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The purpose of this research was to determine whether the transducing phage P1 picks up original bacterial chromosome segments and whether these segments are always the same size. The plan of approach was to grow the donor bacteria Escherichia coli C600 gal$^+$ (galactose) in minimal media containing the heavy isotopes ^{15}nitrogen and deuterium in place of light nitrogen and hydrogen. The heavy isotopes were incorporated into the chromosomes of the donors which were infected with light P1 and allowed to lyse in light media. As a result, only transducing particles contained the heavy DNA picked up from the chromosome. This lysate, plus nontransducing P1 and Streptococcus lactis phage c2 as references, was studied by density-gradient centrifugation in a cesium chloride solution. Drops were collected in individual tubes and assayed for active P1, transducing P1, and c2. The recipient for transduction was E. coli W3350 S^r gal$^-$. On a graph plotted of titer versus drop

number, three peaks were evident: active P1, transducing P1, and c2. The peak of the transducing particles was cone-shaped and denser than the peak of active P1, indicating the following: (1) original heavy chromosome material was incorporated into all the transducing particles; (2) none of the chromosome fragments had replicated, since no half-heavy or light transducing particles were found; (3) all chromosome fragments were the same size or very nearly the same size.

THE SIZE OF THE CHROMOSOME SEGMENT TRANSDUCED BY PHAGE P1

by

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INTRODUCTION

One of the most important revelations of microbial genetics is that the transfer of genetic material occurs not only via classical sexual reproduction but also in other ways. In bacteria several types of genetic transfer permanently modify the hereditary properties of an organism. (1) Transformation, revealed in Pneumococcus by Griffith (1928) and studied by Avery, McLeod, and McCarty (1944), occurs when DNA extracted from a donor cell is adsorbed by a recipient cell. (2) Conjugation, discovered in Escherichia coli by Lederberg and Tatum (1946), involves passage of genetic material through the bridge formed when sexually differentiated cells are in contact. This process bears the closest resemblance to the sexual reproduction in higher forms of life. (3) Lysogeny, investigated by Lwoff (1953), is the peculiar situation in which a temperate phage and bacterium live in symbiotic coexistence and behave as a single reproductive unit. This phage, referred to as prophage, usually occupies a location on the cell chromosome and confers immunity to the cell against subsequent infection by the same phage. (4) Transduction, observed in Salmonella by Zinder and Lederberg (1952), involves phage-mediated transfer of genetic material from a donor cell to a

recipient cell. During vegetative multiplication of some temperate phages, small segments of bacterial chromosome may be incorporated randomly into the progeny phage particles. When these phages are used to infect genotypically different bacteria, the chromosome segments can recombine with the chromosome of the recipient cell. Transduction and lysogenization appear to be similar; however, only some temperate phages can transduce, and transduction can occur without simultaneous lysogenization of the transduced bacteria.

Two types of transduction have been observed: restricted and generalized. In restricted transduction, only genetic markers contiguous to the prophage location on the bacterial chromosome can be transduced. For example, phage λ lysogenizes E. coli K12 between the gal (galactose) and bio (biotin) markers and mediates transduction of those genes only (Rothman, 1965). The gal or bio segment replaces a piece of the phage genome which becomes defective in some properties (λ dg or λ bio). The λ dg particle is derived only from lysogenic cells, never by external lysis of sensitive cells (Fraser, 1962).

In generalized transduction, as seen in phage P1 in E. coli and phage P22 in Salmonella typhimurium, phages can transfer any genetic marker, singly or in short linkage groups. Transduction occurs in the following ways: (1) with phage grown during the lytic

cycle on the donor bacteria; (2) with virulent phage mutants that do not lysogenize; (3) with phage released from lysogenic cells (restricted transduction).

Microorganisms provide an ideal opportunity to study the inheritance of characters known to be simple and unitary and to provide information to help understand the genetic phenomena of higher forms. This research may add a brick to the pyramid of knowledge that may some day solve the mysteries of life itself.

HISTORICAL REVIEW

Following the discovery of transduction by Zinder and Lederberg (1952), extensive research has been done to elucidate this mechanism. The discovery of the temperate phage P1 by Bertani (1951) has provided a convenient generalized transducing system for scientists to study many of the loci on the chromosome of not only E. coli but also strains of Salmonella and Shigella.

One peculiarity of phage P1 is that two head sizes are found even in single plaque isolations (Anderson and Walker, 1960; Hayes, 1965; Ikeda and Tomizawa, 1965c). The smaller particles consist of defective phages with incomplete genomes and transducing phages with bacterial DNA. If a high multiplicity of the defective phages are put in a bacterial cell, lysis will result because of mutual complementation. The total amount of DNA in the smaller particle is 40 percent of the total DNA in the larger particle; consequently, the smaller phage cannot transduce as long a segment of DNA as can the larger phage. The smaller particle has about 33 percent less protein than the larger particle; consistently, the head sizes of the larger and smaller particle are 900 Å and 650 Å respectively. The lengths of the tails are the same. Since no larger protein heads are found containing the smaller length of DNA, it is suggested that the size of the protein head regulates the length of DNA within the

phage (Ikeda and Tomizawa, 1965c).

Lennox (1955) showed that a wide range of characters can be transduced into E. coli and Shigella dysenteriae. When phage P1 is grown on Sh. dysenteriae Sh/s, characters can be transduced into E. coli strains B, C, and W. The phage mutant P1kc transduces characters from E. coli K12 into E. coli B as well as into Shigella. The fact that transduction occurs among organisms in both the coli and the dysentery groups indicates genetic homology in these groups. Further study might provide insight into their evolutionary history. Transduction of the lac^+ (lactose) marker into Sh. dysenteriae Sh, never known to mutate spontaneously to lac^+, creates a unique bacterial strain.

Lennox also showed that the transducing activity of the P1 lysate comes from the phage itself by the following experiments:

1. The transducing titer of a lysate is not decreased by treatment with deoxyribonuclease. There is in fact an increase by a factor of two, possibly due to unclumping of the phage or to cleaning of some phage so as to improve adsorption to the bacteria.

2. Centrifugation decreases the phage plaque titer and the transducing titer in the supernatant at the same rate.

Transduction experiments with bacteria whose markers had been mapped already by sexual crosses (conjugation) were used by Lennox to study the relation between transduction and linkage. He obtained the following results:

1. Closely linked markers can be transduced together.

2. The frequency of joint transduction of two markers decreases with increasing distance between markers as measured by linkage in sexual crosses.

3. For the markers T^+ (threonine) and L^+ (leucine), the frequency of joint transduction is roughly independent of the mode of selection, whether it be for L^+ or T^+ or T^+L^+ together.

While studying whether lysogeny and transduction occur concomitantly, Lennox observed the following:

1. For K12 strains and for transduction of amino acid markers, the frequency of lysogenization seems independent of the character and of the number of jointly transduced markers.

