radioactivity. The results of the biological assays are shown in Figure 4(a) and the radioactive profile is shown in Figure 4(b). It is clear that the densities of the transducing particles and the plaque-forming units in this lysate are different. The plaque-forming units form a peak at the normal light density of 1.506 g/cm<sup>3</sup>, confirming the statement made earlier that little bacterial DNA is broken down and re-utilized to synthesize new phage DNA. There is some <sup>3</sup>H, however, associated with the light phage peak, but the calculated specific activity of the vegetative phage (i.e. <sup>3</sup>H cts/min/plaque-forming unit) is more than 100 times lower than the calculated specific activity of the transducing particles (see Table 2 and Discussion), suggesting a specific activity difference of the same magnitude. The small amount of <sup>3</sup>H which is incorporated into phage DNA could result from limited hydrolysis of the bacterial DNA followed by the re-incorporation of the heavy nucleotides into phage DNA; this would explain the broadening on the heavy side of the phage peak. Alternatively, this <sup>3</sup>H could represent the label which was not removed by our procedure for transferring cells from radioactive to non-radioactive medium.

The transducing particles in this lysate have a much higher density than the plaque-forming units. Particles carrying the *his*<sup>+</sup> gene form a peak at a density of 1.523 g/cm<sup>3</sup>, those active in the *pur*<sup>+</sup> transduction are found at a density of 1.527 g/cm<sup>3</sup> and those particles which carry the *gal*<sup>+</sup> gene are found in a bimodal distribution with densities of 1.526 g/cm<sup>3</sup> and 1.518 g/cm<sup>3</sup>. The average density of all transducing particles, as defined by the position of the major <sup>3</sup>H peak in Figure 4(b), is 1.525 g/cm<sup>3</sup>.

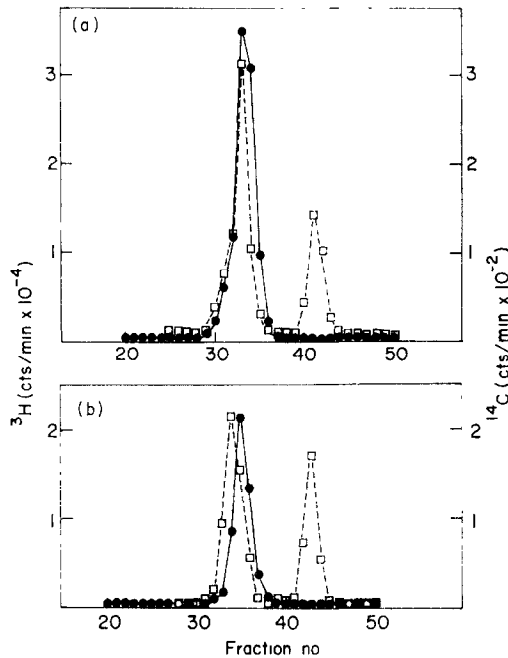


FIG. 5. Equilibrium density-gradient analysis of <sup>3</sup>H-labeled *S. typhimurium* DNA and <sup>3</sup>H-labeled transducing DNA from a Heavy-Light lysate. (a) Density-labeled <sup>3</sup>H-labeled *S. typhimurium* DNA with two reference DNA's: <sup>14</sup>C, <sup>2</sup>H, <sup>15</sup>N-labeled *S. typhimurium* DNA (heavy marker) and <sup>14</sup>C-labeled P22 DNA (light marker). The gradient was run at 37,000 rev./min in a Spinco 50 rotor and two-drop fractions were collected. The total number of fractions collected was 78. (b) Density-labeled <sup>3</sup>H-labeled transducing DNA was isolated from purified transducing particles from the Heavy-Light lysate described in Fig. 4. The <sup>3</sup>H-labeled transducing DNA was centrifuged as described above with the same reference DNA's. The total number of fractions collected was 78. —●—●—, <sup>3</sup>H radioactivity; --□--□--, <sup>14</sup>C radioactivity.

