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Generalized Transduction by Bacteriophage P22 in *Salmonella typhimurium*

II. Mechanism of Integration of Transducing DNA

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Generalized transduction by bacteriophage P22 occurs by the transfer of DNA from one bacterium to another by means of a phage-like particle (i.e. a transducing particle) which contains bacterial DNA formed before infection (preceding paper). Transduction of a ^{32}P-labeled, light recipient with purified transducing particles whose DNA is labeled with ^{3}H, ^{2}H, ^{15}N, ^{13}C results in the physical association of 12 to 15% of the transducing DNA with the DNA of the recipient bacteria. The remainder of the transducing DNA persists as unreplicated phage-size fragments during further bacterial growth. Physical and genetic analysis of the DNA integrated as large fragments reveals that recombination in generalized transduction occurs by a displacement mechanism, often resulting in the integration of double strand fragments of transducing DNA into the DNA of the recipient bacteria. The molecular weight of most of the large integrated fragments is greater than 2 to 4×10^6 daltons but substantially less than 27×10^6 daltons, the molecular weight of the transducing DNA injected into the bacteria. The integrated large double strand fragments of transducing DNA can be detected in DNA isolated soon after transduction, but not in DNA isolated late after transduction, when the transductants have been permitted to replicate.

1. Introduction

There are three main modes of genetic exchange between bacteria. These are generalized transduction (Ozeki & Ikeda, 1968), bacterial transformation (Hotchkiss & Gabor, 1970) and bacterial conjugation (Curtiss, 1969). Although these are very different processes they all have the same end-result: the transfer of heritable properties from one bacterium to another. In transformation in *Pneumococcus*, *Bacillus subtilis* and *Haemophilus influenzae*, donor DNA molecules enter recipient bacteria and become stably integrated into the recipient chromosome by a displacement mechanism. In these systems, a single strand fragment of the donor DNA becomes integrated into the recipient chromosome at the site of the displacement event(s) (Fox & Allen, 1964; Notani & Goodgal, 1966; Dubnau & Davidoff-Abelson, 1971).

Because of the small numbers of generalized transducing particles present in phage lysates, it has, until recently, been impossible to undertake a physical examination of

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the fate of the DNA of these particles in recipient bacteria. Our studies on the formation of P22 transducing particles, presented in the preceding paper, show that P22 transducing particles, like P1 transducing particles (Ikeda & Tomizawa, 1965), contain predominantly bacterial DNA which was synthesized before phage infection, and little or no phage DNA. Similar observations describing the structure of P22 generalized transducing particles have also been reported by Schmieger (1968,1970) and Ozeki & Ikeda (1968). Lysates of heavy pre-labeled bacteria which were transferred to light medium just before infection therefore contain light vegetative phage and density-labeled transducing particles. Transducing particles can be separated from the non-density-labeled phage by cesium chloride density gradient centrifugation and these purified transducing particles, whose DNA contains heavy and radioactive labels, can be utilized to study the fate of transducing DNA in recipient bacteria.

In this paper we will show that the DNA of generalized transducing particles becomes covalently joined to the DNA of the recipient bacteria. Furthermore, genetically-recombinant DNA molecules are shown to contain physical material from both the donor and the recipient DNA.

Our analysis of the structure of the long reaches of donor DNA which have become integrated into the DNA of the recipient bacteria indicates that, for a major fraction, both donor strands have been integrated. This result is to be contrasted with the results of similar experiments on integrated transforming DNA (Fox & Allen, 1964; Notani & Goodgal, 1966; Dubnau & Davidoff-Abelson, 1971) where the integrated DNA was found to displace only a single recipient strand.

2. Materials and Methods

Most of the materials and methods are the same as those described previously (Ebel-Tsipis & Botstein, 1971; Ebel-Tsipis, Botstein & Fox, 1972).

(a) *Bacterial strains*

Salmonella typhimurium DB25 and PV78 (P22*sie* $1m_3$) were described in the preceding paper. In addition, the following strains of *S. typhimurium* were provided from the collection of J. Roth: *hisD1*, a *his*$^-$† auxotroph with a missense mutation in the *hisD* gene; TA470 (*hisD2252hisT1504*), a *his*$^-$ auxotroph containing a frameshift mutation in the

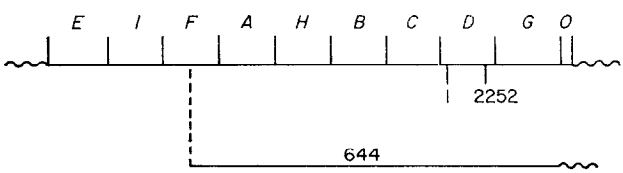

FIG. 1. A summary of the genetic map of the *his* operon in *S. typhimurium* (Whitfield, Martin & Ames, 1966; Roth, personal communication).

hisD gene in addition to a regulatory mutation in the *hisT* gene; *his-644*, a *his*$^-$ auxotroph which contains a deletion covering most of the *his* operon. A partial genetic map of this region is shown in Figure 1.

(b) *Phage strains*

P22 $m_3c_2h_{21}$ and P22 $c_1ts19{\cdot}1$ were described previously (Ebel-Tsipis *et al.*, 1972).

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

(c) *Medium*

Heavy minimal medium was modified to include ^{13}C-labeled glucose and contained: 0·1 M-Tris (pH 7·4), 0·01 M-$^{15}NH_4Cl$, $2·5 \times 10^{-3}$ M-$MgSO_4$, 0·012 M-NaCl, 0·1% (w/v) D-[U-^{13}C]glucose, phosphorus (as phosphate) 20 μg/ml., 10 μg thymine/ml. in D_2O. Two drops of a trace metals solution were added per 100 ml.

(d) *Enzyme solution*

H. influenzae endonuclease R (Smith & Wilcox, 1970) was the kind gift of H. O. Smith. A generous amount of this enzyme was also provided by V. Pirrota.

(e) *Chemicals*

NCS Solubilizer was purchased from Amersham/Searle, Des Plaines, Illinois. D-[U-^{13}C]-glucose (52·5 atom % ^{13}C) was obtained from Merck, Sharpe & Dohme of Canada, Ltd, Montreal, Canada.

Carrier-free [^{32}P]phosphoric acid was obtained from New England Nuclear Corp., Boston, Mass.

(f) *Preparation of purified density-labeled transducing particles*

(i) *Preparation of transducing particles carrying the* hisD1 *allele*

S. typhimurium hisD1 was adapted to heavy minimal medium as described in the preceding paper. The cells (100 ml.) were then grown to a density of 5×10^8/ml. in heavy minimal medium containing [^{13}C]glucose and infected with P22 $c_1ts19·1$ (grown on *his-644*) at a multiplicity of 5 phage per cell. Two min later, NaCN ($2·5 \times 10^{-3}$ M) was added and the infected cells were kept at 39°C for 8 min without shaking. They were then filtered on Millipore filters (GSWPO4700) and resuspended in 200 ml. LCG20 medium (Ebel-Tsipis *et al.*, 1972); after 150 min incubation at 39°C the cells were lysed with lysozyme (1 mg/ml.) and EDTA (0·01 M). The phage were concentrated and treated with a purified P22 base plate preparation and the transducing particles were separated from the plaque-forming units by CsCl density-gradient centrifugation as described previously (Ebel-Tsipis *et al.*, 1972). The fractions including the peak of transducing particles were pooled and fractionated again on a CsCl density gradient in order to further reduce the level of contaminating infective phage. The final solution of transducing particles was dialyzed against buffered saline. The final suspension of transducing particles contained approximately 2×10^6 *pur*$^+$ transducing particles, as assayed on PV78(P22*sie* $1m_3$), and 2×10^5 *his*$^+$ transducing particles, as assayed on TA470(P22*sie* $1m_3$); as described previously, only complete transductants were scored.

(ii) *Preparation of heavy radioactive transducing particles*

S. typhimurium DB25 was grown overnight in 100 ml. of heavy minimal medium containing [*methyl*-^3H]thymine at a specific activity of approximately 40 μCi/μg (total radioactivity added is 20 mCi). When the culture reached a density of 5×10^8 cells/ml., an excess of cold thymine (200 μg/ml.) in D_2O was added. After 15 min the cells were infected and treated as described previously for the preparation of radioactive transducing particles (Ebel-Tsipis *et al.*, 1972). The phage were purified, concentrated and treated with a preparation of purified base plate parts. Transducing particles were separated away from plaque-forming units by repeated preparative CsCl equilibrium density-gradient centrifugations. After 2 such centrifugations, the relative frequency of plaque-forming units was estimated to be less than 1% of all particles. The purified transducing particles were dialyzed against buffered saline and were used as soon as possible. This procedure yields approximately 2×10^{10} transducing particles, containing 2×10^6 cts/min ^3H in density-labeled transducing DNA.