2. The frequency of lysogenization depends on the phage strain.

3. The frequency of lysogenization is higher when selection is made for sugar fermentation characters.

Another question concerning the relation between transduction and lysogeny is the transduction of lysogeny itself. Jacob (1955) showed that a phage coat can contain the genetic material of one or more other phages. The λ-lysogenic character segregates (linked to gal) in crosses between lysogenic and nonlysogenic bacteria (Jacob, 1955). Transduction with phage P1 shows that the λ prophage is located between the gal and bio (biotin) markers (Rothman, 1965).

Transduction of lysogeny offers a new way of analyzing the relation between prophage and the bacterium as well as the size and orientation of the prophage. Adams and Luria (1958) showed that

transduction by a single P1 particle usually is not accompanied by lysogenization and proposed three possible explanations: (1) transducing particles contain no phage genome; (2) they contain an abnormal phage genome that cannot lysogenize; (3) they carry both a complete phage genome and a transducing segment; however, transduction and lysogenization cannot occur within the same recipient cell. Since infection at multiplicities higher than one often leads to lysogeny, the third explanation is ruled out. Since unstable, *lac*$^+$ *Sh. dysenteriae* Sh cells carrying defective phage P1 are found, the most probable explanation is that the phage genome is abnormal in some cases, although it may be missing completely in others.

In the λ phage system, an LFT (low frequency of transduction) lysate is formed when λ-lysogenic, *gal*$^+$ cells are induced with ultraviolet light. When this lysate is used to transduce *gal*$^-$ cells and then those transductants are induced, an HFT (high frequency of transduction) lysate is obtained. LFT particles require an active phage genome in the recipient cell in order to transduce, whereas HFT particles transduce at a low rate without active phage (Fraser, 1962).

Wilson (1960) demonstrated an LFT-HFT system in phage P1 with the *thr* (threonine) and *leu* (leucine) markers, although he did not know whether the P1 lysates consisted of phage defective for a portion of the phage genome. Luria, Adams, and Ting (1960) showed that when *E. coli* is both donor and recipient, transduction gives stable

lac$^+$ transductants (transduction by integration). Transduction from *E. coli lac*$^+$ donors to *Sh. dysenteriae* Sh gives mainly unstable transductants (Luria, *et al.*, 1958) in which the *lac*$^+$ genes apparently are associated with some phage P1 genes in a prophage called P1dl (transduction by lysogenization). Evidently HFT particles contain genetic elements with various combinations of phage genes and bacterial genes. The transductant type that is obtained by integration or by lysogenization depends both on the ability of the transducing elements to lysogenize and on the degree of genetic homology between the donor and the recipient of the transduced bacterial genes. High genetic homology favors integration; low genetic homology hinders integration and allows detection of the transductants carrying the transducing element as prophage (Luria, Adams, and Ting, 1960).

Transduction of the *lac* marker by phage P1 was used by Franklin and Luria (1961) to study the structure of the *lac* genetic region in *E. coli* and *Sh. dysenteriae*. The *lac*$^+$ property requires the function of two loci: *y* (permease gene) and *z* (β-galactosidase gene). In transduction with *E. coli* donors and *Sh. dysenteriae* recipients or vice versa, many transductants are *lac*$^+$ heterogenotes, in which a *z*$^+$ gene from *Sh. dysenteriae* and a *y*$^+$ gene from *E. coli* are present as endogenote and exogenote. The exogenote is often the defective prophage P1dl. Other transductants possess, integrated in their chromosome, a hybrid *lac* region partly from *E. coli* and partly

from Sh. dysenteriae. These hybrids have various levels of β-galactosidase, from the high values of E. coli to the low values of Sh. dysenteriae. Most mutants in the z locus of E. coli have suppressed permease, controlled by the adjacent y locus, as well as suppressed β-galactosidase.

Transduction with phage P1 has been used in other ways also. Yanofsky and Lennox (1959) showed that the clustered tryp (tryptophane) genes of E. coli are arranged in the same relative order as the steps in the biosynthesis of tryptophane which they control. Adler and Kaiser (1963) found that the most likely sequence of the genes within the galactose region is kinase-transferase-operator and epimerase-prophage 82. Gross and Englesberg (1959) demonstrated that the series of linked loci that control L-arabinose utilization is arranged linearly. According to Nakaya, Nakamura, and Murata (1960), the transduction by phage P1 of drug RTA's (resistance transfer agents) is also successful in E. coli K12. The RTA shows no linkage to ordinary bacterial characters and no correlation to the sex factor F of E. coli K12, yet the frequency of transfer from a resistant strain to a sensitive strain by contact is high.

Bacterial pili are long, thin proteinaceous structures that grow out from the surface of many kinds of bacteria. Brinton and Gemski (1961) performed transduction experiments with phage P1

and learned that the piliation marker is closely linked to the thr marker. The exhibition of transducibility and linkage of this marker indicates chromosomal control of this phenomenon.

Although much research had been done with phage P1, no one had yet endeavored to study the densities of transducing and non-transducing particles. Ting (1962) used the CsCl density-gradient technique to show that the peak of unlabelled transducing phages is only slightly heavier than the peak of the active phages. This result shows that the total amount of DNA in transducing and nontransducing particles is almost the same.

Ikeda and Tomizawa (1965a) grew the donor bacteria on the density label bromouracil, infected the bacteria with light phage, and allowed lysis to occur in light medium. Because of this procedure, only the transducing particles carried a piece of heavy chromosome. They studied these lysates using the CsCl density-gradient technique and showed that the piece of bacterial chromosome transduced is always the same size and that both strands of the DNA in the chromosome are picked up.

When the bromouracil-labelled cells were infected with ^{32}P-labelled P1 and lysed in ^{32}P medium, no peak of ^{32}P corresponding to the peak of transducing phages was found in the CsCl gradient. This suggests that there actually is no phage genome in these particles.

Ikeda and Tomizawa (1965b) grew E. coli thy⁻ (thymidine) in ^{3}H thymidine, allowed lysis to occur in media with ^{32}P, and prepared DNA from the purified phages. The bacterial and phage DNA types differed slightly in density. The ^{3}H DNA contained equal amounts of bacterial and phage DNA, while ^{32}P DNA was almost all phage DNA. They found that the density of the transducing phage DNA is lighter than that of the bacterial DNA but increases to that of bacterial DNA after treatment with proteolytic enzymes, which indicates association of protein with transducing phage DNA. Since the group of small P1 phages also contains transducing particles in which the DNA is not associated with protein, Ikeda and Tomizawa suggested, but did not conclusively prove, that the protein is not essential for transduction.

The work reported here was designed to find out whether original chromosomal material is actually incorporated into transducing particles, and whether the transducing fragments are always of the same size.[1]

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[1] The papers published by Ikeda and Tomizawa (1965a, b, and c) appeared during the course of this research.

METHODS AND MATERIALS

Media

LB broth: 1.0 percent Bacto-tryptone, 0.5 percent yeast extract, 0.5 percent NaCl, 1 ml of 1 N NaOH per liter (pH 7.4).