In order to determine the density of the DNA in these heavy transducing particles, fractions 34 to 40 from this gradient were pooled and DNA was isolated from these particles. The density of the DNA from these fractionated transducing particles was compared with the density of <sup>3</sup>H-labeled *S. typhimurium* DNA isolated from the bacteria at the time of infection. Each DNA sample was centrifuged with <sup>14</sup>C-labeled DNA density markers in both the heavy and light positions. The results are shown in Figure 5. The <sup>3</sup>H-labeled *S. typhimurium* DNA (Fig. 5(a)) has a density of 1.728 g/cm<sup>3</sup>, which is slightly lower than the density of the heavy marker, while the DNA isolated from the <sup>3</sup>H-labeled transducing particles (Fig. 5(b)) forms a peak at a density of 1.726 g/cm<sup>3</sup>. This indicates that DNA isolated from transducing particles has a density very similar to that of DNA isolated from the bacteria at the time of phage infection.

Transducing particles are therefore formed primarily by the encapsulation of unreplicated bacterial DNA which was made before infection with P22. There is some evidence though that not all transducing particles contain exclusively unreplicated bacterial DNA. For example, the *gal*<sup>+</sup> transducing particles form two peaks at densities of 1.526 g/cm<sup>3</sup> and 1.518 g/cm<sup>3</sup>, densities which correspond to particles containing heavy and hybrid DNA, respectively (the density of heavy bacterial DNA in this experiment was 0.031 g/cm<sup>3</sup> heavier than light DNA). The *his*<sup>+</sup> transducing particles, although they fall in a single peak, also appear to have a shoulder on the light side of this peak, suggesting that some *his*<sup>+</sup> transducing particles contain replicated DNA.

(iv) *Size of transducing particles and transducing DNA*

In order to be certain that transducing particles and phage particles have the same sedimentation properties, <sup>14</sup>C-labeled P22 and <sup>3</sup>H-labeled transducing particles, isolated from a CsCl density-gradient of a Heavy-Light lysate, were sedimented together in a linear 5 to 20% sucrose gradient. As seen in Figure 6(a), the two types of particles co-sediment, indicating that they have the same size and shape.

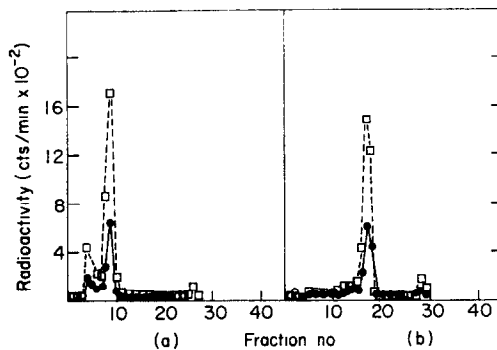


FIG. 6. Zone sedimentation analysis of transducing particles and transducing DNA. (a) Purified transducing particles whose DNA was labeled with <sup>3</sup>H, <sup>2</sup>H, <sup>15</sup>N were run in a neutral sucrose gradient with <sup>14</sup>C-labeled P22 as a position marker. The gradient was centrifuged for 30 min at 25,000 rev./min and five-drop fractions were collected from the bottom. (b) DNA isolated from <sup>3</sup>H-labeled transducing particles was run with <sup>14</sup>C-labeled P22 DNA as a position marker. The gradient was centrifuged for 2 hr at 35,000 rev./min and five-drop fractions were collected from the bottom. —●—●—, <sup>3</sup>H radioactivity; --□--□--, <sup>14</sup>C radioactivity.

DNA isolated from <sup>14</sup>C-labeled P22 and <sup>3</sup>H-labeled transducing particles was also analyzed on a sucrose gradient (Fig. 6(b)) and it can be seen that the two species of DNA are indistinguishable. Thus, transducing particle DNA has a molecular weight of  $27 \times 10^6$  daltons as shown for P22 phage DNA by Rhoades, MacHattie & Thomas (1968).