(g) *Transduction procedure*

Ordinarily, the method was the same as that described in the preceding paper. However, when the DNA of the transduced culture was destined for physical analysis, the following method was used. PV78 (P22*sie* $1m_3$) was grown in LCG20 medium containing 1·0 to 1·5 μCi ^{32}P/ml. When the culture reached a density of 3×10^8 cells/ml., 12 ml. of the culture

were centrifuged at room temperature and resuspended in 5 ml. pre-warmed (39°C) LCG20 medium without ^{32}P. After 15 min incubation, pre-warmed purified transducing particles were added and allowed to adsorb without shaking for 15 min at 39°C. The transduced culture was then diluted into 60 ml. LCG20 medium ($t = 0$) and the culture was incubated with shaking at 39°C. The culture was sampled at later times to measure the viable count and the number of transductants, and DNA was isolated from the transduced culture at the times indicated in each experiment.

(h) *Reference DNA*

^{14}C-labeled P22 DNA (mol. wt. 27×10^6 daltons; $S^0_{20,w} = 33$ S) is isolated from ^{14}C-labeled P22 by lysis with Sarkosyl at 65°C (Botstein, 1968).

^{14}C-labeled *Escherichia coli* DNA, sheared at 44,000 rev./min to a molecular weight of $1{\cdot}7 \times 10^6$, was kindly provided by P. Rosenthal. The $S^0_{20,w}$ of this reference DNA is 12·5 S (Rosenthal, 1968).

^{14}C-labeled *S. typhimurium* DNA, density labeled with ^2H, ^{15}N and ^{13}C, was isolated from DB25 and was purified on a CsCl equilibrium density gradient. The density of this DNA is 1·747 g/cm^3.

(i) *Preparation of low molecular weight DNA*

(i) *Sonication*

One-ml. samples of DNA in 0·01 M-EDTA were sonicated in an MSE Sonicator. Samples were placed in a 2·5 in. × 0·5 in. polyallomer centrifuge tube, suspended in an ice-bath, and were sonicated twice, 10 sec each time, at 1·5 A.

(ii) *Treatment with endonuclease R*

Endonuclease R is a restriction endonuclease isolated from *H. influenzae* (Smith & Wilcox, 1970). A saturating amount of enzyme was added to a solution containing DNA in $6{\cdot}6 \times 10^{-3}$ M-Tris (pH 7·4), $6{\cdot}6 \times 10^{-3}$ M-$MgCl_2$, $6{\cdot}6 \times 10^{-3}$ M-mercaptoethanol, $6{\cdot}6 \times 10^{-2}$ M-NaCl. The DNA was incubated with the enzyme for 15 min at 37°C and the reaction stopped by the addition of 5×10^{-2} M-EDTA. Further incubation does not decrease the size of the DNA.

(j) *Alkali denaturation of DNA*

A solution of DNA (1·5 ml.) in 0·01 M-Tris, 0·01 M-EDTA was gently mixed with 0·6 ml. 0·5 M-Na_3PO_4 (final pH 12·1). The mixture was incubated for 5 to 10 min at 42°C, chilled on ice and then neutralized by gently adding 0·39 ml. of 1·1 N-HCl in 0·2 M-Tris to a final pH of 7·6.

(k) *Determination of molecular weight of DNA*

Sedimentation coefficients were determined by zone centrifugation of the experimental sample together with a reference DNA of known size (Burgi & Hershey, 1963). The molecular weight of a sample of DNA was calculated from its $S^0_{20,w}$ according to the equation of Studier (1965).

(l) *Measurement of radioactivity*

All samples were counted as described previously (Ebel-Tsipis *et al.*, 1972). When the number of counts was small, the dry filter papers were placed in scintillation vials, 0·1 ml. NCS was added, and the vials were covered. After 4 hr at room temperature, scintillation fluid was added and the vials were stored overnight in the cold before counting. The counting efficiencies of ^{14}C and ^{32}P are not affected.

Since many CsCl and sucrose gradients contain low levels of radioactivity all samples were counted 3 times, 5 min each time, and the median value for each sample was then used for all calculations.

(m) *Analysis of data obtained from CsCl density-gradient centrifugation of integrated transducing DNA*

All density gradients for integrated transducing DNA contain two reference DNA's: ^{14}C, ^{15}N, ^2H, ^{13}C-labeled *S. typhimurium* DNA which serves as a heavy position marker

(indicated by the arrows in Figs 6 to 10) and ^{32}P-labeled light *S. typhimurium* DNA from the transduced bacteria which serves as a marker for the light density position. The integrated ^{3}H-labeled transducing DNA is found within the boundaries of these two density markers. If one calculates the ratio ^{3}H/^{32}P across the light DNA peak in any of these gradients, one finds that the ratio ^{3}H/^{32}P decreases gradually as one goes from the heavy side to the light side of the ^{32}P peak; it then reaches a constant value on the light side of this peak. We interpret this as indicating that there are two classes of [^{3}H]DNA: one which is fully-light and isodense with the [^{32}P]DNA and which probably arose from some breakdown and re-incorporation of donor DNA as very small pieces (perhaps nucleotides), and one which is heavier than the light bacterial DNA. On the basis of this assumption, the amount of all [^{3}H]DNA which is heavier than light DNA present in each fraction is calculated. A plot of the density distribution of this DNA is included as an inset in Figs 6 to 10. This plot allows one to visualize the distribution of donor DNA which is integrated as large pieces.

3. Results

(a) *Generalized transduction occurs by a breakage and joining mechanism*

In order to show that DNA which is recombinant for two genetic markers contains physical material from both donor transducing DNA and the DNA of the recipient bacterium, we transduced strain TA470 (*hisD2252*, a point mutation) in light medium with purified density-labeled transducing particles grown on a recipient carrying another point mutation, *hisD1*. One hour after transduction, the newly transduced cells were infected with P22c_1*ts*19·1 (grown on *his-644*, a deletion covering all of *hisD*) at a multiplicity of 5 plague-forming units/cell in order to prepare a lysate containing transducing particles carrying the newly formed *his*$^{+}$ recombinant DNA. After 4 hours incubation at the non-permissive temperature of 39°C, the infected cells were lysed and the lysate was concentrated and analyzed by CsCl density-gradient centrifugation. Fractions were assayed for plaque-forming units, *pur*$^{+}$ transducing activity and *his*$^{+}$ transducing activity (Fig. 2). If the *his*$^{+}$ transducing particles contain both heavy donor and light recipient DNA, their density will be greater than that of the plaque-forming units whereas *his*$^{+}$ transducing particles containing only light DNA will have the same density as that of the plaque-forming units (Ebel-Tsipis *et al.*, 1972).

Although the absolute densities of the different types of particles were not determined in this experiment the density of the *his*$^{+}$ particles can be calculated from the known densities of the phage ($\rho = 1{\cdot}506$ g/cm^{3}) and the *pur*$^{+}$ transducing particles ($\rho = 1{\cdot}511$ g/cm^{3}). The average density of the *his*$^{+}$ transducing particles in this gradient is calculated to be approximately 1·512 g/cm^{3} (as opposed to 1·507 g/cm^{3}, the normal density of *his*$^{+}$ transducing particles (Ebel-Tsipis *et al.*, 1972)). Since the particles are approximately 50% DNA by weight, the DNA carried by *his*$^{+}$ transducing particles is therefore about 0·01 g/cm^{3} more dense than the DNA in light *his*$^{+}$ transducing particles. Since fully-heavy *S. typhimurium* DNA ($\rho = 1{\cdot}747$ g/cm^{3}) is 0·05 g/cm^{3} heavier than light *S. typhimurium* DNA ($\rho = 1{\cdot}697$ g/cm^{3}), a density difference of 0·01 g/cm^{3} corresponds to a content of about 20% fully-heavy DNA. If transducing DNA is commonly integrated as double strand fragments this density difference would result from the integration of a piece of DNA of about 5 to 6×10^{6} daltons, one-fifth the size of the transducing DNA. A similar density shift would result from the integration of a single strand of transducing DNA twice this length (approximately half the single strand length of the transducing DNA). The size of the integrated piece calculated in this way agrees with the direct physical measurements described below.

