

# STAPHYLOCOCCAL TRANSDUCING PARTICLE

C. E. DOWELL<sup>1</sup> AND E. D. ROSENBLUM

*Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas*

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## ABSTRACT

DOWELL, C. E. (The University of Texas, Dallas) AND E. D. ROSENBLUM. Staphylococcal transducing particle. *J. Bacteriol.* **84**:1076-1079. 1962.—When novobiocin-resistant transductants were isolated under conditions that permitted superinfection, almost all the clones were lysogenic for the transducing phage. If superinfection was prevented, then the transductants isolated were nonlysogenic, suggesting the defective nature of the transducing particle. It was noted that the transducing and plaque-forming particles showed no appreciable difference in buoyant density. No difference was found in transduction rates when either sensitive or lysogenic cells were used as recipients. Transduction rates as high as one transductant per  $7 \times 10^4$  phage particles were obtained for novobiocin resistance.

Morse (1959), in a study of transduction in staphylococci, found that 461 of 462 transductants tested were lysogenic for the transducing phage. In later work, Pattee and Baldwin (1961) reported the recovery of nonlysogenic transductants. During a study of the role of lysogenization on the expression and recovery of transductants, we became interested in the nature of the staphylococcal transducing particle, and in comparing it with transducing phages of the enteric bacteria.

## MATERIALS AND METHODS

The materials and methods used in this study have been described previously (Dowell and Rosenblum, 1962), with the following additions.

*Preparative density-gradient centrifugation.* Phage stocks were concentrated by centrifugation in the no. 40 rotor of a Spinco (model L) preparative ultracentrifuge for 90 min at  $10,300 \times g$ . A portion ( $1-2 \times 10^{12}$  particles) of this stock was added to a solution of cesium chloride

<sup>1</sup> Present address: Division of Biology, California Institute of Technology, Pasadena.

to give a final density of  $1.50 \pm 0.01$  g/cm<sup>3</sup>. This suspension (2 to 3 ml) was overlaid with mineral oil in a Lusterloid tube, and then centrifuged in a SW 39L rotor in the preparative ultracentrifuge for 16 hr at  $58,000 \times g$ . The rotor was allowed to come to rest without braking. The tube was removed, and fractions were collected by piercing the bottom of the tube with a fine needle and collecting each successive drop in 0.5 ml of Trypticase Soy Broth. The fractions were then assayed for plaque-forming and transducing titers.

*Transductional rate determination.* This method of transduction was used when it became apparent that the transductant clones remained sensitive to infection by exogenous phage, and when precise transductional rate determinations were desired. In this procedure,  $10^9$  transducing particles were mixed with approximately  $5 \times 10^9$  cells of the appropriate recipient strain in 10 ml of adsorption medium. After a 25-min adsorption period at 37 C, a 2-ml sample was removed for assay of phage adsorption, and 15% (w/v) sodium citrate in Trypticase Soy Broth was added to the remainder to give a final concentration of 0.5% (w/v). Samples were then plated in citrated Brain Heart Infusion-soft agar on citrated Brain Heart Infusion plates containing novobiocin for assay of transductants. Controls consisted of infecting cells in exactly the same manner with nontransducing phage.

This type of determination did not give maximal rates, since no allowance was made for phenotypic lag in the expression of novobiocin resistance. The procedure does, however, eliminate difficulties due to background growth, and serves to compare rates obtained with various preparations and recipient cells.

## RESULTS

*Lysogenization and transduction.* When a broth culture of novobiocin-sensitive cells was infected with transducing phage C (Dowell and Rosenblum, 1962) and hourly samples were plated on

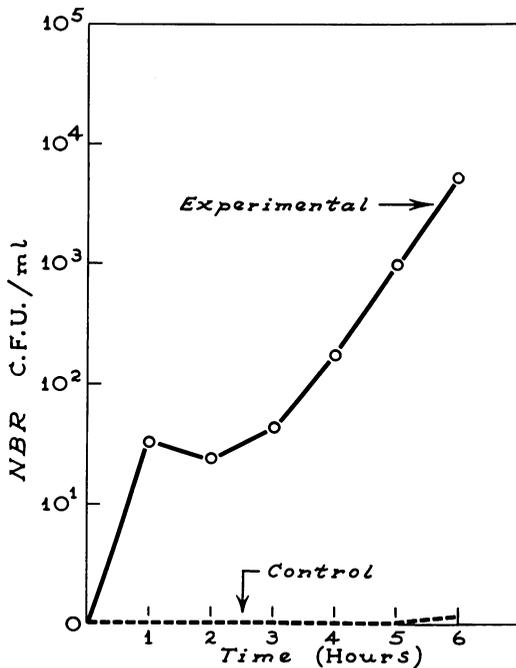


FIG. 1. Novobiocin-resistant colony-forming units (NBR C.F.U.) recovered from broth culture at intervals after infection of sensitive cells with transducing phage C.

