Restriction of a Transducing Bacteriophage in a
Strain of Proteus mirabilis

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SUMMARY

Phage 34.13 adsorbs well to Proteus mirabilis strain N but does not form plaques on it. The DNA of the phage is severely degraded in strain N. Phage which does emerge plates on P. mirabilis strain 13 but not on N. The phenotype of strain N is r+m-. Restriction by stationary phase organisms is weaker than in early log. phase cells. The acceptor ability of Nstr-r for a p-lac+ plasmid from strain 13 is less than that of strain 13str-r. Spheroplasts of 13 plate φ34.13 DNA with an efficiency of 10^-7. The efficiency on strain N spheroplasts is <10^-11. The transduction rate of markers by φ34.13 into N is only reduced to about 10^-1 of the rate into strain 13. Transductants are non-lyso-
genic for the phage, are stable and may be retransduced. Small doses of ultraviolet radiation do not increase the transduction rate. This is interpreted to mean that φ34.13 transduces strain N by integration of the bacterial exogenote, which like φ34.13 DNA and possibly strain 13 p-lac+ DNA, is degraded in strain N. NH mutants of strain N were isolated on which φ34.13 has an e.o.p. of 5×10^-1 and into which φ34.13 transduces markers at the same rate as into strain 13. Phage 34μl mutants plate on strain N. A strain N-induced host specificity was discovered which must be carried by φ34μl for it to form plaques on N. Strain N and its NH mutants are lysogenic for φ13vir and produce a phage tail-like bacteriocin but neither of these factors account for the restricting and modifying properties of the strains. Phages 34.13 and 34μl.N do not affect one another in mixed infections of strain N.

INTRODUCTION

Bacteriophage DNA carries the non-heritable additive specificity which is imparted without change of its genetic information by the last bacterial host in which it has grown. The host imparts its specificity (Arber & Dussoix, 1962) by a process best named host-controlled modification to the phage (Arber, 1965). If the phage now infects a second organism with a different specificity the absence of this latter specificity on the phage DNA is recognized by restricting enzymes of the host and phage multip-
lication is blocked in this host by a process of restriction which degrades the incoming DNA (Dussoix & Arber, 1962; Kellenberger, Symonds & Arber, 1966). The phenotype
of a bacterial strain which imparts host specificity to phage DNA and also restricts DNA which does not bear its specificity is r+m+ (Glover et al. 1963; Colson et al. 1965; Kerszman, Glover & Aronovitch, 1967).

Host-controlled modification has been encountered for phages of Escherichia coli

J. Virol. 4
and Shigella dysenteriae, Streptococcus lactis, Rhizobium, Pseudomonas aeruginosa (see Holloway & Rolfe, 1964), staphylococci (Ralston & Krueger, 1952) and Salmonella typhosa (Anderson & Felix, 1952). The host genome may govern this modification either as a locus on the bacterial chromosome (Zinder, 1960; Holloway & Rolfe, 1964; Colson et al. 1965) or by the presence of prophage containing the controlling loci (Lederberg, 1957; Arber & Dussoix, 1962; Glover & Kerszman, 1967; Kerszman et al. 1967). Like prophage, R factors present in E. coli or S. typhimurium may also be responsible for modification and/or restriction of phage DNA (Watanabe et al. 1964; Watanabe et al. 1966; Bannister & Glover, 1968; Takano, Watanabe & Fukasawa, 1968) and the sex factor F and colicinogenic factor B may also be implicated with restriction of phage W-31 in E. coli (Watanabe & Okado, 1964). Arber (1962) suggested that bacterial DNA could also be provided with specificity and that the processes of modification and restriction would apply to this DNA when transferred to cells possessing different host specificities. This has been confirmed for conjugation and transducing systems in E. coli (Arber, 1964; Arber & Morse, 1965). Restriction may also act against the acceptance of the fertility factor F, its F⁺ derivatives, colicinogenic factor I and R factors (Glover et al. 1963; Arber & Morse, 1965).

For the genus Proteus cases of apparent host-controlled modification of phages by P. rettgeri and Providence strains were encountered by Coetzee (1963a) in an investigation of the host range of P. rettgeri and P. hauseri phages. In an investigation of the growth of phage MS2 in P. mirabilis harbouring F-genotes from Escherichia coli (Horiuchi & Adelberg, 1965) no host-controlled modification of the phage was detected. Gemski, Wohlheiter & Baron (1967) admit the possibility that host modification and restriction may influence chromosome transfer between E. coli and P. mirabilis.

In attempts to discover more hosts for the Proteus mirabilis transducing phage 34/I3 (Coetzee & Sacks, 1960a, b) this phage was tested for plaque formation on many P. mirabilis strains. By this means a strain (named N) was encountered which possesses the phenotype r+m⁻ with regard to phage 34/I3. This is a rare phenotype for a wild-type organism (Kerszman et al. 1967) and results of an investigation of this phage-bacterial system are presented.

**METHODS**

**Bacteriophage.** Phage 34/I3 (Coetzee & Sacks, 1960c) was used. The phage (renamed φ34) produces generalized transduction in Proteus mirabilis strain 13 (Coetzee & Sacks, 1960a; Coetzee 1963b) and is dependent on Ca²⁺ for adsorption. The guanine +cytosine molar content of φ34.13 is 39.6% (unpublished observations). The notation of Arber & Dussoix (1962) is used to indicate the host strain upon which a phage lysate was prepared. Thus φ34.13 denotes a lysate of φ34 produced on strain 13.