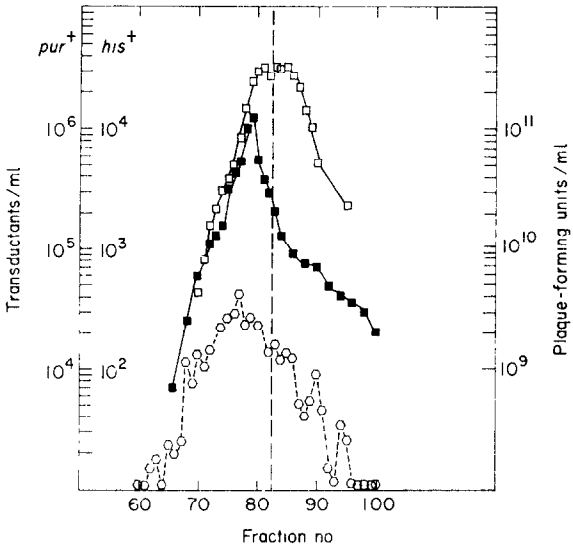

FIG. 2. CsCl equilibrium density-gradient centrifugation of P22c_1*ts*19·1 lysate containing recombinant *his*$^+$ transducing particles. Phage were grown on newly transduced TA470 as described in Results, concentrated and centrifuged in a Spinco SW39 rotor. One-drop fractions were collected into 0·2 ml. of buffered saline and each fraction was assayed for plaque-forming units, *pur*$^+$ transducing activity [on PV78 (P22 *sie* 1m_3)] and *his*$^+$ transducing activity [on *his-644* (P22 *sie* 1m_3)]. The total number of fractions is 150. —□—□—, Plaque-forming units; —■—■—, *pur*$^+$ transducing activity; – –○– –○– –, *his*$^+$ transducing activity.

Since all *his*$^+$ transducing particles capable of transducing the deletion must contain recombinant DNA and since the majority of the *his*$^+$ transducing particles also appear to contain some heavy DNA, it seems that recombination in generalized transduction occurs by a physical displacement mechanism. However, the possibility that a fraction of the *his*$^+$ recombinants derive from some alternative mechanism cannot be excluded.

(b) *Physical studies on the fate of transducing DNA*

(i) *The decision to integrate*

In order to examine the fate of transducing DNA in recipient bacteria, purified P22 transducing particles whose DNA is both ^{3}H-labeled and density-labeled are prepared. Recipient bacteria are grown in light medium containing ^{32}P. Adsorption and subsequent growth of the transduced cells occurs in light medium in the absence of ^{32}P. At various times after infection, DNA is isolated from the transduced cells by a gentle lysis procedure as described by Ebel-Tsipis *et al.* (1972) and analyzed by CsCl equilibrium density-gradient centrifugation. In this way, heavy ^{3}H-labeled DNA which has become associated with high molecular weight, light ^{32}P-labeled DNA of the recipient bacteria can be distinguished from transducing DNA which has not become associated and hence remains at the heavy density position. The ^{3}H-label which becomes stably associated with the light DNA of the recipient bacteria (mol. wt. 70 to 100×10^6 daltons) is said to represent integrated transducing DNA.

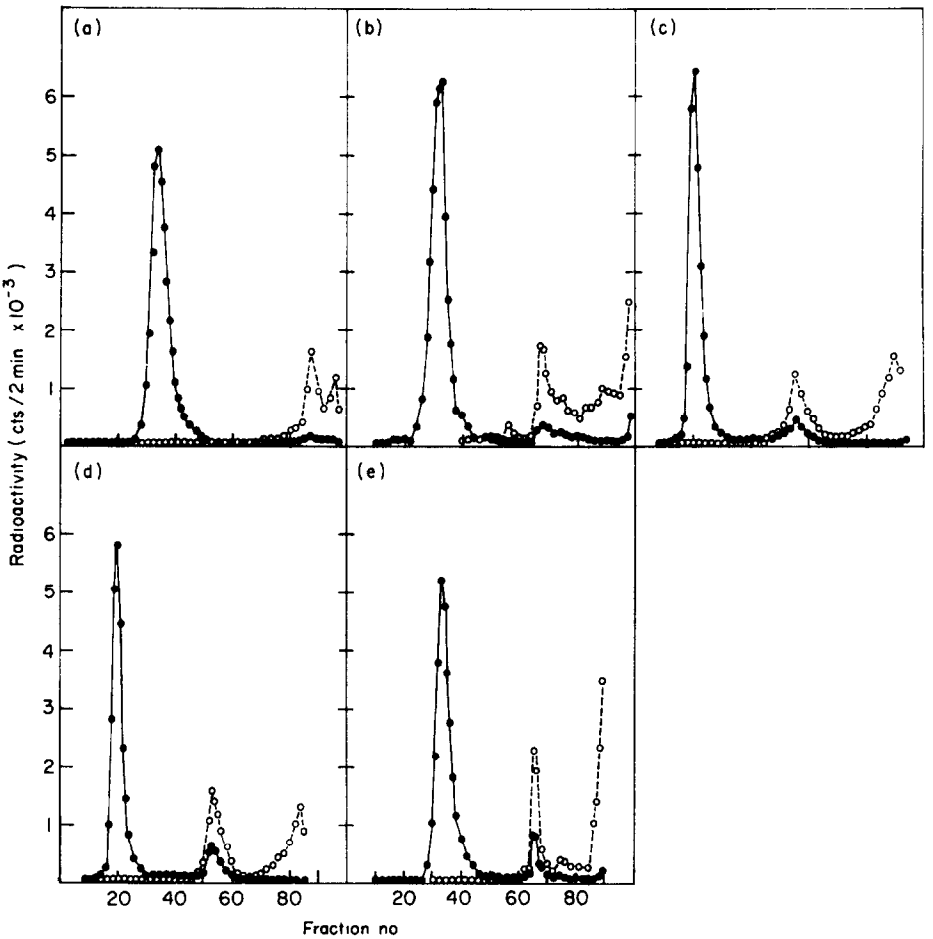

FIG. 3. CsCl equilibrium density-gradient centrifugation of DNA isolated from ^{32}P-labeled PV78 (P22 *sie* 1m_3) following transduction with purified ^{3}H-labeled transducing particles. DNA was isolated at (a) 0 min; (b) 20 min; (c) 60 min; (d) 120 min; and (e) 180 min after infection. The DNA was centrifuged in a Spinco 50 rotor and 2-drop fractions were collected. —●—●—, ^{3}H-radioactivity; --○--○--, ^{32}P-radioactivity.

The results of a typical experiment, in which DNA was isolated at 0, 20, 60, 120 and 180 minutes after transduction, are shown in Figure 3. The results can be summarized as follows:

(1) at most, 12 to 15% of the adsorbed transducing DNA becomes associated with light bacterial DNA.

(2) The quantity of ^{3}H label associated with the DNA of the recipient bacteria reaches a maximum within the first hour following infection. The time-course for this association can be calculated in two ways as outlined in the legend of Figure 4. Both calculations give similar results and both curves extrapolate back to 0 at approximately — 15 minutes, the time at which transducing particles were added to the bacteria.

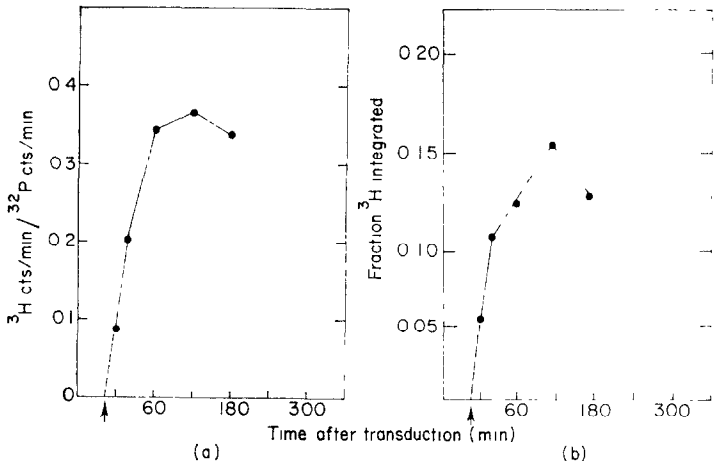

FIG. 4. The association of transducing DNA with the DNA of the recipient bacteria as a function of time after transduction. (a) The ratio ^{3}H cts/min/^{32}P cts/min was calculated for the light ^{32}P peak for each of the gradients shown in Fig. 3.

