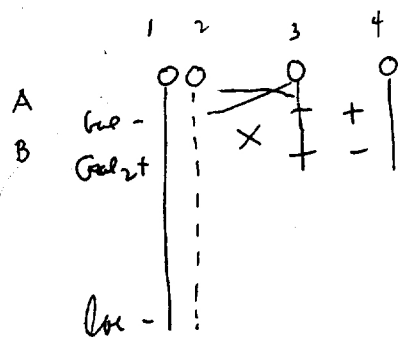
Cavalli - Expt

Shared segregation



Crossover	1,3	1,4	2,3	2,4
A	− − + + − −	− + + − −	+ − − + −	+ + lethal? − −
B	− + + + − −	− + + − −	− + + − −	− + lethal? − −

A 1,3 is only homozygous (−) possible in single crossover. Identify gal- must be gal$_2^-$ in this model to detect defects for loc by reversion test

This methodology should also give 1 lethal segregant per HFT (ie A, 1,3 is HFT, and there seems to be no other simple way to obtain homozygous (−)). Since HFT was between 10-20% then lethals must run this amount also.

To obtain allelyic HFT

	1,3	1,4
A	—	

S	S	R	R
1⁻	1⁻	1⁺	1⁺
4⁺	4⁺	4⁻	4⁻

①

1, 3		1, 4		2, 3		2, 4	
S	R	S	R	S	R	S	R
1⁻	1⁻	1⁻	1⁺	1⁺	1⁻	1⁺	1⁺
4⁺	4⁺	4⁺	4⁻	4⁻	4⁺	4⁻	4⁻
∅		1⁻		≠ (+)		≠ (+)		4⁻	
Seq		S 1⁻		S 1⁻		S 4⁻		S 4⁻	

②

S	R	S	R	S	R	S	R
1⁻	+	−	+	−	+	−	+
4⁺	+	+	−	−	+	−	−
∅		(+)		≠ (+)		(+)		(−)	
Seq		S 1⁻		S 1⁻		S(− −)		S − −	

Uniclés

①

1, 3, 4			2, 3, 4		
S	R	L	S	R	R
1⁻	−	+	+	−	+
4⁺	+	−	−	+	−

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Strains

- W1895 Hfr M⁻ Lac⁺ Lp⁺
  - ↓ W2252 Hfr M⁻ Lac⁺ Lp^S
    - [HFT 1⁻] W2345 Hfr Gal₁⁻ Lp⁺
    - [HFT 2⁻] W2341 Hfr Gal₂⁻/Gal₂⁻ Lp^R/S
      - ↓ W2344 Hfr Gal₂⁻ Lp^S V₁^R
        - ↓ W2694 Hfr Gal₂⁻ Lp⁺ V₁^R H+
    - [HFT 4⁻] W2487 Hfr Gal₄⁻ Lp⁺
      - ↓ ? W2656 Hfr Gal₄⁻ S^R Lac⁺ Lp⁺ M⁻
        - ↓ ? see Below W2662 Hfr Gal₄?⁻ Lac⁺ Lp^S
    - [HFT 4⁻] W2657

- W1503 TLB₁ ≡ Het Lp^S Lac⁺ V₁^R
  - [HFT 1⁻] W2430 Het Gal₁⁻ Lp^S
    - ↓ mut. W2593 Het Gal₁⁻ Lp^S F⁻
      - ↓ λ₂ W2606 Het Gal₁⁻ F⁻ Lp⁺ Mal⁻ Lp₂^R
      - [λ₂] W2607 Mal+ Lp⁻
        - ↓ λ W2608 Lp⁺
  - ↓ HFT 2⁻ W2428 Gal₂⁻ Lp^S
    - ↓ mut. W2591 Gal₂⁻ Lp^S F⁻
  - [HFT 2⁻] W2429 Gal₂⁻ Lp⁺
    - ↓ mut W2592 Gal₂⁻ Lp⁺ F⁻
      - [λ₂] W2605 Mal⁻ Lp₂^R
      - ↓ λ₂ W2604 Mal+ seg col. λ^R

Idiotype S = 1⁻

Case	①	②	③	④
①	+	+	−	−
	S	S	R	R
②	−	−	+	+

Crossover ①

Strands

1, 3	2, 3	2, 4
+ −	+ −	+ −
S S	R S	R R
− −	+ −	+ +

Phenotype: (−) (+) (+)

Segregant: — both probably mistaken for stable (+) because of selective action of gal.

