GENETIC STUDIES OF LYSOGENICITY IN ESCHERICHIA COLI[1]

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RECENT research on *Escherichia coli* phages has outlined the biology of viruses that promptly lyse their bacterial hosts (DELBRÜCK 1950). In addition to the progressive parasitic relationship that these studies have analyzed, many phage-bacterium complexes persist in a more enduring symbiosis, lysogenicity. The experiments to be described in this paper were designed to probe two related questions: how is the virus of a lysogenic bacterium transmitted in vegetative and sexual reproduction? and how is a symbiotic complex established following infection by the virus, as an alternative to the parasitization and lysis of the host bacterium? Complementary problems, especially concerning the growth and release of virus in lysogenic bacteria have received more emphasis from other workers (BERTANI 1951; LWOFF and GUTMANN 1950; WEIGLE and DELBRÜCK 1951).

Our interest in lysogenicity was provoked by the discovery that *E. coli* strain K-12 was lysogenic. On two occasions, mixtures of certain mutant stocks appeared to be contaminated with bacteriophage. The plaques were unusual in showing turbid centers, suggesting those figured by BURNET and LUSH (1936). It soon became apparent that practically all K-12 cultures carried this latent phage. The novelty consisted of two exceptional mutant substrains, W-435 and W-518 which were sensitive to the phage, now referred to as λ. These two strains had been maintained in our stocks as nonfermenting mutants for lactose (Lac_3^-) and galactose ($Gal_4^-Lac_1^-$), respectively, isolated from ultraviolet-treated suspensions. Both cultures are derived from 58-161, a methionine-requiring auxotroph previously used in many recombination experiments (TATUM 1945; TATUM and LEDERBERG 1947). The lysogenicity of strain K-12 had remained unsuspected despite its maintenance for over 25 years and close study as the subject of mutation and recombination experiments since 1944. However, the only objective criterion of a lysogenic symbiosis is the lysis of another sensitive strain that functions as an indicator. Thus, in the absence of an appropriate conjunction of strains the virus carried by the K-12 subline would remain undetected. Because of the low frequency of sensitive strains, such opportunities are rare. The development of crossing techniques in strain K-12 has allowed the virus to be studied as a genetic factor. Intercrosses among strains differing with respect to λ and the development of lysogenic from sensitive strains are the main subjects of this report.

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TERMINOLOGY

Although the adoption of a fixed terminology would be premature, for convenience, a few terms will be defined for use in this account. Lysogenicity will be understood as the regular and persistent transmission of virus potentiality during the multiplication of a bacterium, without overt lysis. When tested directly with the phage, a bacterial culture is *sensitive* (lysed) or *resistant* (not lysed). When tested with a sensitive indicator strain, the bacteria are *lysogenic* (carriers of λ) if the indicator is lysed, or *nonlysogenic* if not. Bacteria that are resistant to λ but nonlysogenic are termed *immune*. The virus as transmitted in lysogenic bacteria will be referred to as *latent virus*.

MATERIALS AND METHODS

Preparation of free phage

Suspensions of λ were first obtained from filtrates of 6–8-hour bacterial cultures developed from mixed inocula of λ-sensitive and λ-lysogenic strains in nutrient broth. Thereafter, further batches were prepared by growing λ-sensitive cells with virus according to the usual methods (ADAMS 1950). Lysis in broth is indicated by decreased turbidity rather than marked clearing. A convenient method for obtaining high titer λ directly from lysogenic bacteria has been developed by WEIGLE and DELBRÜCK (1951) from the methods described by LWOFF *et al.* (1950). A lysogenic strain grown in a yeast-extract broth is subjected to a dose of ultraviolet irradiation which kills only a small fraction of a genetically comparable λ-free strain. After 40–50 minutes incubation in yeast-extract broth the majority of lysogenic cells lyse with a burst of about 100 phage particles each. Virus titrations were made by established methods (ADAMS 1950; DELBRÜCK 1950). All lysates were sterilized by filtration through nine- or fourteen-pound test Mandler candles.

Some pertinent physical and morphological characteristics of λ have been described by other investigators (WEIGLE and DELBRÜCK 1951).

Media

The media recommended for observing phage-bacterium interactions are less useful with the λ system because of the presence of bacterial survivors (which prove to be either resistant or sensitive) in the plaques. The lysed areas are, however, accentuated by their discoloration on an eosin–methylene blue agar medium without the fermentable sugar customarily added (EMB base, LEDERBERG 1950). Plaques from free λ suspensions were counted on TSA (tryptone saline agar, WEIGLE and DELBRÜCK 1951). It was sometimes supplemented with ten percent citrated bovine blood to test the release of hemolysins during bacterial lysis by phage (SCHIFF and BORNSTEIN 1940). A positive reaction is the clearing of the blood at the zone where sensitive bacteria are exposed to λ or to lysogenic bacteria. It must be cautioned, however, that occasional cultures are normally hemolytic, perhaps owing to a high rate of spontaneous lysis.