**Bacteria.** The following strains of Proteus mirabilis were used: 13 (Coetzee & Sacks, 1960c), 13str-r (Coetzee & Sacks, 1960b), 13suc⁺ (Coetzee & Sacks, 1960d), 13 P-lac⁺, 13 (φ34) (Coetzee & Sacks, 1960b), N and Nstr-r. Strain 13 carrying the plasmid P-lac⁺ (Falkow et al. 1964) was kindly prepared by Dr S. Falkow. Strain 13 (φ34), lysogenic for φ34, does not adsorb φ34.13 owing to lysogenic conversion (Coetzee, 1961). Strain Nstr-r was selected for as previously described (Coetzee & Sacks, 1960b). Strains N and 13 have identical biochemical reactions but their swarms do not spread into one another on agar. They thus show a positive Dienes phenomenon (Dienes, 1946, 1947).
Proteus phage restriction

The two strains also differ in reactions to a number of Proteus typing phages (unpublished observations). Strains I3 and N have an identical guanine+cytosine molar content of 38.5% (unpublished observations). In addition, a large number of unnamed strains of *P. mirabilis* were used to detect bacteriocin production by strain N. Cultures were maintained on agar at 4° and incubation temperature was 37°. As stationary phase cells a 24 hr unaerated broth culture was used. These cultures contain $2 \times 10^9$ viable organisms/ml. Early logarithmic (log.)-phase cells were prepared by diluting stationary phase cells 1:30 in prewarmed broth and incubating with aeration for 1 hr. When necessary these cultures were concentrated by centrifugation to the same titre.

*Media.* Difco nutrient broth, brain heart infusion broth, nutrient agar, MacConkey agar and SS agar were used. For certain experiments 1% filtered sucrose was added to SS agar at 45° before pouring plates. The minimal medium was that of Grabow & Smit (1967) and this medium with sucrose substituted for glucose and containing 0.01% (w/v) tetrazolium chloride was used to select *suc*+ transductants. Streptomycin sulphate (1 mg./ml.) was added to molten agar at 45° when required. When lysogenization of transductants was being studied 2% sodium citrate was added to media. The medium for preparing $^{32}$P-labelled phage was the glycerol-lactate medium of Hershey & Chase (1952) modified to contain 7.5 μg./ml. nicotinic acid and 0.3% (w/v) casein hydrolysate (British Drug Houses Ltd). Carrier-free $^{32}$P-phosphoric acid (CEA, 91-Gif-sur-Yvette, France) was added in a concentration of 5 μc./ml. This medium was solidified by the addition of 1.5% or 0.5% agar for solid and top-layer media respectively.

*Phage techniques.* Phage lysates were prepared by the modification (Adams, 1959) of the double-agar-layer method of Hershey, Kalmanson & Bronfenbrenner (1943). The general phage techniques were those of Adams (1959). Lysogenization procedures were those of Watanabe *et al.* (1966). Overnight broth cultures of strains were infected with phage. After incubation for 15 min. cultures were diluted 1:200 in ice-cold broth and washed three times in a refrigerated centrifuge to remove free phage and suitable dilutions plated on SS agar. After overnight incubation the plates were replicated to SS agar seeded with strain I3. The replica plates were incubated overnight and colonies with surrounding lytic zones were regarded as lysogenic. The Hanovia sterilamp and the method used to irradiate phage suspended in T2 buffer have been described (Coetzee & Sacks, 1960b).

**Single cycle growth of phage in Proteus mirabilis strain N.** Phage was adsorbed to $2 \times 10^9$ organisms for 10 min. at 37°. A sample was then diluted 1:100 in broth containing 0.1 vol. chloroform and titrated for unadsorbed phage. Another sample was diluted 1:1 in potent phage antiserum. After 5 min. at 37° two samples were removed from the serum tube. One sample was diluted into chloroform broth for assay of free phage. The other was diluted 1:100 in nutrient broth and 0.1 ml. titrated on 200 plates for infective centres. The transmission of the phage through N was expressed as the fraction of infected bacteria which produce infective centres on strain I3.

**Preparation of $^{32}$P-labelled φ34.** Strain 13 was grown overnight in liquid $^{32}$P-labelled medium. This growth was then used with the radioactive solid media to prepare lysates of φ34 by the double agar method. Lysates were purified and concentrated by alternate high (22,000 g for 90 min.) and low-speed (3000 g for 10 min.) centrifugation in isotonic saline. Final purification was done by caesium chloride density gradient centrifugation (Sheppard, 1962) and ten-drop fractions collected after puncture of the bottom of the tube. The fractions were titrated and high titre samples pooled.
Fate of $\phi$34.13 DNA upon infection of bacterial hosts. Overnight broth cultures of organisms were centrifuged and starved by suspending in equal volumes of 0.01 M-MgSO$_4$.7H$_2$O for 2 hr. Two ml. of the suspension were then infected at a final m.o.i. of about 1 with the highly purified $^{32}$P-labelled $\phi$34.13 and total radioactivity measured. After 15 min. incubation the mixtures were diluted threefold in prewarmed broth and incubation with aeration continued for a further 15 min. Samples of the mixture were then lysed by exposure to 2% sodium lauryl sulphate (specially pure, British Drug Houses Ltd) for 2 min. at 70° followed by treatment with 2% (v/v) cold perchloric acid (Dussoix & Arber, 1962). Radioactivity was measured in a Packard Tri-carb scintillation counter model 3375 according to Davila, Charles & Ledoux (1965). Control samples without phage were similarly treated and counted with an internal standard. Sufficient counts were recorded to give an error less than 2%.

Transduction procedures. These were as described by Coetzee, Smit & Prozesky (1966). Adsorption mixtures were filtered through membrane filters and these then placed on suitable agar plates. With experiments which involved selection for the str-r marker, membranes were incubated on nutrient agar for 4 hr before transfer to streptomycin MacConkey agar. When lysogenization of transductants was studied, recipient cells were infected at m.o.i. of about 1 and, after filtration, 2% trisodium citrate.2H$_2$O was passed through the filter. The membrane was then transferred to agar plates containing sodium citrate which had previously been spread with potent phage antiserum. These procedures prevented secondary lysogenization of transductants by phage on the plates (see Coetzee & Sacks, 1960b).

