THE ESTABLISHMENT OF LYSOGENICITY IN ESCHERICHIA COLI

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Received for publication November 17, 1952

When a population of sensitive bacteria is exposed to infection by a temperate phage, a large number of cells are not lysed and give rise to colonies containing lysogenic cells. Some of the factors influencing the probability that an infected cell of *Escherichia coli*, strain K12S, will give a lysogenic response are reported here. In addition, the present study deals with the change of free phage particles into the intracellular prophage form which is transmitted to all cells in a lysogenic culture. Information was obtained on specific questions regarding the establishment of lysogenicity, as, for example: When is the decision as to the type of response, i.e., lytic or lysogenic, made by the infected cell? How much time is required for transformation (reduction) of the infecting phage into prophage and what changes take place in the host cell? Is the prophage a cytoplasmic constituent of the lysogenic cell or linked to the genetic mechanism?

MATERIALS AND METHODS

The temperate phage, λ, used in these studies was derived from a lysate from ultraviolet induced K12 cells (Weigle and Delbrück, 1951). A variant, λl, which gives relatively large plaques, was selected and used in the following experiments. The indicator strain, K12S, was derived from strain W1294 kindly furnished by Dr. E. Lederberg. K12S is not lysogenic for λ, requires thiamin for growth, and is resistant to phages T1 and T5. Cultures were grown in tryptone broth with aeration at 37 C. The standard media and plating methods have been described by Adams (1950). For phage assays, K12S cells always were obtained from nutrient broth cultures which had just reached a concentration of about 10^9 cells per ml. Colonies were tested for lysogenicity by transferring a sample of cells to a plate previously coated with K12S cells and dried in the incubator for 15 to 30 minutes. The transfer was by sterile needle or by the replica plating method (Lederberg and Lederberg, 1952). The streaks or prints were given a small dose of ultraviolet to induce phage development. After several hours of incubation, the lysogenic streaks were surrounded by an area of lysis where the liberated phage had killed the background K12S cells. To determine whether a nonlysogenic colony was composed of λ-sensitive cells, cells from a colony were cross-streaked on a plate which previously had been streaked with λ.

[1] Aided by a fellowship from the National Foundation for Infantile Paralysis.

RESULTS

Types of bacterial response after exposure to λ. From the results of experiments which will not be presented in detail here, it was evident that five different types of response could be elicited from cells of a K12S culture exposed to 3 to 50 λ per cell:

1. *Lytic* (or *productive*). Virus multiplication occurs within the cell, resulting in lysis and the production of new phage. λ gives a burst size of 35 to 100 particles after a latent period of about 35 minutes.

2. *Lysogenic.* The infected cell gives rise to a colony containing lysogenic cells. These cells can be induced to form free phage by ultraviolet irradiation and are not lysed by λ although the phage is adsorbed at the same rate as on nonlysogenic sensitive cells.

3. *Refractory.* The cell is unchanged and gives rise to a colony of K12S cells that may give any of the 5 responses. Refractory cells adsorb λ poorly, but there is no evidence that they inactivate λ.

4. *Lethal.* The cell is killed and the adsorbed phage is inactivated. This phenomenon occurs most frequently at high multiplicities and may be analogous to "lysis from without" which has been observed with virulent phages under certain conditions (Benzer *et al.*, 1950).

5. *Resistant.* The cell gives rise to a colony of nonlysogenic λ-resistant cells. These cells do not adsorb λ and also are not killed by virulent mutants of λ. This type of response is relatively

rare and may be due to mutation to Lp2^r (Lederberg and Lederberg, 1953).

For convenience, cells giving the various types of responses will be referred to as lytics, lethals, etc.

Plating efficiency. A large proportion of K12S cells exposed to λ gives a lysogenic response. When lysogenic cells are plated on K12S as indicator, only about 10 per cent of the cells are detected as plaques. Thus, it is probable that in phage assays the majority of phage which is adsorbed by cells giving a lysogenic response will not be detected. To determine the actual proportion of phage particles which would escape detection in this manner, λ was adsorbed on a 10-fold excess of cells for 25 minutes at 37 C to simulate the conditions for adsorption on the plate. The adsorption mixture then was plated on agar seeded with K12S to determine the number of plaque formers, which would be analogous to the plaque count by the regular plate assay method. The number of plaques was 167, as opposed to 174 when λ was plated directly. When the mixture was plated on plain agar and the resultant colonies tested for lysogenicity, 30 lysogenic colonies were found. Thus, only about 15 per cent of the phage particles present were not detected as plaques, giving a plating efficiency of at least 85 per cent.