Scoring for sensitivity and lysogenicity

Susceptibility to λ is tested by streaking a phage suspension across a dry EMB agar plate with a broad loop. A small loopful of the cells to be tested is then streaked at right angles to the phage. To test for lysogenicity, the bacteria are similarly streaked against a sensitive indicator. As a precautionary measure, the tested cells are also deposited at a control spot. As shown in figure 1, positive tests consist of the interruption in the continuity of growth of the indicator, or plaques and discoloration at the conjunction of phage with sensitive bacteria. The technique of replica plating (LEDERBERG and LEDERBERG 1952) facilitates large-scale tests of lysogenicity. Instead of individual tests on bacterial colonies from a plate, these are transferred en masse by means of velveteen fabric to a TSA plate previously layered with 10 ml of TSA seeded with about 10^8 indicator cells. On the replica, each lysogenic colony is surrounded by a zone of lysis.

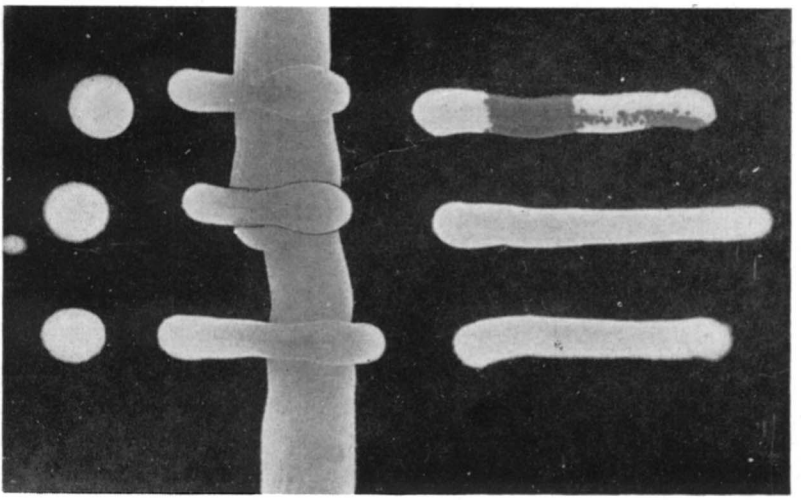

FIGURE 1.—Reactions of sensitive, lysogenic and immune. Extreme left: control spots. Left center: cross-streaks against sensitive indicator bacteria. Right: cross-streaks against λ. From top to bottom Lp_1^s, Lp_1^+, and Lp_1^r respectively.

Crosses

Crosses are carried out by plating washed cultures differing in nutritional characters on minimal agar (TATUM and LEDERBERG 1947; LEDERBERG *et al.* 1951) or with added streptomycin where streptomycin sensitive (S^s) prototrophs are crossed with resistant (S^r) diauxotrophs (LEDERBERG 1951a). The resulting progeny are picked and purified by streaking on a complete medium, and from this, single colonies are isolated for further characterization of segregating markers, including lysogenicity.

Selection of lysogenic and nonlysogenic cultures

Lysogenic bacteria may be routinely isolated from turbid plaques on sensitive bacteria plated with λ or from the residual growth after mixed inoculation

of sensitive bacteria and phage in broth on agar. Successive single colony purifications result in stably lysogenic isolates free of extraneous λ and sensitive bacteria. By isolating one lysogenic derivative from a series of single plaques, it was demonstrated that the transfer of λ from well-marked lysogenic to previously sensitive stocks occurs without any alteration of the known genetic markers of the new host other than its reaction to λ.

The isolation of nonlysogenic (immune or sensitive) types from lysogenic bacteria is less predictable but they have been obtained by the following procedure. EMB or blood agar plates were spread with 10^8 cells and exposed to ultraviolet light so that about 100 to 200 colonies survived. New types have been sporadically detected either by testing large numbers of normal-appearing colonies or by the partially lysed appearance of phage-contaminated sensitive colonies. These consist of lysogenic and sensitive sectors, and free λ. Immune mutants have arisen from sensitive bacteria after selection with phage. The various occurrences of nonlysogenic derivatives are listed in table 1.

RESULTS

Intercrosses of various phenotypes

Crosses among the sensitive, lysogenic, and immune strains are all fully fertile. They have been repeated many times with the following qualitative results, based on 200 or more tests for each cross.

1. Lysogenic × lysogenic: all progeny lysogenic.
2. Sensitive × sensitive: all progeny sensitive.
3. Sensitive × lysogenic: the progeny segregate into sensitive and lysogenic, with ratios depending on the nutritional markers of the parents.

The total number of tests of crosses 1 and 2 is actually much larger, for exceptional progeny would have been apparent upon inspection of similar crosses conducted for other purposes.

Since only the parental types are found in cross 3, it might be inferred that lysogenic differs from sensitive only by one factor, the presence of the λ. However, the consideration of λ as a cytoplasmic factor leads to a possible paradox: when λ is contributed by just one parent, it segregates among the progeny, but it always appears when contributed by both parents. It should be emphasized that the same segregation ratios for other markers have been obtained regardless of the presence or absence of λ in the parents. No evidence has been found to date for the functioning of λ as a gamete or other sexual form (cf. HAYES 1952; LEDERBERG, CAVALLI and LEDERBERG 1952).