LB agar: LB broth with 1.5 percent agar. $CaCl_2$ was added to a final concentration of 2.5×10^{-3} M before pouring.

Soft λ agar for overlays: 1.0 percent Bacto-tryptone, 0.25 percent NaCl, 0.75 percent agar.

EMB-galactose agar: 1.0 percent Bacto-tryptone, 0.5 percent yeast extract, 0.25 percent NaCl, 1.5 percent agar. The following were added separately: 0.04 percent eosin y, 0.0065 percent methylene blue, and 1.0 percent galactose.

Streptomycin + EMB-galactose agar: Seven drops of a solution of 2×10^3 γ/ml streptomycin were spread on EMB-galactose plates. Plates with porcelain covers were dried in a 37° C incubator for about 30 minutes.

Density-labelled minimal medium: 0.695 percent Na_2HPO_4, 0.3 percent KH_2PO_4, 0.029 percent NaCl, 0.025 percent $MgSO_4$, 3.0×10^{-5} percent $FeCl_3$. The desired volume was evaporated to dryness and the following were added: 0.1 percent $^{15}NH_4Cl$, appropriate volume of D_2O, 1.0 percent of a stock solution of 20 percent

glucose, 0. 5 percent 6 N NaOH (pH 7. 0), and 1. 0 percent of a solution containing 0. 2 percent threonine, 0. 2 percent leucine, and 0. 2 percent vitamin B_1.

Anderson's lactic broth: 2. 0 percent Bacto-tryptone, 0. 5 percent glucose, 0. 4 percent NaCl, 0. 15 percent sodium citrate· 5 H_2O, 0. 05 percent ascorbic acid, 0. 5 percent yeast extract, 0. 015 percent $CaCl_2$· 2 H_2O, 0. 02 percent $MgSO_4$· 7 H_2O, 0. 005 percent $MnSO_4$· 1 H_2O. The pH was adjusted to 7. 0 with HCl or NaOH.

Anderson's soft lactic agar: Anderson's lactic broth with 0. 75 percent agar.

Anderson's lactic agar: Anderson's lactic broth with 1. 5 percent agar.

Peptone diluent: 0. 5 percent Bacto-peptone.

121 buffer: 1. 05 percent KCl, 1. 07 percent NH_4Cl, 0. 35 percent Na_2SO_4, 14. 5 percent tris, 3. 2 x 10^{-5} percent $FeCl_3$, HCl (pH 7. 0).

Saline: 0. 85 percent NaCl.

Nutrient agar: 0. 8 percent Bacto-nutrient broth, 1. 5 percent agar.

Tween 80: 5. 0 percent Tween 80.

Bacteria and Phage Strains

E. coli strains C600, C600(P1), and W3350 S^r were obtained from Dr. Dorothy K. Fraser. Strain C600 (Appleyard, 1964) was *gal*$^+$ and S^s (streptomycin sensitive); it was used to assay phage P1 and to act as the donor in transduction. Strain C600(P1) (Hattman, 1964), lysogenic for phage P1, was the original source of the phage. Strain W3350 *gal*$^-$ S^r (streptomycin resistant), was the recipient in transduction.

Phage P1, obtained by induction of strain C600(P1) with ultraviolet light, was the vehicle of transduction. Phage P1 does not form plaques on *Streptococcus lactis*.

S. lactis C_2S^s was used to assay phage c2 and was obtained from the Oregon State University Microbiology Department. (Since strain C_2S^s was the only *S. lactis* used, the organism will be referred to as *S. lactis* in this thesis.) Phage c2, which forms plaques on *S. lactis* but not on *E. coli* K12, was used as a reference in the CsCl density gradient and was obtained from Gerry Anderson.

E. coli strains were stored on nutrient agar slants at 10°C; *S. lactis* was maintained by daily transfers in Anderson's lactic broth incubated at 30°C. All phage lysates were stored at 10°C; lysates of P1 had a few drops of chloroform or 1.0 percent of a Tween 80 solution added. Addition of Tween 80 seemed to help the lysates keep up

their active titer over extended periods of storage.

Procedures

Original Isolation of Phage P1

A culture of E. coli C600(P1) in LB broth was shaken overnight at 37°C, diluted 1:100 in LB broth, and shaken three more hours to obtain a log culture. The cells were centrifuged, resuspended in saline, and irradiated with ultraviolet light in 5-ml aliquots in a petri dish for ten seconds at a distance of 50 centimeters. The light source was two 15-watt General Electric germicidal lamp bulbs placed in parallel. Each 5-ml aliquot was diluted 10^{0}, 10^{-1}, and 10^{-2}, and 0.1 ml of each dilution was plated on LB agar with a soft overlay consisting of 2.5 ml soft λ agar, three drops of an overnight culture of E. coli C600, and 2.5×10^{-3} M $CaCl_2$. After overnight incubation at 37°C, the plates with the final dilution of 10^{-2} were confluently lysed so the soft agar was scraped off and suspended in 5 ml LB broth. These broth tubes were shaken for one hour at 37°C, centrifuged, and chloroformed. This lysate had a titer of 1×10^{7} particles/ml.

Preparation of Non-dense Transducing and Nontransducing Lysates

E. coli C600 or W 3350 S^r was shaken overnight in LB broth at 37°C, diluted 2:100 in LB broth, and shaken two more hours. $CaCl_2$ was added to a final concentration of 2.5×10^{-2} M and phage P1 was added to a final titer of 6×10^6 particles/ml. The flask was shaken at 37°C for five to six hours when clumps of cellular debris became visible. The lysate was clarified by low speed centrifugation, and a few drops of chloroform or 1.0 percent of a Tween 80 solution were added. Such lysates had titers of about 1×10^9 particles/ml.

Preparation of Dense Transducing Phage

E. coli C600 was inoculated into a small amout of minimal media containing ^{15}nitrogen and deuterium and was shaken for 12 hours at 37°C. Subsequent transfers were made into fresh media every 12 hours until cells were well-adapted and grew rapidly. The lysate was started by diluting the adapted cells 2:100 in dense media and shaking them about five hours at 37°C until the titer was about 1×10^8 cells/ml. The cells were counted in a Petroff-Hauser counting chamber, centrifuged, and resuspended in 121 buffer with the final titer in the adsorption tube equal to 1×10^9 cells/ml. A multiplicity of about ten nontransducing phages was added, and $CaCl_2$ was added to a final concentration of 2.5×10^{-3} M. The tube was

incubated for 30 minutes in a 37° C waterbath to allow adsorption, warm LB broth was added to make a final titer of 2×10^8 cells/ml, and the flask was shaken at 37° C for 1.5 hours. Secondary adsorption was inhibited by lack of addition of $CaCl_2$ to the warm LB broth, so that all transducing particles would be produced by the original labelled bacteria. The lysate was clarified by low-speed centrifugation; a few drops of chloroform or 1.0 percent of a Tween 80 solution were then added. Such lysates had a titer of 5×10^7 - 1×10^9 plaque-forming units/ml.