Two basic types of response. To facilitate the study of factors responsible for the various types of responses, it was decided to consider first the two types of cell reaction to phage exposure that are distinguished most easily: survival, as evidenced by colony formation, and death of the cell. The former includes response types 2, 3, and 5 whereas cell loss includes responses 1 and 4. A "standard resistance assay" method was used in which 0.1 ml containing about 10^3 to 10^3 cells was plated in 2 ml of soft agar inoculated with 2×10^8 λ particles. This agar was poured on a bottom layer of about 20 ml of plain agar. The total number of cells plated was determined by an assay on a control plate without phage. By comparing the number of colonies formed on the two plates one determines what proportion of the cells is sensitive to killing by the phage, or "Sens", and what proportion is not killed, or "R".

Possible genetic factors and cellular response. If K12S cultures consist of balanced populations of genetically different types of cells giving the various responses to λ, single cell cultures from a K12S population should come only gradually to genetic equilibrium. An experiment was set up to observe the variation in the number of R and Sens cells in individual clones. A culture giving 31 per cent R responses was diluted in broth to give about 0.6 cells per ml, and 1 ml aliquots were put into small tubes and incubated at 37 C. When the clones had grown to about 100 cells, one-half of the contents of each tube was plated on plain agar and the remainder on agar inoculated with λ. Cells were found in 24 out of 60 tubes, and although there was considerable variation in clone size, the numbers of colonies on matched plates were almost identical for every clone. The average total number of cells was 91, of which 89 were R cells. Similar results were obtained in a duplicate experiment. The parental Sens cells as well as the R cells gave rise to similar clones, containing R cells only. This finding is incompatible with any known system of spontaneous mutation, and we infer that primarily nongenetic or physiological factors determine the response of a K12S cell to temperate phage. Heterogeneity of the phage population is excluded also as the factor determining cellular response since the same phage stock was used in all tests.

Physiological factors and cell response. When young cultures of K12S, i.e., cultures not nearing the end of growth, are tested for their resistance to phage, 100 per cent of the cells respond as R. To determine when Sens cells appear in a growing culture, aliquots were removed at various times and tested for the proportion of R and Sens. It was found that in tryptone broth the cultures are 100 per cent R until the cellular concentration reaches about 5×10^7 cells per ml. The per cent then decreases and reaches a minimum of 20 per cent at the time that cell multiplication has practically ceased. In figure 1 we use the per cent R as an index of the physiological state of the culture and plot the proportions of response types 1, 2, 3, and 4 versus R. The highest per cent of lysogenic responses usually is obtained at cellular concentrations above 2×10^8 per ml.

The effect of ultraviolet radiation on K12S. Bacteria were removed from a growing culture at various times and irradiated after centrifugation and resuspension in buffer at 0 C. The per cent R was determined by the standard resistance

assay. In the experiment shown in figure 2, and in many similar tests, the per cent R dropped much more rapidly with increasing doses of ultraviolet than did the total number of survivors. For example, after irradiation, less than 1 per cent of the surviving cells were resistant to λ, a condition never obtained in unirradiated cultures. In the culture that was less resistant initially, the R fraction decreased much more rapidly after irradiation than in the 87 per cent R culture.

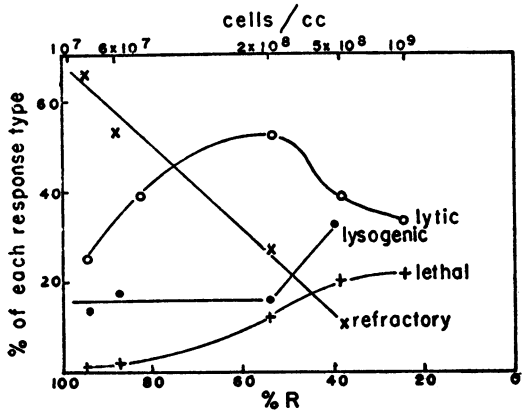

Figure 1. Proportions of four types of cell response at different times during growth of a culture. Samples were tested for per cent R by plating with λ. In addition, λ was added to aliquots of broth suspensions of cells to give a multiplicity of 2 to 4 phages per cell. After treatment with λ and antiserum, the cells were diluted and plated on plain agar and on agar seeded with K12S. Colonies appearing on the unseeded plates were tested for lysogenicity by the transfer methods.

