The Infection of *Escherichia coli* with Lambda (λ_{22}) Phage and the Establishment of Lysogeny

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SUMMARY

Infection of *Escherichia coli* by phage λ_{22} in 0.02 M-Sr(NO₃)₂, CaCl₂ or MnSO₄ occurred as readily as in 0.02 M-MgSO₄. The response of bacteria infected in the presence of Sr²⁺, Ca²⁺ and Mg²⁺ was predominantly lysogenic, but infection in 0.02 M-Mg²⁺ gave a higher lysogenic response (90%) than with the other cations tested. With Mn²⁺ in optimal conditions, at least 25% of the infected bacteria always gave the lytic response. When the newly infected bacteria were transferred to a growth medium, a period of about 40 min. had to elapse before the prophage could be detected by zygotic induction by mating with a non-lysogenic *F*⁻ organism. A period of about 100 min. was required before all the bacteria giving the lysogenic response could be induced by ultraviolet radiation.

INTRODUCTION

Lambda (λ) phage will adsorb well to washed organisms of *Escherichia coli* in the presence of magnesium sulphate (Kaiser, 1957), and for *E. coli* strain K112 and phage λ_{22} conditions have been found (0.02 M-MgSO₄ at about pH 6.5) in which about 99% of the bacteria were infected (Fry, 1959); 10% of the infected bacteria eventually lysed and 90% became lysogenic for phage λ_{22} . The factors which control the decision of whether a bacterium gives a lytic or lysogenic response and the biochemical events which lead to the establishment of the prophage in those bacteria which give a lysogenic response are largely unknown. It appeared from earlier work that the composition of the adsorption medium may affect the response of the cell to infection with phage λ_{22} (Fry, 1959). Since more than one type of cation will aid the irreversible adsorption of various T phages to *E. coli* strain B (Puck, Garen & Cline, 1951), the adsorption of phage λ_{22} in the presence of ions other than Mg²⁺ was therefore studied.

After infection with a temperate phage, the metabolism of bacteria which are to become lysogenic seems to be profoundly disturbed and when the bacteria are placed in a growth medium one reflexion of this disturbance is a long delay before division occurs (Lieb, 1953; Fry & Gros, 1959). Moreover some bacteria may divide more than once before the prophage is fully established when no more non-lysogenic progeny segregate (Luria, Fraser, Adams & Burrows, 1958). It would be of interest to know how soon after infection the genetic material of the phage is established as a prophage on the bacterial chromosome. By using λ phage labelled with ³²P and then studying the effect of ³²P decay on the establishment of lysogeny, Stent & Fuerst (1956) found that 30 min. after infection the genome of the phage

was in a state such that it was no longer susceptible to ^{32}P decay. However, this does not prove directly whether the prophage has in fact become associated with bacterial chromosome at this time. In order to establish when the latter event has taken place, other properties of the prophage might be exploited. For example, samples of the bacteria can be taken at intervals after infection and irradiated with ultraviolet (u.v.) light in order to determine whether those bacteria which ultimately give the lysogenic response are in an inducible state at the time of sampling. Use may also be made of the phenomenon of zygotic induction (Jacob & Wollman, 1956), which is observed when an Hfr strain lysogenic for phage λ is mated with a non-lysogenic F^- strain, since on entry into the F^- organism, the prophage undergoes spontaneous induction. Jacob & Wollman (1956) established that only phage genetic material attached to the bacterial chromosome, i.e. in the form of prophage, can be transferred to the F^- strain. By using such techniques, studies have now been made to see how soon after infection the establishment of the prophage can be recognized.

METHODS

Organisms. *Escherichia coli* strains $\kappa 112$, HfrC-Wollman, w678 and the indicator organisms for phage λ (strains c600 Sr^+ and $\kappa 112$ Sr^+ , streptomycin resistant) were from the culture collection of Drs F. Jacob and E. Wollman of the Pasteur Institute, Paris. Strain HfrC-Wollman requires thiamine and is sensitive to streptomycin and phage T_6 (HfrC B_1^- Sr^+ T_6^+); strain w678 is F^- , requires threonine + leucine + thiamine, and is resistant to streptomycin and to phage λ (w678 T^- L^- B_1^- Sr^+ λF^-); a mutant resistant to phage T_6 (T_6^+) was isolated by the author; strain $\kappa 112$ is F^+ .

Phage. The λ phage used was a wild type designated as λ_{22} (see Fry, 1959).

Growth medium. Nutrient broth (medium A), pH 7, contained (% w/v): 1, Oxoid peptone; 0.1, Liebig's meat extract; 1, NaCl. For plating procedure, 1% agar (Parke Davis) was added (medium B). For the detection of lysogeny (medium C), medium B was supplemented with 1% lactose and an indicator solution (2 ml./100 ml. medium). The indicator solution contained 5 mg. thymol blue, 12.5 mg. methyl red, 60 mg. bromthymol blue/100 ml. Defined medium, for the assay of T^+L^+ recombinants in the mating experiments, was that described by Hayes (1957) supplemented with 100 μg . streptomycin/ml.