The lysates used are described in Table 1.

Assay of Phage P1

The phage lysate was diluted in peptone to an appropriate titer, and 0.1 ml of the dilution was placed in a small test tube in a 45° C waterbath. Three drops of an overnight culture of E. coli C600 were added, followed by $CaCl_2$ to a final concentration of 2.5×10^{-3} M and 2.5 ml soft λ agar. The overlay was poured on thick plates of LB agar with 2.5×10^{-3} M $CaCl_2$. The plates were incubated overnight at 37° C. Plaques were counted after overnight incubation.

Concentration of Phage Lysates

Transducing phage lysates were concentrated with carrier non-transducing lysates by centrifugation at 25,000 g for three hours.

Table 1

Description of lysates used to obtain Figures 1, 2, 3, and 4

	total number of bacteria	total input non-transducing phages	fraction unadsorbed phage	active titer (pfu/ml)	transducing frequency
lysate for Figure 1	8.7×10^8	8.7×10^9	1.08	5×10^7	1.3×10^{-6}
lysate for Figure 2	2.3×10^9	1×10^{10}	0.89	7×10^7	7×10^{-5}
lysate for Figure 3	2.8×10^9	2.8×10^{10}	0.72	1.4×10^9 before concentration with helper phage 4×10^{10} after concentration with helper phage	4.8×10^{-5}
lysate for Figure 4	5.1×10^9	2×10^{10}	0.79	5.6×10^8 before concentration with helper phage 2×10^{11} after concentration with helper phage	1.2×10^{-6}

The supernatant was removed and the pellet was soaked in 121 buffer overnight. After the tubes were shaken gently, low speed centrifugation (6, 000 rpm) was employed to remove any bacteria present. These suspensions had titers of about 1×10^{11} particles/ml and were treated with 1. 0 percent of a Tween 80 solution to maintain the active titer.

Transduction and Analysis of Transductants

A culture of E. coli W 3350 S^r (the recipient) was shaken overnight at 37° in LB broth (about 2×10^9 cells/ml). This culture was diluted 2:100 and shaken for two more hours. After centrifugation, the cells were resuspended in 1/10 volume of 121 buffer and distributed in tubes as follows:

1. Transduction tubes with helper (nontransducing) phage: resuspended cells + phages from transducing lysate at a multiplicity of seven + multiplicity of three helper phages + 2.5×10^{-3} M $CaCl_2$.
2. Transduction tubes without helper phage: resuspended cells + phages from transducing lysate at a multiplicity of seven + 2.5×10^{-3} M $CaCl_2$.
3. Reversion control: resuspended cells + 2.5×10^{-3} M $CaCl_2$.

4. Control:

   resuspended cells + multiplicity of three helper phages + 2.5×10^{-3} M $CaCl_2$. (Helper phage is added to provide a complete phage genome which is lacking in transducing particles.)

All tubes were adsorbed at 37° C for 30 minutes; the contents were mixed with 2.5 ml soft λ agar, poured on EMB-galactose plates, left at room temperature for two hours, and incubated at 37° C for one week. Transducing and helper lysates were streaked on nutrient agar to assure sterility.

On EMB-galactose plates the transductants appeared as pink colonies against a maroon background. The transductant colonies were picked with sterile toothpicks and resuspended in saline. Colonies of E. coli C600 and W 3350 S^r were tested identically to serve as controls. These suspensions were inoculated into minimal media with galactose as the only carbon source and were grown overnight at 37° C. The positives were spotted on streptomycin + EMB-galactose agar, and those that grew were Gram-stained to check for contamination. Thus the gal^+ character of strain C600 was carried by phage P1 to change strain W 3350 S^r from gal^- to gal^+.

Gram Stain

Smears were made on clean glass slides and fixed in a flame. Crystal violet was added for one minute, then Gram's iodine for one minute, then 95 percent alcohol until no more color came off, followed by safranin for 30 seconds. *E. coli* strains, short Gram negative rods, appeared pinkish-red.

Preparation of Phage c2 Lysate

S. lactis was shaken for 12 hours in Anderson's lactic broth at 30°C, diluted 1:100 in fresh media, and grown for 2.5 hours. Phage c2 was added to a final concentration of 3×10^7 particles/ml. Shaking was continued until the lysate cleared, about 1.5 hours. The lysate was clarified by low speed centrifugation and filtered through Millipore HA membranes (0.45 μ pore size). Such lysates had titers of about 1×10^9 particles/ml.

Assay of Phage c2

The phage lysate was diluted in peptone to an appropriate titer and 0.1 ml of the dilution was placed in a small test tube in a 45°C waterbath. Two-tenths ml of an overnight culture of *S. lactis* was added, followed by 2.5 ml of Anderson's soft lactic agar. The overlay was poured on thick plates of Anderson's lactic agar. Plaques

were counted after overnight incubation at 30° C.

Cesium Chloride Density-gradient Centrifugation

One and eight-tenths ml of saturated CsCl was evaporated. Two ml of dense transducing phage P1 and 1.0 ml of c2 lysate were added to the residue. The mixture was placed in a cellulose nitrate tube, overlaid with 2.0 ml of mineral oil, and centrifuged at 10° C in an SW 39 swinging-bucket rotor of a Beckman Model L-2 centrifuge at 29,000 rpm. After 20 hours, the rotor was allowed to come to rest without braking. The tubes were removed and pierced at the bottom with a size 00 insect pin. Sucessive drops, two per tube, were collected in 0.3 ml of LB broth. A 3-ml sample yielded 80-220 drops (depending on the size of the hole), each drop comprising a successive density layer in the gradient. These drops were diluted appropriately and checked for active phage P1 titer by the phage P1 assay procedure, for P1 transduction titer by the procedure for transduction, and for phage c2 titer by the phage c2 assay procedure.

RESULTS AND DISCUSSION

Four experiments were performed (Figures 1-4) in which two controls were used: recipient cells plated alone and recipient cells plated with nontransducing phage. No transductants ever appeared on these controls, which showed that only phages carrying bacterial chromosome can transduce. In the first two experiments, (Figures 1 and 2), no extra nontransducing phage was added and the following conclusions were reached: (1) no active phage was dense; (2) all transducing particles were heavier than the active particles. Non-transducing particles were added in order to concentrate the transducing particles so that more transductants could be obtained. The mixed lysate was centrifuged at high speed. This mixed lysate was studied by CsCl density-gradient centrifugation and the results are shown in Figures 3 and 4. The following conclusions were reached: (1) Again, all transducing particles are heavier than the active phages so that all of them must contain fragments of the original dense bacterial chromosome. This shows that the transducing particles have not been formed by a copying process, but by recombination of phage DNA with bacterial DNA or complete replacement of phage DNA by bacterial DNA (Ikeda and Tomizawa, 1965a). (2) The transducing particles have not replicated during vegetative growth of the phage, because no half-heavy or light transducing peak is observed.