This ultraviolet induced increase in the sensitivity of cells to phage may be due to the fact that cells which normally would give a lysogenic response, and be included in the R fraction, are "pre-induced" by irradiation and give a lytic response. These cells would appear among the total survivors after ultraviolet, but not in the R fraction. To determine whether cells of the R fraction of the culture were induced by ultraviolet radiation to become capable of giving a lytic response, the number of plaques obtained when the cells were infected with λ was compared before and after irradiation. As shown in figure 2, there was a large increase in plaque formers after a low dose of ultraviolet. The increase varied in different experiments, but this was not unexpected since the "competence" of lysogenic cells to induction is highly dependent on their nutritional condition (Lwoff, 1951).

Can one phage cause lysogenization? The proportions of each of the four most frequent responses detected after exposure of K12S to λ are influenced greatly by the multiplicity. Since, as will be described below, the lysogenic response

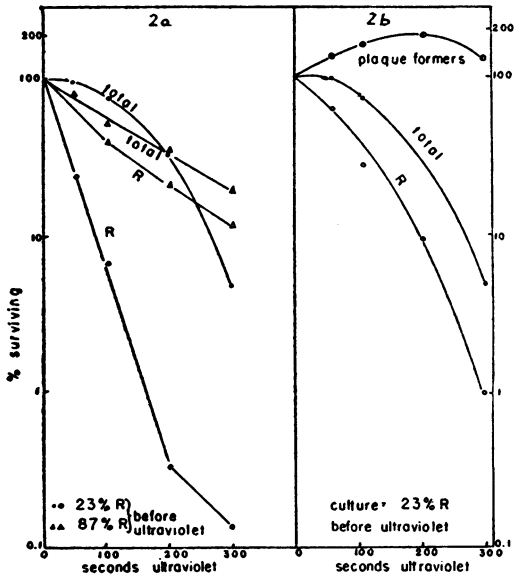

Figure 2. Killing of bacteria and changes in the size of the R fraction produced by irradiation.

2a: K12S cultures were grown to 2 × 10^7 and 1 × 10^9 cells per ml; cells were assayed for per cent R before and after ultraviolet by the standard plating method. *2b:* The same 1 × 10^9 per ml culture as used in *2a* was assayed for per cent R before and after ultraviolet by exposure to 5 λ per cell for 5 minutes, followed by 5 minutes in antiserum. The λ-treated cells were plated on plain and K12S-seeded agar. The total number of cells, the number giving a lytic response and the number giving an R response *before* irradiation, have each been taken as 100 per cent, and the per cent survival plotted against the dose.

is elicited more frequently at high multiplicities, it might be supposed that this response was dependent on the adsorption of two or more phage particles per cell. To test this hypothesis, K12S cells were exposed to low multiplicities of λ in three different experiments (table 1). In each case, the observed frequency of lysogenic responses was much higher than expected if the adsorption of two or more phage particles was

required. Thus, the adsorption of a single λ particle can lysogenize a cell, but the possibility remains that a cell has a higher probability of giving rise to a lysogenic colony when more than one particle has been adsorbed.

Effect of multiplicity on the per cent of lysogenic and lytic responses. In table 2 are tabulated the results of several experiments in which cultures of K12S were exposed to different multiplicities of λ. "Multiplicity" given as the number of phage per bacterium present in the adsorption tube or on a plate is the *multiplicity of exposure*, not the number of phage particles actually adsorbed per cell. Cells of K12S cultures which have reached different levels of growth adsorb at different rates

TABLE 1

The "lysogenization" of cells at low phage multiplicities

EXPERIMENT	AVERAGE NUMBER PHAGE PER CELL	PROBABILITY OF 2 OR MORE PHAGE PER CELL	PROPORTION OF TOTAL CELLS LYSOGENIC	PROPORTION OF TOTAL CELLS LYTIC
C253	0.12	0.007	0.019	0.11
C230a	0.037	0.0007	0.014	0.022
C230b	0.034	0.0006	0.013	0.021

Cells were exposed to phage at a ratio of about 0.1 phage per cell. After 5 minutes, free phage was removed by diluting into antiserum for 5 minutes. Cells then were plated on plain and K12S seeded agar. Colonies were tested for lysogenicity by the printing method. The number of phage adsorbed per cell was determined by dividing the sum of plaque formers and lysogenic cells by the total number of cells.

(Lieb, *unpublished*) so that it is necessary to determine in each individual experiment how many phage particles were adsorbed during the time of exposure to λ. In the experiments in table 2, adsorption multiplicity has been calculated on the basis of the highest virus dilution, at which the per cent refractory has been taken to be the per cent of cells that are uninfected. The multiplicity at higher phage concentrations has been assumed to be a multiple of this multiplicity proportional to the λ concentration in the adsorption tubes.

