Conditions for the Infection of *Escherichia coli* with Lambda Phage and for the Establishment of Lysogeny

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SUMMARY: Optimal conditions for the adsorption of bacteriophage λ_{22} by *Escherichia coli* strain K112 require 0.02 M-MgSO₄ and a pH value of the order 6.5. With a multiplicity of input of 20 phage particles per bacterium, 99 % of the bacteria are infected and at least 90 % are lysogenized. Irradiation of the cells with ultra-violet light immediately prior to infection causes them to give the lytic rather than the lysogenic response.

When a bacterial cell is infected with a temperate phage, there are two major characteristic types of response, the lytic and the lysogenic. In the lytic response, the phage passes into the vegetative state, and a large number of infective particles are eventually synthesized and the cell lyses. In the lysogenic response, the genome of the virus becomes attached to the bacterial chromosome and thereafter this bacterium and its descendants are endowed with the ability to produce, in certain circumstances, infective phage particles similar to those involved in the original infection. At the present time, little is known about the processes which decide whether a cell will give a lytic or lysogenic response, and there is no detailed information about the sequence of events between infection and the establishment of lysogeny. Lieb (1953) was the first to make a comprehensive study of the establishment of lysogeny by λ phage in *Escherichia coli*. In her experiments, the phage and cells were mixed in a broth medium, consequently several processes such as the adsorption of the phage, infection, development of vegetative phage and the establishment of the prophage were all going on at the same time in different cells. With biochemical studies as the ultimate aim, it was desirable to achieve infection of *E. coli* with λ phage in a simple mineral medium and then transfer the infected bacteria to a complete growth medium: in these conditions all the infected cells should begin to respond at about the same time. Kaiser (1957) has reported good adsorption of λ phages to *E. coli* in the presence of 0.01 M-MgSO₄. With *E. coli* K112 and λ_{22} , adsorption in 0.01 M-MgSO₄ was relatively poor, and often less than 50 % of the cells were infected. However, the concentration of MgSO₄ has a profound effect on the total number of bacteria which can be infected and conditions were eventually found in which about 99 % of the bacteria were infected, about 90 % giving the lysogenic response. A preliminary report of these experiments and the effect of the establishment of lysogeny on the metabolic activities of the cells has been made (Fry & Gros, 1957).

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METHODS

Organisms. *Escherichia coli* strains K112, HfrC, (λ_{22}) (lysogenic for λ_{22} and source of phage stock) and C600 Sr^r (streptomycin resistant and indicator bacteria for λ) were from the culture collection of Drs F. Jacob and E. Wollman of the Pasteur Institute, Paris.

Growth media and assay methods. The media and plating procedures were based on those of Adams (1950). Nutrient broth (medium A) contained 1% (w/v) peptone, 1% (w/v) Liebig's meat extract and 0.5% (w/v) NaCl. Medium B for plating procedures was similar to medium A, but with 1% (w/v) NaCl and agar as required (2% (w/v) for colony counts; 1% (w/v) for assay of phage and detection of cells giving a lytic or lysogenic response; 0.7% (w/v) for small tubes in which samples were dispersed prior to plating). For the detection of lysogeny, medium B was supplemented with lactose (2%, w/v) and Prolabo Universal Indicator (5 ml./100 ml.), thus giving a green coloured medium (medium C, cf. Kaiser, 1957). The strains of *Escherichia coli* K112 and HfrC used in these experiments can utilize lactose and when plated on this green medium the resultant colonies are stained yellow and are readily visible. Consequently, when λ -infected cells of strains K112 and HfrC are plated with the indicator bacteria (free phage having been removed by anti- λ serum), it is easy to distinguish those cells giving a lytic response (large turbid plaques), cells not affected by the experimental procedures—refractory or resistant cells (yellow colonies) and cells giving a lysogenic response (formation of yellow colony surrounded by halo of lysis due to release of λ by spontaneous induction in a few cells during growth of the colony). After overnight incubation of the plates at 37°, the plaques from the cells giving the lytic response are fully formed, but a further 24 hr. at 37° is required for all the lysogenic cells to give well-defined haloes round the colonies. In trial experiments with *E. coli* strain K112 (λ_{22}) at least 95% of the cells gave haloed colonies in these conditions. In general, conditions were arranged such that the responses of about 100 cells were tested on each plate. All platings were done in triplicate and results expressed as the average of the three plates.