(b) The fraction of total [^{3}H]DNA associated with the light DNA of the recipient bacteria was calculated for each of the gradients presented in Fig. 3.

(ii) *Studies on the integrated transducing DNA*

Isolation and purification of integrated DNA. DNA was isolated from transduced cultures by a gentle lysis procedure followed by extensive RNase and pronase treatment and dialysis. In order to examine the molecular structure of the integrated transducing DNA, the integrated DNA must first be separated from the relatively large amount of heavy non-associated transducing DNA; in general, two purifications on angle-head CsCl equilibrium density gradients are adequate to completely remove all unassociated heavy DNA from the light bacterial DNA. All steps in the isolation and purification of the DNA are carried out with minimum agitation, resulting in a molecular weight of purified DNA of 70 to 100×10^6 daltons. This DNA is referred to as high molecular weight DNA.

In order to "dissect" out the structure of the integrated transducing DNA, the DNA is then broken into small pieces and the density of the donor [^{3}H]DNA (for both native and denatured DNA samples) is examined by CsCl density-gradient centrifugation. If the size of the high molecular weight DNA sample is much greater than the size of the integrated DNA, the integrated DNA would be expected to be found initially at or near the light density position in the unbroken DNA sample. However, as the size of the DNA molecules is reduced, the relative density contribution of the heavy transducing fragment would be expected to increase if the DNA were integrated as large fragments. If one reduces the molecular weight of the DNA to a size which is, on the average, smaller than the size of the integrated fragments of transducing DNA then it becomes possible to infer the original structure of the integrated DNA within the bacterial chromosome. If the DNA had integrated as single strand fragments, then a reduction of molecular weight would cause a density shift of the molecules containing transducing fragments to a position approaching the hybrid density position; denatu-

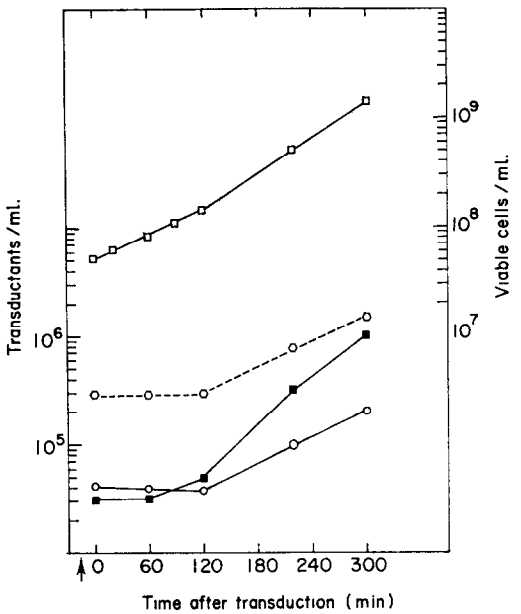

FIG. 5. Growth curve of newly transduced bacteria in LCG20 medium. PV78 (P22 *sie* $1m_3$) grown before infection in LCG20 containing 1 μCi ^{32}P/ml., was transduced with purified ^{3}H, ^{2}H, ^{15}N, ^{13}C-labeled transducing particles at a multiplicity of 6 particles/cell. The transduced cells were then diluted into 60 ml. of warmed LCG20 medium and the growth of the *pur*$^+$ transductants, the *his*$^+$ transductants, the *gal*$^+$ transductants and the viable cells was followed. —■—■—, *pur*$^+$ transductants; --○--○--, *his*$^+$ transductants; —○—○—, *gal*$^+$ transductants; —□—□—, viable cells.

ration of this low molecular weight DNA would separate the transducing DNA from light recipient DNA and it would then assume the position of fully-heavy denatured DNA on a CsCl gradient. On the other hand, if DNA had integrated as double strand fragments then a reduction of molecular weight would cause the appearance of double strand fragments of transducing DNA at the heavy density position. With this rationale in mind, we analyzed the structure of purified integrated DNA isolated at several times after transduction. In the experiment described below, DNA was isolated at 20 minutes after transduction in order to examine the initial structure of the integrated transducing DNA and at 300 minutes after transduction in order to examine the structure of the integrated transducing DNA under conditions in which transductants have replicated.

PV78 (P22 *sie* $1m_3$) was transduced with purified ^{3}H-labeled heavy transducing particles at a multiplicity of 6 particles/cell. DNA was isolated from half of the transduced culture at 20 minutes, while the rest of the culture was incubated for 300 minutes before it was lysed. Zero time is defined as the time at which the infected cells are diluted into fresh growth medium. The growth of *pur*$^+$ transductants, *gal*$^+$ transductants, *his*$^+$ transductants and viable cells was followed during this time and the results are shown in Figure 5. It is clear that at 20 minutes no replication of transductants is apparent, whereas at 300 minutes all of the transductants appear to have

replicated at least once. It is not clear why newly formed transductants take such a long time to begin to replicate but in all our experiments a lag of at least three division times was observed (Ebel-Tsipis, 1971).

The integrated DNA from each sample was then purified away from the heavy non-associated DNA and was analyzed by CsCl density-gradient centrifugation. Samples of the high molecular weight DNA, both native and alkali-denatured, from both samples were run on CsCl equilibrium density gradients. The average molecular weight of these species is given in the Figure legends. In both native samples (Figs 6(a) and 7(a)) all of the ^{3}H label is found in the light region of the gradient indicating that the integrated transducing DNA has been completely separated from the heavy non-associated DNA.

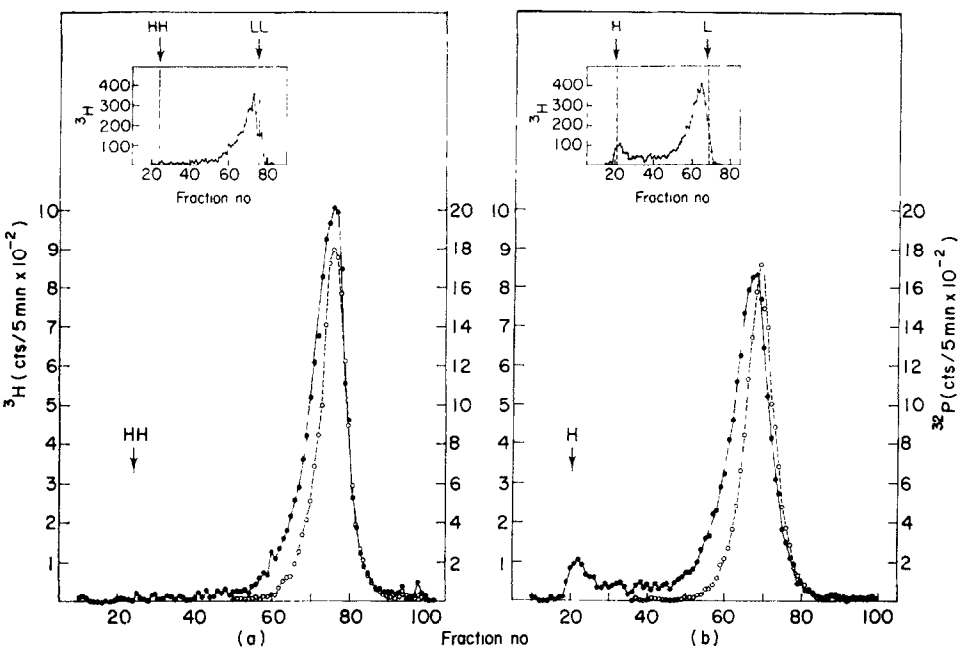

FIG. 6. CsCl equilibrium density-gradient centrifugation analysis of purified high molecular weight DNA isolated from ^{32}P-labeled recipient bacteria 20 min after transduction with ^{3}H, ^{2}H, ^{15}N, ^{13}C-labeled transducing particles. Samples (0·9 ml.) of this DNA (to which ^{14}C, ^{2}H, ^{15}N and ^{13}C-labeled *S. typhimurium* DNA had been added as a heavy position marker) were run on CsCl density gradients as (a) native and (b) alkali-denatured. The molecular weights of these samples were 7×10^{7} and $2{\cdot}3 \times 10^{7}$ daltons, respectively. Samples were centrifuged in a Spinco 50 rotor and 2-drop fractions were collected and counted for radioactivity.