Extraction of $\phi$34.13 DNA. This was done according to the method of Mandell & Hershey (1960). DNA concentration was determined by ultraviolet absorption at 260 nm.

Preparation of spheroplasts. Ten ml. of an overnight broth culture of the organism was added to 100 ml. of prewarmed broth supplemented with 0.5 M-sucrose, and 0.2% MgSO$_4$.7H$_2$O and incubated for 1 hr. Penicillin G was then added to a final concentration of 700 μg./ml. and the mixture incubated for a further 150 min. Spheroplasts were counted in a Petroff-Hauser chamber with the use of a phase-contrast microscope.

Transfection techniques. Penicillin spheroplasts at a final concentration of $5 \times 10^8$ ml. were incubated for 15 min. in minimal medium supplemented with 0.5 M-sucrose and 2% bovine serum albumin (Difco). Phage DNA was then added to a final concentration of 50 μg./ml. and incubation continued. This concentration of DNA corresponds to about $10^{14}$ $\phi$34.13 equivalents. Controls consisted of spheroplasts and DNA added individually to the medium. After 120 min. incubation the mixtures were diluted 1:10 in sterile distilled water to disrupt spheroplasts and plated on strain 13 for infectious units.

Transfer of $\beta$-lac to strains 13str-r and Nstr-r. Strains 13 $\beta$-lac$^+$, 13str-r, Nstr-r were grown in nutrient broth to concentrations of about $5 \times 10^8$ organisms/ml. One ml. of recipient and 0.5 ml. of donor cultures were then mixed and incubated for 1 hr. Mating mixtures were then plated on streptomycin SS agar to yield about 100 colonies/plate after incubation. Donor and recipient cultures were also plated individually as controls. The acceptor ability of a strain for the $\beta$-lac$^+$ element from strain 13 was expressed as the percentage of red (lac$^+$) colonies amongst 3000 colonies.

Mutagenic treatment of bacteria and phage. This was done by treating washed log. phase cultures for 30 min. with N-methyl-N'-nitro-N-nitrosoguanidine (nitroso-
guanidine) (Aldrich Chemical Co., Inc., Milwaukee, Wis., U.S.A.) in phosphate buffer at pH 6-0 according to the method of Adelberg, Mandel & Chen (1965). When bacterial mutants were being selected the organisms were washed and allowed to complete two to three generations in nutrient broth before plating on SS agar to yield about 200 colonies/plate. Auxotrophs were isolated by replication to minimal medium and identified auxanographically (Lederberg, 1950). Mutants of strain N which allowed productive growth of ϕ34.13 were sought by picking off colonies from the SS plates into broth. The broth cultures were then tested for plaque formation by ϕ34.13 by spotting suitable dilutions of the phage on agar plates which contained the cultures in top-layer agar. When mutants of ϕ34.13 were sought which could productively lyse strain N, strain 13 (ϕ34) was treated with nitrosoguanidine as above (see also Gottesman & Yarmolinsky, 1968) and then diluted 100-fold in broth in a number of tubes. After incubation for 3 hr chloroform was added and the supernatant plated on strain N.

Curing bacteria of prophages. This was done according to the method of Lederberg & Lederberg (1953). MacConkey plates were spread with about 10^8 cells and exposed to ultraviolet light so that about 50 colonies survived after incubation. These colonies were then tested for productive lysis by ϕ34.13 by spotting dilutions of the phage on plates containing the organisms in top-layer agar.

Detection of bacteriocin production by Proteus mirabilis strain N. This was done according to the methods of Coetzee et al. (1968).

Electron microscopy. The methods of Prozesky, de Klerk & Coetzee (1965) were used.

RESULTS

Action of ϕ34.13 on Proteus mirabilis strain N

The initial observation was that, when high concentrations of ϕ34.13 were spotted on strain N, the bacteria were killed but serial dilutions of the phage never gave plaques. More than 99% of the phage adsorbed to strains 13 and N within 10 min. at 37°C. Transmission experiments (Table 1) showed that only 9 × 10^-6 of ϕ34.13 infected cells of strain N burst to yield phage which formed plaques on strain 13 but not on N. With the use of early log. phase cells the figure was reduced to 2 × 10^-6. The growth of ϕ34.13 in strain N was thus restricted and the phage which escaped restriction did not receive the host specificity of strain N and had to face the same restriction in new strain N hosts. Even when about 10^{11} p.f.u. of ϕ34.13 were plated with an excess of N cells (early log. or stationary phase) no plaques were seen. The phenotype of strain N is thought to be r^+m^-m. The absence of plaque formation was not due to extensive lysogenization. Lysogenization of strain N with ϕ34.13 has never been demonstrated.

<table>
<thead>
<tr>
<th>Growth phase of strain N</th>
<th>M.o.i.</th>
<th>Adsorbed phage (%)</th>
<th>Free phage after antiserum</th>
<th>No. infective centres on</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary</td>
<td>1·2</td>
<td>99·4</td>
<td>1·1 × 10^8</td>
<td>1·1 × 10^4</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Early log.</td>
<td>1·4</td>
<td>99·6</td>
<td>1·5 × 10^8</td>
<td>3·2 × 10^3</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>
The transmission rate encountered is lower than that of phage λ in *Escherichia coli* strain w (Kerszman *et al.* 1967) where under certain circumstances λ does form a few plaques.