Preparation of phage stocks. *Escherichia coli* strain K112 (λ_{22}) was grown with gentle shaking in medium A at 37° for 3 hr. to $1.5\text{--}2 \times 10^8$ cells/ml. The cells were collected by centrifugation, suspended in sterile distilled water ($2\text{--}4 \times 10^8$ cells/ml.) and then irradiated with ultraviolet (u.v.) light (from a G.E.C. germicidal lamp giving an intensity of illumination at 2570 Å. of approx. 500 ergs/sq.mm. at a surface 1 m. below the lamp): the amount of irradiation was sufficient to bring about at least 95% induction. The suspension of irradiated cells was mixed with one-fifth of its volume of medium A and then shaken at 37° until optical density measurements showed that lysis was nearly complete. Bacteria were removed by centrifuging at 3000 g. The supernatant was centrifuged at 20,000 g for 1 hr. in a Spinco centrifuge and the pellet resuspended in 20% medium A in distilled water (debris was removed by centrifugation at 3000 g). These phage stocks (about 3×10^{11} particles/ml.)

were stable for several months at 4°: CHCl_3 was added to prevent bacterial contamination.

Preparation of cell suspensions. Cells of *Escherichia coli* in the late log phase of growth adsorb λ phage well (Lieb, 1953) and such cells were used in the experiments described below. Cultures in medium A were incubated in Erlenmeyer flasks (50 ml./500 ml. flask) shaken in a water bath at 37°. After about 4 hr., when the cell density was $3-4 \times 10^9$ cells/ml., the bacteria were collected by centrifugation, washed twice in water and suspended in distilled water at a concentration of 2×10^8 cells/ml.

Measurement of phage adsorption. Cells of strains K112 or HfrC (both strains sensitive to streptomycin) were mixed with phage in the required adsorption medium; samples were taken at intervals, and after appropriate dilution in broth, 0.1 ml. was plated with streptomycin (0.05 ml. of a solution containing 100 mg./ml.) and streptomycin-resistant indicator bacteria (Bertani, 1951). This method enables the unadsorbed phage at the time of sampling to be readily determined.

Detection of type of response of cells after treatment with phage. At the end of the time allowed for adsorption, free phage was removed by diluting into anti- λ serum in broth (1/200, v/v). After 10 min. at 37°, a sample was diluted in broth and incubated at 37° for a further 10 min. Appropriate amounts were then taken for plating with the indicator bacteria, *Escherichia coli* C600 on medium C.

Terminology. 'Multiplicity of exposure' refers to the average number of phage per bacterial cell in the adsorption tube at the beginning of infection. In order to calculate the 'multiplicity of infection', the total number of phage adsorbed by a given number of cells was determined by experiment and it was assumed that cells giving the refractory response (i.e. neither lytic nor lysogenic responses) did not adsorb phage. The concentrations of cells refer to viable counts determined after dilution in medium A and plating on medium A plus 2% (w/v) agar.

RESULTS

Effect of concentration of MgSO_4 on type of response to infection with λ_{22} phage

Samples of the same washed cell suspension were mixed with an equal volume of phage suspension in different concentrations of MgSO_4 solution. After 10 min. at 37°, samples were transferred to anti- λ serum in broth so that the infected cells were able to metabolize for 20 min. before being plated with the indicator bacteria. From Table 1, it can be seen that increasing the concentration of MgSO_4 in the adsorption medium to 0.02 M has two marked effects—a decrease in the number of refractory bacteria and an increase in the number giving the lysogenic response. It would appear from these results that the optimal concentration of MgSO_4 for the adsorption of λ_{22} phage is 0.02 M since at concentrations greater or less than this, there is a progressive increase in the number of bacteria giving the 'refractory response', i.e. bacteria apparently not

infected by λ phage in the conditions being studied. As the concentration of MgSO_4 approaches 0.02M, not only is there an increase in the number of infected bacteria but also a change in the type of response as between lytic and lysogenic. In the presence of the lower concentrations of MgSO_4 , the number of bacteria giving the lytic response is equal to or greater than the number giving the lysogenic response. At concentrations above 0.01M and especially at 0.02M, the proportion of bacteria giving the lysogenic response is much greater than that giving the lytic response. With HfrC, too, infection