In all experiments the highest per cent of lytic responses among infected cells is obtained at the lowest multiplicities. As more cells become infected at higher multiplicities, an increasing proportion of the infected cells gives a lysogenic response. At least two explanations are possible for this increase in the per cent of lysogenics: a, the less easily infected cells, those infected only at high λ concentration, have a greater probability of giving a lysogenic response, and b, all cells have a greater probability of giving a lysogenic response when infected with more than one phage particle. If the latter hypothesis were true, the same cells that give a lytic response at low multiplicities could give a lysogenic response at higher multiplicities. Since these cells would be detected in the lytic class at one multiplicity and in the lysogenic class at another, the sum (highest per cent of cells giving lytic response) plus (highest per cent of cells giving lysogenic response)

TABLE 2

Effect of multiplicity of infection on cell response

EXPERIMENT NUMBER	MULTIPLICITY	% OF CELLS GIVING EACH RESPONSE:				% LYTIC	% LYSOGENIC
		Lytic	Lysogenic	Refractory	Lethal	% INFECTED	% INFECTED
235	0.6	23	23	54	0	0.50	0.50
	1.2	31	29	35	5	0.48	0.45
	2.4	34	51	13	2	0.39	0.59
	4.8	33	55	7	5	0.35	0.59
	9.6	12	30	1	57	0.12	0.30
232	0.7	7	3	49	41	0.14	0.06
	2.1	17	5	36	42	0.27	0.08
	6.3	30	30	5	35	0.32	0.32
	18.9	9	24	2	65	0.09	0.24
	56.7	20	25	<1	55	0.20	0.25
202-Y	0.3	23	4	73	0	0.85	0.15
	0.9	29	4	39	28	0.47	0.06
	2.7	57	20	15	8	0.67	0.23
	8.1	27	37	12	24	0.31	0.42
	24.3	24	50	5	21	0.25	0.53
	72.0	33	45	11	12	0.37	0.50

Adsorption tubes containing increasing dilutions of λ were inoculated with aliquots of a K12S culture at about 5×10^8 cells per ml. After 5 or 10 minutes, aliquots were transferred to antiserum for 5 to 7 minutes. Samples then were diluted in broth and plated on plain and K12S-seeded agar. A sample of the colonies arising on the plain agar plates was tested for lysogenesis. The "% infected" = total input (100 per cent) minus the per cent refractory (nonlysogenic) survivors. The "% lethal" = 100 per cent minus the per cent lytics, lysogenics, and refractories.

might well be greater than 100 per cent in any experiment. With one exception in eight experiments, this total was not over 100 per cent. Although these data do not permit explanation b to be ruled out, additional unpublished evidence on adsorption favors the first hypothesis. Among the first 40 to 50 per cent of cells infected, the average proportion giving a lysogenic response was 27 per cent. Assuming that an infected cell cannot change its response from lytic to lysogenic, the proportion of lysogenic responses in the cells infected only at higher multiplicities must have been significantly higher to account for the overall increase in lysogenic frequency.

TABLE 3

Occurrence of free phage and cells in single clones

	EXPERIMENT NUMBER								TOTAL
	1	3	5	6	7	8a	8b	13	
Incubation temperature	37 C	37 C	37 C	37 C	25 C	37 C	25 C	42 C	
Culture density $\times 10^{-8}$	6.8	6.5	4.9	9.7	6.4	9.5	9.5	6.4	
Phage adsorbed per cell	3	6	20	13	10	5	5	11	
Total number of tubes with free phage only	17	13	7	6	12	10	13	17	92
Number of tubes with nonlysogenics only (± free phage)	17	17	7	2	5	23	21	19	111
Number of tubes with lysogenics (± free phage)	8	7	11	11	31	9	11	10	98
Number of tubes with nonlysogenics + free phage	5	7	0	0	1	5	5	8	31
Expected number	4	5	1	0	1	4	4	5	24
Number of tubes with lysogenics + free phage	2	4	0	3	2	6	2	5	24
Expected number	2	1	1	1	6	2	2	3	18

Total number of tubes in each experiment = 100.