(b) *Effect of bacterial and phage recombination systems on the formation of generalized transducing particles*

We have investigated the possible roles of the bacterial and phage recombination systems on the formation of transducing particles. If transducing particles contain only bacterial DNA made before infection and if this DNA is packaged into phage heads in the same way that concatenated P22 DNA is packaged, then one might expect

the formation of transducing particles to be independent of these recombination systems.

We therefore examined the formation of transducing particles under conditions in which the bacterial recombination system (*rec*) and/or the phage recombination system (*erf*) are inactive (Botstein & Matz, 1970). The results are presented in Table 3. P22 $m_3c_2h_{21}$  lysates grown on *rec*<sup>+</sup> and *rec*<sup>-</sup> bacteria are very similar and the yields of vegetative phage and transducing particles are approximately the same in the presence or absence of the bacterial *rec* function.

TABLE 3  
*Effect of bacterial and/or phage recombination systems on the formation of transducing particles*

Phage	Bacteria	Multiplicity of infection	Multiplicity				
			p.f.u./†/ml.	<i>pur</i> <sup>+</sup> /ml.	<i>his</i> <sup>+</sup> /ml.	<i>pur</i> <sup>+</sup> /p.f.u.	<i>his</i> <sup>+</sup> /p.f.u.
$m_3c_2h_{21}$	DB21 <i>rec</i> <sup>+</sup>	1	$5.5 \times 10^{10}$	$5.2 \times 10^2$	$3.9 \times 10^4$	$9.5 \times 10^{-9}$	$7.1 \times 10^{-7}$
		5	$1.1 \times 10^{11}$	$5.9 \times 10^2$	$3.7 \times 10^4$	$5.4 \times 10^{-9}$	$3.3 \times 10^{-7}$
		10	$9.0 \times 10^{10}$	$9.7 \times 10^2$	$4.1 \times 10^4$	$1.1 \times 10^{-8}$	$4.5 \times 10^{-7}$
	DB47 <i>rec</i> <sup>-</sup>	1	$4.9 \times 10^{10}$	$8.8 \times 10^2$	$9.6 \times 10^4$	$1.8 \times 10^{-8}$	$2.0 \times 10^{-6}$
		5	$3.8 \times 10^{10}$	$7.4 \times 10^2$	$1.0 \times 10^5$	$2.0 \times 10^{-8}$	$2.6 \times 10^{-6}$
		10	$5.2 \times 10^{10}$	$1.1 \times 10^3$	$9.7 \times 10^4$	$2.1 \times 10^{-8}$	$1.8 \times 10^{-6}$
<i>erf</i> 3 $c_2$	DB21 <i>rec</i> <sup>+</sup>	1	$2.5 \times 10^{10}$	$5.4 \times 10^2$	$8.4 \times 10^4$	$2.2 \times 10^{-8}$	$3.4 \times 10^{-6}$
		5	$2.5 \times 10^{10}$	$5.1 \times 10^2$	$8.4 \times 10^4$	$2.0 \times 10^{-8}$	$3.4 \times 10^{-6}$
		10	$1.1 \times 10^{10}$	$2.5 \times 10^2$	$4.3 \times 10^4$	$2.3 \times 10^{-8}$	$3.9 \times 10^{-6}$
	DB47 <i>rec</i> <sup>-</sup>	1	$7.6 \times 10^8$	$7.5 \times 10^2$	$8.9 \times 10^4$	$9.9 \times 10^{-7}$	$1.2 \times 10^{-4}$
		5	$1.6 \times 10^9$	$1.6 \times 10^3$	$2.1 \times 10^5$	$1.0 \times 10^{-6}$	$1.3 \times 10^{-4}$
		10	$4.1 \times 10^8$	$4.8 \times 10^2$	$6.9 \times 10^4$	$1.2 \times 10^{-6}$	$1.7 \times 10^{-4}$