Further crosses involving two immune parents gave the following results:

4. Immune-1 × sensitive: parental only.
5. Immune-2 × sensitive: parental only.
6. Immune-1 × lysogenic: parental only.
7. Immune-2 × lysogenic: parental and sensitive.
8. Immune-1 × immune-2: parental and sensitive.

TABLE 1

Principal stocks used in lysogenicity studies.

Strain number	Source strain	History	Genotype[1]
		Sensitive (Lp_1^s)	
W-435	58-161	UV (ultraviolet)	$M^-Lac_3^-$
W-518	Y-87	UV	$M^-Lac_1^-Gal_4^-$
W-1267		W-518 × W-588, f-1 segregant	$T^-L^-Lac_1^-Gal_4^-$
W-1485	K-12	UV; blood agar	wild type sensitive
W-1486	W-811	plating with streptomycin	$M^-Lac_1^-Gal_4^-S^r$
W-1487	W-1405	plating with streptomycin	$T^-L^-Lac_1^-Gal_4^-S^r$
W-1502	W-1245	spontaneous variation	M^-
W-1503	W-1296	spontaneous variation	T^-L^-
W-1655	58-161	UV	M^-
W-1872	K-12	UV	wild type sensitive
		Immune-1 and -2 (Lp_1^r and Lp_2^r)	
W-1027	Y-70	UV	$T^-L^-\ Lac_1^-Lp_1^r\ Lp_2^s$
W-1924	W-518	selection with λ	$M^-Lac_1^-Gal_4^-Lp_1^r\ Lp_2^s$
W-1248	W-518	selection with λ	$M^-Lac_1^-Gal_4^-Lp_1^s\ Lp_2^r$
W-1603	W-1177	UV	T^-L^- etc., $Lp_1^s\ Lp_2^r$
W-1245	W-478	UV	M^-; unstable immune
W-1296	W-588	UV	T^-L^-; unstable immune
		Lysogenic (Lp_1^+: Lp_2^r or Lp_2^s)	
58-161		standard parent	$M^-\ Lp_2^s$
W-1177		multiple marker parent	$T^-L^-\ Lac_1^-Mal_1^-Xyl^-Gal_5^-S^rLp_2^r$
W-811	W-518	infection with λ	$M^-\ Lac_1^-Gal_4^-\ Lp_2^s$
W-1439	W-811	selection with λ-2	$M^-\ Lac_1^-Gal_4^-\ Lp_2^r$

[1] The significance of the genotypic symbols, and further details of ancestry of many stocks are given in LEDERBERG *et al.* (1951) and LEDERBERG (1952).

The appearance of a sensitive recombination class in cross 8 implicates two loci in resistance to λ. Sensitive can be described as $Lp_1^s\ Lp_2^s$, immune-1 as $Lp_1^r\ Lp_2^s$ and immune-2 as $Lp_1^s\ Lp_2^r$. From the result of cross 6, in contrast to cross 7, lysogenicity is also determined at the Lp_1 (latent phage) locus. Evidence for two kinds of lysogenic, Lp_2^s (those so far discussed) and Lp_2^r, respectively, will be presented in another section.

Occasional sensitive progeny would have been anticipated in cross 6 on the hypothesis that lysogenic is genotypically equivalent to sensitive, and differs only by the presence of cytoplasmic λ, but were not found. The independent segregation of λ (cross 3) and of the genetic factor Lp_1 (cross 4) would have resulted in some λ-free recombinants sensitive to the virus. The results of all these crosses hinted at a primarily " chromosomal " determination of lysogenicity.

Linkage behavior of lysogenicity

The concept of an Lp_1 locus was strengthened by the outcome of linkage tests in which various markers were segregating. A loose linkage of Lp_1 to *Xyl* and to *S* was indicated in preliminary crosses with a multiple marker stock. However, Lp_2 was also segregating, thus doubling the number of genotypic classes, and perhaps confusing the issue. The closest linkage of Lp_1 thus far found has been to Gal_4, as shown in table 2. As it happens, this is the distinctive marker of W-518, in which λ-sensitivity was first noticed.

The linkage of Lp_1 with Gal_4 has been verified by crosses with various combinations of lysogenic stocks resynthesized from sensitive auxotrophs. Some of the latter were newly developed from W-1485, a λ-sensitive directly derived from strain K-12. There can be little doubt, therefore, that the segregating

TABLE 2

Linked segregation of Gal_4 and Lp_1 among prototrophic recombinants.