Response of individual cells. The following procedure was used to study the fate of individual cells of a K12S culture after exposure to λ: A culture was grown to a concentration of 5×10^8 to 1×10^9 cells per ml. A 1:10 dilution was made into λ suspended in broth at a concentration of 10^9 to 10^{10} particles per ml, the multiplicity of exposure varying in different experiments. Five minutes at 37 C were allowed for adsorption, and an aliquot was diluted into anti-λ serum at 37 C. After 5 to 7 minutes' exposure to serum, the culture was diluted further in broth at room temperature, and 0.5 or 1.0 ml aliquots were distributed into small tubes. The dilution was usually such that the probability of a tube being inoculated with two or more cells was small. After a period of incubation allowing several cellular divisions, each tube was assayed for free λ and the remaining liquid in each tube was plated to determine the number of cells present. The colonies formed by these cells were tested for lysogenicity by streaking or by replica plating.

Most of the tubes, with the exception of blanks, contained either a clone of cells or from 30 to 80 λ particles. There was only a small number of tubes that contained both free phage, due to a lytic response, and a clone of cells. We wish to know whether all of these doubles are due to chance inoculation of tubes with both a lytic and a nonlytic cell or whether some arise from single cells which divide after infection to produce two daughter cells, one giving a lytic response while the other forms a clone of cells. Such a response will be called a mixed lytic-clone response. The expected chance doubles were calculated in the following manner: The average number of lytic cells per tube was determined, omitting from the calculation all tubes which

contained a clone of cells. In this way, one avoids biasing the calculation by inclusion of tubes in which the mixed lytic-clone response might have taken place. The average number of lytics per tube was used to determine how many of the tubes containing clones of cells were expected also to contain free phage due to inoculation with both types of cells.

In table 3, the number of tubes containing both free phage and cells is compared with the number expected on the basis of chance. When all the data are pooled, it is found that the frequency of doubles among tubes containing either lysogenic cells or nonlysogenic cells is about 30 per cent

TABLE 4

Occurrence of lysogenic and nonlysogenic cells in single clones

	EXPERIMENT NUMBER							TOTAL
	1	2	3	5	6	8a	9	
Total number of tubes with lysogenics	8	9	7	11	11	9	32	87
Number with lysogenics only	2	1	0	2	2	0	12	19
Number with mixed clones	6	8	7	9	9	9	20	68
Expected number	0.6	0.4	0.3	0.3	0.2	0.5	4.0	6.3

Total number of tubes in each experiment = 100.

higher than expected. This excess is not statistically significant. We conclude that the mixed lytic-clone response occurs with a probability of less than 0.05 per infected cell, and that a λ-infected K12S cell gives *either* a lytic *or* a nonlytic response.

When the colonies derived from single cells in the individual clones were tested for lysogenicity, a large proportion of the clones was found to consist of mixtures of lysogenic and nonlysogenic (sensitive) cells. Here again, we wish to know whether we are dealing with chance double inoculation or with different responses by the offspring of single cells. The average number per tube of cells giving a lysogenic response was calculated omitting all tubes containing nonlysogenic (sensitive) cells, for the reason stated in connection with table 3. The average number of lysogenics per tube was used to determine how many of the tubes containing sensitive cells were expected to contain lysogenic cells also, due to inoculation with both types of cells. As seen in table 4, the number of mixed clones is larger by a factor of 10 than the expected number of doubles. We are dealing, therefore, with genuine mixed clones containing lysogenic and sensitive cells derived from a single parent.

Temperature and the establishment of lysogenicity. How was λ prevented from infecting all the progeny of an infected cell? Before this can be answered, more must be known about the processes taking place in a potentially lysogenic cell after infection. Cells of K12S were exposed to λ as in the previous experiments and inoculated into separate tubes which were incubated at different temperatures for varying lengths of time. Then the contents of the tubes were plated, and the colonies arising from the cells in the clones were tested for lysogenicity.

The graphs in figure 3 give the per cent of clones which contain various proportions of lysogenic cells when incubated at various temperatures after infection. Since the total number of clones in any experiment was quite small, similar experiments have been averaged and the clones have been grouped into classes of those containing 100 per cent, 66 to 99 per cent, etc. lysogenic cells. Comparing diagrams A, B, and C, one observes that at the higher temperatures the clones containing *any* lysogenic cells contained a smaller proportion of lysogenic cells but that there was little increase in the class of completely nonlysogenic clones. The temperature effect is less pronounced at a multiplicity of 10 (diagrams D and E), but here also decrease in the per cent of lysogenic cells in the clones grown at 37 C is unmistakable. At a multiplicity of 10, incubation at 37 C does not increase the number of completely nonlysogenic clones. If, however, the cells are incubated at 42 to 44 C, there is a dramatic drop in the proportion of lysogenic cells in the clones and a great increase in the number of completely nonlysogenic clones recovered. Such an increase must be due to the loss or destruction of λ or prophage in the originally infected cells and/or in all their progeny.