Experimental techniques. The detection of response of λ -infected bacteria, preparation of phage stocks and of washed bacterial suspensions and the measurement of phage adsorption have been described previously (Fry, 1959). The response to infection was calculated from the mean of two plates, and refers in each experiment to the same dilution of the original suspension. The percentage response is expressed relative to the total number of bacteria giving the lytic + lysogenic + refractory response. The mating experiments for obtaining zygotic induction followed the procedures of Jacob & Wollman (1956). In the u.v. irradiation of bacteria, a Hanovia Chromatolite low pressure Hg. lamp (Hanovia Ltd., Slough, Bucks.) without the filter, was used at 75 cm. from the sample (about 7 ml.) contained in an open Petri dish (9 cm. diam.). Immediately after irradiation, some of the bacteria appear to be in an unstable state, and unless they are allowed to metabolize in broth for a period, the outcome of the irradiation procedure can be reversed, e.g. by plating. Thus

bacteria which would otherwise appear to be induced, will survive, remain viable and form lysogenic colonies. Consequently, after irradiation, samples were usually not plated immediately but incubated in broth for 45 min. in order to stabilize the response to u.v. irradiation (cf. Weigle & Delbrück, 1951). The procedure for single step curves was that of Ellis & Delbrück (1939). Concentrations of solutes refer to final concentrations. All incubations were done at 37°.

RESULTS

Assay of response to infection with λ phage

Bertani (1960) suggested that plate assays for the detection of response to phage infection would be upset if an appreciable number of the bacteria occurred in chains of two or more organisms. If, for example, only one organism of a chain were infected and gave the lytic response and lysed on the plate, one or more of the remaining bacteria of the chain might thus undergo multiple infection and these are conditions which favour the establishment of lysogeny. In these circumstances a true lytic response would in fact be recorded as a lysogenic response. Examination of cultures of *Escherichia coli* κ 112 at the time of harvesting for the preparation of washed

Table 1. *Response of Escherichia coli* κ 112 to infection with λ phage in different concentrations of MnSO_4 , SrNO_3 and CaCl_2 .

In each experiment samples of the same washed suspension (about 2×10^8 bacteria/ml.) of *E. coli* κ 112 were infected with λ phage in MnSO_4 , SrNO_3 or CaCl_2 at final concentration and input multiplicities shown in the table. Adsorption period: 10 min. Numbers are the mean of three plates and refer to same dilution of original suspension of infected bacteria. Percentage response is expressed relative to the total number of cells giving the lytic, lysogenic and refractory response. Expt. 4 shows effect of multiplicity of infection (in 0.02 M- MnSO_4) on type of response. Multiplicities of infection shown in parentheses and calculated from phage remaining unadsorbed at end of 10 min. in sample from mixture with lowest input multiplicity (found: 14 % phage unadsorbed) and assuming that bacteria giving refractory response did not adsorb λ phage.

Expt. no.	Infection medium	Input multiplicity (λ particles/bacterium)	Type of response					
			Lytic		Lysogenic		Refractory	
			No.	%	No.	%	No.	%
1	0.02 M- MgSO_4	10	7	6	104	91	3	3
	0.02 M- MnSO_4	10	54	46	52	45	11	9
	0.01 M- MnSO_4	10	41	38	58	54	9	8
	0.004 M- MnSO_4	10	9	7	11	9	101	84
2	0.02 M- MgSO_4	17	6	6	85	90	4	4
	0.02 M- $\text{Sr}(\text{NO}_3)_2$	17	15	15	82	81	4	4
	0.01 M- $\text{Sr}(\text{NO}_3)_2$	17	13	12	63	58	32	30
	0.004 M- $\text{Sr}(\text{NO}_3)_2$	17	3	3	8	7	97	90
3	0.02 M- MgSO_4	12	21	17	100	79	6	4
	0.02 M- CaCl_2	11	32	22	109	76	3	2
	0.01 M- CaCl_2	11	56	37	88	58	7	5
	0.004 M- CaCl_2	11	9	5	3	2	166	93
4	0.02 M- MnSO_4	16.8 (16.4)	31	34	50	54	11	12
		8.4 (8.0)	20	21	63	69	9	10
		4.2 (4.4)	22	23	56	58	19	19
		0.8 (1.8)	9	9	32	31	62	60

suspensions showed that less than 5% of the total count of organisms was in chain form. Further evidence that the plate assay technique used gave a true record of response was obtained by determining the response by plate assay, and also by incubating a sample in liquid medium at 37°, and taking samples at intervals for a standard one-step curve of the number of plaque-forming units present. Phage released by lysis of bacteria in the second growth tube (nomenclature of one-step curve technique) should remain free and could be assayed accurately. If an appreciable number of the bacteria present were in chains and were giving a true lytic response, but appeared as lysogenic on the lactose + indicator plates (medium C) or if samples of the first growth tube were plated on medium B, then one would