Comment: not fun (+) —

Crossover ②

1, 3	2, 3	2, 4
+ −	+ −	+ −
S R	S R	S R
− −	+ −	+ +

Phenotype: (−) (+) (+)

Segregant: — As above

Comment: not fun (+) — —

C.o outside the requi

strands	1, 3	2, 3	2, 4
	+ −	− +	− −
	S R	R S	R R
	− +	+ −	+ +
Phenotype	≠ (+)	≠ (+)	(−)

Two disjunctions

Strands: 1, 3, 4 | 2, 3, 4

Crossover ①

1, 3, 4	2, 3, 4
+ − −	+ − −
S S R	R R R
− − +	+ − +

Phenotype: ≠ (+) ? (+)

Segregant: — probably mistaken for stable (+)

Given

	①	②	③	④
Lg	S	S	R	R
	4⁻	4⁻	4+	4+
	2+	2+	2⁻	2⁻

idio R

allo R

allo R

(−) Closed Region ① — ϕ Seq

1,3	S 4⁻ 2+	R 4⁻ 2+	(−)	idio S
1,4	S 4⁻ 2+	R 4+ 2⁻	(+)	idio S
2,3	S 4+ 2⁻	R 4⁻ 2+	(+)	allo S
2,4	S 4+ 2⁻	R 4+ 2⁻	(−)	al. S

Region ②

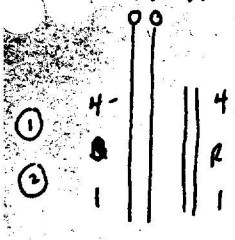
1,3	S 4⁻ 2+	R 4+ 2+	(+)	idio S
1,4	S 4⁻ 2+	R 4+ 2⁻	(+)	idio S
2,3	S 4⁻ 2⁻	R 4+ 2+	(+)	amphi S
2,4	S 4⁻ 2⁻	R 4+ 2⁻	(−)	amphi R

Order of segregation

1. simple loss yielding idio S
2.

Qdistype S = 4⁻

Model



Case

	−	−	+	+
①	S	S	R	R
②	+	+	−	−

Examining (−) segregants from (+)

Crossover ①

Strands	1, 3	2, 3	2, 4
	− +	− +	− +
	S S	S S	R R
	+ +	− +	− −
Phenotype	(+)	(+)	(−)
Segregant	Qdis S	Qamphi S	—
Comment	could not give parental (−)	could give parental (−)	would be overlooked since not from (+)

Crossover ②

	− +	− +	− +
	S R	S R	S R
	+ +	− +	− −
Phenotype	(+)	(+)	(−)
Segregant	Qdis S	Qamphi S	—
Comment	as above	as above	as above

C.O. outside the region

Strands	1, 3	2, 3	2, 4
	− −	+ −	+ +
	S S	R S	R R
	+ +	− +	− −
phenotype	(−)	≠ (+)	(−)

Non disjunction

Crossover ①

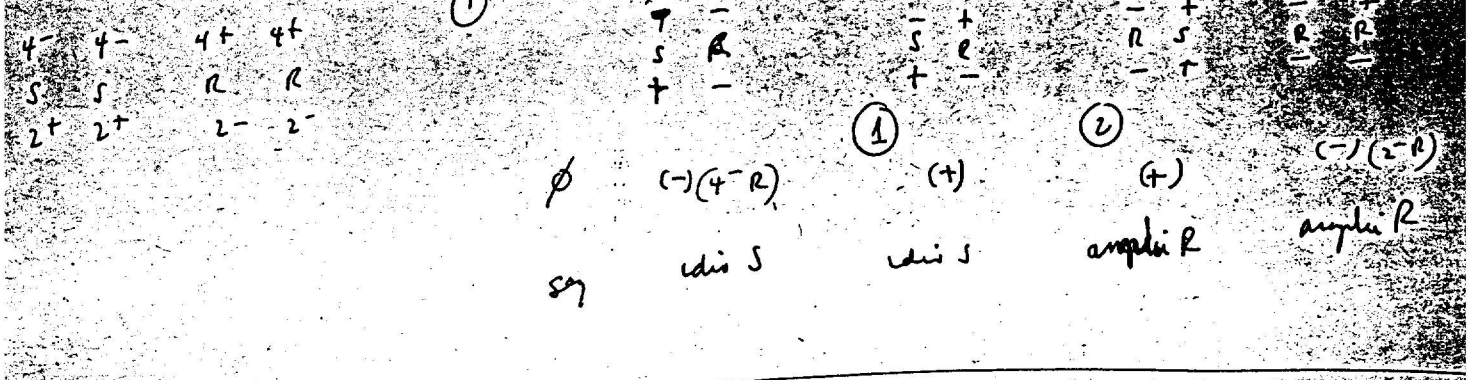
Strands	1, 3, 4	2, 3, 4
	− − +	− + +
	S R R	R S R
	+ − −	− + −
Segregant	Qdis S	Qamphi R