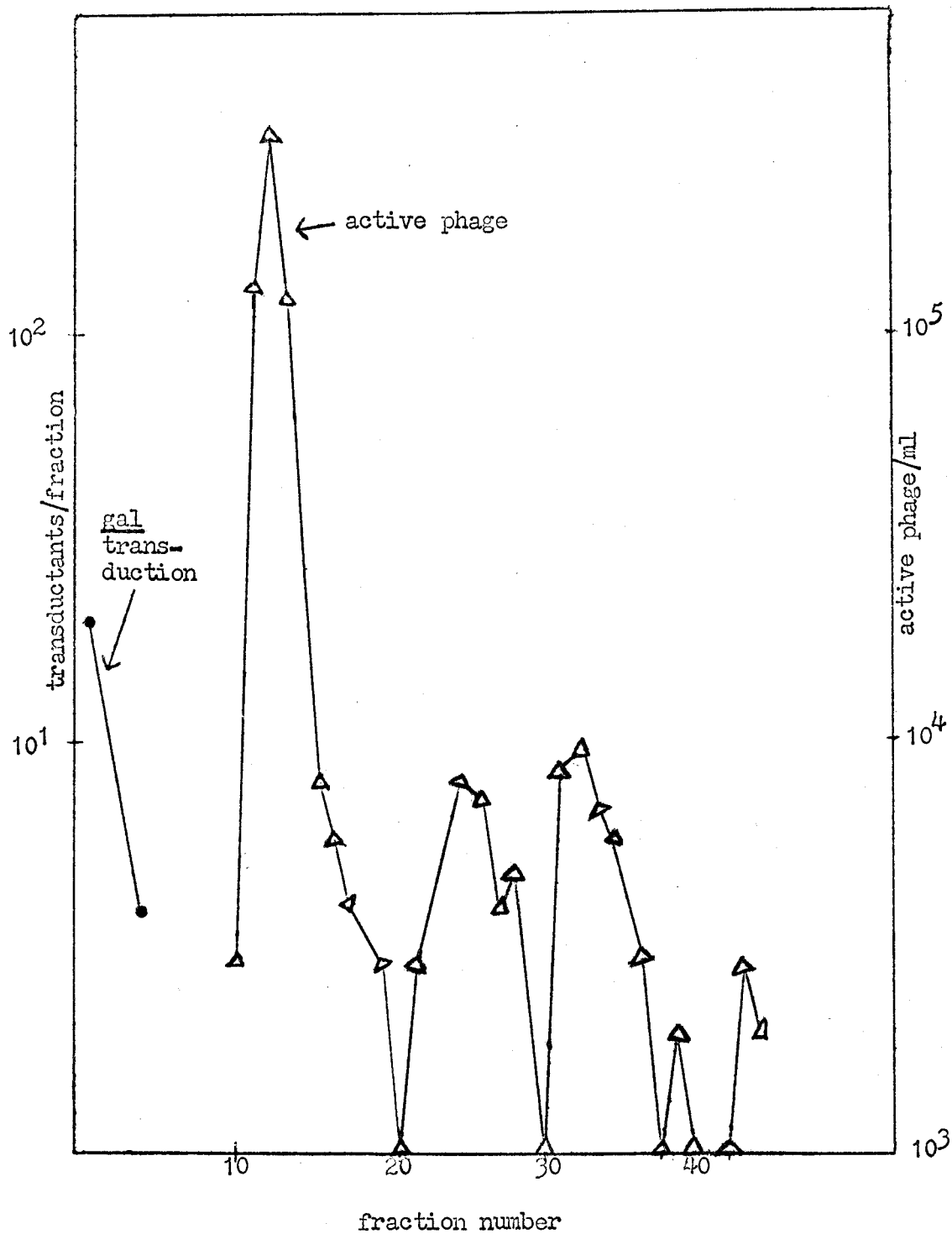

Figure 1. CsCl density-gradient centrifugation analysis of phage P1 transducing lysate with no nontransducing phage added.

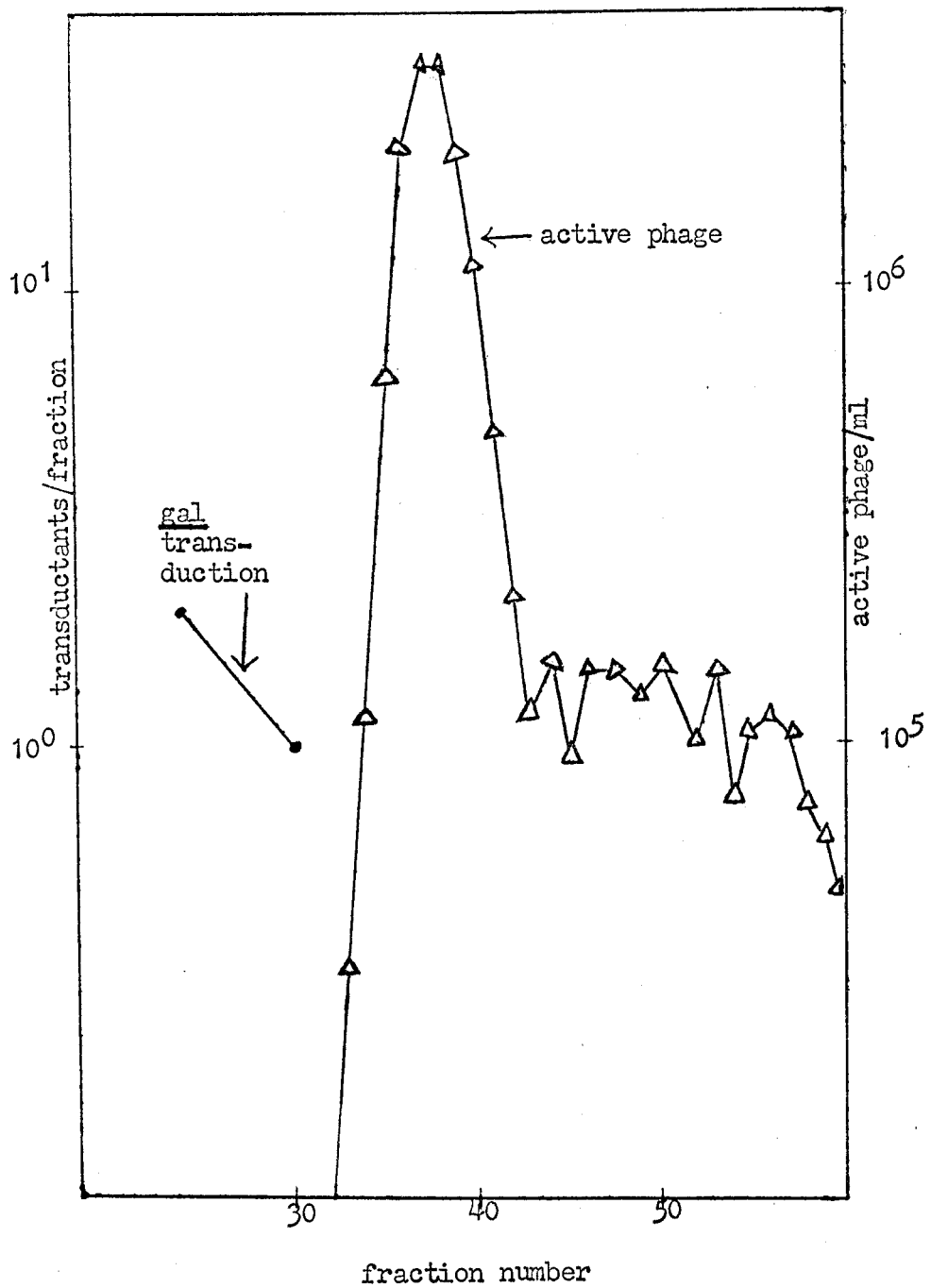

Figure 2. CsCl density-gradient centrifugation analysis of phage P1 transducing lysate with no nontransducing phage added.