Fig. 1

Fig. 2

Fig. 1. Determination of yield of phage particles/bacterium giving the lytic response. Washed suspension of *Escherichia coli* κ 112 (1×10^8 bacteria/ml.) infected in 0.02 M-MgSO₄ with λ phage (1.6×10^8 particles/ml.). After 10 min., sample of infected bacteria transferred to λ phage antiserum in broth (10 min.) and then first and second growth tubes prepared (dilutions of 10^{-4} and 2×10^{-6} respectively). Samples taken at intervals and plated with indicator bacteria on medium B. Results are expressed as plaque-forming units corresponding to 0.1 ml. of first growth tube (FGT). Overall response to infection in an equivalent sample determined after 20 min. showed (bacteria/sample) 90 lytic, 820 lysogenic and 20 refractory.

Fig. 2. Rate of adsorption of phage λ to *Escherichia coli* κ 112 in 0.01 M-MnSO₄ (×), 0.02 M-MnSO₄ (○) or 0.02 M-MgSO₄ (●). Washed suspension (2.5×10^8 bacteria/ml.) mixed at 37° with equal volume of phage suspension in MnSO₄ or MgSO₄ solution. Samples taken at intervals to determine unadsorbed phage, P , at time t ; P_0 = concentration of phage at time 0. Bacteria+phage suspension at pH 6.6; input multiplicity 2. Bacteria lytic:lysogenic:refractory response (%) at 15 min. was: in 0.02 M-Mn²⁺, 46:19:35; in 0.02 M-Mg²⁺, 18:64:28; in 0.01 M-Mn²⁺, 17:14:69.

expect that the calculated yield of phage particles/bacterium giving the lytic response would be greater than normal, since the number of lytic bacteria would be underestimated. In fact the yield was found to be 84 λ particles/bacterium (Fig. 1),

which is similar to that found by other workers (Jacob & Wollman, 1954). After infection, the decision of a bacterium to give the lysogenic response or the lytic response may be altered by cultural conditions (cf. Lieb, 1953) and it is possible that the small percentage of bacteria which show the lytic response after infection in 0.02M-MgSO₄ (cf. Table 1) is in fact due to bacteria which would become lysogenic if they were maintained in liquid medium and not subjected to the temperature shocks, etc., involved in plating procedures. However, the production of free phage by such a population of newly infected bacteria during their first 80 min. in broth, and the order of the phage yield/bacterium giving the lytic response, together provide evidence for the conclusion that the plate assay gives a true measure of the response to infection with phage λ .

Response to adsorption of phage λ in the presence of Mn²⁺, Sr²⁺ and Ca²⁺

Samples of the same washed bacterial suspension were infected with λ phage in the presence of different concentrations of Mn²⁺ or in 0.02M-MgSO₄ (control). About 90 % of the bacteria were infected when the absorption medium was 0.02M- or 0.01M-MnSO₄, and 16 % when the concentration was 0.004M (Table 1, expt. 1). After adsorption in the presence of MgSO₄, 91 % of the bacteria were lysogenic but only about half of the bacteria infected in MnSO gave a lysogenic response; the remainder gave the lytic response (Table 1). Irrespective of the multiplicity, infection in the presence of Mn²⁺ always yielded an appreciable percentage of bacteria which gave the lytic response (Table 1, expt. 4). Studies of the rate of uptake of phage λ in the presence of MnSO₄ (0.01M and 0.02M) compared with uptake in the presence of 0.02M-MgSO₄, showed that uptake in 0.02M-MnSO₄ took place more quickly (60 % faster) than in 0.02M-MgSO₄ (Fig. 2). Once again the bacteria infected in Mn²⁺ tended to give the lytic response whereas those infected in Mg²⁺ were mainly lysogenic. Uptake of λ phage in the presence of 0.01M-MnSO₄ was comparatively poor (Fig. 2). The effect of different concentrations of Sr²⁺ (as SrNO₃) and of Ca²⁺ (as CaCl₂) was also studied. Bacteria infected in presence of either of these cations tended to respond like bacteria infected in Mg²⁺, i.e. when the cation was 0.02M about 95 % of the bacteria were infected and most of these (75–80 %) gave the lysogenic response (Table 1, expts. 2 and 3), when exposed to a high multiplicity of λ phage. There has always been the possibility that infection in the presence of a cation other than Mg²⁺ might yield a lysogenization rate of nearly 100 %, but conditions for this ideal situation have not yet been found. No evidence has been found that the type of anion affects the adsorption of λ phage.