The fraction of the total ^{3}H represented in the inset is 0·33 and 0·48 for (a) and (b), respectively. —●—●—, ^{3}H-radioactivity; – – ○ – – ○ – –, ^{32}P-radioactivity. H, heavy; L, light.

Centrifugation of the alkali-denatured DNA (Figs 6(b) and 7(b)) reveals that 90% of the integrated donor DNA isolated at 20 minutes and 95% of the integrated donor DNA isolated at 300 minutes after transduction remain associated with the light recipient DNA indicating covalent bonds between most of the transducing DNA and

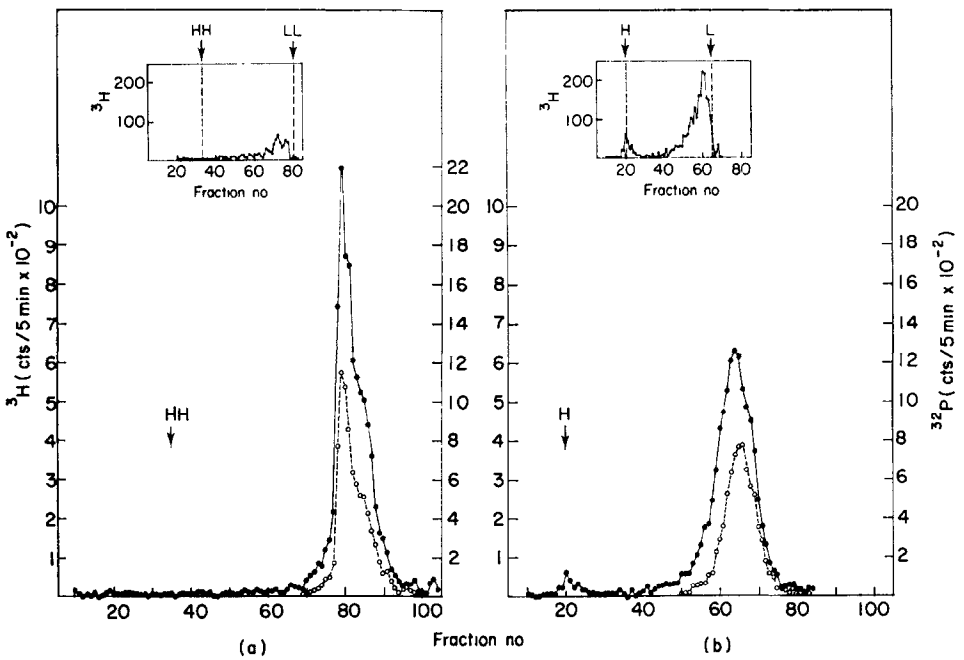

FIG. 7. CsCl equilibrium density-gradient centrifugation analysis of purified, high molecular weight DNA isolated from ^{32}P-labeled recipient bacteria 300 min after transduction with ^{3}H, ^{2}H, ^{15}N, ^{13}C-labeled transducing particles. Samples (0·9 ml.) of this DNA (to which ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* DNA had been added as a heavy position marker) were run on CsCl density gradients as (a) native and (b) alkali-denatured DNA. The molecular weights of the samples were $9{\cdot}3 \times 10^7$ and $2{\cdot}7 \times 10^7$ daltons, respectively. Samples were centrifuged in a Spinco 50 rotor and 2-drop fractions were collected and counted for radioactivity.

The fraction of the total ^{3}H represented in the inset is 0·10 and 0·31 for (a) and (b), respectively. —●—●—, ^{3}H-radioactivity; – –○– –○– –, ^{32}P-radioactivity. H, heavy; L, light.

the DNA of the recipient bacteria. The ^{3}H label which shifts in density after alkali denaturation is found at both the heavy and intermediate density positions. The fact that some heavy ^{3}H label is released by denaturation from both high molecular weight DNA isolates suggests that it does not represent an intermediate in the recombination process.

Most of the remaining DNA from both samples was treated with *H. influenzae* endonuclease R to reduce its molecular weight to about 2×10^6 daltons. This enzyme is a restriction enzyme which produces double strand breaks at specific sites on foreign (i.e. not *H. influenzae*) DNA (Smith & Wilcox, 1970; Kelley & Smith, 1970).

Cesium chloride density-gradient analysis of native enzyme-treated DNA isolated 20 minutes after transduction (Fig. 8) reveals that the reduction in the size of the DNA from 70×10^6 to 2×10^6 daltons frees some fully-heavy double strand transducing DNA from the molecules of the recipient DNA. Since there was no such heavy material present in the high molecular weight native DNA sample (Fig. 6(a)), intact double strand segments of transducing DNA must have been present in the light unbroken bacterial DNA. The amount of [^{3}H]DNA appearing at the fully-heavy position is small (0·10 of the total [^{3}H]DNA) but, as will be seen later, the total fraction of the

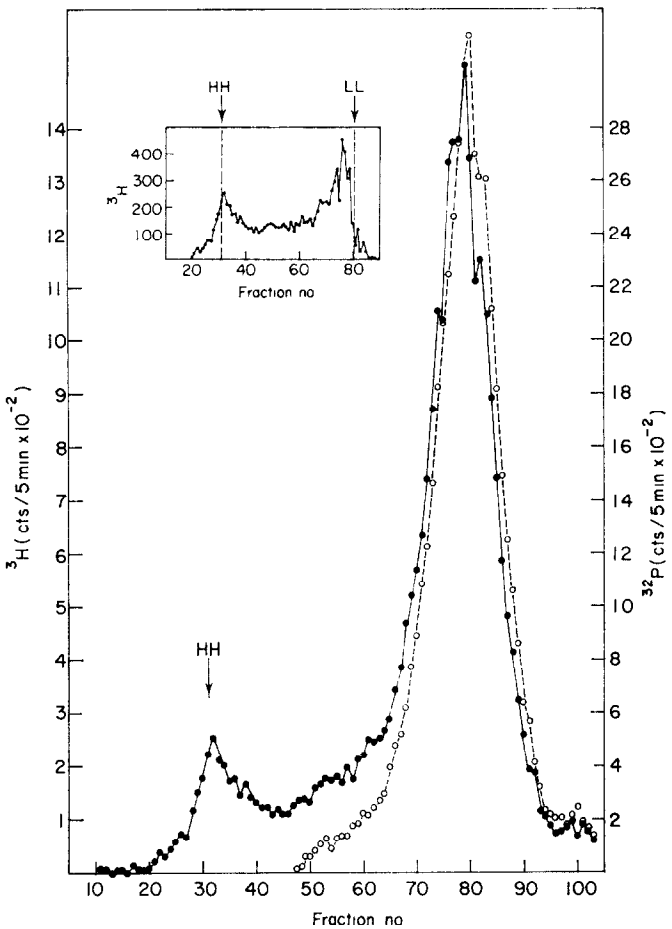

FIG. 8. CsCl equilibrium density-gradient centrifugation analysis of purified, endonuclease R-treated DNA isolated from transduced bacteria 20 min after transduction with ^{3}H-labeled heavy transducing particles. This native DNA (mol. wt. $2{\cdot}1 \times 10^6$ daltons) was run without further treatment together with ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* DNA as a heavy marker. The DNA was centrifuged in a Spinco 50 rotor and 2-drop fractions were collected and counted for radioactivity. All samples were solubilized with NCS solubilizer before counting.

The fraction of total ^{3}H represented in the inset is 0·32. —●—●—, ^{3}H-radioactivity; --○--○--, ^{32}P-radioactivity. H, heavy; L, light.

integrated ^{3}H label that can be separated as sizeable pieces from the recipient DNA is not very much larger (0·28).