DB21 and DB47 were grown in LB medium to a density of  $2 \times 10^8$ /ml. Two-ml. samples of cells were infected with phage at the indicated multiplicities; the infected cells were incubated at 37°C for 2 hr, and then sterilized with CHCl<sub>3</sub>. The crude lysates were titered for plaque-forming units on DB21 indicator and were assayed for *his*<sup>+</sup> and *pur*<sup>+</sup> activities on PV78 (P22 *vie*  $Im_3$ ); multiplicities of viable phage were never greater than 5 per cell (Ebel-Tsipis & Botstein, 1971).

† Abbreviation used: p.f.u., plaque-forming unit.

P22 *erf*<sup>-</sup> phage were used to examine the effect of a phage recombination system on the formation of transducing particles. These mutants, which were isolated and described by Botstein & Matz (1970), lack an essential recombination function which is necessary for P22 growth on *rec*<sup>-</sup> bacteria. The *erf* gene is located in the "early" region of the phage genetic map but *rec*<sup>-</sup> cells infected with P22 *erf*<sup>-</sup> express late functions (Botstein & Matz, 1970). As shown in Table 3, P22 *erf*<sup>-</sup> behaves approximately the same as P22 *erf*<sup>+</sup> when infecting *rec*<sup>+</sup> bacteria. However, the P22 *erf*<sup>-</sup> lysate grown on *rec*<sup>-</sup> cells is markedly different from the other three lysates examined. Under these conditions the yield of plaque-forming units is grossly reduced while the yield of transducing particles is normal. Thus, the frequency of *pur*<sup>+</sup> and *his*<sup>+</sup> transducing particles per plaque-forming unit in these lysates is more than 20 times higher than in any of the other lysates examined.

These experiments indicate that neither of the known general recombination systems in *S. typhimurium* and P22 are necessary for the formation of transducing particles. They also demonstrate that the formation of transducing particles does not

depend upon the formation of vegetative phage. That is, P22 *erf*<sup>-</sup>-infected *rec*<sup>-</sup> bacteria synthesize little phage or phage DNA (0.3 to 2 copies/cell; Botstein & Matz, 1970), yet they are capable of producing transducing particles at the normal levels.

#### 4. Discussion

##### (a) *The structure of transducing particles*

###### (i) *Transducing particles are physically very similar to phage particles*

Phage P22 transducing particles are very similar in their physical properties to phage particles. The particles are the same size, as judged from zone sedimentation. Likewise their DNA appears to have the same molecular weight as that of P22 DNA. The density of transducing particles in CsCl is somewhat different from the phage density, in agreement with the results of Sheppard (1962) and Roth & Hartman (1965). This appears to reflect a difference in density between P22 DNA and *Salmonella* DNA.

Similar conclusions regarding the properties of generalized transducing particles have been reached for coliphage P1 (Ikeda & Tomizawa, 1965).

###### (ii) *Generalized transducing particles contain bacterial DNA and little or no phage DNA*

Starlinger (1959) found that growth of P22 on <sup>32</sup>P-labeled bacteria in unlabeled medium resulted in lysates which contained transducing particles which were sensitive to <sup>32</sup>P decay and vegetative phage which were not. These results demonstrated that transducing particles contain bacterial DNA which was made before phage infection while vegetative phage contain newly-synthesized DNA. Starlinger was unable to determine, however, whether transducing particles contain only bacterial DNA or whether they contain segments of both bacterial and phage DNA. Our experiments indicate that P22 transducing particles contain primarily bacterial DNA which was synthesized before phage infection, and little or no phage DNA. Examination of the buoyant densities of transducing DNA and bacterial DNA in the Heavy-Light lysate reveals that the two DNA species have similar buoyant densities (1.726 and 1.728 g/cm<sup>3</sup>, respectively). The small density difference between the transducing DNA and the bacterial DNA could be due to one or more of the following factors.