At any particular temperature, an increase in multiplicity is correlated with an increase in the proportion of clones containing a high percentage of lysogenics. Another fact shown by these dia-

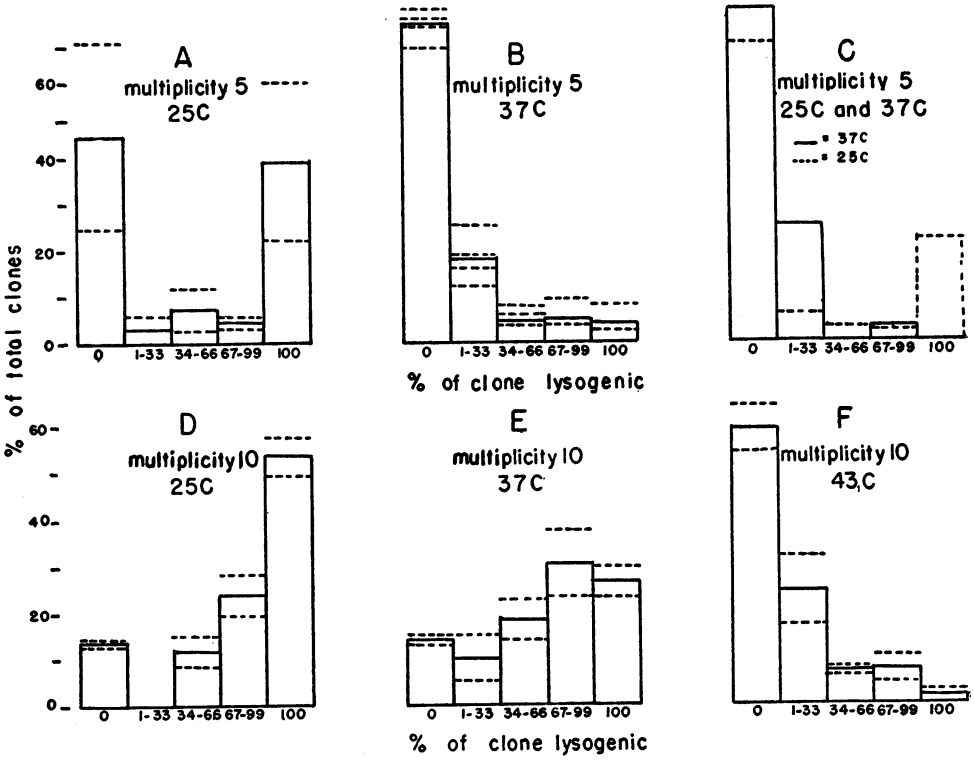

Figure 3. The per cent of lysogenic cells in clones from cells incubated at various temperatures. Diagrams A, B, D, E, F: cells of K12S were exposed to λ as in the previous single clone experiments and inoculated into tubes which were incubated at various temperatures for 3 hours. Individual experiments are represented by dotted lines; solid lines show the average of 2 or more experiments. Diagram C: cells of a single culture were grown at 25 or 37 C.

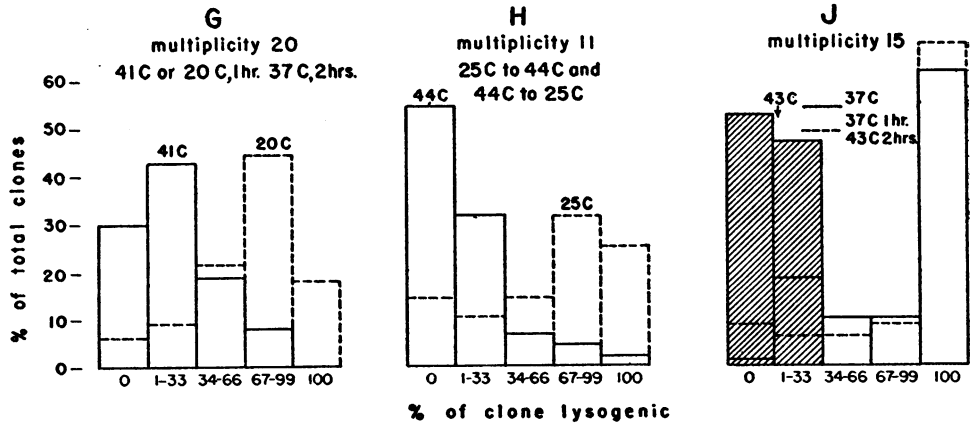

Figure 4. Effect of temperature during the first hour of incubation on the per cent of lysogenics in a clone. G: Cells incubated at 41 or 20 C for 1 hour, then at 37 C for 2 hours. H: Solid line indicates results from cells incubated at 44 C for 1½ hours, then at 25 C 1½ hours; dotted line indicates results from cells incubated at 25 C 1½ hours, then at 44 C 1½ hours. J: Shaded portion indicates results from cells incubated 3 hours at 43 C; solid line, cells incubated 3 hours at 37 C; dotted line, cells incubated at 37 C 1 hour, then at 43 C 2 hours.