Table 1. *Effect of concentration of MgSO_4 during adsorption of λ_{22} phage on the subsequent response of the cells*

In each experiment, samples of the same washed cell suspensions of *Escherichia coli* strain K112 were infected with λ_{22} in MgSO_4 solution: input multiplicities and final concentration of MgSO_4 in adsorption mixture are shown in the table. Adsorption period, 10 min. and then samples transferred to anti- λ serum in broth (1/200) and after 10 min. to broth. After a further 10 min., samples diluted in broth and plated with indicator bacteria on medium C. All incubations at 37°. Numbers in table are the mean of three plates for each sample. The viable count of the suspension was determined in each experiment. Within experimental error, the sum of the cells giving the lytic, lysogenic and refractory response equalled the initial viable count. The percentage response is expressed relative to the total number of cells giving the lytic, lysogenic and refractory response.

Expt. no.	MgSO_4 concn.	Multi- plicity of exposure	Type of response					
			Lytic		Lysogenic		Refractory	
			No.	%	No.	%	No.	%
1	0.005	4.6	23	19	8	7	91	75
	0.01		44	44	40	40	16	16
	0.015		27	26	70	69	5	5
	0.02		17	17	73	74	9	9
2	0.005	11.0	7	8	4	5	72	87
	0.01		28	33	15	18	42	49
	0.015		18	22	54	65	11	13
	0.02		21	23	62	68	8	9
3	0.02	2.4	31	23	71	53	32	24
	0.03		24	18	44	33	67	49
	0.04		20	15	22	17	90	68
4	0.02	3.8	12	9	94	73	23	18
	0.05		7	5	24	19	97	76

appears to occur more readily in the presence of 0.02M- MgSO_4 than in 0.01M- MgSO_4 , e.g. in Table 2, when the phage input is 6.5 in 0.01M- MgSO_4 , 47% of the bacteria give a refractory response, whereas in 0.02M- MgSO_4 , only 7% of the bacteria are refractory. The multiplicity of phage input was greater than one in all these experiments, hence if the phage was more easily adsorbed in 0.02M- MgSO_4 , it is probable that in such conditions many cells became infected with more than one phage. Previous work with *Salmonella typhimurium* has shown that increasing the multiplicity of infection leads to an increased lysogenic response (Boyd, 1951).

Kinetics of the uptake of λ_{22} phage by Escherichia coli strain K112

The uptake of λ_{22} phage by *Escherichia coli* K112 was measured in the presence of two concentrations of MgSO_4 , 0.01 M and 0.02 M, using samples of the same cell suspension in each experiment. After mixing the pre-warmed suspensions of bacteria and phage, samples were taken at 4 min. intervals and the number of unadsorbed phage determined. The rate of uptake was faster and more prolonged in the presence of 0.02 M- MgSO_4 (Fig. 1 A), so that in a given time more phage was adsorbed than in 0.01 M- MgSO_4 (92 % as compared with 52 %). Similar results were obtained with HfrC (Fig. 1 B).

Fig. 1. Rate of adsorption of lambda phage to *Escherichia coli* strains K112 and HfrC. Washed cell suspension of *E. coli* (a) strain K112 (1.4×10^8 /ml.), (b) strain HfrC (2.9×10^8 /ml.) mixed at 37° with equal volume of phage suspension in MgSO_4 , final concn. either 0.02 M or 0.01 M. Samples taken at intervals to determine unadsorbed phage. P_0 = concn. of phage at time 0; P = concn. of phage unadsorbed at time t . pH value of cells + phage = 6.65. Initial multiplicity (i.e. phage/cell) in (a) = 1.4; in (b) = 1.8.

In the foregoing experiments no attempt was made to control the hydrogen-ion concentration of the adsorption medium. It was in fact found that the pH of the mixture of cells and phage suspensions was about pH 6.5 as measured by a Beckman glass-electrode pH meter. Electrostatic forces are believed to be operative in the first stages of the attachment of phage to a bacterial cell (Puck, 1953) and since the electrical charges of the surfaces of both the bacteria and the phage will be affected by the pH value of their environment, the rates of adsorption of λ_{22} phage were determined in different conditions of hydrogen ion concentration. The adsorption media were buffered with weak potassium phosphate buffers (0.0025 M), and although the initial rates of adsorption were very similar in the pH range 5.9–7.4, the best overall adsorption (94 %) was obtained at pH 6.6 (Fig. 2). Thus, the optimal conditions for the adsorption of λ_{22} phage by *Escherichia coli* K112 are 0.02 M- MgSO_4 and a pH of the order 6.5.