Use of zygotic induction to detect when prophage becomes established in bacteria giving a lysogenic response

Organisms of *Escherichia coli* strain HfrC B₁⁻ Sr⁺ T₆⁺ were infected with phage λ_{22} in conditions such that a large proportion of the infected bacteria gave the lysogenic response (assayed by the usual method; the response was found to be 9 % lytic, 80 % lysogenic, 11 % refractory). The infected bacteria were then incubated in broth + antiserum to λ phage and samples taken at intervals and incubated with *E. coli* w678-T⁻L⁻B₁⁻Sr⁺T₆⁺F⁻ in broth + λ phage antiserum and mating allowed to continue for 20 min. A sample of the mating mixture was then transferred to

broth + 0.02M-KCN + λ phage antiserum. After 30 min., samples were diluted and plated (a) for T⁺L⁺ recombinants and (b) with the indicator organism *E. coli* K112 SrF⁺ for plaques. Meanwhile other samples of the infected Hfr bacteria were taken at intervals, transferred directly to broth + KCN + λ phage antiserum for 30 min., and then diluted and plated with indicator organisms to give the number of plaques produced by unmated Hfr bacteria. The number of T⁺L⁺ recombinants produced by uninfected HfrC bacteria taken through the same experimental procedure

Table 2. *Zygotic induction with Escherichia coli strain HfrC in which lysogeny for λ phage is being established*

Washed suspension (1×10^8 bacteria/ml.) of *E. coli* HfrC infected in 0.02M-MgSO₄ with λ phage (multiplicity 10 λ particles/bacterium), and after 10 min. a sample transferred to broth + λ phage antiserum (culture A). Samples (0.1 ml.) of latter taken at intervals and mated with F⁻ strain in broth + antiserum (0.9 ml. containing 2×10^7 *E. coli* W678/ml.). After mating for 20 min., a sample was transferred to broth + KCN + antiserum for 30 min., and then diluted in saline and plated for (a) plaque formation, (b) recombinants (T⁺L⁺) able to synthesize threonine and leucine. Uninfected = T⁺L⁺ recombinants produced by uninfected Hfr bacteria taken through same procedure. Samples of infected Hfr bacteria taken at intervals from culture A, incubated in broth + KCN + antiserum for 30 min., and then plated for plaque formation. Time after infection is the time from when infected bacteria were placed in culture A. Plaques and T⁺L⁺ recombinants = no./plate. Other details in text.

Bacteria mated			Bacteria not mated		Plaques due to zygotic induction (A - B)
Time after infection (min.)	Plaques (A)	T ⁺ L ⁺ recombinants	Time after infection (min.)	Plaques (B)	
0	123	65	20	150	—
10	121	140	30	145	—
20	172	164	40	197	—
30	146	174	50	135	9
40	182	223	60	71	111
50	203	251	70	64	139
60	177	295	80	65	112
	Uninfected	198			

(i.e. incubation in 0.02M-MgSO₄, mated for 20 min. and then cyanide broth treatment), was used to control the effect of infection with λ phage on the mating properties of strain HfrC. The broth containing KCN was used to eliminate Hfr bacteria containing mature phage particles (method of Doerman, 1952; used for λ phage by Weigle & Delbrück, 1951), since in preliminary experiments without this step, lysis of some of the Hfr bacteria giving the lytic response occurred during the plating procedure and caused unexpectedly large numbers of plaques on the plates. Plating Sr⁻-Hfr bacteria infected with λ phage without mating (column B, Table 2), leads to the formation of some plaques, even in presence of streptomycin. This was not due to mating on the plate, and it is concluded that phage development in some Hfr bacteria was not halted by plating on streptomycin media. The mating period was 20 min. in nutrient broth, and during this time it is reasonable to expect that development of λ phage still continues in Hfr bacteria giving the lytic response. Consequently, plaque formation due to zygotic induction is taken as the difference between the number of plaques produced by mating a sample of the Hfr bacteria taken *t* min. after infection and the number produced by unmated Hfr bacteria

taken $t+20$ min. after infection. If the prophage is established in the Hfr bacteria very soon after infection (i.e. within a few minutes), then the number of plaques produced by zygotic induction after a mating period of 20 min. should be maximal, and should not vary with the time after infection at which the Hfr sample was taken for mating with the F^- organism. If, however, an appreciable period must elapse after infection before the prophage is established in the Hfr bacteria, then the number of plaques should increase to a maximum, as the samples of Hfr bacteria are taken for mating at progressively longer intervals after infection. The number of plaques reached a maximum when the Hfr bacteria had been allowed to metabolize for some 40 min. in broth before being mated with the F^- organism (Table 2). After 10 min. in broth, the infected bacteria yielded 70 % of the number of T^+L^+ recombinants produced by uninfected bacteria (Table 2). It is therefore concluded that the mating properties of Hfr bacteria newly infected with phage λ were not disturbed to any appreciable extent.