The effect of enzyme treatment on the 300-minute native DNA isolate is different from on the 20-minute native sample. Instead of causing the appearance of transducing DNA at the heavy density position, a reduction of the molecular weight of DNA isolated 300 minutes after transduction causes the appearance of donor [^{3}H]DNA at approximately the hybrid density (Fig. 9). The distribution of ^{3}H label of heavier densities in both DNA samples can be seen most clearly by comparing the insets for Figures 8 and 9. Whereas the 20-minute lysate shows density-shifted [^{3}H]DNA at both the fully-heavy and intermediate densities, the 300-minute lysate shows no density-shifted [^{3}H]DNA at the heavy density position but instead appears to contain

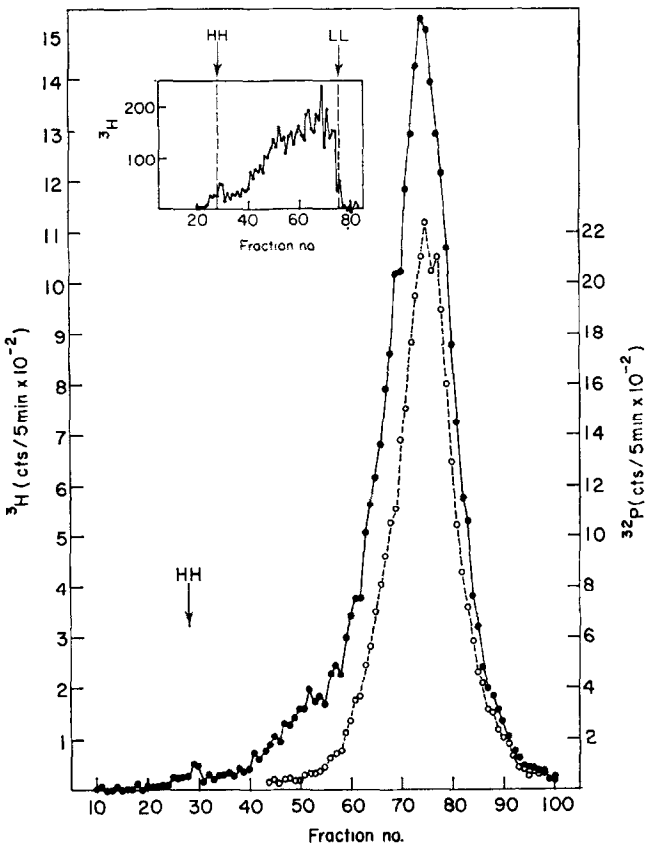

FIG. 9. CsCl equilibrium density-gradient centrifugation analysis of purified, endonuclease R-treated DNA isolated from transduced bacteria 300 min after transduction with ^{3}H-labeled heavy transducing particles. This native DNA (mol. wt. $1{\cdot}9 \times 10^6$ daltons) was run without further treatment together with ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* DNA as a heavy position marker. The DNA was centrifuged in a Spinco 50 rotor and 2-drop fractions were collected and counted for radioactivity. All samples were solubilized with NCS before counting.

The fraction of total ^{3}H represented in the inset is 0·20. —●—●—, ^{3}H-radioactivity; --○--○--, ^{32}P-radioactivity. H, heavy; L, light.

only [^{3}H]DNA of hybrid to light densities. This observation is consistent with the proposal that some transducing DNA is integrated as double strand fragments and that most of this integrated transducing DNA has replicated by 5 hours after transduction, as expected from the growth curves of the transduced cells (Fig. 5).

In order to further reduce the molecular weight of each DNA sample to 4×10^5 daltons, samples of high molecular weight DNA were sonicated. For the 20-minute DNA isolate little additional fully-heavy DNA is released (Fig. 10(a)). However, those ^{3}H-containing molecules that are more dense than light DNA do exhibit an increased density as compared with the enzyme-treated sample. Denaturation of the sonicated 20-minute DNA (Fig. 10(b)) yields some additional fully-heavy DNA but, again, most (70%) of the associated ^{3}H label remains in the light density position.

Portions of the 300-minute DNA sample were also sonicated and denatured. Following this treatment some ^{3}H counts appear in the heavy region of the gradient

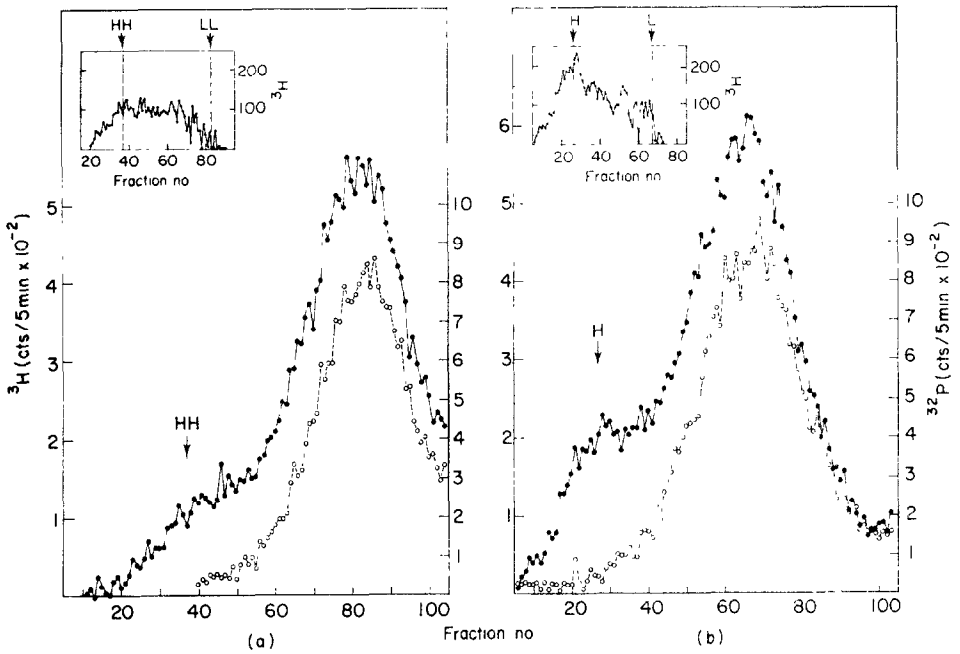

FIG. 10. CsCl equilibrium density-gradient centrifugation analysis of purified, sonicated DNA isolated from transduced bacteria 20 min after transduction. Equal fractions of the sonicated sample were run on CsCl density gradients as (a) native and (b) alkali-denatured DNA. ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* DNA, which had also been sonicated before centrifugation, was used as a heavy position marker. The molecular weight of the native DNA was $4{\cdot}6 \times 10^5$ daltons. Centrifugation was in a Spinco 50 rotor and 2-drop fractions were collected. All samples were solubilized with NCS before counting.

The fraction of total ^{3}H represented in the insets is 0·23 and 0·27 for (a) and (b), respectively. —●—●—, ^{3}H-radioactivity; --○--○--, ^{32}P-radioactivity. H, heavy; L, light.

(Fig. 11). Since the number of counts in this gradient was small, calculations are uncertain but it appears that about 0·14 of all ^{3}H is present in the bottom half of the gradient, thereby demonstrating that some large segments of integrated transducing DNA are still present in the DNA of the recipient bacteria 5 hours after transduction.

The results of these experiments can be summarized as follows:

(1) most (90 to 95%) of the [^{3}H]DNA which has become associated with the DNA of the recipient bacteria is covalently associated.

(2) Double strand fragments of transducing DNA are found integrated into the DNA of the recipient bacteria when DNA is isolated 20 minutes after transduction.

(3) Very little, if any, double strand donor DNA is evident in DNA isolated 300 minutes after transduction.

(4) About 30% of the transducing DNA which has become associated with the light bacterial DNA by 20 minutes after transduction exhibits a density heavier than that of light bacterial DNA after sonication and denaturation. A smaller fraction (15 to 20%) of the transducing DNA which has become associated with the light bacterial DNA by 300 minutes after transduction can be shown to undergo a shift to higher density by similar treatment.