Effect of multiplicity of infection on the lysogenic response

Samples of the same washed cell suspensions of *Escherichia coli* K112 were mixed with λ_{22} phage at four different multiplicities of input. After 10 min. at 37° samples were transferred to anti- λ serum in broth as in previous experiments, and after 20 min. at 37° the cells were plated with indicator bacteria on medium C. In addition a sample of the suspension with the lowest multiplicity of input was taken at the end of the 10 min. adsorption period and the

Table 2. *Effect of multiplicity of infection on the type of response*

Samples of the same washed cell suspension of *Escherichia coli* strain K112 (A) or *Escherichia coli* strain HfrC (B) infected with different multiplicities of input of λ_{22} phage in 0.02 M-MgSO₄. Adsorption period 10 min. Samples then transferred to anti- λ serum (10 min.) and broth (10 min.) and plated on medium C with indicator bacteria. All incubations at 37°. Multiplicities of infection calculated from phage remaining unadsorbed at the end of the adsorption period in a sample from the mixture with the lowest multiplicity of input: assumed that cells giving the refractory response did not adsorb λ . (Found; in A 26 % and in B, 18 % unadsorbed phage.)

	Multi- plicity of input	Multi- plicity of infection	Type of response					
			Lytic		Lysogenic		Refractory	
			No.	%	No.	%	No.	%
A	1.8	2.9	17	19	23	26	49	55
	8.8	7.1	12	13	71	78	8	9
	17.5	13	7	8	71	90	2	2
	35	26	8	9	81	90	1	1
B	1.3	1.6	22	22	42	42	37	37
	6.5	5.6	7	8	76	85	6	7
	12.9	10.9	7	8	80	89	3	3
	6.5*	3.2	27	24	34	30	53	47

* Adsorption of λ_{22} in 0.01 M-MgSO₄.

unadsorbed phage measured. The average multiplicity of infection per cell was calculated assuming that, whatever the input multiplicity, the same proportion of phage was adsorbed and also that cells giving a refractory response did not adsorb phage. By using high multiplicities of input and thus high multiplicities of infection, it is possible to obtain populations of infected bacteria in which the number of refractory bacteria is reduced to only 1 or 2 % and the number of bacteria giving the lysogenic response is about 90 % (Table 2). Similar results were obtained with *E. coli* strain HfrC (Table 2).

Effect of pretreatment with ultra-violet light before infection with λ_{22} phage

Portions (5 ml.) of the same washed cell suspensions of *Escherichia coli* K112 were irradiated with u.v. light for different periods of time, and then a sample of each irradiated suspension was diluted in broth and allowed to metabolize at 37° for 30 min. before being plated to determine the percentage survival, i.e. ability to form colonies. Other samples of the irradiated cells were mixed with λ_{22} phage in 0.02 M-MgSO₄ at 37° for 10 min. and then transferred to anti- λ serum and broth at 37°. After 20 min. samples were diluted and plated

on medium C for the determination of type of response of the cells. In Fig. 3, it can be seen that the ability to give the lysogenic response appears to decrease more rapidly than the ability to form colonies, and these results support the idea that prior exposure to u.v. light disorganizes the bacterial nucleic acid and thus forces the phage into the lytic pathway. It is to be noted that many of the irradiated bacteria are able to give a lytic response, even though they are unable to form colonies. These results are essentially similar to those of Lieb (1953).

Fig. 2

Fig. 3

Fig. 2. Effect of pH value on the adsorption of lambda phage by *Escherichia coli* strain K112. Washed cell suspension of *E. coli* K112 (1.9×10^8 /ml.) mixed at 37° with equal volume of phage suspension (4.6×10^8 /ml.) in 0.005 M-phosphate buffer. Samples taken at intervals to determine phage unadsorbed. pH value of cells plus phage measured by glass electrode. P_0 = concn. of phage at time 0; P = concn. of unadsorbed phage at time t . Initial multiplicity = 2.4.