	Parents		Prototroph recombinants: $M^+T^+L^+$...			
	A $M^-T^+L^+$	× $M^+T^-L^-$	Gal^+Lp^+	Gal^+Lp^s	Gal^-Lp^+	Gal^-Lp^s
1	Gal^+Lp^s	× Gal^-Lp^+	1	83	90	2
2	Gal^+Lp^+	× Gal^-Lp^s	33	1	3	41
3	Gal^-Lp^s	× Gal^+Lp^+	55	0	5	53
4	Gal^-Lp^+	× Gal^+Lp^s	1	42	44	1
	B $M^-H^+L_2^+$	× $M^+H^-L_2^-$				
1	Gal^-Lp^+	× Gal^+Lp^s	0	34	40	1
	C $M^-M_2^+G^+$	× $M^+M_2^-G^-$				
1	$Gal^-Lp_1^+$	× Gal^+Lp^s	0	40	39	1
2	Gal^-Lp^s	× Gal^+Lp^+	64	2	1	67

The crosses were conducted on EMS galactose medium, from which approximately equal numbers of Gal^+ and Gal^- prototrophs were picked for further test. Similar results were obtained when the proportion of Gal^+ and Gal^- was not thus fixed, as on non-indicator glucose minimal agar, but the preponderance of one parental type among the prototrophs limited the usefulness of unselected isolates for linkage tests. The $H^+L_2^-$ and $M_2^-G^-$ parents indicated in B and C are histidine-leucine and methionine-glycine auxotrophs, respectively, recently derived from W-1485. All parents in these crosses were Lp_2^s, but V_1, Lac_1, and *S* were segregating in their usual patterns.

factor is directly associated with lysogenicity. The linked segregations justify the assignment of a new allele, Lp_1^+, characteristic of lysogenicity. The result indicated for cross 6 points to this as a third allele at the same locus as the contrasting Lp_1^r (immune-1) and Lp_1^s (sensitive).

Segregation of λ from diploids

Heterozygotes selected as Lac^+/Lac^- or Gal^+/Gal^- were obtained and shown to be segregating for a number of other factors (LEDERBERG 1949), but these selections were either λ-sensitive or λ-lysogenic. Similar results were obtained in immune, *Het* crosses. It was thought, however, that the λ-determinant might be hemizygous in these diploids, like the *Mal* and *S* factors previously studied (LEDERBERG *et al.* 1951). This difficulty has been circumvented by the use of diploid × haploid crosses, in which the segmental elimination

(of *Mal* and *S*) apparently does not occur. A lysogenic diploid parent (T^-L^- Gal^+ Lac^+ Mal^+/Lac^- Mal^-) was crossed with a sensitive, haploid auxotroph (M^- Gal_4^- Lac^- Mal^+) on minimal agar. The resulting prototrophs were almost all diploid, and several were identified as lysogenic, but segregating Gal^+/Gal^- as well as other factors. As shown in table 3, presence vs. absence of λ segregated in the same coupling as shown by the parents: Gal^+ lysogenic/Gal^- nonlysogenic. Unfortunately, this diploid is also segregating Lp_2, so that the nonlysogenic segregants include immune-2 as well as λ-sensitive.

The linkage and segregation evidence shows that a chromosomal factor is altered when a cell becomes lysogenic. In addition, a cytoplasmic factor (λ itself) may be postulated, but genetic evidence for it is entirely inconclusive. Two possible interpretations may be considered: 1) The virus or provirus occupies a definite niche on the chromosome, near Gal_4. Lysogenicity results from the cellular or even chromosomal fixation of the latent virus. 2) The chromosomal factor is a gene, Lp_1^s, which mutates spontaneously to an allele Lp_1^+ that potentiates a symbiotic relationship of λ in the bacterial cytoplasm.

TABLE 3

Segregation of Gal_4 and Lp_1 from heterozygous diploids.

	Gal^+Lp^+	Gal^+Lp^s	Gal^-Lp^+	Gal^-Lp^s
H-295	36	1	1	39 (19 Lp_2^s)
H-297	29	0	0	11 (3 Lp_2^s)

Segregant (pure) Gal^+ and Gal^- colonies were picked from EMB galactose agar at random, and tested for susceptibility to λ and λ-2, and for lysogenicity. The phenotypically λ-sensitive (Lp_2^s) moiety of the Lp_1^s segregants is shown in parentheses. Almost all of the Lp_1^+ were Lp_2^r.

On this hypothesis, the role of λ in the induction of lysogenicity is confined to the selection of the pre-adaptive mutation, Lp_1^+. A similar dilemma in the determination of the killer trait in *Paramecium aurelia* has been resolved in terms similar to the second interpretation (SONNEBORN 1950), although the first was originally favored (SONNEBORN 1945). Its substantiation for lysogenicity would require the recognition of the possible genotypes: Lp_1^s no-λ (presumably the sensitives); Lp_1^s infected with λ (presumably lethal); Lp_1^+ with lambda (the lysogenic); and a new combination, Lp_1^+ no-λ. This last type, genetically pre-conditioned for lysogenicity, would presumably be recognized as an apparently immune form that would promptly absorb λ to become lysogenic. It has not yet been identified among immune stocks of K-12, or immune progeny collected from a variety of strain intercrosses.