grams is that the distribution of clones among classes of lysogenic frequency is bimodal with a relatively large proportion of clones falling into the "O" class. In D and E, for example, 15 per cent of the cells fail to give clones containing lysogenic cells even at the high multiplicity. These cells are the "die-hard" refractory portion of the population which resists infection, probably because of poor adsorption (Lieb, *unpublished*).

The data in figure 4 show conclusively that the temperature to which the cells are exposed during the first 1 to 1½ hours after infection has a predominant influence on the proportion of lysogenics in clones.

Lysogenicity and the growth rate of cells. A comparison was made of the average sizes of clones containing lysogenic cells and clones without lysogenic cells in 11 experiments. After 5 to 9 cellular divisions, clones without lysogenic cells were, on the average, twice as large as those containing lysogenics (128 cells versus 56). To determine whether the presence of prophage decreased the growth rate of K12S cells, lysogenic and nonlysogenic clones derived from cells of the same culture were grown in broth to about 5×10^7 cells at which time turbidimetric measurements were made of their further growth. No differences could be detected among the growth rates of 7 lysogenic and 3 nonlysogenic clones, and a second experiment gave identical results.

The possibility must be considered that the smaller size of lysogenic clones is not primarily the result of infection: a temporarily slower growth rate or longer lag might be correlated with a tendency for a cell to give a lysogenic response. In this case, the average size of lysogenic clones derived from infected cells of a culture should be no smaller than the average size of an equal number of the smallest clones derived from uninfected cells of the same culture. Three experiments were performed to investigate this point; in one experiment the smallest lysogenic clones averaged one-half the size of the smallest clones from the uninfected portion of the culture, and in the remaining experiments the smallest lysogenic clones were almost twice as large as the smallest uninfected clones. Thus, the lysogenic clones do not appear to arise from a selected class of cells in a culture. A more likely explanation for the smaller size of lysogenic clones is a temporary slowing of the division rate or the death of some cells in a clone during the reduction period.

DISCUSSION

When a culture of K12S cells is exposed to a temperate phage, different cells give different responses. The proportions of cells giving the various responses change when the culture ages. The cause for the differences in response given by different cells does not lie in genetic factors. The cause may lie in physiological differences between the cells, or it may lie in differences arising only as a result of infection. In *Salmonella typhimurium* (Boyd, 1951; Lwoff, *personal communication*) cellular response is influenced by multiplicity of infection, but this factor does not appear to affect the response type in K12S.

Once λ enters a K12S cell, the phage either initiates lysis or changes into a carried form, prophage. The term *reduction* has been suggested to describe the change of λ into prophage (Delbrück, *personal communication*). Clones resulting from several divisions of cells in which λ reduction has taken place do not contain free phage. Prophage is transmitted to every cell of a lysogenic culture and so its division must keep pace with cellular division. In addition, it is as resistant as the host to inactivation at high temperatures and reproduces to form infective phage if the host is given a low dose of ultraviolet light. The time required for the attainment by the infecting phage of these properties may be defined as the reduction period. The reduction period may not be the same for all properties, but the most easily obtained estimate for the time involved comes from a study of the temperature sensitivity.

During the reduction period, intracellular λ is more easily temperature inactivated than free λ. While 80 per cent of free phage particles survived heating to 43 C in broth for 2 hours, less than 20 per cent of the λ particles survived in cells that had just been infected. Thus, during the reduction period, cells can be cured of their potential prophage, but once established, the prophage is even more resistant than free phage to inactivation at 43 C. The inactivation of the phage apparently does not affect the host adversely since the cured cells give rise to clones of cells. If infected cells are incubated for about 1 hour at 37 C and then heated to 43 C, there is no indica-

tion of intracellular phage inactivation. The reduction time measured as the time required for the attainment of heat stability thus is less than 1 hour at 37 C. At 20 or 25 C, the reduction time appears to be longer. Observations show that spontaneous lysis very rarely occurs among cells of a new lysogenic clone while such lysis might be expected if reduction and phage multiplication leading to lysis involved the same steps. The process of reduction probably involves changes in the virus different from those which accompany phage multiplication in a lytic cell.