**Mechanism of restriction of φ34.13 in Proteus mirabilis strain N**

Within 15 min. of phage adsorption to strain N about 57% of phage DNA was extractable from these bacteria in acid-soluble form (Table 3). The corresponding result with strain 13 was 6% and 1% for strain 13 (φ34). The 1% acid-soluble DNA corresponds to the amount of soluble 32P found in purified preparations of 32P-labelled φ34.13. The 6% breakdown in strain 13 is higher than Kerszman *et al.* (1967) encountered for λ.C DNA in the non-restricting host *Escherichia coli* strain c but agrees with results of Arber, Hattman & Dussoix (1963) for the latter system. Results of these experiments indicate that φ34.13 DNA penetrates strain N but is rapidly and extensively degraded in the organism.

### Table 2. Frequency of lysogenization of various Proteus mirabilis strains by φ34 and φ34n-I.N

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phage</th>
<th>M.o.i.</th>
<th>No. of lysogenized colonies/no. studied</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>34·13</td>
<td>1</td>
<td>413/1188</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>723/1279</td>
<td>57</td>
</tr>
<tr>
<td>N</td>
<td>34·13</td>
<td>1</td>
<td>0/1770</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0/1823</td>
<td>0</td>
</tr>
<tr>
<td>NH1</td>
<td>34·13</td>
<td>1</td>
<td>422/1263</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>479/1071</td>
<td>45</td>
</tr>
<tr>
<td>N</td>
<td>34n-I</td>
<td>1</td>
<td>336/1238</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>406/1197</td>
<td>34</td>
</tr>
</tbody>
</table>

### Table 3. Fate of φ34.13 DNA labelled with 32P after infection of various Proteus mirabilis hosts

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Non-restricting</th>
<th>Restricting</th>
<th>Adsorption resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency of plating of phage 34.13</td>
<td>13</td>
<td>N</td>
<td>13 (φ34.13)</td>
</tr>
<tr>
<td>Multiplicity of infection</td>
<td>1</td>
<td>1·2</td>
<td>1·4*</td>
</tr>
<tr>
<td>Adsorption 15 min. (%)</td>
<td>99·8</td>
<td>99·8</td>
<td>0</td>
</tr>
<tr>
<td>Cells lysed with sodium lauryl sulphate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adsorption mixture 0 min. (counts/min.)</td>
<td>17,758</td>
<td>18,217</td>
<td>17,679</td>
</tr>
<tr>
<td>Insoluble 32P 30 min. (counts/min.)</td>
<td>16,710</td>
<td>7,321</td>
<td>16,389</td>
</tr>
<tr>
<td>Soluble 32P 30 min. (counts/min.)</td>
<td>1,048</td>
<td>9,919</td>
<td>157</td>
</tr>
<tr>
<td>Perchloric acid soluble 32P (%)</td>
<td>5·9</td>
<td>57·5</td>
<td>0·9</td>
</tr>
</tbody>
</table>

* Multiplicity of input.

**Lysogenicity and bacteriocinogeny in strain N**

Supernatant medium from irradiated cultures of strain N occasionally contained a phage which forms large clear plaques on strain 13 and N but not on other strains of *Proteus mirabilis*. This phage was never detected in unirradiated cultures of strain N.
Proteus phage restriction

Morphologically the phage was identical to phage 13vir (Prozesky et al. 1965), a virulent mutant of a prophage present in strain 13. Phage 13vir was liberated spontaneously by strain 13 and forms large clear plaques on both strains. A phage 13vir antiserum with a k value of 120 min.−1 neutralized the phage from strain N at a rate of 110 min.−1 and the phages are thought to be identical. 'Cured' colonies of strain N were readily obtained by radiation. Broth cultures of these colonies never produce phage on radiation but they do not differ from the parent strain N in reactions to φ34.13 or phage 34n, to be described later. A search for bacteriocinogeny in strain N by the cross-streak method, with irradiation of the N streak, revealed that strain N killed two strains of Proteus mirabilis which were then named P and Q but had no effect on strain 13. The killing effect was not transmissible. Differential centrifugation of irradiated brain heart infusion broth cultures produced a preparation with a killing titre of >1:1000 on strains P and Q but no action on strain 13. Electron microscopy of such preparations revealed an abundance of phage tail-like structures identical to those encountered by Coetzee et al. (1968) in bacteriocinogenic strains of P. vulgaris. These structures differ in many respects from the tails of phage 13vir. A number of colonies of strain N were obtained by heavy irradiation which no longer had an action on strains P and Q. These clones were regarded as 'cured' of bacteriocinogeny but, like the variants 'cured' of prophage 13vir, they still restricted φ34.13 to the same extent. Neither the prophage nor the bacteriocinogenic factor appears to play a role in the restriction of φ34.13 by P. mirabilis strain N.

Transfection experiments

Experiments with φ34.13 DNA and strain 13 spheroplasts yielded about 10⁴ plaques (an efficiency of about 10⁻⁷). Controls never showed plaques. Transfection of strain N spheroplasts with the same DNA as used above did not produce plaques (efficiency < 10⁻¹¹). In these experiments the DNA-treated spheroplasts were plated on strain 13 and the transmission rate of φ34.13 DNA in strain N spheroplasts thus approaches zero. Dussoix & Arber (1965) showed that phenol extraction does not affect the host specificity of λ DNA. They demonstrated that in the K 12 (P1) strain of Escherichia coli phenol extracted λ. K DNA is severely restricted. The results presented here suggest that the restricting principle of strain N was not released by spheroplasting. Spheroplasts of the non-restricting mutant NH1 to be described later plated φ34.13 DNA with an efficiency of about 10⁻⁷.