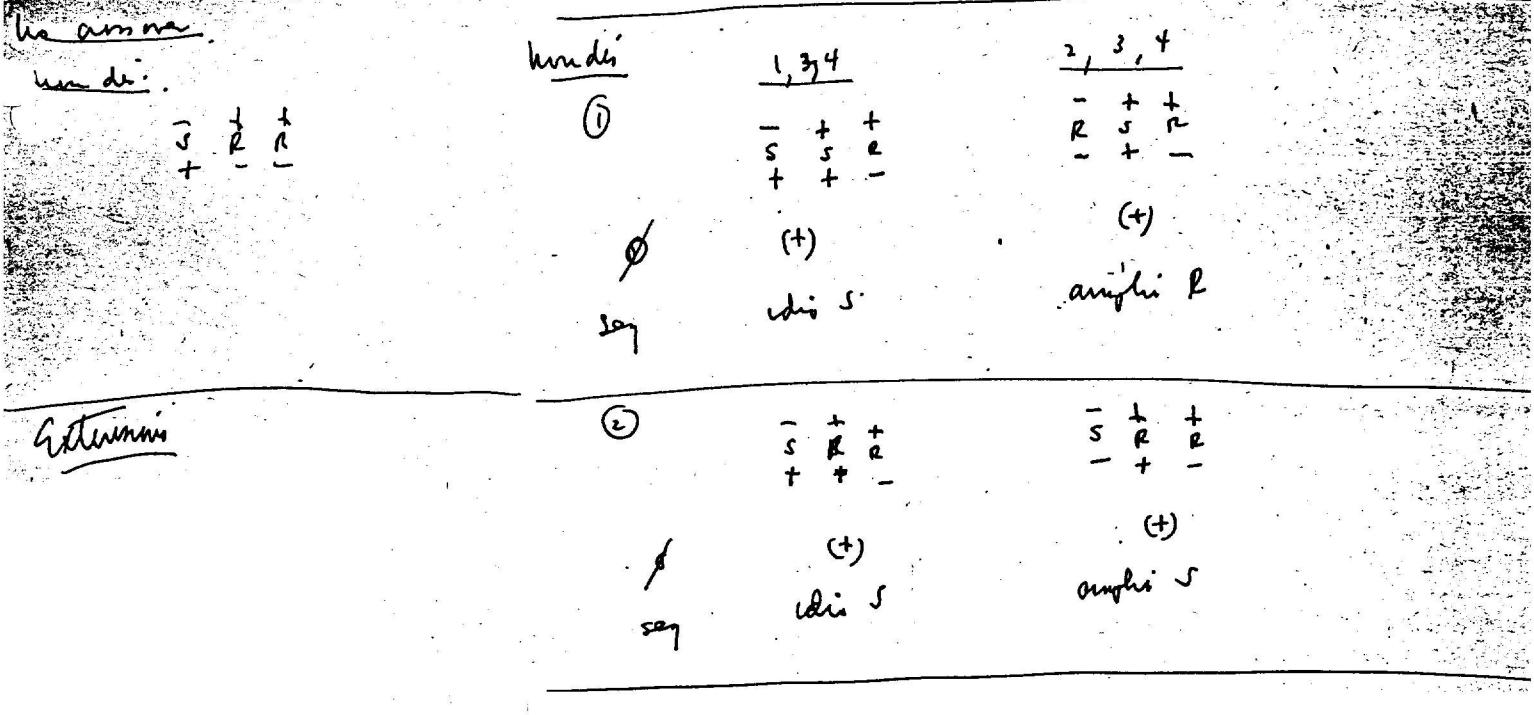
Crossover ②

	− + +	− + +
	S R R	S R R
	+ + −	− + −
Segregants	Qdis S	Qamphi S

General Comment.

1. Principle segregants from (+) are Qdis by S as observed
2. The exceptional, not observed cases, involve the passage into one pair of crossover members.