Mechanism of infection

When λ-sensitive bacteria are plated with λ, survival ratios in the range of ten to fifty percent are usually encountered. Many of the survivors are apparently lysogenic. The hypothesis of spontaneous variation at the Lp_1 locus

would be untenable if, as these facts appear to show *prima facie,* several percent of sensitive bacteria became lysogenic under the direct influence of the virus. Only preliminary experiments have been done on this aspect of the problem, with results that are not yet conclusive. A striking feature of platings of diluted bacteria-virus mixtures of varying relative multiplicity has been the development of contaminated colonies, similar to those figured by BOYD (1951). These colonies displayed a very characteristic appearance on EMB agar. They were often delayed in their development, lagging a few hours behind their neighbors, and later show either a central " necrosis " or plaquing, or often single or multiple pericentric plaques. When the contaminated colonies were restreaked, they typically gave rise to a mixture of contaminated, sensitive and lysogenic colonies.

Many of the latter are only apparently lysogenic, for they include sensitive bacteria as shown by serial restreaking of single colonies. It is not unlikely (though not yet proven) that contaminated colonies may arise from single infected cells. If this is the case, the determination of lysis versus lysogenicity is effected during the development of a contaminated clone, and there would be greater opportunity for genetic variation and natural selection. On the other hand, if a fair proportion of infected cells are actually converted directly into lysogenics, it would be concluded that λ itself induces or fixes the mutation from Lp_1^s to Lp_1^+.

Virus and host mutations

Following irradiation of a type lysogenic, a self-lysed colony was noted from which a distinctive virus was isolated. This virus, λ-2, differs from λ in its ability to destroy Lp_1^+ bacteria. Attempts to develop a symbiosis of λ-2 with each of a variety of bacterial stocks have been unsuccessful. Its relationship to λ as a " host range mutant " is supported by the concurrent development of resistance to λ with mutations from sensitivity to resistance to λ-2. Several recurrences of λ-2 have been detected in lysed colonies after ultraviolet irradiation, and in λ stocks grown on sensitive cells. It has not, however, been observed in routine bacterial cultures, although it would presumably have been conspicuous. This is in contrast to the rapid accumulation of comparable virus mutants in cultures of the lysogenic staphylococci studied by BURNET and LUSH (1936).

Immune bacterial mutants have been observed among survivors of both irradiated lysogenic cultures and sensitive cultures exposed to the viruses. Immune-1 has occurred very infrequently, and is resistant to (and nonlysogenic for) λ, but sensitive to λ-2. Immune-2 is resistant to both phages, showing neither lysis nor the development of lysogenicity. As already mentioned, different loci, Lp_1 and Lp_2, appear to be involved. Although immune-2, $Lp_1^s\, Lp_2^r$, does not respond to free λ, selection for resistance to λ-2 in a lysogenic stock gives the genotype $Lp_1^+\, Lp_2^r$ which remains lysogenic for λ. Crosses of such lysogenics with sensitive ($Lp_1^+\, Lp_2^r \times Lp_1^s\, Lp_2^s$) gave all four of the expected types: immune-2 ($Lp_1^s\, Lp_2^r$) and type lysogenic ($Lp_1^+\, Lp_2^s$), in addition to the parents. Current stocks of K-12 are mixed populations with respect to Lp_2.

It is not surprising, therefore, that several mutant derivatives, notably W-1177 extensively used in crossing experiments, are already Lp_2^r. Two λ-immune selections have been found, both sensitive to λ-2, which were unstable and frequently engendered λ-sensitive colonies. Tests for allelism with Lp_1^r were inconclusive owing to this instability.

Other mutants of the virus have been sought, but only plaque variants not readily scored were observed. Resistance to λ and λ-2 is concomitant with resistance to p-14, a phage isolated from sewage. Morphologically, the plaques of p-14 are intermediate between those of λ and λ-2, with turbid centers associated with a spurious or unstable lysogenicity which persisted in slow-growing isolates at 30° and was rapidly lost at 37°. Despite its initial promise as a selective agent for other bacterial mutations related to λ, p-14 did not elicit any otherwise unrecognized types.

A " weakly lysogenic " bacterium was recovered after ultraviolet irradiation of a typical lysogenic form. When inoculated with the indicator strain, the variant induced very few plaques, so that it was not readily distinguished from immune nonlysogenic forms. When the virus was transferred from the weakly lysogenic form to sensitives normal lysogenicity ensued. This suggests that reduced lysogenicity was a property of the host rather than of the phage. It was conceivable, however, that the plaques of free virus represent reverse-mutants from a virus population that otherwise remains entirely latent within the infected variant bacterium. To eliminate this possibility, sensitive recombinants from crosses of the weak lysogenic with sensitive were infected individually with type λ. Both types of lysogenicity were expected on the hypothesis of bacterial mutation, and this was actually observed. A modifier locus is thus revealed, but its relationships with other factors have not been explored.

Another intermediate reaction type was isolated from plates spread with 10^8 bacteria and λ-2. Most of the survivors were fully resistant to both λ and λ-2, but some exhibited a partial resistance to λ and λ-2, which was reflected in overgrowth of cross-streaks and reduced efficiency of plating and plaque size for both viruses, similar to the expression of V_1^p (partial resistance to T_1, LEDERBERG 1951b; WAHL and BLUM-EMERIQUE 1952). λ-lysogenic derivatives were prepared which were still semi-resistant to λ-2. The mutation thus involved either a third allele, Lp_2^p, at the Lp_2 locus or mutation at another locus.