Transduction of markers by φ34.13 into strain N

Lysates of φ34.13str-r (5 × 10⁶ p.f.u./ml.) transduced the str-r marker or prototrophy to tyr, trp and met auxotrophs of strain N at a rate of 1 × 10⁻⁷/p.f.u. adsorbed. Experiments done simultaneously with strain 13 auxotrophs gave a rate of 3 × 10⁻⁶/p.f.u. adsorbed (see Coetzee & Sacks, 1960b). With the use of early log. phase cells of strain N the transduction frequency was reduced by about half to 5 × 10⁻⁶/p.f.u. of φ34.13 str-r adsorbed (Table 4). U.v. irradiation of φ34.13str-r affected the number of transductants obtained in strain 13 differently from strain N. While small doses produced an initial increase in strain 13 transductants (Coetzee & Sacks, 1960b) the number of strain N transductants simply declined exponentially (Fig. 1). Two hundred strain Nstr-r transductants produced by phage lysates which had been submitted to small doses of radiation and 200 str-r transductant clones resulting from lysates sub-
mitted to larger doses of radiation were picked off into 1 ml. of φ34.13 antiserum (k value, 430 min⁻¹). After 1 hr at 37° the suspensions were plated on SS agar. After overnight incubation a colony of each of the original 400 clones was tested for lyso-

Table 4. Summary of results of various transduction experiments

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Growth phase</th>
<th>Transducing phage</th>
<th>Selected marker</th>
<th>Transduction rate/p.f.u. adsorbed</th>
<th>Lysogenicity of transductants</th>
<th>Retransduction of transductants</th>
</tr>
</thead>
<tbody>
<tr>
<td>13met-I</td>
<td>Stationary</td>
<td>34.13str-r</td>
<td>met⁺</td>
<td>3 x 10⁻⁶</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Nmet-I</td>
<td>Stationary</td>
<td>34.13str-r</td>
<td>met⁺</td>
<td>1 x 10⁻⁷</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Ntrp-I</td>
<td>Early log.</td>
<td>34.13str-r</td>
<td>met⁺</td>
<td>5 x 10⁻⁸</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>N</td>
<td>Stationary</td>
<td>34.13str-r</td>
<td>str⁻</td>
<td>6 x 10⁻⁷</td>
<td>+ *</td>
<td>+</td>
</tr>
<tr>
<td>NH I</td>
<td>Stationary</td>
<td>34.13suc⁺</td>
<td>suc⁺</td>
<td>1 x 10⁻⁷</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Ntyr-I</td>
<td>Early log.</td>
<td>34.13str-r</td>
<td>str⁻</td>
<td>2 x 10⁻⁶</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Ntyr-I</td>
<td>Stationary</td>
<td>34n-1.13</td>
<td>tyr⁺</td>
<td>1 x 10⁻⁷</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

M.o.i. of transducing phages was about 1·4.

* Transductants lysogenic for φ34n-1.

† Non-restricted helper φ34n-1. N was added simultaneously at a m.o.i. of 1·1.

Fig. 1. Effect of u.v. irradiation on plaque-forming and transducing abilities of φ34.13str-r. Samples (6 ml.) of φ34.13str-r (5 x 10⁹ p.f.u./ml.) suspended in phage T2 buffer were irradiated at 10 ergs/mm²/sec. for different periods of time. These samples were used in quantitative transduction experiments with strain Ntrp-I as recipient. The m.o.i. was 1·4. In the experiment with selection for the str⁻ marker the recipient was simultaneously infected at a m.o.i. of 1·1 with φ34n-1. N. ■, trp⁺ transductants; ▲, str⁻ transductants with helper φ34n-1. N; ●, plaque-forming titre.
Proteus phage restriction

Proteus phage restriction 601
genicity. None of the clones was lysogenic for \( \phi 34 \) although all were still \( str^{-r} \). Twenty clones of each of the two types were tested for \( \phi 34.13 \) adsorption. All adsorbed the phage as avidly as the wild strain \( N \). These transductants could be retransduced with a second marker at a slightly lower rate than before (Table 4). None of the transductant clones were susceptible to productive lysis by \( \phi 34.13 \). It might have been expected that transduction with a restricted phage would have been a strong selection factor for the isolation of bacterial mutants which do not restrict non-adapted DNA. However, Arber (1964) was also unsuccessful in isolating restrictionless mutants from \( gal^{+} \) transductants produced by \( \lambda \ dg \) in the restricting strain \( K12 \) of \( Escherichia coli \). In another experiment \( \phi 34.13 suc^{+} \) was used to transduce the \( suc^{+} \) marker into \( N \). Four hundred \( suc^{+} \) transductant clones were streaked on to separate SS sucrose agar plates. After incubation one \( suc^{+} \) colony from each plate was again spread on SS sucrose agar. No pale segregants were detected after overnight incubation. It is concluded that the transductants are all stable \( suc^{+} \) clones. Two hundred of the \( suc^{+} \) transductants were subsequently tested for lysogenicity with exactly the same results obtained with the \( str^{-r} \) transductants (Table 4). These results differ markedly from those obtained with strain 13 where the vast majority of transductants are lysogenic for \( \phi 34 \) (Coetzee & Sacks, 1960b) and do not adsorb the phage due to lysogenic conversion (Coetzee, 1961). As in other Proteus transducing systems (see Coetzee, de Klerk & Smit, 1967) no abortive transductants were encountered. Mixed infection of strain \( N \) with \( \phi 34.13str^{-r} \) and its non-restricted mutant \( \phi 34n^{-1}.N \) (see later) increased the transduction rate of the \( str^{-r} \) marker up to \( 6 \times 10^{-7} \) p.f.u. adsorbed. Many of these transductants are lysogenic for \( \phi 34n^{-1} \) (Tables 4 and 5). Prior u.v. irradiation of \( \phi 34.13str^{-r} \) for different periods of time before simultaneous infection with \( \phi 34n^{-1}.N \) also resulted in a general increase in transduction frequencies and imposed a shoulder on the resulting curve (Fig. 1). Arber (1964) observed an increase in transduction frequency of the \( gal^{+} \) marker in \( Escherichia coli \) with a restricted high frequency \( \lambda \) lysate in the presence of a non-restricted helper \( \lambda \). The effect was most noticeable when the helper was adsorbed to the host prior to the restricted transducing phage. He suggested the result was probably due to rescue of the \( gal^{+} \) marker.