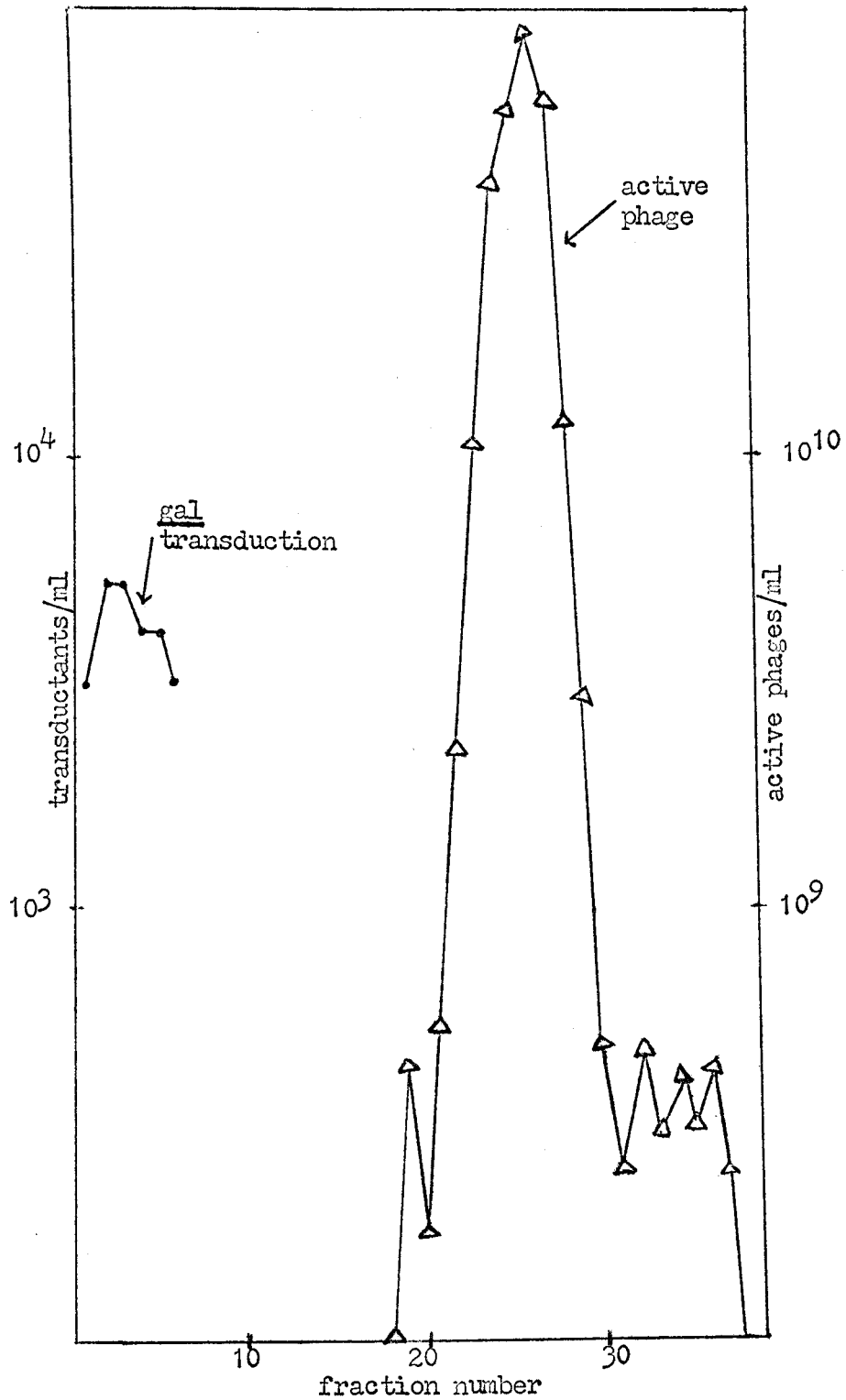

Figure 3. CsCl density-gradient centrifugation analysis of phage P1 transducing lysate with nontransducing phage added.