4− 4− 4+ 4+
S S R R
2+ 2+ 2− 2−
①
1, 3
1, 4
2, 3
2, 4
(−)(4−R)
(+)
(+)
(−)(2−R)
ampli R
ampli R
②
③
④
⑤
(+)
(+)
(+)
(−) R−R
ampli S
ampli S


Ha senso.
Non dà.
S R R
+ − −
Non dà
1, 3, 4
2, 3, 4
①
(+)
(+)
ampli R
Sing
ridu S
Estremi
②
(+)
(+)
ridu S
ampli S
Sing

(108)

1 2 3 4

① S S R R
4^- 4^- 4^+ 4^+
② 1^+ 1^+ 1^- 1^-

① — ∅ seg

SR
1^-1^-
4^+4^+

1, 3 S R
4^- 4^-
1^+ 1^+
(−) 4^- S

SR
1^-1^+
4^+4^-

1, 4 S R
4^- 4^+
1^+ 1^-
(±) parental 4^- S

SR
1^+1^-
4^-4^+

2, 3 S R
4^+ 4^-
1^- 1^+
(±) parental 4 S

SR
1^-1^-
4^+4^+

2, 4 S R
4^+ 4^+
1^- 1^-
(−) 1^- S

---

②

SR
1^-1^+
4^+4^+

1, 3 S R
4^- 4^+
1^+ 1^+
(−) 4^- S

1 3

S S R R
− − + +
② X
+ + − −

② 1, 3
S R
− +
+ +

S, R,
1^-4^+
4^+1^-

1, 4 S R
4^- 4^+
1^+ 1^-
(−) parental 4^- S

S R
1^-1^+
4^-4^+

2, 3 S R
4 4^+
1^- 1^+
(+)

2, 4 S R
4^+ 4^+
1^- 1^-
(−) 1^- S

Two (+) by
single cross over

Triple interactions

4	1	2	
−	−	+	
+	+	−	
+	+	+	= 130
−	−	+	= 14
+	+	+	= 2

	1	2	3	4
①	+	+	−	−
②	+	+	−	−
③	−	−	+	+

① 1,3 | 1,4 | 2,3 | 2,4

	+ +	+ −	− +	− −
	+ +	+ −	− +	+ +
	− −	− +	+ −	− −
ϕ	−	(+)	(+)	−
Allele	2	−	−	−
Seq	2−	2−	(−)	(−)

Two disjunctions

	1	5	4	2	3	4
①	+	+	−	−	+	−
	+	+	−	−	+	−
	−	−	+	+	−	+
②	+	−	−	+	−	−
	+	+	−	−	+	−
	−	−	+	+	−	+
③	+	−	−	+	−	−
	+	−	−	+	−	−
	−	−	+	+	−	+

② 1,3 | 1,4 | 2,3 | 2,4

	+ +	+ −	+ −	+ −
	+ −	+ −	+ −	+ −
	− +	− +	+ −	+ +
ϕ	(+)	(+)	≠(+)	(−)
Allele	−	−	−	1−
Seq	2−	2−	1−	1−

③ 1,3 | 1,4 | 2,3 | 2,4

	+ −	+ −	+ −	+ −
	+ −	+ −	+ −	+ −
	− −	− +	+ −	+ +
ϕ	(−)	(+)	(+)	(+)
Allele	2−	−		
Seq	2−	2−	apparently stable ⊕	

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GENETIC TRANSDUCTION IN *ESCHERICHIA COLI*

By

MELVIN LAURANCE MORSE

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF WISCONSIN

1955

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INTRODUCTION

Exchanges of genetic material between bacterial cells can be classified into two main categories (Lederberg, J., 1954). The first category is exemplified by the recombinational process found in Escherichia coli K-12 by Tatum and Lederberg (1947). This form of gentic change includes a syngamic process, that is, the conjunction of large blocks of genetic material, and there is evidence of linkage groups, linearity of genes, and requirement for intact cells (Lederberg, J., et al, 1951, Lederberg, J., 1954).

Under the second main category are found the exchanges where one of the participating cells is not found in intact form, but whose genetic material is presented as a solution or suspension of particles much smaller than the cell. This category has been given the general title of transduction (Zinder and Lederberg, 1952, Lederberg, J. 1954), and is readily subdivided into two classes on the basis of the vector of recombination. The first subclass of transduction is exemplified by the pneumococcus transformation system (Austrian, 1952), where the genetic changes are brought about by means of purified preparations of desoxyribonucleic acid (DNA). In the second subclass the genetic changes are mediated by bacterial viruses or bacteriophages (Zinder and Lederberg, 1952, [redacted]). In contrast to syngamy, genetic transduction usually results in monofactorial genetic changes, although dual changes have been noted (Stocker, Zinder and Lederberg, 1953, Hotchkiss, 1954).