Effect of ultraviolet radiation on bacteria in which lysogeny for λ phage is being established

When the genome of λ phage in an infected bacterium has reached the prophage state then, provided the aptitude of the bacterium has not been upset (Lwoff, 1953), it is to be expected that exposure of such a bacterium to u.v. radiation would lead to the induction of phage development. Irradiation of the bacteria after infection with phage λ_{22} , but before they were placed in a growth medium, showed that the effect of u.v. radiation was to cause a decrease in the number of bacteria which gave the lysogenic response and, especially for the lower doses of u.v. radiation, most of this decrease was correlated with an increase in the number of bacteria which gave the lytic response (Table 3). Though at first sight this result might appear to be due to the induction of truly lysogenic bacteria, this is regarded as being unlikely, since irradiation of newly infected bacteria after they had been for 20 min. in a growth medium showed that although the irradiation caused a decrease in the number of bacteria which gave the lysogenic response, this was only correlated with a relatively small increase in the number of bacteria which gave the lytic response (Table 3). Moreover it has already been shown (Fry, 1959) that when the bacteria are u.v. irradiated before infection, then depending on the dose received, some of the bacteria which are no longer capable of giving the lysogenic response when infected with phage λ_{22} give a lytic response. It is therefore likely that the effect of u.v. irradiation on the phage+bacterium complex in newly infected bacteria before transfer to a growth medium is the same as that found when the bacteria are first u.v. irradiated and then immediately infected with phage λ_{22} , i.e. in both cases, the u.v. radiation acts on a target in the bacterium which is distinct from phage material, and thus disposes the bacterium to the vegetative reproduction of phage.

In a further experiment, bacteria infected in $0.02M$ - $MgSO_4$ with phage λ_{22} were transferred after 10 min. to complete medium containing λ phage antiserum. Samples were taken at intervals and u.v.-irradiated with a dose known to give 90–100 % induction of the parent *Escherichia coli* K112 lysogenic for phage λ_{22} . Inspection of Table 4 reveals that not until 100 min. had elapsed did the number of bacteria giving a lytic response after irradiation become equivalent to the number

Table 3. *Effect of exposure of λ -infected Escherichia coli to ultraviolet radiation immediately after infection with λ phage and after a short period in complete medium*

Washed suspension of *Escherichia coli* κ 112 (2.6×10^8 bacteria/ml.) mixed with an equal volume of λ phage (2.7×10^9 particles/ml.) in 0.02M-MgSO₄ (average multiplicity 10 λ phages/bacterium). After 10 min. sample A of infected bacteria was taken and irradiated with doses of u.v. radiation and then incubated in λ phage antiserum in broth for 45 min., diluted in broth and plated. Meanwhile sample B of the infected bacteria was transferred to broth+antiserum, incubated for 20 min. and then diluted 1/10 with mineral salts medium and irradiated as required. Samples were taken and incubated in broth for 45 min. and then plated. Unirradiated controls were not incubated for the additional 45 min. Platings were made with indicator bacteria on medium C. 'Induction' is percentage of lytic responses relative to lysogenic bacteria in corresponding unirradiated control. The effects of the same dose of u.v. radiation on the survival of uninfected *E. coli* κ 112 and on the induction of *E. coli* κ 112 (λ_{22}) in equivalent conditions are given for comparison.

Time of u.v. irradiation (sec.)	Type of response			Survival of lysogenic bacteria (%)	'Induction' (%)	Survival of strain κ 112 (%)	Induction of strain κ 112 (λ_{22}) (%)
	Lytic	Lysogenic	Refractory				
Sample A							
0	9	115	2	—	—	—	—
10	34	77	10	67	30	84	17
20	60	51	4	44	52	69	34
30	52	35	13	30	45	61	58
60	87	5	6	4	76	34	97
Sample B							
0	6	103	2	—	—	—	—
10	13	84	2	82	13	—	—
20	20	63	11	61	19	—	—
30	22	52	2	51	21	—	—
60	44	22	2	21	43	—	—

Table 4. *Effect of optimal induction dose of u.v. radiation on Escherichia coli κ 112 in which lysogeny for λ phage is being established*

Washed suspension of *Escherichia coli* κ 112 (2.6×10^8 bacteria/ml.) mixed with an equal volume of phage λ_{22} suspension (2.3×10^9 particles/ml.) in 0.02M-MgSO₄ (average multiplicity 9 λ phages/bacterium), and after 10 min. sample of infected bacteria transferred to λ phage antiserum in broth and incubated. Samples of the latter were diluted at appropriate times in broth and one portion diluted further and plated immediately ('unirradiated' in table) whilst another portion was irradiated with a dose of u.v. radiation which would normally induce at least 90% of strain κ 112 (λ_{22}). The irradiated bacteria were transferred to broth+antiserum, incubated for 45 min. to stabilize response to irradiation and then plated. Other details as in Table 3.