### Table 5. Transduction of strain \( N \)trp-1 with restricted \( \phi 34.13str^{-r} \) superinfected with non-restricted \( \phi 34n^{-1}.N \)

<table>
<thead>
<tr>
<th>M.o.i. of ( \phi 34.13str^{-r} )</th>
<th>M.o.i. of ( \phi 34n^{-1}.N )</th>
<th>Time of addition of ( \phi 34.13str^{-r} ) with respect to ( \phi 34n^{-1}.N ) (min.)</th>
<th>Transduction frequency/p.f.u. ( \phi 34.13str^{-r} ) adsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>1.1</td>
<td>—</td>
<td>( 7 \times 10^{-8} )</td>
</tr>
<tr>
<td>1.2</td>
<td>1.1</td>
<td>—15</td>
<td>( 1 \times 10^{-7} )</td>
</tr>
<tr>
<td>1.2</td>
<td>1.1</td>
<td>0</td>
<td>( 3 \times 10^{-7} )</td>
</tr>
<tr>
<td>1.2</td>
<td>1.1</td>
<td>+15</td>
<td>( 6 \times 10^{-7} )</td>
</tr>
</tbody>
</table>

Transmission of \( P-lac^{+} \) to strain \( N \)

The acceptor ability for \( P-lac^{+} \) from strain 13 \( P-lac^{+} \) by strain \( 13str^{-r} \) was 19.5%. The corresponding value for strain \( Nstr^{-r} \) was 2% and 1% for log.-phase organisms (Table 6). Strain 13 \( P-lac^{+} \) never appeared on control plates and recipient controls did not produce red \( (lac^{+}) \) colonies. \( Lac^{+} \) recipients of both strains segregated \( lac^{-} \) colonies at a frequency of about \( 2 \times 10^{-3} \). \( Lac^{+} \) colonies of strain \( N \) (and \( lac^{-} \) segregants) would
possibly lack the restriction principle and could conceivably also accept \( \phi \) 34.13 DNA. Cultures of ninety \( lac^+ \) and 24 \( lac^- \) segregant colonies of strain \( nstr-r \) were spotted with serial dilutions of \( \phi \) 34.13. No plaques were seen. Four of the 24 \( lac^- \) segregants were also tested for \( P-lac^+ \) acceptor ability from strain 13 \( P-lac^+ \). Acceptor ability was again only about 1%. Mutants of strain N (NH mutants, see later) which accept \( \phi \) 34.13 have an acceptor ability for \( P-lac^+ \) from strain 13 of 17% (Table 6). The restriction of the acceptor ability of strain N for the \( P-lac^+ \) plasmid from strain 13 has not been proved to be due to degradation of the \( P-lac^+ \) DNA but the fact that NH mutants, which do not restrict \( \phi \) 34.13, accept the plasmid as readily as strain 13\( str-r \) supports this possibility.

Table 6. Transmission of \( P-lac^+ \) to strains of Proteus mirabilis

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Acceptor ability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13( P-lac^+ )</td>
<td>13( str-r )</td>
<td>19.5</td>
</tr>
<tr>
<td>13( P-lac^+ )</td>
<td>13( str-r ) stationary phase</td>
<td>2.3</td>
</tr>
<tr>
<td>13( P-lac^+ )</td>
<td>13( str-r ) log. phase</td>
<td>0.9</td>
</tr>
<tr>
<td>13( P-lac^+ )</td>
<td>13( str-r ) P-lac(^-) segregant</td>
<td>1.1</td>
</tr>
<tr>
<td>13( P-lac^+ )</td>
<td>NH1( str-r )</td>
<td>17.0</td>
</tr>
</tbody>
</table>

Table 7. Plating efficiency of phages on various Proteus mirabilis strains

<table>
<thead>
<tr>
<th>Proteus mirabilis strains</th>
<th>Phage</th>
<th>13</th>
<th>N</th>
<th>NH1</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.13</td>
<td>1</td>
<td>&lt;10(^-11)</td>
<td>5\times10(^{-1})</td>
<td></td>
</tr>
<tr>
<td>34( n-1 )--13</td>
<td>1</td>
<td>10(^{-8})</td>
<td>5\times10(^{-3})</td>
<td></td>
</tr>
<tr>
<td>34( n-1 )--N</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>34( n-2 )--N</td>
<td>&lt;10(^-11)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>34( n-1 )--NH1</td>
<td>1</td>
<td>10(^{-3})</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>34--NH1</td>
<td>1</td>
<td>5\times10(^{-4})</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

The e.o.p. is expressed as the ratio of titre on heterologous to that on the homologous host.