The frequency of occurrence of these exchange processes among the various genera of bacteria is not known. Genetic recombination of the E. coli K-12 type has been observed in about 50 additional strains of E. coli of over 2000 examined (Lederberg and Tatum, 1953). Transductions similar to that of pneumococcus [redacted] have been observed in Hemophilus influenzae

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(Alexander and Leidy, 1951), Neisseria menigitidis (Alexander and Redman, 1952), and Escherichia coli (Boivin, 1947). While strains of E. coli are reported to show syngamy and transduction, Boivin's culture has been lost and further studies with it are impossible. Attempts to transfer genetic material via desoxyribonucleic acid preparations in E. coli K-12 have been unsucessful. (Lederberg, J., 1947 Atchly, 1951). In Salmonella, Zinder and Lederberg (1952) demonstrated phage mediated transductions but failed to show the occurrence of syngamic recombination. Thus, of the three forms of recombination considered, no one culture has previously been observed to exhibit more than one of the exchange processes. It is the purpose of this thesis to describe a limited system of transduction in E. coli mediated by the lysogenic phage of strain K-12, lambda. The occurrence within the same strain of syngamic recombination and of phage mediated transduction promises to improve our understanding of both processes.

MATERIALS AND METHODS

The principal cultures used are listed in table 1. In summary they represent mutations at three distinct loci which lead to the loss of ability to ferment galactose. Such mutations have been obtained by irradiating galactose positive cultures on an indicator medium, EMB galactose agar. The different loci have been distinguished by intercrossing the various stocks and finding galactose positive recombinants in certain crosses (Lederberg, E. 1950). The Gal_1- and Gal_4- stocks are the result of a single mutation to (-) in each case, while Gal_2- stocks represent two independent mutations to (-) whose identity is based upon the observation that no galactose positive recombinants have been observed in more than 11,000 prototrophic recombinants from crosses between them, and upon the synonymous behavior of the stocks in transduction experiments. These three loci are closely linked to one another as indicated by the data in table 2, but the order of the loci is not specified.

In addition, each of these loci is known (Lederberg, [redacted] and Lederberg, [redacted] 1953) to be closely linked to the Lp, [redacted] (latent phage) locus of E. coli K-12. Three alleles are known to exist at the Lp locus: (1) Lp^+ is overtly lysogenic [redacted] (showing evidence of free phage in cross brushes with Lp^s forms) and resistant to lysis by free lambda phage, (2) Lp^r is not overtly lysogenic [redacted] but is resistant to lysis by free lambda phage, (3) Lp^s is not lysogenic, and [redacted] is lysed or lysogenized by free phage.

At least two other loci affect the interaction of lambda with E. coli K-12. and are scored by resistance to lambda-2, the lytic mutant of lambda. One of these shows a coincidence change in maltose fermentation. Both mutations result in a loss by the cell of the ability to adsorb either lambda or lambda-2 regardless of the state at the Lp locus.

Methods and media were as detailed in Lederberg, J.(1950). Liquid cultures were in penassay broth, with or without aeration; solid media were of EMB base, either with or without added sugar, or Difco nutrient agar with 0.5 percent NaCl. For crosses, a synthetic form of EMB, EMS, was used.

High titered lambda phage lysates were prepared by two methods. The first and most commonly used was that of Weigle and Delbrück(1951) in which induction by ultraviolet radiation (UV) is used. The UV was administered to penassay grown cells resuspended in saline at a density of about 10^9 per ml. After irradiation the cells were diluted with double strength penassay broth and incubated at 37C with aeration until maximal clearing was obtained. " Lytic " lambda was prepared by infecting lambda sensitive cells with UV-induced lambda; the infected cells were resuspended in nutrient saline broth. These suspensions were then incubated at 37C with aeration until maximal clearing was obtained. Lysates prepared by UV induction had titers in excess of 10^{10} per ml, whereas the lysates prepared by the other method had slightly lower titers. Unless otherwise specified, the lambda used in the following experiments was obtained by UV induction of lysogenic bacteria.