Time (min.)	Unirradiated		u.v. irradiated		'Induction' (%)
	Lytic	Lysogenic	Lytic	Lysogenic	
10	10	77	38	11	49
35	7	85	44	2	52
60	19	97	61	1	63
80	16	121	95	1	79
100	5	140	139	0	99
120	4	202	206	4	102
140	0	348	336	8	111
160	0	606	562	4	93

of bacteria giving the lysogenic response in the unirradiated samples. Thus an appreciable time appears to be required before the prophage is in an inducible state in all the bacteria which become lysogenic. With samples taken earlier than 100 min. the decrease in the number of lysogenic bacteria in the irradiated culture was less than the increase in the number of bacteria which gave the lytic response. This indicates that for about 80 min. after infection, some bacteria which gave the lysogenic response were in such a state that u.v. irradiation produced a lethal effect and did not induce bacteria in the normal manner.

Effect of chloramphenicol on the establishment of lysogeny for λ phage

In certain systems, treatment with chloramphenicol is reported to alter the response of bacteria infected with temperate phages. Thus with *Salmonella typhimurium* and phage P22, chloramphenicol added early after infection caused 'curing' of the bacteria, but when added several minutes later, it brought about an increase in the number of bacteria which became lysogenic (Ting, 1960). With

Table 5. *Effect of chloramphenicol on the establishment of lysogeny in Escherichia coli* κ 112

Washed suspension of *Escherichia coli* κ 112 infected with λ phage in 0.02M-MgSO₄ and one sample transferred to broth and one to broth containing chloramphenicol (30 μ g./ml.). Samples taken at appropriate times, diluted ten times with 0.9% NaCl and one portion plated immediately whilst another was irradiated and then incubated for 45 min. in broth to stabilize response and then plated. All broths contained λ phage antiserum. Other details in table. Dose of u.v. radiation for 60 sec. will give 90–100% induction of *E. coli* strain κ 112 (λ_{22}) in similar conditions.

Treatment after infection	Time of u.v. irradiation (sec.)	Type of response		
		Lytic	Lysogenic	Refractory
Broth (10 min.)	0	2	76	3
	60	9	22	1
Broth + chloramphenicol (45 min.)	0	64	14	2
	60	45	17	2
Broth + chloramphenicol (45 min.) then broth (10 min.)	0	18	49	3
Broth (30 min.)	0	1	73	3
	60	9	26	2
Broth (30 min.) then broth + chloramphenicol (45 min.)	0	4	56	5
	60	1	28	3
	120	3	10	2

Shigella dysenteriae infected with phage P1, the addition of chloramphenicol at the time of infection or 10–15 min. later caused the prompt establishment of lysogeny (detected by immunity to superinfection by phage T₁) in those bacteria which gave the lysogenic response to infection (Christensen, 1960). Studies of the effect of chloramphenicol on *Escherichia coli* infected with phage λ_{22} did not show any comparable effects. When *E. coli* infected in 0.02M-MgSO₄ were transferred to broth + chloramphenicol (+ λ phage antiserum) for 45 min. and then plated to determine their response to infection, although there was no curing of the bacteria from phage infection, there was a marked increase in the number of bacteria which gave the lytic response and a decrease in those which were lysogenic (Table 5). This result indicates that immediately after infection, the bacteria were

in a labile state in the sense that environmental conditions could still affect their type of response. When newly infected bacteria were transferred to broth (i.e. with no chloramphenicol) and plated after only 2 min., the lytic response was greater than with samples taken after 5 min. or later. (A period less than 2 min. in broth is difficult to study, since sufficient time must be allowed for inactivation of unadsorbed phage by antiserum before plating.) Evidently when bacteria can metabolize normally, a change occurs in the first few minutes which stabilizes the decision to become lysogenic and nullifies the adverse effects of the plating procedure. The predominantly lytic response of bacteria plated immediately after exposure to broth + chloramphenicol for 45 min. might mean that either this change had not taken place in these bacteria or it had not been irreversibly established. Since chloramphenicol inhibits protein synthesis but not the synthesis of ribo- and deoxyribonucleic acids (Gros & Gros, 1958), it may tentatively be concluded that the first step in the decision to become lysogenic involves the synthesis of protein. Treatment with chloramphenicol for 45 min. immediately after infection had no long lasting detrimental effect, since such bacteria after incubation for only 10 min. in broth in the absence of chloramphenicol showed the expected predominantly lysogenic response (Table 5). Treatment of infected bacteria with chloramphenicol for 45 min. after they had first been incubated in broth alone for 30 min. was without significant effect; most of the bacteria still give the lysogenic response on plating. When u.v. irradiation is used to detect an established prophage, then it is clear that treatment of bacteria with chloramphenicol even 30 min. after infection did not lead to the immediate establishment of lysogeny, since although the irradiation caused a decrease in the number of bacteria which gave the lysogenic response, this was not correlated with a corresponding increase in those which gave the lytic response (Table 5). Indeed the results were identical with those discussed previously (p. 303). Furthermore, it may be concluded that even after 30 min. in a complete growth medium, the processes which enable the bacteria giving the lysogenic response to become fully inducible could not take place (or continue) in the presence of chloramphenicol.