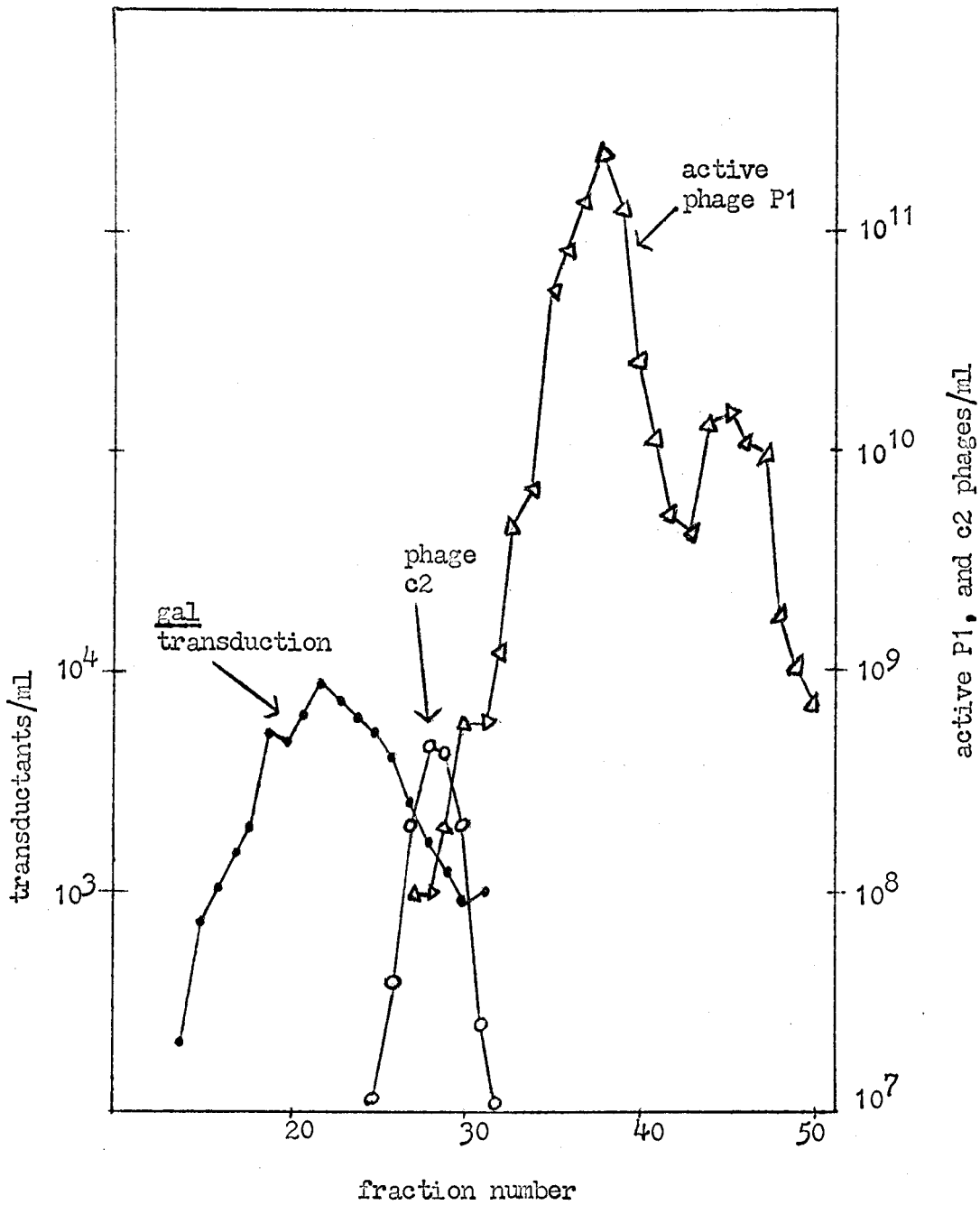

Figure 4. CsCl density-gradient centrifugation analysis of P1 active, P1 transducing, and c2 particles with nontransducing P1 added.

HFT λ-gal does replicate, but LFT λ-gal probably does not (Fraser, 1966). For this reason, the transducing particles used in this research are more similar to LFT λ-gal than HFT λ-gal. (3) All transducing particles are in a single peak, although this peak is somewhat wider than the peaks of the active phages P1 and c2. There could be two close, overlapping peaks, suggesting two sizes of transducing particles as found by Ikeda and Tomizawa (1965c). Therefore, the conclusion was drawn that all the transducing particles have the same or nearly the same density.

This fact means that they contain the same proportion of heavy bacterial chromosome to light phage chromosome. In fact, it appears likely from the work of Ikeda and Tomizawa (1965a) that the transducing phages of P1 carry no phage genome at all. If this is so, then the transducing particles contain equal or nearly equal lengths of pure bacterial DNA. The meaning of these observations is still not clear. There appears to be a mechanism in the case of phage replication for cutting off defined lengths of phage genome to be included in the head of the finished phage particle (Streisinger, 1964). Since Ting (1962) has shown that approximately the same amount of DNA is present in both transducing and nontransducing particles of P1, perhaps the same "cutting" mechanism occasionally cuts off equal-sized pieces of bacterial genome instead and these become accidentally wrapped into phage coats. The wrapping and cutting

of DNA lengths may be concurrent results of a single maturation process.

Ting (1962) showed that unlabelled LFT P1 particles have a density close to that of active phage (Figures 5 and 6), whether one or more markers are transduced. This means that the total length of DNA (chromosomal plus phage DNA, if any) is nearly the same in transducing and nontransducing phages.

Figure 7 (Ting, 1962) shows the peaks in an HFT lysate where the lac marker is being transduced. The transducing peak is considerably denser than the active phage peak in this case. This shows that in the HFT strain a change has occurred in the hybrid particle so it has a greater density than the LFT particle from which it was derived. In HFT phages there is definitely P1 genome material from which P1 markers can be recovered (Luria, Adams and Ting, 1960).

Ikeda and Tomizawa (1965a) studied transducing particles of phage P1 using density labels. The main differences in their work from that presented here is that they used bromouracil (BU), ^{3}H, and ^{32}P as labels instead of ^{15}N and ^{2}H and they studied transduction of the R factor (drug resistance) from Sh. flexneri 2b, lac, try, his (histidine), arg (arginine), leu, pro (proline), and the λ prophage instead of gal.

They prepared four sets of P1 lysates, which differed by the presence or absence of bromouracil in the pre- and post-infection

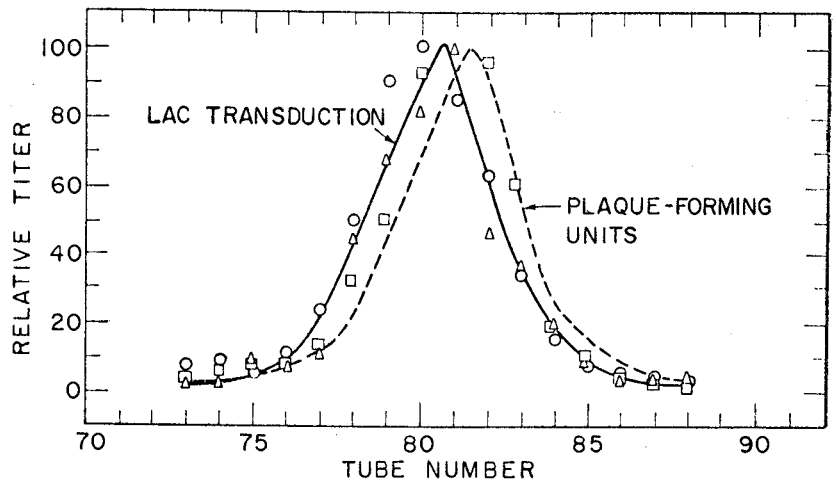

Figure 5. CsCl density-gradient centrifugation of LFT lysate analyzed for lac transduction. (△ is Sh. dysenteriae Sh recipient; o is E. coli 2.0S0 recipient; □ is E. coli W4032 recipient.) The solid line fits the values obtained with Sh. dysenteriae Sh as recipient.

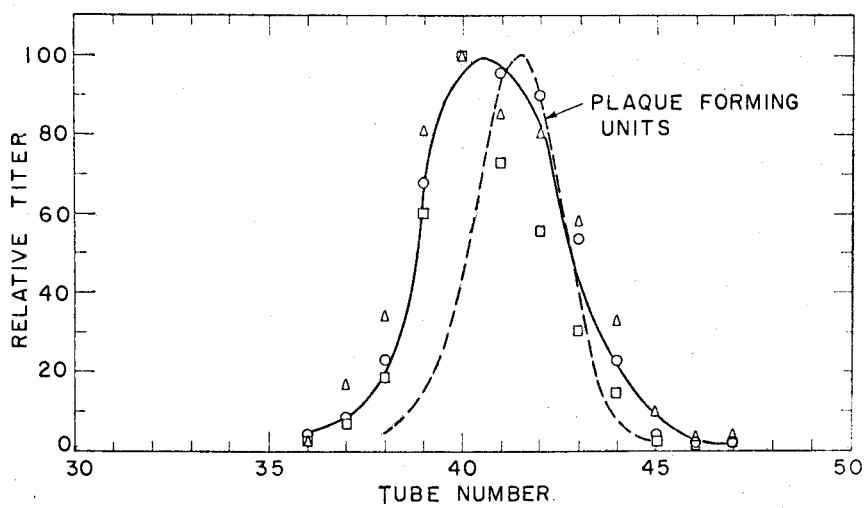

Figure 6. CsCl density-gradient centrifugation of LFT lysate analyzed for single and joint transduction. E. coli 2.0G0 lac⁻, leu⁻, thr⁻ is the recipient. (o is lac transduction; △ is leu transduction; □ is thr-leu joint transduction.)