In view of speculation concerning the dispersion of lytic phages into genetic subunits during intracellular growth, the possibility that fragments of λ might persist in apparently nonlysogenic cells was considered. The reconstitution of lytically active λ from components carried in different nonlysogenic recombinants or variants would be relevant evidence. However, such a recurrence of phage from appropriate mixtures and crosses has hitherto not been demonstrated.

Disinfection

Two lysogenic streptomycin-sensitive (S^s) cultures plated on streptomycin agar have been observed to yield large numbers of resistant (S^r) mutant colo-

nies which showed the characteristically mottled margins of phage attack. These colonies gave rise to S^r λ-sensitive isolates. Reconstruction experiments with these mutants or their re-infected derivatives failed to establish any foundation for either a selective advantage or a specific inductive effect of streptomycin to explain the accumulation of λ-sensitive. By indirect selection (LEDERBERG and LEDERBERG 1952), it was possible to extract the S^r components, and show their λ-sensitive character without exposing them to streptomycin. The λ-sensitive and S^r characters were not distinguishable from mutations previously isolated in single steps. No explanation for this remarkable association can be offered.

Systematic attempts were made to remove λ from lysogenic bacteria by a number of other methods. As none were successful, details will be omitted. The treatments that were tried included cultivation at limiting temperatures and pH ranges (as originally suggested by D'HERELLE 1926), and exposure to antibiotics and antiviral chemicals, including streptomycin, aureomycin, chloromycetin, Phosphine GNR, 2-nitro-5-aminoacridine, citrate ion, cobaltous ion, and desoxypyridoxine. A serious limitation to this type of investigation is the inadequacy of earlier methods of detecting disinfected variants, if they occur infrequently. Replica plating should help to surmount this problem, but was not available at the time of these experiments.

Almost all of our original λ-sensitive stocks in strain K-12 have been noticed following exposure to treatment with ultraviolet light. Inasmuch as this agent, under certain conditions, preferentially kills lysogenic cells by inducing lysis (WEIGLE and DELBRÜCK 1951), it cannot be concluded whether a selective or inductive (disinfective) action is involved.

Lysogenicity and other E. coli strains

The λ reaction of about 2000 strains under investigation for intercrossability has been routinely tested. No recurrence of λ itself has been identified, but five new strains are sensitive to λ and λ-2. One apparently unstable immune strain gave rise to sensitive subtypes, which, however, could not be made lysogenic on K-12 line indicators for either virus. All of the new sensitive lines, including NTCC 123 (CAVALLI and HESLOT 1949) are fertile with K-12, suggesting a statistical correlation of λ receptors with compatibility. Most of the 50 or so interfertile strains that have been screened are, however, immune to λ.

Although a large proportion of the strains tested produced an antibiotic or colicin (FREDERICQ 1948) active on K-12, less than one percent were lysogenic. The lysogenic cultures (which include, for example, the Waksman strain used in biochemical genetic studies, DAVIS 1950) carry what appear to be quite distinctive phages, judging from plaque type and resistance patterns. Two of the new latent phages have been successfully transferred to the K-12 line. Triply lysogenic K-12 strains were maintained without any overt effects on the λ system or other characters of the bacteria. The genetic determination of lysogenicity for other phages may differ from that of λ, however, in so far as clear-cut segregation for them was not observed in crosses or from diploids also segregating λ.

DISCUSSION

This work was initiated in the expectation that λ would behave as an extranuclear factor, and might indeed provide a favorable model system for studies of cytoplasmic heredity. Phenotypic changes associated with the transfer of λ have, so far as known, been confined to the direct consequences of virus infection. For example, lysogenic bacteria are more susceptible to ultraviolet light, owing to the "induction" of the latent phage and lysis of the bacterium (WEIGLE and DELBRÜCK 1951). In other systems, latent viruses have been shown to determine the pattern of susceptibility to other viruses, the "lysotype" (NICOLLE and HAMON 1951; WILLIAMS-SMITH 1948; ANDERSON 1951), by a mutual exclusion effect. With one dubious exception, no phages that would differentiate λ-sensitive from λ-lysogenic were found in tests of some thousands of coliphage plaques from sewage. In principle, however, a virus-symbiosis might be detected in terms of the intercellular transfer of a genetically active agent not readily recognizable as a lytic phage (LOMINSKI 1938).

This view of λ may have to be qualified in view of the genetic tests discussed in this paper. No genetic evidence of λ as a cytoplasmic agent was found. In the most critical tests, segregation from heterozygote diploids, lysogenicity behaved precisely as if it were controlled by a nuclear factor, linked to other segregating factors. This result provides strong support for the "provirus" concept of the symbiosis. The segregation of uninfected, virus-sensitive haploids from a lysogenic diploid is not readily compatible with the presence of free, mature virus in the latter. It is not, however, conclusive against a cytoplasmic provirus. The segregation of lysogenicity/sensitivity may reflect the overriding control by a segregating nuclear factor which is concerned with the maintenance of the pro-λ. The mutational origin of this segregating factor is, however, still in question.