DISCUSSION

The experiments concerned with the adsorption of phage λ_{22} to *Escherichia coli* in the presence of inorganic cations showed that Sr^{2+} , Ca^{2+} and Mn^{2+} can replace Mg^{2+} (Table 1). It is well known that although these ions are related and have several properties in common, the similarities between Mg^{2+} , Sr^{2+} and Ca^{2+} are greater than those between Mg^{2+} and Mn^{2+} . It is therefore not surprising that whilst all of them will aid the infection of *E. coli* by phage λ_{22} , the results with Sr^{2+} and Ca^{2+} are more akin to those obtained with Mg^{2+} than with Mn^{2+} . However, whereas 75–80 % of the bacteria infected in the presence of Ca^{2+} and Sr^{2+} become lysogenic, infection in optimal conditions in the presence of Mg^{2+} usually gives a 90 % lysogenic response, and Mg^{2+} is still the cation of choice for infection in defined conditions. Adsorption of phage λ to sensitive bacteria can take place in broth (cf. Jacob & Wollman, 1954), which usually contains Mg^{2+} , though not at a concentration of 0.02M. Unpublished experiments by the author with single amino acids at different concentrations have not shown any amino acid which by itself promoted the adsorption of phage λ_{22} to *E. coli* strain K112.

Bacteria which are becoming lysogenic for λ phage show a delay in cell division and disturbance of their metabolism (Fry & Gros, 1959). These findings could be explained on the basis that they reflect the time required for the prophage to become established or, that the prophage may be established soon after infection but the events connected with this process cause a profound upset in the metabolism of the host bacterium which takes an appreciable time to recover. From the results of the chloramphenicol experiments (Table 5) it is tentatively suggested that in the decision to become lysogenic there is a primary reaction which involves protein synthesis rather than the synthesis of nucleic acid.

The two tests used here to detect establishment of the prophage, namely zygotic induction and induction of prophage by u.v. irradiation, are admittedly indirect. Meaningful results are only obtained if, immediately after establishment of the prophage, the physiological state and metabolism of the host bacteria are not abnormal. The mating potential of newly infected Hfr bacteria is somewhat less than that of non-infected bacteria as shown by the yield of T⁺L⁺ recombinants. Nevertheless, 10 min. after infection it is 70 % of that produced by the uninfected control bacteria, and since the prophage is carried at a definite locus on the bacterial chromosome (Jacob & Wollman, 1958), it is difficult to understand how, if the prophage were established very soon after infection (i.e. within 5–10 min.), transfer of prophage genetic material could not take place whilst recombination for T⁺L⁺ genes proceeded more or less normally. From the zygotic induction experiment it is therefore concluded that establishment of the prophage takes place about 40 min. after infection (Table 2). The time is not precise, because prophage material may be added to the Hfr chromosome during the mating period up to the time of entry of the prophage locus into the F[−] organism. The results of the zygotic induction experiment would be more convincing if all the Hfr bacteria could have been eliminated from the sample plated with the indicator organism, so that any plaque formation observed in such conditions would unequivocally be due to phage development in the F[−] bacteria alone. This was attempted by using a phage T₆ Hfr strain and a w678 T₆ F[−] mutant, and treating samples of the mating cultures with a high multiplicity of phage T₆ (sufficient to cause lysis from without) in the presence of λ phage antiserum (method of Hayes, 1957) before plating for recombinants and plaque formation. However when this method was used in trial experiments with strain T₆ HfrC lysogenic for phage λ_{22} , the number of plaques due to zygotic induction was markedly less than in the control where the procedures of Jacob & Wollman (1956) were followed and platings were made directly on media containing streptomycin. There are two possible explanations of these results. With this Hfr strain the gene for T₆ sensitivity precedes entry of genetic material of the prophage into the F[−] bacterium (Jacob & Wollman, 1958), and a large number of the F[−] bacteria may thus become sensitive to phage T₆ and would consequently be eliminated by the Hayes-T₆ method before being plated. However, if this were so, one would also expect the number of T⁺L⁺ recombinants to show a significant decrease in the T₆-treated samples, and this was not observed. It is therefore possible that the T₆ phages inject their DNA into the T₆ F[−] organism, and although incapable of T₆ production, this DNA interferes with the vegetative development of λ phage in those bacteria in which zygotic induction is taking place (cf. Weigle & Delbrück, 1951).