**Isolation of mutants of strain N which accept \( \phi \) 34.13**

While nitrosoguanidine proved very effective for obtaining various auxotrophs of strain N (about 50% of colonies on SS agar did not replicate to minimal medium) 4500 colonies were screened to obtain three mutants on which \( \phi \) 34.13 formed plaques. Kerszman et al. (1967), working with Escherichia coli w phage \( \lambda \) system, employed the same methods and found about 2% of colonies susceptible to \( \lambda \). The reason for the discrepancy between the two systems is unknown. The possibility that a double mutation is involved was not tested experimentally. The mutants named NH1, NH2 and NH3 adsorbed \( \phi \) 34.13 as efficiently as the parent strain N and the e.o.p. of \( \phi \) 34.13 was \( 5\times10^{-4} \) for all three variants (Table 7). It is unlikely that this small reduction in e.o.p. is due to restriction by the NH strains (Lederberg, 1957), although Arber (1966) has shown that phage fd.B plates on E. coli B(P1) with an e.o.p. of \( 3\times10^{-3} \) and is host-modified by it (also see Bannister & Glover, 1968). The plaque morphology of \( \phi \) 34.13 on strain 13 and the NH mutants is identical. Mutant NH1 was further investigated. It still carried the prophage of \( \phi 13vir \) and was bacteriocinogenic for Proteus mirabilis strains P and Q. Phage 34.NH1 adsorbed (99%) within 10 min. to N but had an e.o.p. of \( 5\times10^{-4} \) on this strain. On strain 13 phage 34.NH1 plated with an efficiency...
Proteus phage restriction

of 1. The transduction rate of the \( \text{str-r} \) marker by \( \phi 34.13 \text{str-r} \) into \( \text{NH} \) is \( 2 \times 10^{-6} \) p.f.u. adsorbed. This value is higher than the rate with strain \( \text{N} \) as recipient and approaches the value obtained with strain 13. Transductants are lysogenic for \( \phi 34 \) and do not adsorb \( \phi 34.13 \). A 'cured' strain of \( \text{NH} \) which did not liberate \( \phi 13 \text{vir} \) or the bacteriocin behaved like \( \text{NH} \) in the above experiments.

The isolation of mutants of \( \phi 34.13 \) able to lyse strain \( \text{N} \)

The one experiment involving nitrosoguanidine treatment of the lysogenic strain 13 (\( \phi 34 \)) yielded three phages which formed plaques on strain \( \text{N} \). These phages are named \( \phi 34n-1, \phi 34n-2, \phi 34n-3 \). Phage \( \phi 13 \text{vir} \) antiserum did not neutralize the phages but a \( \phi 34.13 \) antiserum with a \( k \) value of 230 min.\(^{-1} \) neutralized all three phages at a rate of 220 min.\(^{-1} \). Electron microscopy of these three phages proved them to be morphologically identical to \( \phi 34.13 \) (see Prozesky et al. 1965). The three phages are regarded as mutants of \( \phi 34.13 \). Phages \( 34n-1 \text{N} \) and \( 34n-3 \text{N} \) plaque on strains 13, \( \text{N} \)

Table 8. Mixed infection of strain \( \text{N} \) with \( \phi 34n-2 \) and \( \phi 34.13 \)

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of bacteria</td>
<td>( 2 \times 10^9 )</td>
<td>( 2 \times 10^9 )</td>
<td>( 2 \times 10^9 )</td>
<td>( 2 \times 10^9 )</td>
</tr>
<tr>
<td>No. of p.f.u.</td>
<td>( 3 \times 10^{10} )</td>
<td>( 3 \times 10^{10} )</td>
<td>( 3 \times 10^{10} )</td>
<td>( 3 \times 10^{10} )</td>
</tr>
<tr>
<td>( 34n-2 \text{N} )</td>
<td>( 4'4 \times 10^8 )</td>
<td>( 4'4 \times 10^8 )</td>
<td>( 4'4 \times 10^8 )</td>
<td>( 4'4 \times 10^8 )</td>
</tr>
<tr>
<td>Unadsorbed phage</td>
<td>( 1'2 \times 10^8 )</td>
<td>( 1'8 \times 10^8 )</td>
<td>( 3'3 \times 10^7 )</td>
<td>( 3'3 \times 10^7 )</td>
</tr>
<tr>
<td>Free phage after antiserum treatment</td>
<td>( 8'8 \times 10^6 )</td>
<td>( 7'9 \times 10^6 )</td>
<td>( 6'6 \times 10^6 )</td>
<td>( 6'6 \times 10^6 )</td>
</tr>
<tr>
<td>Infective centres assayed on</td>
<td>( 1'6 \times 10^4 )</td>
<td>( 1'8 \times 10^4 )</td>
<td>( 1'3 \times 10^4 )</td>
<td>( 1'3 \times 10^4 )</td>
</tr>
<tr>
<td>Transmission</td>
<td>( 8 \times 10^{-6} )</td>
<td>( 9 \times 10^{-6} )</td>
<td>( 8'5 \times 10^{-6} )</td>
<td>( 8'5 \times 10^{-6} )</td>
</tr>
</tbody>
</table>

Stationary phase cells of strain \( \text{N} \) were used.

and \( \text{NH} \) while \( \phi 34n-2 \text{N} \) plates on the latter two strains but not on strain 13. The reason for this has not been investigated. The plaques of the former two phages on strains 13 and \( \text{N} \) are identical and resemble those of \( \phi 34.13 \) on strain 13. Phages \( 34n \) escape restriction in strain \( \text{N} \) and it was decided to investigate whether \( \text{N} \) bacteria impose a host specificity on the phages. Phage \( 34n-1 \) was grown on strains \( \text{N}, 13 \) and \( \text{NH} \) and the e.o.p. of the lysates tested on three strains (Table 7). An \( \text{N} \)-specific host-induced modification which must be carried by \( \phi 34n \) for it to form plaques on strain \( \text{N} \) was evident. This was not conferred upon \( \phi 34n \) when it was grown on strains 13 or \( \text{NH} \). Strain \( \text{NH} \) accepted \( \phi 34n-1.13 \) more efficiently than did strain \( \text{N} \) but it did not confer \( \text{N} \) host-specificity of \( \phi 34n-1 \). Phage \( 34n-1.13 \) transduced various markers to auxotrophs of strain \( \text{N} \) at a frequency equal to that of \( \phi 34.13 \text{str-r} \), i.e. \( 10^{-7} \) p.f.u. adsorbed although the former phage had an e.o.p. of \( 10^{-8} \) on strain \( \text{N} \) (Table 7), while the latter formed no plaques on it. Many of the \( \text{N} \) transductants were lysogenic for \( \phi 34n-1 \) and were able to adsorb the phage. It has not been demonstrated that the \( n \) mutation affects phage conversion (Coetzee, 1961) as well as host-controlled modification. Phage \( 34n-1 \) may carry two mutations which control the two properties separately.
Mixed infections of strain N with restricted and unrestricted φ34