It should not be assumed that these results can be generalized to other lysogenic symbioses. In *Salmonella typhimurium,* BOYD (1951) has shown that the multiplicity of infection is an important element in the determination of lysogenicity. This would leave little room for bacterial variability, but a closer analysis of the incidents immediately related to the development of lysogenic cells might reveal a situation more comparable to that in *E. coli.* In preliminary studies of the transmission of other viruses, transferred to K-12 from other lysogenic strains, diploids lysogenic for two phages showed segregation for λ but not for the second phage. The apparent difference with respect to nuclear determination may be a consequence of the antiquity of the association of K-12 with λ in contrast to the newly introduced phages.

It may be noteworthy that λ has not recurred in extensive samplings of other *E. coli* strains and of sewage. The occurrence of λ-sensitive isolates has already been mentioned. It is rather striking that all five of these isolates should be cross-fertile, compared to the four to five percent of the whole population. Whether this speaks for a close genetic relationship or for the closer attention given these lines cannot be said. It should be emphasized that all of the evidence argues against any functional relationship between lysogenicity and

sexual fertility. The most decisive point, perhaps, is that nonlysogenic crosses are as fertile as crosses involving one or both lysogenic parents, both within strain K-12, and as between strains.

The biological significance of the lysogenic symbiosis is attested to not only by the behavior of individual examples, but by its prevalence in many groups of bacteria. BURNET (1945) and others have emphasized the biological advantages to the parasite as well as the host of symbiotic adaptation. In addition, the virus genotype represents an additional reservoir of genetic material subject to adaptive variation. This adaptation will often lead to an amelioration of the pathogenic effects of the virus. One can imagine a situation in which a virus remains trapped within a host that it never lyses. A bacterial mutation for weak lysogenicity illustrates this trend, and it has perhaps been realized in LOMINSKI'S (1938) experiments. The extreme case would however restrict the migration of the virus to other genotypes, as well as our ability to recognize it as a virus. It is conceivable that the immune-1 (Lp_1^r) mutation represents such a bound virus, although free λ has not recurred even in its crosses to λ-sensitive.

The most prominent mutation of λ has, according to this picture, only short-term evolutionary advantages. The virulent mutant, λ-2 will rapidly destroy lysogenic bacteria, and thus displace λ from viral populations. The exhaustion of sensitive hosts will, however, limit its long-term survival. The early literature on bacteriophage contains many references to the so-called spontaneous generation of bacteriophage in bacterial cultures. While some of these reports are possibly founded on technical faults, probably most of them represent instances of the mutation of virus in lysogenic bacteria not recognized as such. If it were not for the availability of an indicator strain for λ, the occurrence of lysis due to λ-2 in platings of ultraviolet irradiated K-12 would have passed either for a contamination or such " spontaneous generation."

For technical reasons, the phages of the T series acting on *E. coli* B have received considerable attention. These phages have been used for such work precisely because they are atypical in their prompt destruction of sensitive bacteria, high efficiency of plating, the limited number of secondary resistants, and clear plaques. A plating of sewage with indicator bacteria shows at a glance that phages of this kind are relatively infrequent. Although the analysis of phage-bacterium relationships on a logically sound, particulate basis has demanded systems with these technical properties, it would be a fallacy to generalize too hastily on virus biology from the study of a restricted set of materials.

SUMMARY

Escherichia coli strain K-12 carries a symbiotic phage, λ. This phage was discovered only by the occurrence of " mutant " substrains sensitive to λ, and serving as indicators for it. In addition to the lysogenic (carrier) and sensitive bacteria, two immune types (" 1 " and " 2 ") were found. These are defined as resistant but nonlysogenic.

The various types have been intercrossed to elucidate the genetic basis of lysogenicity. The crosses lysogenic × lysogenic; immune-1 × immune-1 and sensitive × sensitive have yielded only the parental class. Similarly, only the two parental classes were found in lysogenic × sensitive; lysogenic × immune-1; and sensitive × immune-1. The segregation of lysogenicity has been confirmed by the synthesis of diploid stocks heterozygous for lysogenicity, which behaves as a factor linked to Gal_4 (galactose fermentation). Genetic evidence of the transmission of λ as a cytoplasmic factor was not found. A locus for latent phage, Lp_1, which controls the maintenance of λ, or to which λ is bound is postulated. The detailed role of λ in the alteration of the Lp_1 locus that is associated with the resynthesis of lysogenic from sensitive has not been clarified.

Mutation of λ to a more virulent mutant λ-2 has been observed. λ-2 lyses λ-lysogenic as well as λ-sensitive bacteria. Immune-2 confers resistance both to λ and to λ-2. It does not, however, interfere with the maintenance of λ in bacteria already lysogenic. It is genetically separable from immune-1. A few additional *E. coli* stocks sensitive to λ, or lysogenic for other phages, have been found. In an extensive survey, λ itself has not recurred.

