The following is a true copy of my notes, Volume III, labelled "Summaries" which were recorded during the interval 1951-1956 while I was in the Lederberg laboratory in the Department of Genetics at the University of Wisconsin in Madison.

The notes were entered in intervals with some pagination and represent various types of summaries, speculatiuons, etc. I have numbered them sequentially in the upper right hand corner from page 1 to page 253 for convenience.

There are a number of irradiation experiments (UV) plotted which have never been published. I remember communicating some of them to E. Kellenberger who may have giventhem to Werner Arber since I believe there are some similar experiments in Arber's doctoral dissertation. There are also some drafts of my own disseration.

Items of possible interest are the handwritten notes of J. Lederberg (4/10/54) labelled "Remaining Questions" on pages 90-92; the typing bill for my disseration, page 155; a status report of the Lederberg lab for 1953, pages 161-195; some notes of JL on putting the stock book on keysort cards, page 197; a matrix by JL for transduction mapping, page 201; and an index to Volume II of my notes, pages 202-206.

This Volume is a hodge-podge and doesn't represent any temporal order - I believe page 253 is really ahead of page 1 which was a preliminary report leading to my dissertation.

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Summaries

Research

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Materials and Methods

The principal cultures used are listed in table 1. In summary they represent three distinct mutations which lead to the loss of ability to ferment galactose (Lederberg, E., 1950). The Gal_1- and Gal_4- stocks are the result of a single mutation to (-) in each case, while the Gal_2- stocks represent two independent mutations to (-) whose identity is based upon the observation that no(+) 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, E. and Lederberg, J., 1953) to be closely linked to the Lp or latent phage locus of E. coli K-12. Three alleles are known to exist at the Lp locus; (1) Lp+, overtly lysogenic and showing evidence of free phage in cross brushes with Lp^s forms, and resistant to lysis by free lambda phage, (2) Lp^r, not overtly lysogenic and showing the presence of free phage in cross brushes with Lp^s forms, but resistant to lysis by free lambda phage, (3) Lp^s, not lysogenic, and being lysed or lysogenized by free phage.

More extensive delineation of the interrelationship of these loci has not been feasible until recently and it is hoped that with the aid of a new method of distinguishing the minus recombinants that mapping of this region may be accomplished.

Another locus which has a direct bearing upon the problem is the locus controlling resistance to lambda-2, the lytic mutant of lambda. This locus, Lp_2,

has an epistatic effect upon events controlled by Lp (Lederberg, E. and Lederberg, J., 1953). Thus a change from Lp_2^s (lambda-2 sensitive) to Lp_2^r, (lambda-2 resistant) results in a loss by the cell of the ability to adsorb lambda as well as lambda-2. Sensitivity to lambda by a cell therefore can be masked by the presence of the r allele of Lp_2. The Lp_2 locus is not closely ~~is not closely~~ linked to either Lp or to any of the galactose loci in question.

Methods of cultivation and media used were as detailed in Lederberg, J. (1950). Liquid cultuivations were in penassay broth medium, with or without added aeration, solid media used were of EMB base, either with or without added sugar, or for crosses, a synthetic form of EMB, EMS was used.

High titered lysates of cultures were prepared after the method of Weigle and Delbrück (195) by inducing lysis of penassay grown cells by means of irradiation with small doses of ultraviolet. The UV was adinistered to saline x suspehsions of the cells and the cells subsequently diluted with 2X penassay broth and incubated with aeration until maximal clearing was obtained.

Lytically grown lambda was obtained by infecting the sensitive cells by exposures to lambda prepared by the Lwoff technique, discarding the supernatants after the adsorptions and resuspending the sedimented cells in nutrient saline broth. The NSB suspensions were then incubated with aeration until maximal clearing was obtained.