novobiocin agar plates to enumerate novobiocin-resistant transductants, we noticed that the number of transductants expressed in 1 hr declined during the second hour and then increased throughout the remainder of the sampling period (Fig. 1). The decrease between 1 and 2 hr suggested that the transductants might still be susceptible to infection and lysis by free phage in the culture.

This hypothesis was tested by using sodium citrate to bind calcium and thus prevent superinfection by preventing phage adsorption. After a 25-min adsorption period, 5% (w/v) citrate was added to one tube and an equal volume of broth was added to the control tube. The cultures were then sampled as before and gave results as shown in Fig. 2. It is evident that the addition of citrate prevents the loss of transductants between 1 and 2 hr. Phage-specific antiserum also gave the same result, but was less desirable because it tended to clump cells.

When transductants were isolated from a liquid culture system in which superinfection was not prevented, then almost all of them (46 of 47

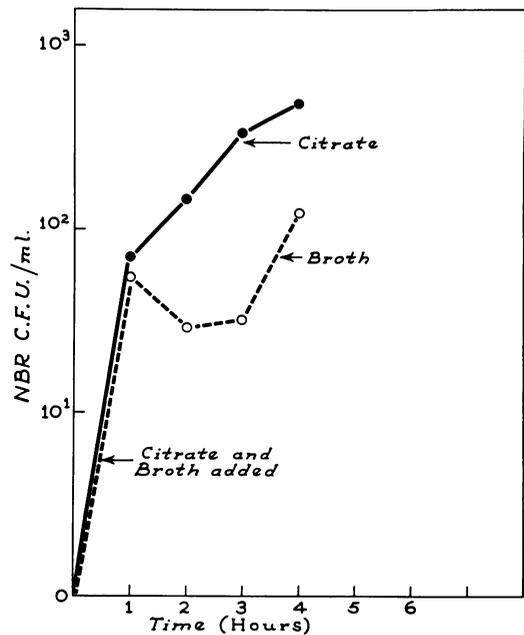
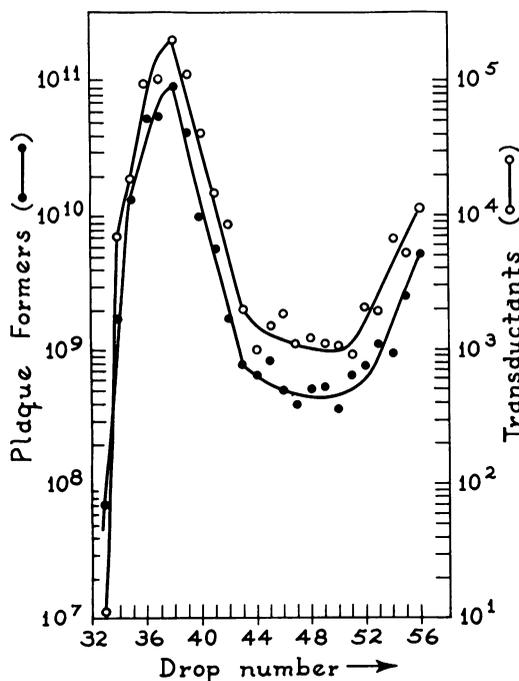


FIG. 2. Effect of citrate on the recovery of novobiocin-resistant colony-forming units (NBR C.F.U.) after infection of sensitive cells with transducing phage C.

tested) were lysogenic for the transducing phage. The lysogenic state of transductants isolated under conditions that prevented superinfection was then examined. Table 1 illustrates the results of a typical experiment when three multiplicities of infection were used and superinfection was prevented by the addition of citrate. Transductants were isolated by plating immediately on selective media after a 25-min adsorption period. Transductant clones, after restreaking for purification, were screened for immunity to and lysogenic carriage of the transducing phage. At a low multiplicity of infection (0.3 and 0.5), no lysogenic transductants were obtained; at the higher multiplicity of infection, a small number of lysogenic colonies were isolated, probably representing dual infection with a transducing and normal phage particle. Unfortunately, we have not been able to obtain an adsorbed multiplicity of infection higher than about 1.4 with this phage-host system. These results imply that the staphylococcal transducing particles are usually defective. Most, if not all, transducing particles are unable to lysogenize the transduced cells.