Crosses were performed by mixing saline suspensions of penassay grown cells either before plating on the EMS synthetic medium (usually with added galactose) or directly upon the plates

Tests of cultures for phage reaction were by the cross brush method in which the culture is streaked across either phage or phage sensitive cells to ascertain whether or not it is carrying phage or sensitive to phage (Lederberg, [redacted] and Lederberg, [redacted] 1953).

Transduction assays were made in the case of lysates giving a normal, low frequency of transduction (NFT) by adding 0.1 ml of lysate to the appropriate cells on EMB galactose agar and incubating the plate for 48 hours. A separate plate with no lysate added served as an estimate of the amount of spontaneous reversion occurring. In other cases the lysate was spread only upon one-half of the plate. With lysates giving a high frequency of transduction (HFT) the lysate was cross brushed on the cells, as for tests of phage sensitivity.

EXPERIMENTAL RESULTS

General Observations on Transduction

Tests of a number of loci selected at random for ability to be transduced gave negative results (table 3). The tests for transduction of the auxotrophic markers were performed by adding lysate to cells on minimal medium, the tests on fermentation markers on EMB medium with the appropriate sugar, the test for transduction of streptomycin resistance was performed by growing the 6- addition a galactose negative cultures unable to ferment an additional carbohydrate such as lactose, xylose, and arabinose (E. Lederberg, unpublished) will give apparent transductions when plated with phage on media containing these substances. Such apparent transductions are not for the fermentation of the carbohydrate in the medium, but for galactose fermentation, since after purification, the transductions clones are found only galactose positive. Media containing these substances have some selective action on galactose fermenting clones.

in the number of galactose fermenting papillae are observed (table 4). The number of galactose fermenting clones is proportional to the amount of lysate added (figure 1). Since each of these mutations to inability to ferment galactose is capable of reverse mutation the data must be corrected. This has been done for the data in figure 1 by subtracting the number of spontaneous reversions as determined from control platings with no added lysate. In addition to indicating proportionality, the data in figure 1 indicate that the cells show the effect irrespective of the Lp genotype of the cell, and that

lambda sensitive cells are more capable of showing the effect of added lysate than lysogenic cultures.

2. Activity of lysates of galactose negative cultures.

When lysates of galactose negative cultures are mixed with the various galactose negative cells results similar to those shown in table 4 are obtained. With the possible exception of the interactions of Gal_1 and Gal_4, each of the lysates is capable of evoking galactose fermenting papillae upon plates spread with non-homologous negative cells. With the usual lysates Gal_1, Gal_4 interactions are erratic, sometimes giving significant differences between control and lysate added plates, sometimes not. This interaction will be dealt with in more detail in a later section, it will be sufficient to state here that such interaction does not produce clones that are phenotypically positive. The differentiation of these loci by lysate interaction corresponds to the differentiation by recombinational analysis.

3. Activity of lysates of reverted galactose negative cultures.

Reverse mutation restores the ability of lysates of a galactose

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A00

Mimic reversals should be able to evoke papillae from cells of the original mutant type only in the improbable event that they are located in the restricted genetic segment that appears to be capable of genetic transduction.

Measurement of the transducing activity of a lysate by the method of mixing lysate and cells on the plates appears to be satisfactory in the case of lysogenic cultures, the variation being less than two-fold over a thousand-fold change in the number of cells plated. Cell concentrations

between 5×10^7 and 5×10^8 appear to give optimal detection of lysat activity. When the assay cells are lambda sensitive the variation is two to three fold greater over the thousand-fold range of cell values from 10^6 to 10^9, with increasing assay values as the number of cells increases. Since the ratio of phage particles to transducing particles in a lysate is very large the interaction between lysate and sensitive cells is complex, with the great probability that the inactive phage particles influence the expression of the transducing particles.

The ratio of transductions to phage content of the lysates varies, approximating 10^{-7} for lysogenic assay cells, about 10^{-6} for sensitive cells, that is, about a ten-fold difference in efficiency.

The necessity of lambda adsorption for transduction

The necessity for lambda adsorption for transduction is illustrated by the results given in table 9. When the various galactose negative cultures are lambda-2 resistant, a combination which is incapable of adsorbing either lambda or lambda-2, transductions are not obtained. The ability to transform a galactose negative locus found coupled with lambda-2 resistance is demonstrable when a suitable out cross is made and the galactose negative lambda-2 sensitive recombinant obtained. Lambda-2 resistance does not effect the ability of a lysogenic culture to give rise to phage and transducing particles after UV induction.