LITERATURE CITED

ADAMS, M. H., 1950 Methods of study of bacterial viruses. Methods in Medical Research **2:** 1–73.

ANDERSON, E. S., 1951 The significance of Vi-phage types F1 and F2 of *Salmonella typhi*. J. Hyg. **49:** 458–470.

BERTANI, G., 1951 Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bact. **62:** 293–300.

BOYD, J. S. K., 1951 Observations on the relationship of symbiotic and lytic bacteriophage. J. Path. Bact. **63:** 445–457.

BURNET, F. M., 1945 Virus as Organism. 134 pp. Cambridge, Mass.: Harvard University Press.

BURNET, F. M., and D. LUSH, 1936 Induced lysogenicity and mutation of bacteriophage within lysogenic bacteria. Austr. J. Exp. Biol. Med. Sci. **14:** 27–38.

CAVALLI, L. L., and H. HESLOT, 1949 Recombination in bacteria: outcrossing *Escherichia coli* K-12. Nature **164:** 1057–1058.

DAVIS, B. D., 1950 Studies on nutritionally deficient bacterial mutants isolated by means of penicillin. Experientia **6:** 41–50.

DELBRÜCK, M. (ed.), 1950 Viruses 1950. Pasadena: California Institute of Technology.

D'HERELLE, F. D. 1926 The Bacteriophage and Its Behavior. Transl. G. H. Smith. Baltimore: Williams and Wilkins. See p. 237.

FREDERICQ, P., 1948 Actions antibiotiques réciproques chez les Enterobactériaceae. Rév. belge path. med. exp. 19 (Supplement 4): 1–107.

HAYES, W., 1952 Recombination in *Bact. coli* K 12: unidirectional transfer of genetic material. Nature **169:** 118.

LEDERBERG, E. M., 1952 Allelic relationships and reverse mutation in *Escherichia coli*. Genetics **37:** 469–483.

LEDERBERG, J., 1949 Aberrant heterozygotes in *Escherichia coli*. Proc. Nat. Acad. Sci. **35:** 178–184.

1950 Isolation and characterization of biochemical mutants of bacteria. Methods in Medical Research **3:** 5–22.

1951a Prevalence of *Escherichia coli* strains exhibiting genetic recombination. Science **114:** 68–69.

1951b Genetic studies with bacteria. Genetics in the 20th Century. viii + 634 pp. New York: Macmillan.

LEDERBERG, J., L. L. CAVALLI, and E. M. LEDERBERG, 1952 Sex compatibility in *Escherichia coli*. Genetics **37**: 720–730.

LEDERBERG, J., and E. M. LEDERBERG, 1952 Replica plating and indirect selection of bacterial mutants. J. Bact. **63**: 399–406.

LEDERBERG, J., E. M. LEDERBERG, N. D. ZINDER, and E. R. LIVELY, 1951 Recombination analysis of bacterial heredity. Cold Spring Harbor Symp. Quant. Biol. **16**: 414–443.

LOMINSKI, I., 1938 Souches lysogènes nonactives (cryptolysogènes). Compt. Rend. Soc. Biol. **129**: 151–153.

LWOFF, A., and A. GUTMAN, 1950 Recherches sur un *Bacillus megaterium* lysogène. Ann. Inst. Pasteur **78**: 711–739.

LWOFF, A., L. SIMINOVITCH, and N. KJELGAARD, 1950 Induction de production de phage dans une bactérie lysogène. Ann. Inst. Pasteur **79**: 815–858.

NICOLLE, P., and Y. HAMON, 1951 Recherches sur les facteurs qui conditionnent l'appertenance des bacilles paratyphiques B aux différents types bacteriophagiques de Felix et Callow. III. Nouvelles études sur les transformations de types par l'action des bactériophages extraits des bacilles lysogènes. Ann. Inst. Pasteur **81**: 614–630.

SCHIFF, F., and S. BORNSTEIN, 1940 Hemolytic effect of typhoid cultures in combination with pure lines of bacteriophage. J. Immunol. **39**: 361–367.

SONNEBORN, T. M., 1945 The dependence of the physiological action of a gene on a primer and the relation of primer to gene. Amer. Nat. **79**: 318–339.

1950 The cytoplasm in heredity. Heredity **4**: 11–36.

TATUM, E. L., 1945 X-ray induced mutant strains of *Escherichia coli*. Proc. Nat. Acad. Sci. **31**: 215–219.

TATUM, E. L., and J. LEDERBERG, 1947 Gene recombination in the bacterium *Escherichia coli*. J. Bact. **53**: 673–684.

WEIGLE, J. J., and M. DELBRÜCK, 1951 Mutual exclusion between an infecting phage and a carried phage. J. Bact. **62**: 301–318.

WILLIAMS-SMITH, H., 1948 Investigations on the typing of staphylococci by means of bacteriophage. J. Hyg. **46**: 74–81, 82–89.

WAHL, R., and L. BLUM-EMERIQUE, 1952 Les bactéries semi-resistantes au bactériophage. Ann. Inst. Pasteur **82**: 29–43.