Fig. 3. Irradiation with ultraviolet light before infection and its effect on response to infection with lambda phage. Washed cell suspensions of *Escherichia coli* strain K112 (1.2×10^8 /ml.) irradiated in thin layer in open Petri dish with ultraviolet light (lamp at 1 m.) for desired time. Samples taken and incubated in broth for 30 min. at 37° , and then plated to determine survival of ability to form colonies. Other samples mixed with equal volume of phage suspension (1.54×10^8), incubated at 37° for 10 min., and then samples taken to determine response of infected cells. Response expressed as % of total number of cells giving the lytic + lysogenic + refractory response in the un-irradiated control.

DISCUSSION

The experiments reported here show that with λ_{22} phage and *Escherichia coli* strain K112, the optimal conditions for adsorption of the phage are 0.02 M- MgSO_4 at a pH value of the order 6.5. This result is in contrast with that of Kaiser (1957) who, in experiments with other λ phages, used a concentration

of 0.01M-MgSO₄. At the present time, it is not known why an increase in the concentration of MgSO₄ above 0.02M should decrease the number of cells becoming infected (Table 1). Such a result may be due to instability of λ_{22} in the presence of high concentrations of MgSO₄. To date no studies have been made of the stability of λ_{22} in such conditions: indeed, although the λ phages have been used in many experiments, especially those involving genetics, there appears to be little published information concerning their basic properties.

With λ_{22} phage, it is clear that in optimal conditions (multiplicity of input about 20 and 0.02M-MgSO₄) a population of infected cells can be obtained in which at least 90 % give the lysogenic response. In general, previous workers have attained no more than a 30 % lysogenic response (Kaiser, 1957); though in some experiments, up to 60 % has been reported (Lieb, 1953), but such results were not obtained consistently. Since a relatively high multiplicity of phage input is a factor influencing the number of cells which become lysogenic, it is probable that as in *Salmonella typhimurium* (Boyd, 1951), the lysogenic response is determined at least in part by the multiplicity of infection. However, the high concentration of MgSO₄ leading to better adsorption, and hence more phage adsorbed per cell, may not be a complete picture of the role of Mg⁺⁺ in the establishment of lysogeny. If it can be assumed that bacteria giving the refractory response do not adsorb any phage, it can be seen from Table 2 that when the multiplicity of infection of HfrC in 0.01M-MgSO₄ is 3.2, then the lysogenic response is 30 %. But though infection in the presence of 0.02M-MgSO₄ at a lower multiplicity of input gave a multiplicity of infection of only 1.6, the percentage lysogenic response was 42 %. Some preliminary experiments have therefore been made to try to determine whether the concentration of Mg⁺⁺ can directly affect the decision of the cell with regard to a lytic or lysogenic response. These experiments have not yet been completed, but it appears that during infection the main, if not the only, function of MgSO₄ is to be an agent for adsorption. However, if after adsorption, the concentration of MgSO₄ is kept at 0.02M whilst the infected cells are metabolizing in broth, there is some evidence that the cells show a change towards the lysogenic response as compared with cells infected in the same conditions and kept for the same time in broth not supplemented with MgSO₄ (Fry, unpublished). Such a result could mean that Mg⁺⁺ may directly aid the coupling of the genome of the phage to the bacterial chromosome. Alternatively, such a concentration of Mg⁺⁺ may inhibit certain cellular processes and thus form an environment favouring the lysogenic pathway. It has been noted that *Escherichia coli* strain K112 grows more slowly in the presence of high concentrations of MgSO₄ and there is some evidence that reduction of metabolic activities does in fact favour lysogeny. If cells of *E. coli* strain K12S (Lieb, 1953) or *Shigella dysenteriae* (Bertani & Nice, 1954) are infected with temperate phage at 37° and then transferred to a lower temperature (20° or 25°) for an hour before plating, there is a marked change towards the lysogenic response as compared with those kept at 37° throughout the experiment. Results have also been obtained which indicate that the presence of chloramphenicol (15 μ g./ml.) in the broth medium after infection of *E. coli* K112 by

λ_{22} causes a change towards lysogeny (Fry, unpublished). In such conditions, chloramphenicol is known to inhibit protein synthesis (at least 95 % inhibition) and DNA synthesis (at least 40 % inhibition) (cf. Neidhardt & Gros, 1957).

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