TABLE 1. *Effect of multiplicity of infection on novobiocin-resistant transductants*

Characteristic	Multiplicity of infection		
	0.3	0.5	1.38
Phage adsorbed.....	$4.2 \times 10^8$	$7.5 \times 10^7$	$2.05 \times 10^9$
Transductants (corrected for spontaneous mutation).....	429	42	550
Rate (plaque-forming particles/transductant).....	$1.0 \times 10^6$	$1.8 \times 10^6$	$3.7 \times 10^6$
Nature of the transductants			
Colonies screened.....	77	42	100
Colonies sensitive.....	77	42	96
Colonies lysogenic.....	0	0	4

FIG. 3. *Densities of infective and novobiocin transducing particles of phage C in a cesium chloride gradient.*

Further evidence for the fact that lysogeny is not a corequisite for staphylococcal transduction comes from the observation that typing phage 80, a nonlysogenizing variant of typing phage 52A (Rountree, 1959; Comtois, 1960), is capable of transduction (Ritz and Baldwin, 1961; Dowell and Rosenblum, 1962).

*Density-gradient studies.* In the  $\lambda$ -galactose transducing system, it has been observed that the transducing particles often differ from the normal  $\lambda$  particles in density (Weigle, 1961). Ting (1962) and Sheppard (1962), using P1 and P22,

TABLE 2. *Rates of transduction of novobiocin resistance with phage C*

Prepn. no.	Recipient cell	MOI*	Rate (plaque-forming particles/transductant)
1	Sensitive	0.3	$1 \times 10^6$
	Sensitive	0.5	$1.8 \times 10^6$
2	Sensitive	0.37	$3.4 \times 10^5$
	Lysogenic for C	0.37	$4.06 \times 10^5$
3	Sensitive	0.24	$7.41 \times 10^4$
	Lysogenic for C	0.21	$1.18 \times 10^5$

\* Multiplicity of infection.

respectively, have reported that the difference in the densities of the plaque-forming and the transducing particles is much less marked than reported for the  $\lambda$  transducing lysate.

We examined the distribution of densities of the novobiocin transducing and plaque-forming particles of phage C in a cesium chloride gradient (Fig. 3). The peak of activity for novobiocin transduction was found to coincide with the peak for infective phage.

*Transductional rate studies.* Table 2 shows the results of several transduction rate studies, using different preparations of phage C and various recipient cells. The phage preparations differed in their transductional ability, and there seemed to be no significant difference in transduction rates when sensitive or lysogenic cells were used as recipients. These rates are generally higher than those previously reported for staphylococcal transducing systems (Morse, 1959; Korman, 1960; Ritz and Baldwin, 1961; Edgar and Stocker, 1961; Pattee and Baldwin, 1961). The higher rates obtained are most probably a result of more efficient recovery of transductants, owing to prevention of superinfection.

## DISCUSSION

It is evident that lysogenization is not a prerequisite for staphylococcal transduction. The fact that most transductants remain phage-sensitive when isolated under conditions that prevent superinfection suggests that the staphylococcal transducing particle is defective in some of its normal phage functions, as has been shown for transduction with P1 (Luria, Adams, and Ting, 1960). The defective nature of a transducing particle was originally demonstrated in phage  $\lambda$  by Arber, Kellenberger, and Weigle (1957). It is not necessary, however, for recipient cells to be sensitive to the transducing phage lysate, since cells lysogenic for the phage are transduced at rates comparable with transduction of sensitive cells. It was previously noted that strains immune to the phage preparation could be transduced (Dowell and Rosenblum, 1962), although the nature of the immunity was not determined.

The densities of the novobiocin transducing and plaque-forming staphylococcal phage particles were not detectably different; this is in essential agreement with the findings for generalized transducing systems in the enteric bacteria (Ting, 1962; Sheppard, 1962). Since the transduced cell is not immune to superinfection, true rates of transduction are most closely approximated when superinfection is rigorously controlled by citrate or antiserum or by Brain Heart Infusion Agar. The rates of transduction we have obtained with staphylococci compare favorably with rates of generalized transduction in *Salmonella* and *Escherichia coli*. Preliminary attempts to derive a high-frequency transducing lysate in the staphylococcal system gave ambiguous results. It would seem that, on the basis of the limited evidence available, generalized transduction in staphylococcal systems corresponds in most of its features to generalized transduction in the enteric bacteria.

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