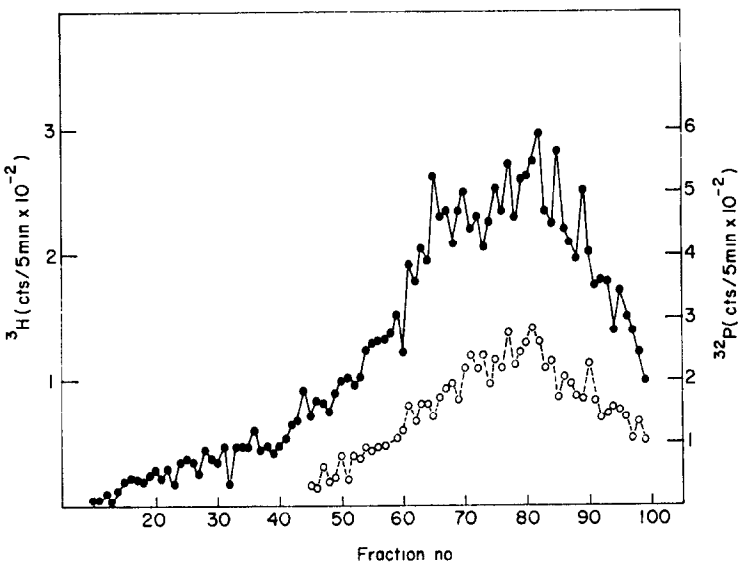

FIG. 11. CsCl equilibrium density-gradient centrifugation of purified sonicated DNA, isolated from transduced bacteria 300 min after transduction with ^{3}H-labeled heavy transducing particles. A portion (1 ml.) of the unsheared DNA was sonicated together with ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* DNA. The DNA was alkali-denatured and was then analyzed on a CsCl density gradient. Centrifugation was in a Spinco 50 rotor and 2-drop fractions were collected. All samples were solubilized with NCS before counting. —●—●—, ^{3}H-radioactivity; --○--○--, ^{32}P-radioactivity.

(5) About 70 to 80% of the label in transducing DNA which has become associated with the light bacterial DNA is indistinguishable in density from that of light recipient DNA. Since this label makes such a small contribution to any particular molecule that contains it, it seems likely that it represents the products of hydrolysis and reutilization of ^{3}H-labeled nucleotides by new DNA synthesis.

(iii) *Studies on the non-integrated transducing DNA*

As seen in Figure 3, most of the transducing DNA entering a cell fails to become integrated into the recipient bacterial chromosome. This non-integrated DNA retains its characteristic molecular weight (27×10^{6} daltons) and its original heavy native density. Zone sedimentation analysis of the non-integrated DNA, isolated from transduced cells 20 minutes after infection, was carried out using ^{14}C-labeled P22 DNA as a reference (Fig. 12(a)). A control gradient (Fig. 12(b)) containing DNA isolated from the transducing particles themselves was also run with the same reference DNA. It can be seen that the transducing particle DNA and the non-integrated DNA have similar sedimentation coefficients. The presence of density labels is probably sufficient to account for the observation that both sediment slightly faster than P22 DNA.

In order to determine the density of the non-integrated transducing DNA, bacteria were transduced with purified transducing particles and DNA isolated from the transduced culture at 20 minutes and 300 minutes after transduction was analyzed by

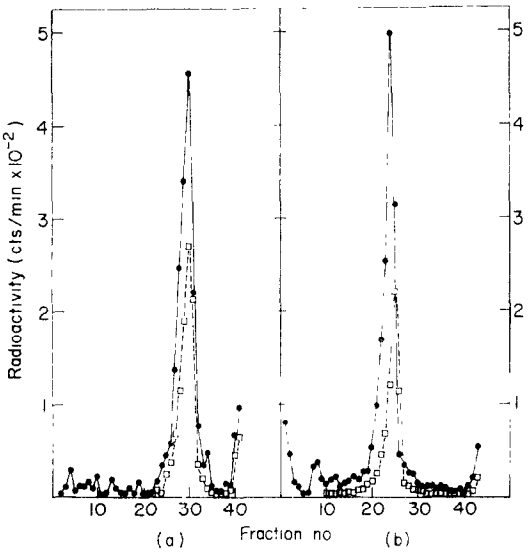

FIG. 12. (a) Zone sedimentation analysis of the non-integrated ^{3}H-transducing DNA isolated from transduced bacteria 20 min after infection with transducing particles, with ^{14}C-labeled P22 DNA as a reference. Centrifugation was for 60 min at 45,000 rev./min and 4-drop fractions were collected and counted for radioactivity.

(b) Zone sedimentation analysis of DNA extracted from purified ^{3}H-labeled transducing particles with ^{14}C-labeled P22 DNA as a reference marker. Centrifugation was for 90 min at 45,000 rev./min and 4-drop fractions were collected and counted for radioactivity. —●—●—, ^{3}H-radioactivity; — □ — □ —, ^{14}C-radioactivity.

CsCl density-gradient centrifugation. The results of the initial CsCl density gradients are shown in Figure 13. The non-integrated ^{3}H-labeled DNA from each gradient was pooled and run again on CsCl density gradients with ^{14}C-labeled heavy *S. typhimurium* DNA ($\rho = 1{\cdot}747$ g/cm^{3}) as a density marker. The results (Fig. 14) indicate that the 20-minute and 300-minute samples have similar densities. Both DNA's are slightly less dense than the ^{14}C-labeled marker, as was the density of the DNA isolated from the transducing particles used in this experiment. The Figure also shows that most of non-integrated DNA observed at 20 minutes is still recovered at 300 minutes. It can be concluded then that the non-integrated DNA in a transduced bacterium retains its initial heavy native density for at least 5 hours after transduction.

4. Discussion

Generalized transduction can be defined as the transfer of genetic information from one bacterium to another by means of a phage vector. The phage vector responsible for P22-mediated generalized transduction is a phage-like particle, physically quite similar to P22, which contains a double strand piece of bacterial DNA rather than the normal phage DNA. Because the DNA in P22 transducing particles, as in P1 transducing particles (Ikeda & Tomizawa, 1965), is bacterial DNA which was synthesized before infection and has little or no phage DNA attached to it, we have been able to

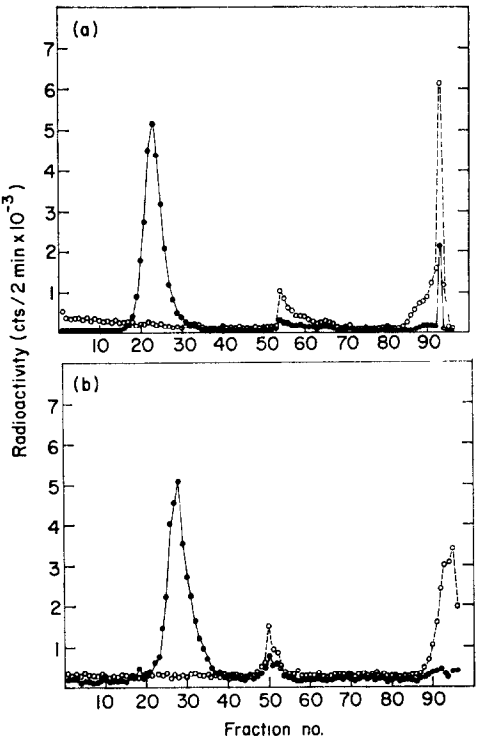

FIG. 13. CsCl equilibrium density-gradient centrifugation of DNA isolated from ^{32}P-labeled recipient bacteria following transduction with purified ^{3}H, ^{2}H, ^{15}N, ^{13}C-labeled transducing particles. DNA was isolated from the transduced bacterial culture at (a) 20 min and (b) 300 min after transduction and the DNA samples were centrifuged in a Spinco 50 rotor. Three-drop fractions were collected into 0·3 ml. of 0·01 M-Tris, 0·01 M-EDTA and 20-μl. portions of each fraction were counted for radioactivity. —●—●—, ^{3}H-radioactivity; --○--○--, ^{32}P-radioactivity.

isolate density-labeled radioactive transducing particles free of vegetative phage; these particles have been used to transduce light recipient bacteria.

We found that the transducing DNA either becomes associated with the DNA of the recipient bacteria or persists in the recipient bacteria as intact, non-integrated, non-replicating double strand molecules. Of the transducing DNA present in the bacteria only 10 to 15% becomes physically associated with the DNA of the recipient bacteria and this association reaches its maximum level within the first hour after transduction. In addition, only 20 to 30% of the donor DNA which normally becomes associated with the recipient DNA is integrated as large fragments (mol. wt. greater than about 10^{6} daltons). Therefore, in the end only 2 to 5% of the total tranducing DNA taken up by a population of bacteria (i.e. PV78 (P22 *sie* $1m_3$)) becomes integrated as large fragments. The rest of the ^{3}H label associated with the light recipient DNA makes a negligible contribution to the density of any molecule that contains it, as shown by the fact that this ^{3}H label is still indistinguishable in density from the light DNA even when the size of the DNA has been reduced by sonication to 4×10^{5} daltons. It therefore seems likely that this label was introduced as the result of hydrolysis of a small amount of the transducing DNA and reincorporation into the light recipient DNA by new synthesis; since the nucleotide pool in the transduced bacteria is light, the

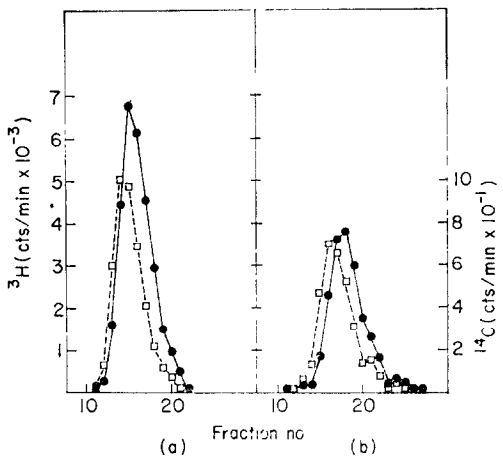

FIG. 14. CsCl equilibrium density-gradient centrifugation analysis of non-integrated ^{3}H-labeled transducing DNA.