(1) A small fraction of the bacterial chromosome might replicate after infection and some transducing particles could then encapsulate this newly replicated DNA, causing a small shift in the average density of the transducing particles. The double peak of *gal*<sup>+</sup> transducing activity (Fig. 4) provides evidence of such replication. (2) Different regions of the bacterial chromosome having different mean buoyant densities might be unequally represented in the population of transducing particles. For example, the relative *his*<sup>+</sup> transducing activity compared to *pur*<sup>+</sup> or *gal*<sup>+</sup> activity may reflect differences in the yield of particles carrying those markers. Since the *his*<sup>+</sup> transducing particles have a lighter density than most transducing particles, an excess of such particles in the lysate would cause the average density of transducing DNA to be less than that of unfractionated *S. typhimurium* DNA. (3) All transducing particles could contain some newly-synthesized phage DNA (i.e. DNA normally found in plaque-forming particles) but the observed density difference allows no more than a few per cent of the DNA in the transducing particles to be phage DNA. Schmieger (1968, 1970) has proposed a model in which transducing particles are formed by covalently joining a piece of phage DNA to a fragment of the bacterial chromosome. It seems to us that the other possibilities outlined above are adequate to account for the density

differences which are observed in our experiments. This conclusion is consistent with the observations of Ozeki (Ozeki & Ikeda, 1968) who found that the density of P22 transducing particles grown on bromouracil-labeled bacteria in light medium was the same as the density of transducing particles grown on bromouracil-labeled bacteria in bromouracil medium. The situation with P22 is therefore similar to that reported by Ikeda & Tomizawa (1965) who found that P1*vir* generalized transducing particles contain bacterial DNA which had been synthesized before infection and little or no phage DNA.

The structure of transducing fragments has also been examined for two *Bacillus subtilis* phages, SP10 and PBS1. Okubo, Stodolsky, Bott & Strauss (1963) demonstrated that SP10 generalized transducing particles contain no phage DNA. This also seems to be the case with PBS1 transducing particles (Yamagishi & Takahashi, 1968). It seems therefore that the absence of phage DNA in generalized transducing particles is a common phenomenon. This property distinguishes generalized transducing particles from specialized transducing particles which are known to contain a particular piece of bacterial DNA inserted into the phage genome (Campbell, 1962; Signer, 1968).

#### (b) *The frequency of transducing particles*

Since transducing particles contain primarily bacterial DNA, which had not replicated after infection, and little or no phage DNA, the specific activity of the DNA in transducing particles should be the same as the specific activity of the bacterial DNA at the time of infection. It is, therefore, possible to calculate the frequency of transducing particles in the total lysate. Such a calculation is difficult from biological data alone because of the existence of abortive transductants, which constitute about 90% of all transductants (Stocker, Zinder & Lederberg, 1953), and because individual markers manifest different transduction frequencies. Using a preparation of fractionated density-labeled radioactive transducing particles (containing DNA of molecular weight  $27 \times 10^6$  daltons;  $4 \times 10^{-11}$   $\mu\text{g}$  DNA/particle), grown on bacteria whose DNA was labeled before infection with [*methyl*- $^3\text{H}$ ]thymine at a known specific activity (10  $\mu\text{Ci}$  [*methyl*- $^3\text{H}$ ]thymine/ $\mu\text{g}$  thymine; 0.96  $\mu\text{Ci}$  [*methyl*- $^3\text{H}$ ]thymine/ $\mu\text{g}$  DNA), we have calculated that the Heavy-Light lysate described in Figure 4 and Table 2 has  $4.2 \times 10^{10}$  transducing particles per  $3.2 \times 10^{12}$  plaque-forming units. If one assumes that P22 plates with an efficiency of 1, then transducing particles make up about 1.3% of the total lysate. Assuming that the formation of transducing particles in the Light-Light lysate also occurs by the encapsulation of DNA which did not replicate after infection, the fraction of transducing particles in the Light-Light lysate would be about 2%.