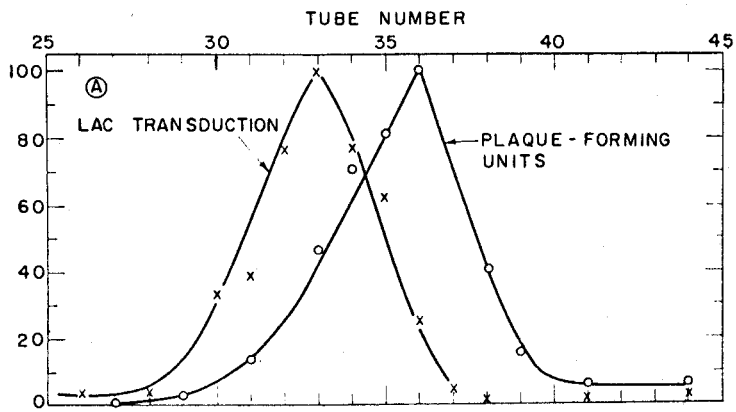

Figure 7. CsCl density-gradient centrifugation of HFT lysate analyzed for lac transduction. E. coli W4032 type W-1 is the donor.

growth media. The lysate called BU-T was prepared by infecting bacteria grown in BU media and subsequently incubating the infected cells in T (thymine). The lysate called T-BU was prepared by reversing the use of T and BU; also, T-T and BU-BU lysates were prepared. Their results are illustrated in Figure 8.

Figure 8a shows that T-T unlabelled leu-transducing phages are similar in density to active P1, as shown previously by Ting (1962).

A comparison of Figure 8a with Figure 8b shows that the active phage peaks of the BU-T and T-T lysates are similar. This result demonstrates that substitution of BU by T in the nucleotide pool of the infected bacteria was rapid and complete.

Figure 8b shows that prelabelling of the bacterial chromosome makes transducing phages heavier than active phages, revealing that dense chromosome DNA has been directly incorporated into the transducing particles. Since the transducing phages are in a single dense peak, the size of the transduced chromosome segments must be exactly or almost the same; and transducing phages do not replicate.

A comparison of Figure 8b with Figure 8c shows that the BU-T and the BU-BU transducing peaks are similar. This result indicates that most BU-T phages are fully heavy and contain no phage genome at all. However, in some cases transducing particles do contain

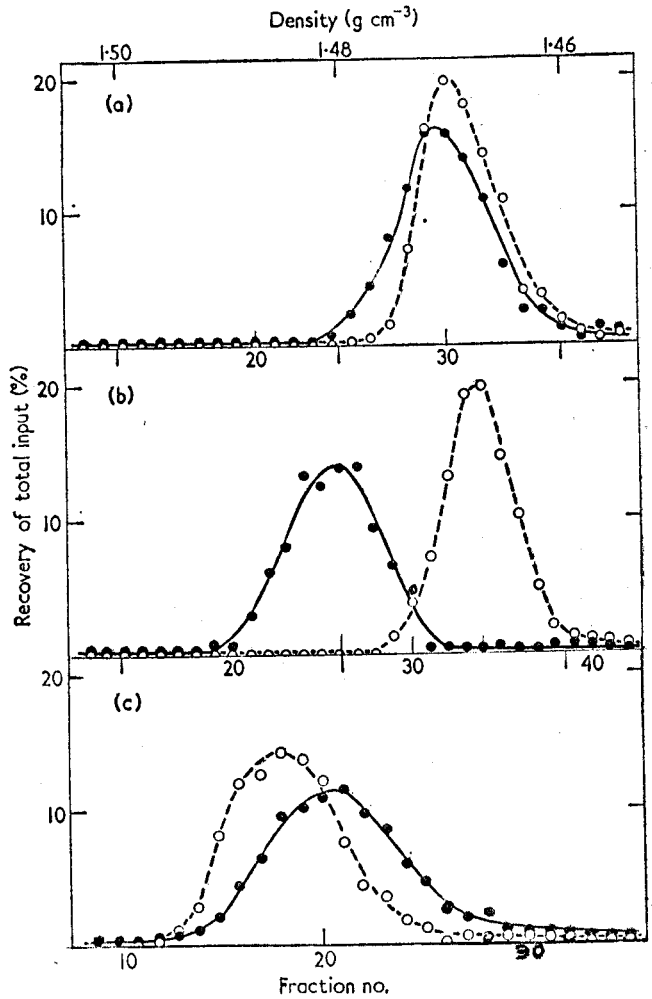

Figure 8. CsCl density-gradient centrifugation analyses of infective and transducing particles (-o-o- for infective particles and -●-●- for transducing particles carrying the leu marker). (a) analysis of T-T lysate; (b) analysis of BU-T lysate; (c) analysis of BU-BU lysate.

phage genome, as previously mentioned (Adams and Luria, 1958).

When phages transducing the R factor were studied, the peak of the BU-T lysate was skewed slightly in the heavy side, indicating half-heavy particles. These half-heavy particles suggest that the R factor transducing phages can replicate, and that the phage must be picking up both strands of the DNA of the bacterial R factor, because if only one strand were being picked up, only fully heavy and fully light particles would appear.

The results of Ikeda and Tomizawa (1965a) agree with the results presented in this thesis.

SUMMARY

The purpose of this research was to determine whether the transducing phage P1 picks up original bacterial chromosome segments and whether these segments are always the same size. A transducing lysate was made by growing phage P1 on cells labelled with ^{15}nitrogen and deuterium, and by lysing the cells in light media so only the transducing particles contained dense DNA from the bacterial chromosome. When this lysate alone was studied by density-gradient centrifugation, a dense, transducing peak and a light, active peak were observed, showing that no active phage was dense and no transducing particles were light.

When a mixture of transducing and nontransducing particles were centrifuged in a density-gradient, the graphs showed no half-heavy or light transducing peak, indicating that transducing particles had not replicated during vegetative growth of the phage. The transducing peak was slightly wider than the active peak, which suggested two sizes of transducing particles. Since transducing particles are heavier than active phages, they must contain a fragment of dense, bacterial chromosome. The transducing peak was sharp and cone-shaped, indicating that the chromosome segments transduced were nearly the same size.

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