The activity of lytic lambda.

The transductions described thus far have been effected by means of lysates prepared by the ultraviolet induction technique. Lysates prepared by lytic growth of the phage on a sensitive culture apparently have no transducing activity and have lost the transducing activity included in the starting phage inoculum (table 8).

The transduction clones

With the exception of the Lp locus in the case of lambda sensitive cells, no changes have been observed in any of the other genetic characteristics of the transformed cells. Many of the galactose fermenting clones produced by transduction are different from the spontaneous reversions in their instability for galactose fermentation and in some cases for lambda reaction. That is, they continue to segregate galactose negative clones in the course of many serial isolations. In addition, in the case of the transductions with Lp^r reaction there is segregation for lambda sensitivity with segregation for galactose fermentation. Lysates from unstable transduction clones also differ from lysates of galactose reversions: in the former the ratio of transductions to plaques is much closer to unity (table 8).

Lysates of the cultures unstable for galactose fermentation when prepared in the manner of the other cultures

have lower phage titers. The reason for this is not known but the production of phage in these lysates is being studied further. With the exception of transductions formed with wild type lysates, the transduction titer of these lysates is dependent on the genotype of the assay culture.

When portions of these lysates are cross brushed on galactose negative cultures the intersection of the streaks is converted principally to galactose positive growth because of the high frequency of transduction (HFT). The problem of the HFT lysates will be dealt with in more detail in a later section.

Incidence of lysogenicity in the transduction clones derived from Lp^s recipient cells.

When HFT lysates are used in transductions to Lp^s recipient cells, about 90 percent of the resultant transduction clones are lysogenic (Lp^+) or Lp^r. There is some slight evidence for lambda sensitive transductions, but these putative transductions have been found stable for galactose fermenattion and it has not been possible to distinguish them from spontaneous reversions except by their frequency of occurrence.

When Lp^r cultures are treated with lysates a small fraction (3-5 percent) of the segregants from the resultant transductions are lysogenic whereas it had not been possible to lysogenize Lp^r cultures with previous methods (Lederberg and Lederberg, 1953).

The high incidence of lysogenicity in the transduction clones may be misleading owing to the excess of phage, and it cannot be ascertained whether lysogenization took place before, concomitant with, or after transduction by the NFT phage. In the section on HFT lysates the relationship between transduction and lysogenization will be shown more clearly.

The segregants from the transductions with Lp^* reaction are Lp^+, while the segregants from the Lp^r transductions are Lp^s and Lp^r.

In speaking of the Lp^r reaction it should be noted that the classification of Lp^r is more subject to quantitative considerations than the other alleles of Lp. The two cultures (W1924, W1027) derived from sources other than transduction that showed no plaque forming phage in cross brushes with sensitive cultures gave plaque forming phage after induction with ultraviolet radiation. The amount of phage was greatly reduced over that obtained from Lp^+ cultures under similar conditions. These two cultures were obtained after separate procedures, one from an ultraviolet irradiated Lp^+ culture, the other from an Lp^s culture treated with lambda (E. Lederberg, unpublished). Both were stable as regards their lambda reactions. The Lp^r clones observed after transduction have not given plaque forming phage after U.V. exposure, but they differ from those which have given phage by instability at the Lp locus

Whether the transductions with Lp^r reaction are the results of heterogeneity among the phage particles, the cells, or as the results of a "defective" ⟶

act of lysogenization is not known, but presumably the problem could be investigated by statistical means.

Existence of transductions stable for galactose fermentation.

The evidence for the occurrence of stable transductions is the increased number of stable galactose positive clones found on lysate plates ~~over that expected from control platings~~ (table 9). Although the increase could also be explained on the assumption of a change in selective conditions favoring spontaneous reversions, the finding that most of them are also lysogenic (in the case of hfr recipient cells), and the fact that heated lysates (56C for 30 minutes), or filtrates of galactose positive, lambda sensitive cultures gave no increase in number of papillae, suggests that change in selective conditions is not the case.

THE SEGREGANTS FROM THE UNSTABLE TRANSDUCTIONS

The non-fermenting segregants from the unstable transduction clones can be classified for the negative alleles that they carry by three separate methods: (1) by testing the segregants against lysates of known galactose negative cultures, (2) by testing known galactose negative cultures against lysates of the segregants, (3) by crosses with known galactose negative types. In classifying the segregants it will be convenient to refer to the parental source of the negative allele or alleles by generalized designations. By idiotype is meant the genotype of the recipient cell parent, by allotype the genotype of the donor source of the transducing lysate. Amphitypic will designate cultures which at some loci are idiotypic and at others are allotypic. Unstable or segregating stocks, as will appear, are heterogenotes and the underlying state is described as heterogenic to distinguish it from euploid heterozygosis for entire genomes.