Table I

List of pertinent cultures

Culture	Genotype
K-12	$F^+ Lp_1^+ Lp_2^s$
W518	$F^+ M^- Lac_1^- Gal_4^- Lp_1^{+s} Lp_2^s$
W750	$F^+ M^- Lac_1^- Gal_1^- Lp_1^+ Lp_2^s$
W811	$F^+ M^- Lac_1^- Gal_4^- Lp_1^+ Lp_2^s$
W902	$F^- T^- L^- Th^- Gal_2^- Lp_1^+ Lp_2^r$
W1210	$F^+ M^- Lac_1^- Gal_2^- Lp_1^+ Lp_2^s$
W1436	$F^+ T^- L^- Th^- Lac_1^- Gal_4^- Lp_1^s Lp_2^s$
W1924	$F^+ M^- Lac_1^- Gal_4^- Lp_1^r Lp_2^s$
W2175	$F^+ gal_2^- Lp_1^+ Lp_2^s$
W2281	$F^+ M^- Lac_1^- Gal_2^- Lp_1^s Lp_2^s$
W2342	$F^+ Lac_1^- Gal_2^- Lp_1^+ Lp_2^s$
W2373	$F^+ Hist^- Leuc^- Gal_1^- Lp_1^s Lp_2^s$

Table 2

Recombination between the Galactose Negatives

Cross	Minimum Number of Prototrophic Recombinants	Percent (+) Recombinants
F^+ Gal_1- X F^-Gal_2-	(1) 1500	0.13
	(2) 6517	0.06
	(3) 3603	0.027
	11620	0.06
F^+ Gal_4- X F^-Gal_1-	4588	0.13
F^+ Gal_4- X F^- Gal_2-	2654	0.23

Results

When varied amounts of high titered lysates of wild type cultures are mixed with Gal_1-, Gal_2- or Gal_4- cells and plated on EMB galactose medium, results such as those in figure 1 are obtained. Since each of these mutations to gal- is capable of reverse mutation the data shown in figure 1 have been corrected for the number of reversions by subtracting this number as determined from control platings with no added lysate. Figure 1 shows that with increasing amounts of added lysate there is a linear increase in the number of galactose fermenting papillae per plate. In addition, figure 1 indicates that lambda sensitive cultures appear to be more capable of showing the effect of added lysate than lysogenic cultures or cultures carrying a non-plaque-forming type of lambda.

When lysates of gal- cultures are mixed with the various gal- cells and plated upon galactose medium results similar to those shown in table 3 are obtained. Each of the lysates of the gal- is capable of evoking galactose fermenting papillae uppn plates spread with the other gal- cell types but not with plates spread with cells of its own type. The ability to induce gal+ clones in other gal- but not with cells of type corresponds to the differentiation of these gal- mutations by recombinational analysis. Evidence complementing this is shown in table 4 which shows that the ability to evoke papillae with cells of type is restored by reverse mutation. Presumably phenotypic reversions can be of two types, reverse mutation at the mutated locus, and mutation at a second locus whose action mimics the action of the first gene. Reversions of this second class should not be able to evoke papillae from cells of type. Such reversions as the latter have not as yet been investigated.

Table 3
Interaction of Gal_1-, Gal_2- and Gal_4-

		Lysates				
	Titer($\times 10^{10}$) -	None	Gal_1- 2.4	Gal_2- 4.9	Gal_4- 1.7	Wild Type 1.4
Cells						
Gal_1- Lp^+	(1)	2*	-	176*	43	a
	(2)	2	2	-	-	405
Gal_2- Lp^+	(1)	14	52	11	43	-
	(2)	20	-	10	-	356
Gal_4- Lp^+	(1)	89	-	202	-	-
	(2)	50	85	-	-	417
	(3)	47	-	-	50	394

* Number of papillae per plate, 0.1 ml lysate plated. Between 10^8 and 10^9 cells plated

Table 4
Restoration by Reverse Mutation of the Ability to Transduce Previously Nontransducible Loci

Locus (all Lp^+)	Reversion	Addition None	Addition Reversion Lysate
Gal_1-	Gal_1^+ #1	0	648*
Gal_2-	Gal_2^+ # 1	10	96
	Gal_2^+ # 2	6	552
Gal_4a	Gal_4^+ # 5	39	204
	Gal_4^+ # 8	25	291

* Number of papillae per plate, 0.1 ml lysate plated Between 10^8 and 10^9 cells plated.

Examination of the other characteristics of the cells transduced to gal (+) by lysate exposure has uniformly shown no changes in any of them with the exception of the induction of lysogenicity in the lambda sensitive forms. Direct attempts to transduce other factors have been uniformly negative. A summary of the avilable data is given in table 5. In connection with the negative results in attempts to transduce xylose and lactose loci it should be noted that both xylose and lactose containing media have some selective value for galactose fermenting clones.