In view of the fact that the mass of the *Salmonella* host genome is about 100 phage-equivalents of DNA, this proportion is less than one might expect. We conclude that the phage encapsulation system packages host DNA less efficiently than phage DNA, since more than half of the intracellular phage DNA is encapsulated in lytic infections (Botstein, 1968), while the fraction of total host DNA in an infected culture which appears ultimately in transducing particles is only about 5% (unpublished observation). This conclusion is in agreement with those of Arber (1960) and Ikeda & Tomizawa (1965) for the *Escherichia coli*-P1 system.

In the Light-Light and Heavy-Light lysates, and presumably in the Heavy-Heavy lysate as well, the total number of transducing particles far exceeds the number of particles that yield complete *his*<sup>+</sup>, *gal*<sup>+</sup> or *pur*<sup>+</sup> transductants. Since P22 DNA is

about one-hundredth the size of the *S. typhimurium* chromosome ( $27 \times 10^6$  versus 2 to  $3 \times 10^9$  daltons; genetic evidence from Sanderson & Demerec, 1965) one might expect that any type of transducing particle would represent approximately 1% of all transducing particles. Assuming that the number of *his*<sup>+</sup> transducing particles is representative, one would predict that the total number of particles, based on transducing activity, in the Heavy-Light lysate would be  $100 \times 2.7 \times 10^7 = 2.7 \times 10^9$  transducing particles. The actual number of transducing particles calculated above is fifteen times this number; calculation of the total number of particles from the measured number of *gal*<sup>+</sup> or *pur*<sup>+</sup> transductants would indicate an even larger discrepancy. It seems that no more than 10% of all transducing particles can form complete transductants (i.e. transductants arising from the integration of donor DNA into the recipient chromosome). The other 90% or more of the transducing particles presumably give rise to abortive transductants. This is in agreement with the results of Stocker *et al.* (1953) and Ozeki (1959), who found that the number of abortive transductants obtained for any marker was approximately ten times higher than the number of complete transductants obtained for that marker.

(c) *The formation of generalized transducing particles*

It is clear that P22 generalized transducing particles contain primarily bacterial DNA which was made before infection and little or no phage DNA. This bacterial DNA is presumed to be packaged into phage heads in the same way that concatenated P22 DNA is packaged. Although this packaging mechanism is not understood, several relevant observations have been made. It appears that the phage head is capable of encapsulating "heads-full" of either phage or bacterial DNA since both phage and transducing DNA are packaged and both DNA's have the same molecular weight. The encapsulation mechanism must, however, have a higher affinity for phage DNA than bacterial DNA. The low affinity of P22 phage heads for bacterial DNA becomes even more apparent in *erf*<sup>-</sup>-infected *rec*<sup>-</sup> bacteria; under these circumstances, in the presence of an excess of empty phage heads and little phage DNA, the number of transducing particles formed is about the same as in *erf*<sup>+</sup>-infected *rec*<sup>+</sup> bacteria. The mechanism responsible for determining the size of the encapsulated DNA is not understood either. It seems likely that it is associated with the packaging process since, in P22-infected bacteria, there is no evidence that the *S. typhimurium* chromosome is broken into smaller fragments before it is packaged (Schmieger, 1971). Over-length phage DNA which is the intracellular precursor to mature phage DNA (Botstein & Levine, 1968) seems also to be cut only as part of the encapsulation process (Botstein, Waddell & King, manuscript in preparation). Whatever the mechanism, these experiments demonstrate that the encapsulation mechanism operating on bacterial DNA requires neither of the general recombination functions (*erf*, *rec*) that have so far been identified in P22-infected *S. typhimurium* (Botstein & Matz, 1970).

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