Two basically different concepts are possible of the property of prophage that cause it to be transmitted to every cell of a culture. λ may become a prophage by attaching to a regularly inherited portion of the cell, or by multiplying to give a number of cytoplasmically carried particles sufficiently large to preclude their transmission to only one daughter cell at division. A study of the proportions of lysogenics and nonlysogenics found in clones might be expected to point to one hypothesis as the more likely one.

If prophage is carried in the cytoplasm and is distributed at random at cellular division, nonlysogenics will arise in infected clones until the concentration of prophage in every cell becomes sufficiently high to ensure its distribution into both daughter cells at division. Since clones containing less than 10 per cent lysogenic cells were very rare, one would have to conclude that the prophage reaches the required concentration after 3 to 4 cellular divisions. A conservative estimate of the number of prophage particles required to prevent the detection of nonlysogenic cells in lysogenic cultures is 10 per cell. Thus, unless 10 or more λ particles infected the parental cell, the number of phage particles would have to increase more rapidly than the number of bacteria during reduction. At 37 C, the rate of increase of phage relative to bacteria would have to be lower than at 25 C to account for the higher proportion of nonlysogenic cells in the 25 C clones.

The hypothesis of λ attachment to a regularly inherited part of the cell does not require multiplication of the phage during the reduction period. To account for the mixed clones, one can suggest that permanent attachment of the prophage, associated with stability at 42 C, requires about an hour in infected cells. Again, one must postulate that at 25 C reduction is more rapid in relation to cell division than at 37 C. The failure to cure lysogenicity in K12 by methods (high temperature, rapid growth) which have been successful in freeing *Paramecium* from cytoplasmic kappa (Sonneborn, 1947) strengthens the notion that the prophage is not a cytoplasmic particle.

If the prophage is, indeed, a regularly inherited cellular constituent, the most obvious location is a bacterial chromosome. Lederberg and Lederberg (1953) have shown that lysogenicity in certain K12 strains is linked closely with an inherited factor, Gal 4. However, no linkage has been observed between lysogenicity and any other genetic factors available for testing in this laboratory. The Lederbergs have suggested that lysogenicity is established only in certain cells of K12S in which a mutation from Lp^s to Lp^+ has occurred. The cytoplasmic prophage presumably is maintained by the Lp^+ gene, and segregation of this locus results in segregation of lysogenicity in crosses. The data presented here have shown that all K12S cells are, genetically, potentially lysogenic, and thus the linked segregation of lysogenicity and a known chromosomal genetic marker must involve the prophage itself. The prophage may become part of one of the well marked chromosomes of *E. coli* (Fried and Lederberg, 1952) or be part of an independent gene complex like the transducing phage in *Salmonella typhimurium* (Zinder and Lederberg, 1952).

ACKNOWLEDGMENTS

The author wishes to express thanks to Dr. J. J. Weigle for his interest and help during the course of these investigations and also to Professors M. Delbrück and R. Dulbecco for their many valuable suggestions.

SUMMARY

Cells of a strain of *Escherichia coli*, K12, sensitive to the temperate phage λ may give any of 5 different responses after exposure to the phage: lytic, lysogenic, refractory (unaffected by the phage), lethal, and hereditarily resistant.

With the exception of the rare resistant type, these responses are governed by nongenetic factors. The proportions of the 4 chief response types are determined by the physiological condition of the culture, and thus of the individual cells, and by the multiplicity of phage to which the cells are exposed. In any culture, there is a tendency in cells which become infected most rapidly to

give a lytic response and for cells that become infected less readily to give a lysogenic response.

Individual clones derived from cells of *Escherichia coli*, strain K12S, infected with λ were analyzed for the presence of lysogenic cells, nonlysogenic cells, and free phage. Cells which gave rise to clones of cells did not, in addition, produce free phage. The decision as to whether lysis or cellular multiplication is to take place after exposure to the phage is made by the bacterium either before or immediately after infection. However, during the first hour after infection, the establishment of lambda prophage leading to lysogenesis can be suppressed in some or all of the cells in a clone by growing the cells at temperatures above 20 C. Such temperature treatments have no effect on cells of established lysogenic cultures. The observations are compatible with the hypothesis that the infecting phage which leads to lysogenesis acts as a cytoplasmic particle immediately after infection of the cell and later becomes attached to a regularly inherited cell constituent.

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