In the induction experiment, bacteria which showed the lysogenic response were not all inducible until about 100 min. after infection. Though this time differs from that obtained in the mating experiment, it is in keeping with the observation (Fry & Gros, 1959) that bacteria in which lysogeny is being established do not grow and metabolize normally until 60–80 min. after infection. So that, even though the prophage may be established in about 40 min., detection by induction might not be possible because the aptitude of at least some of the bacteria for induction and phage development was not normal (cf. Lwoff, 1953). The mating experiment and the induction experiment were done with different strains of bacteria, and it is possible that if they had been done at the same time with the same bacterial suspension closer agreement in times for the establishment of the prophage might have been found.

Chloramphenicol did not seem to promote the establishment of lysogeny (determined by inducibility of prophage), even when added after 30 min. growth in complete medium (Table 5). On the other hand, with *Shigella dysenteriae* infected with phage P₁, the addition of chloramphenicol immediately after infection or within 10–15 min., caused the prompt establishment of lysogeny (Christensen, 1960). However, here the detection of lysogeny depended on the expression of immunity to infection by phage T₁, and it is possible that immunity is expressed before the prophage is in fact established. The relationship of immunity to establishment of the prophage for phage λ is being studied in *Escherichia coli* and will be reported separately.

REFERENCES

- BERTANI, G. (1960). On the interpretation of Boyd's multiplicity effect in lysogenization. *Virology*, **12**, 13.
- CHRISTENSEN, J. R. (1960). Effect of chloramphenicol on the establishment of lysogeny by bacteriophage P₁. *Bact. Proc.* p. 46.
- DOERMAN, A. H. (1952). Intracellular growth of bacteriophages. 1. Liberation of intracellular bacteriophage T₄ by premature lysis with another phage or with cyanide. *J. gen. Physiol.* **35**, 645.
- ELLIS, E. L. & DELBRÜCK, M. (1939). The growth of bacteriophage. *J. gen. Physiol.* **22**, 365.
- FRY, B. A. (1959). Conditions for the infection of *Escherichia coli* with lambda phage and for the establishment of lysogeny. *J. gen. Microbiol.* **21**, 676.
- FRY, B. A. & GROS, F. (1959). The metabolic activities of *Escherichia coli* during the establishment of lysogeny. *J. gen. Microbiol.* **21**, 685.
- GROS, F. & GROS, F. (1958). Rôle des acides aminés dans la synthèse des acides nucléiques chez *Escherichia coli*. *Exp. Cell Res.* **14**, 104.
- HAYES, W. (1957). The kinetics of the mating process in *Escherichia coli*. *J. gen. Microbiol.* **16**, 97.
- JACOB, F. & WOLLMAN, E. L. (1954). Étude génétique d'un bactériophage tempéré d'*Escherichia coli*. 1. Le système génétique du bactériophage λ . *Ann. Inst. Pasteur*, **87**, 653.
- JACOB, F. & WOLLMAN, E. L. (1956). Sur le processus de conjugaison et de recombinaison chez *Escherichia coli*. 1. L'induction par conjugaison ou induction zygotique. *Ann. Inst. Pasteur*, **91**, 486.
- JACOB, F. & WOLLMAN, E. L. (1958). The relationship between the prophage and the bacterial chromosome in lysogenic bacteria. In *Recent Progress in Microbiology*, Symp. VII Internat. Congr. for Microbiol. Stockholm: Almquist and Wiksell.
- KAISER, A. D. (1957). Mutations in a temperate phage affecting its ability to lysogenize *Escherichia coli*. *Virology*, **3**, 42.

- LIEB, M. (1953). The establishment of lysogenicity in *Escherichia coli*. *J. Bact.* **65**, 642.
- LURIA, S. E., FRASER, D. K., ADAMS, J. N. & BURROWS, J. W. (1958). Lysogenization, transduction and genetic recombination in bacteria. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 65.
- LWOFF, A. (1953). Lysogeny. *Bact. Rev.* **17**, 269.
- PUCK, T. T., GAREN, A. & CLINE, J. (1951). The mechanism of virus attachment to host cells. 1. The role of ions in the primary reaction. *J. exp. Med.* **99**, 481.
- STENT, G. S. & FURST, C. R. (1956). Decay of incorporated radioactive phosphorus during the development of a temperate bacteriophage. *Virology*, **2**, 737.
- TING, R. C-Y. (1960). A curing effect of chloramphenicol on bacteria infected with bacteriophage. *Virology*, **12**, 68.
- WEIGLE, J. J. & DELBRÜCK, M. (1951). Mutual exclusion between an infecting phage and a carried phage. *J. Bact.* **62**, 301.