(a) Fractions 20 to 26 from Fig. 13(a) were pooled and recentrifuged with ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* reference DNA. The DNA was centrifuged in a Spinco 50 rotor and 3-drop fractions were collected and counted for radioactivity. The total number of fractions is 83.

(b) Fractions 26 to 30 from Fig. 13(b) were pooled and recentrifuged with ^{14}C, ^{2}H, ^{15}N, ^{13}C-labeled *S. typhimurium* reference DNA. The DNA was centrifuged and collected as described for (a). The total number of fractions is 87. —●—●—, ^{3}H-radioactivity; --□--□--, ^{14}C-radioactivity.

incorporation of a few heavy nucleotides into a predominantly light DNA molecule would not be expected to alter the density of the newly synthesized DNA.

Our "retransduction" experiment (Fig. 2) demonstrates that recombination between a density-labeled transducing DNA molecule and a light recipient DNA molecule results in the substantial physical association of heavy DNA with the light DNA in the region of the recombination event. We therefore believe that it is the 2 to 5% of the total transducing DNA integrated as large fragments which gives rise to the recombinant DNA molecules and complete transductants. It appears that the association of the ^{3}H-labeled nucleotides and small fragments of transducing DNA with the recipient DNA does not contribute significantly to the formation of complete transductants. Fox & Allen (1964) reported the presence of similar transforming DNA label, free of donor transforming activity, associated with the recipient DNA following transformation in *Pneumococcus* and they concluded that this label resulted from hydrolysis and reincorporation of nucleotides into the recipient DNA by new synthesis.

The majority of the transducing DNA, however, does not become associated with the DNA of the recipient bacteria. It retains its original size and its heavy native density. It is this DNA which is thought to be responsible for the formation of abortive transductants. This hypothesis is supported by two lines of evidence. The genetic experiments of Stocker, Zinder & Lederberg (1953) and Ozeki (1956,1959) demonstrate that the number of abortive transductants formed is about 10 to 20 times the number of complete transductants formed for any marker. The experiments of Ozeki (1959) clearly show that abortive transductants do not increase in number with further growth of the culture, whereas complete transductants begin to replicate shortly after transduction. It is not at all clear why such a small fraction of the available trans-

ducing DNA is integrated. Arber (1960), investigating P1-mediated generalized tranduction in *E. coli*, has described observations which suggest that the two systems are similar. This observation contrasts with results obtained from transformation experiments in *B. subtilis*, *Pneumococcus*, and *H. influenzae* in which a larger fraction of the adsorbed transforming DNA becomes associated with the bacterial chromosome while the remainder is degraded to trichloroacetic acid-soluble material.

Examination of the transducing DNA which has become associated with the DNA of the recipient bacteria as large pieces reveals the following.

(1) Covalently bound double strand fragments of transducing DNA are present in the recipient bacterial DNA soon after transduction. A rough estimate of the size of these double strand fragments would be about 4 to 8×10^6 daltons. The lower limit is set at about 4×10^6 daltons since a large fraction of the integrated double strand fragments can be released as fully-heavy DNA by reducing the size of the DNA to 2 to 4×10^6 daltons. An upper limit on the size of the integrated DNA can be set at about 8 to 10×10^6 daltons. This calculation is based on the fact that the high molecular weight DNA has a molecular weight of 70 to 100×10^6 daltons and that the peak of the non-light ^3H is displaced from the light ^{32}P peak by a distance corresponding to a 10% substitution of heavy DNA.

Reduction of the molecular weight of the DNA isolated 20 minutes after transduction also causes the release of some [^3H]DNA of intermediate densities. Such molecules could represent either heteroduplex structures or molecules having a fragment of heavy DNA attached end-to-end to a piece of light DNA.

The size of the integrated pieces estimated by these physical methods is in agreement with the size estimated from the genetic experiment described in Results. This experiment showed that genetically-recombinant (i.e. *his*$^+$) DNA, encapsulated in transducing particles, contains a fragment of heavy transducing DNA of approximately 5 to 6×10^6 daltons associated with the DNA of the light recipient bacteria. The results of Takebe & Hartman (1962) and Benzinger & Hartman (1962), based on the X-ray sensitivity and ultraviolet sensitivity of the capacity to form complete transductants, are also consistent with an estimated molecular weight of 5 to 6×10^6 daltons for the integrated fragments of transducing DNA. The size of the integrated transducing DNA is therefore not unlike the sizes of integrated DNA found in transformation. Fox & Allen (1964) and Gurney & Fox (1968) found that transforming DNA in *Pneumococcus* is integrated as single strands of approximately 2×10^6 daltons. Notani & Goodgal (1966) reported the integration of fragments of 6×10^6 daltons, single strand molecular weight, into the DNA of *H. influenzae*.

(2) Although double strand fragments are found in the recipient DNA when DNA is isolated either at 20 minutes or 180 minutes after transduction (Ebel-Tsipis, 1971), they are no longer present in DNA isolated 300 minutes after transduction. This indicates that most of the integrated elements have not been replicated during the first 3 hours of incubation after transduction. By 300 minutes after transduction, however, most of the integrated elements appear to have replicated.

(3) There appear to be two modes of association of transducing DNA with the DNA of the recipient bacteria: most of the integration of large fragments occurs at early times after transduction and the increased association observed at later times (i.e. after 20 min) appears to be in the form of reincorporated ^3H-labeled nucleotides or very small fragments of transducing DNA. This conclusion is based on a set of experiments which demonstrate that the fraction of the total associated ^3H label which exhibits a

density increase after sonication and denaturation is lower in DNA isolated at either 180 or 300 minutes after transduction than in DNA isolated at 20 minutes after transduction.

S. typhimurium is therefore capable of integrating double strand fragments of transducing DNA into its chromosome. This finding differs in some respects from the results obtained in other systems in which recombination has been examined. For example, recombination following transformation in *Pneumococcus* (Fox & Allen, 1964), *H. influenzae* (Notani & Goodgal, 1966) and *B. subtilis* (Dubnau & Davidoff-Abelson, 1971) results in the single strand insertion of donor transforming DNA into the recipient chromosome. The reason for this difference between transduction and transformation is not understood, but it may be a reflection of the fact that in transduction both strands of donor DNA are injected into the bacteria and are available for recombination while in transformation in *Pneumococcus* and *B. subtilis* the donor transforming DNA may be converted to single strand molecules soon after (or during) entry into the recipient (Lacks, 1962; Piechowska & Fox, 1971).

It is difficult to suggest a detailed mechanism for the integration of a double strand fragment of transducing DNA into the DNA of the recipient bacteria. One might propose that the transducing DNA circularizes before recombining with the host DNA and then becomes integrated by a single cross-over event, as occurs in the integration of prophage into the host chromosome (Signer, 1968). This would result in the introduction of transducing DNA of 27×10^6 daltons and the net addition of genetic material into the bacterial chromosome. However, our physical experiments indicate that the size of the integrated fragment is substantially smaller than 27×10^6 daltons; furthermore, Stocker *et al.* (1953) showed that transduction in *S. typhimurium* occurs by gene substitution and not by gene addition. These two pieces of data would seem to rule out the simplest form of integration of circular transducing DNA. An alternate model would be one in which two or more exchange events are needed for the integration of a fragment of transducing DNA. This would satisfy the physical and genetic data. However, this mechanism would seem to require that the bacterial chromosome be broken at some step intermediate in the formation of a recombinant molecule.

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