The inability of φ34n-2.N to plate on strain 13 was used to study the fate of this unrestricted phage and φ34.13 in mixed infections of strain N. Transmission of φ34n-2.N remained unchanged despite a great excess of φ34.13 (Expts 3 and 4, Table 8). Furthermore φ34n-2.N did not affect the transmission of φ34.13 in strain N.

DISCUSSION

The restricting principle of strain N was apparently not removed by converting cells to spheroplasts. Takano et al. (1968) found the restricting enzyme of φ-R factors in Escherichia coli K12 for phage λ to be retained by spheroplasts and the DNA's of bacteriophage fd are restricted by spheroplasts of E. coli F' + and E. coli (φ1) strains (Benzinger, 1968). Molholt & Fraser (1965) and Schell & Glover (1966) conclude that a surface-localized enzyme plays an essential role in the host-controlled restriction of phage λ.C by E. coli strain K (φ1) (see also Kerszman et al. 1967). Watanabe et al. (1966) also implicate an enzyme located near the cell surface to explain restriction of phages λ and φ22 by φ-R factors.

The two phages 34.13 and 34n-1.13 do not affect each other in mixed infection. This situation may be accounted for by the ‘passive’ model of Kerszman et al. (1967) which would have it that the DNA of φ34.13 carries nucleotide sequences susceptible to restricting enzyme(s) of strain N. The n mutation in φ34.n-1 changed the sequence and rendered its DNA resistant to degradation. Stationary phase cells appeared to restrict the phage less severely than log. phase cells. Particular physiological conditions of cells may cause weakness in restriction of phages (Bertani & Weigle, 1953; Uetake, Toyama & Hagiwara, 1964; Holloway, 1965; Rolfe & Holloway, 1968) and Luria & Human (1952) encountered this condition with the use of stationary phase cells. Benzinger (1968) explains the weaker restriction of phage fd infectious DNA by spheroplasts compared to that of intact fd phage in cells of E. coli as due to the greater age of spheroplasts. Reference to Tables 1 and 2 shows that while strain N confers its specificity on φ34n-1 it does not do so to φ34.13. In Escherichia coli strain W a superficially similar situation exists where λ.C is restricted without being modified but w mutants are restricted and host-modified. Kerszman et al. (1967) have shown that the restriction of λ.C in w cells is a property of a prophage wφ while the fate of λw mutants is controlled by the genome of strain W. In Proteus mirabilis strain N a search for plasmids which could account for its restricting and modifying properties has revealed an inducible prophage and a phage tail-like bacteriocin, neither of which appears to be implicated. The mutant NH1 of strain N accepts φ34n-1.13 only about as efficiently as strain N (5 x 10⁻⁵) and does not confer N host specificity on either φ34 or φ34n-1. It is obviously a complex mutant affecting the processes of restriction and modification normally controlled by the N genome.

Thorne (1962) isolated a generalized transducing phage SP-10 which transduces Bacillus subtilis strain 168 (indole-) to prototrophy at a rate of 4.8 x 10⁻⁵/p.f.u. but does not form plaques on it. In this respect it resembles the system described here. Arber (1964) and Watanabe et al. (1966) found that the transduction frequency to restricting hosts was less severely affected than reduction of e.o.p. of transducing lysates on these hosts.
Proteus phage restriction

The degradation of strain 13 bacterial chromosomal fragments associated with the transducing particles of φ 34.13 in strain N has not been proved but the fact that the transduction rate (and p-lac + acceptor ability) is restored to normal in non-restricting mutants of strain N would favour such a view. The initial rise in transduction frequencies (Coetzee & Sacks, 1960b) may be interpreted to mean that slight irradiation facilitates the process of integration (Jacob & Wollman, 1958; Takebe, 1968). If donor fragments are simultaneously degraded by restricting principles in the recipient cell the former process may be annulled by lack of available fragments (Fig. 1). The fact that the transduction frequency by φ 34.13str-r is increased by mixed infection of N with φ 34n-1.N (Table 5) may mean that the str-r bacterial marker is rescued by the non-restricted phage (Arber, 1964). In Escherichia coli (Arber, 1964; Lederberg, 1965; Wood, 1966; Eskridge, Weinfeld & Paigen, 1967) and in Salmonella typhimurium (Watanabe et al. 1966) host-controlled modification is a general condition, in the sense that all genetic material from a donor strain is restricted in recipient strains which restrict the DNA of phages grown on the donor strain. The same situation may apply to the strains of Proteus mirabilis studied here.

The high rate of transduction of markers by φ 34.13 into N could mean that at any particular time a large number of N cells must be physiologically r-. Although transductant formation (and p-lac + acceptance) does not involve phage transmission in the sense that plaque formation does, the complete absence of the latter is surprising. Another explanation may be that bacterial chromosomal genes in transducing phage particles and p-lac + DNA have a greater chance of surviving the restriction process than φ 34.13 DNA. Watanabe et al. (1966) have invoked such an explanation to account for results obtained with restricting fi-R factors for the transducing phages λ and ϕ 22.

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Proteus phage restriction


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