For further analysis it will ultimately be desirable to construct single cell pedigrees. The following observations on colony isolations are made with due regard to the complexities of colony formation.

ADD TOP 13

(123)

Various segregants were tested by one of the three possible methods, and some cases (table 10) by all three methods. Tables 11 and 12 present summaries of the analysis as transduction recipients and as transduction donors. The pattern of segregation in the various transduction experiments can be obtained from table 11. Gal_1- segregants have not been tested in crossing experiments because no suitable stock is available for this purpose.

is, a culture classified by the first method was Gal_4- was also classified as this by the other two tests.

Three segregants obtained were classified as amphitypic in tests against lysates of known cultures. Two were Gal_1- Gal_2-, and one was Gal_2- Gal_4-. The former were prototrophic and it was not possible to examine their behavior in crosses. The Gal_2- Gal_4- culture is crossable but has not been tested as yet.

Because of the Gal_1- Gal_4 interaction it is not possible to test any of the amphitypic segregants using only the three loci so far considered. Attempts were made to analyse the amphitypes further by the action of their lysates on an additional locus, Gal_6-. Lysates of the two Gal_1-Gal_2- were plated with cells of a Gal_6- culture. Both lysates had little action in producing papillae . (This perhaps might have been expected since NFT lysates ? Gal_1- have questionable activity on Gal_6-). Several unstable galactose fermenting clones were obtained from each interaction, however, and a number of segregants were tested. Of 16 segregants from the transductions by the lysate of one amphitypic culture, 15 were Gal_2-, and one was classified as Gal_1- Gal_2-. From the action of the lysate of the second amphitypic culture five Gal_1- and two Gal_2- segregants were obtained. Although both lysates

transmitted Gal_1^- and Gal_2^-, confirming the existence of these negative alleles in the parental cultures, the failure to recover the idiotypic Gal_6^- locus among the segregants is disturbing. Beyond the fact that Gal_6 is a locus transduced by lysates nothing is known of its behavior.

Although the amphitypic (double mutant) cultures are not transformed to wild type by the action of a single pure lysate, galactose positive clones are obtained by the action of a mixture from the two single mutants genotyped. The statistics of the interaction of cells and lysate have not been investigated but the greatly reduced number of transductions produced by the mixed lysate is expected on the assumption of independent interaction between the cells and each of the transducing activities.

The transductions produced by the action of mixed lysates on amphitypic segregants appear to be less stable than transductions of cultures negative at a single galactose locus. In addition they give rise to "intermediate" segregants in which only one of the two transducing activities has been lost from the clone. These "intermediate" segregants in turn give rise to segregants from which both transducing activities have been lost.

Galactose negative cultures giving lysates with HFT property.

Under the section on transformed cells it was noted that in lysates of the unstable galactose positive clones the ratio of transduction titer to plaque titer was remarkably high. In fact, these cultures were not the first that were discovered to give HFT lysates. In the course of examining segregants from transductions by means of lysates of them, several exceptional cultures were encountered, which gave a high frequency

of transduction. [redacted] An examination of a number of HFT cultures was made, the results of which are shown in table 12. [redacted]

Except for the HFT property [redacted], these exceptional cultures appeared to be no different from the other segregants. That is, they reacted in tests against lysates in the same manner as HFT segregants, [redacted] preliminary crosses with them gave no [redacted] lysates.

[redacted]

HFT cultures [redacted] were unstable for this property and unstable on rare occasions for galactose genotype. Regarding the latter instability, HFT cultures which were negative at a single locus segregated HFT segregants that were negative at this locus and [redacted] negative at an additional locus as well. In most instances, however, the HFT segregants were of the same negative [redacted] AT THE SAME LOCUS as the parent galactose negative HFT culture.

The galactose positive reversions of the HFT cultures that have been studied are still capable of giving HFT lysates, but are unstable for galactose fermentation. The galactose negative segregants from the reverted HFT cultures are HFT, are either negative at the same locus as the original negative HFT segregant, or negative at this locus and negative at an additional locus, one which proved to be the original idiotypic locus in the formation