Transduction in K-12 thus far has been found to be limited to several galactose loci closely linked to the latent phage locus, Lp. These loci include Gal_1, Gal_2, Gal_3, Gal_4, Gal_6, and possibly several more that have not as yet been classified. The experiments reported here will concern only Gal_1, Gal_2 and Gal_4 although some observations on Gal_3 and Gal_6 have been made. Not all loci controlling galactose fermentation are transducible. One occurring in W2312 will be mentioned later, and another induced by copper treatment by Helen Bayers has been found.

The transductions described above have been effected by means of lysates perpared by the Lwoff technique of inducing lysis with a small dose of ultraviolet. 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 inoculum. The inability of this type of lysate to transduce is demonstrated by the results given in table 6.

The necessity for lambda adsorption for transduction is illustrated by the results given in table 7. When the various gal- are found coupled with the r allele of Lp_2, a combination which is incapable of adsorbing either lambda or lambda-2, transductions are not observed. The presence of this allele of Lp_2 does

not interfer in the capacity of a culture to give rise to transducing lysates and the transducibility of a transducible λ gal- locus found coupled with Lp_2^r is demonstrable when a suitable λ cross is made and a gal- Lp_2^s recombinant obtained.

Recovery of the transducing activity of a lysate by the method of mixing lysate and cells on plates appears to be good in the case of lysogenic cultures, the variation being less than two-fold over a thousand-fold change in the number of cells plated (figure 2). This is not the case when the added cells are lambda sensitive, the variation being in this case two or three-fold greater over a similar range of cell concentrations. It should be noted again that the lambda sensitive cultures give approximately ten-fold or more transductions at any cell density, and that ~~that~~ the relationship of the activities on the two types of cells is not known. The ratio of number of transductions to phage content of the lysates approximates 10^{-7} for lysogenic assay cells, about 10^{-6} for sensitive cells.

Alternatively to mixing cells and lysate on plates the transducing activity of the lysates may be adsorbed upon cells and the cells then plated out on agar. Table 8 gives some indication of the adsorption of the transducing activity and also some indication of the adsorption of the phage under the same conditions.

Table #1
Effect of the Lp_2 Locus on Transducibility

Galactose Locus	No Addition	Wild Type Lysate
Gal_1- Lp_{1+}^{+} Lp_{2r}^{s}	1*	426*
Gal_1- Lp_1 Lp_2	1	2
Gal_2- Lp_{1+}^{+} Lp_{2r}^{s}	20	356
Gal_2- Lp_1 Lp_2	14	14
Gal_4- Lp_{1+}^{+} Lp_{2r}^{s}	89	296
Gal_4- Lp_1 Lp_2	50	57

* Numbers of papillae per plate, 0.1 ml lysate plated
Between 10^8 an 10^9 cells plated.

Table #5
Other Loci tested but not found Transducible

Locus	Number of Experiments	Cultures Involved
Lac_1	4	W112
(ser or glyc)	1	W1678
Leuc	3	W1736, W1436
Methionine	4	58-161, W811, W1821, W518
Xylose	3	W1821
S	1	W518
Prol	7	W1692, W1920, W2062
Mal_x	1	W2071

Table R6

Action of Lytically Grown lambda in Transduction

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Experiment	Culture	No Addition	Lytic Lambda Lysate (2.4×10^{10} lambda/ ml)
228	W750	3*	2*
	W 518	9	8
	W2175	7	8
239	W750	2	0
	W518	13	8
	W2175	6	2
254	W750	-	3**
	W518	-	6**
	W2281	-	9**
	W2373	-	6**
	W811	-	39**

* Numbers of papillae per plate, 0.1 ml lysate plated. 10^8-10^9 cells plated.

** these papillae picked and streaked out, all found stable.

Lytically grown λ as follows. W811 gal$^-$ λ adsorbed on W1485 gal$^+$ in two exposures. Centrifuged and resuspended in NSB. Aerated 4-5 hours with aeration. Control tube consisted of both exposed cells.

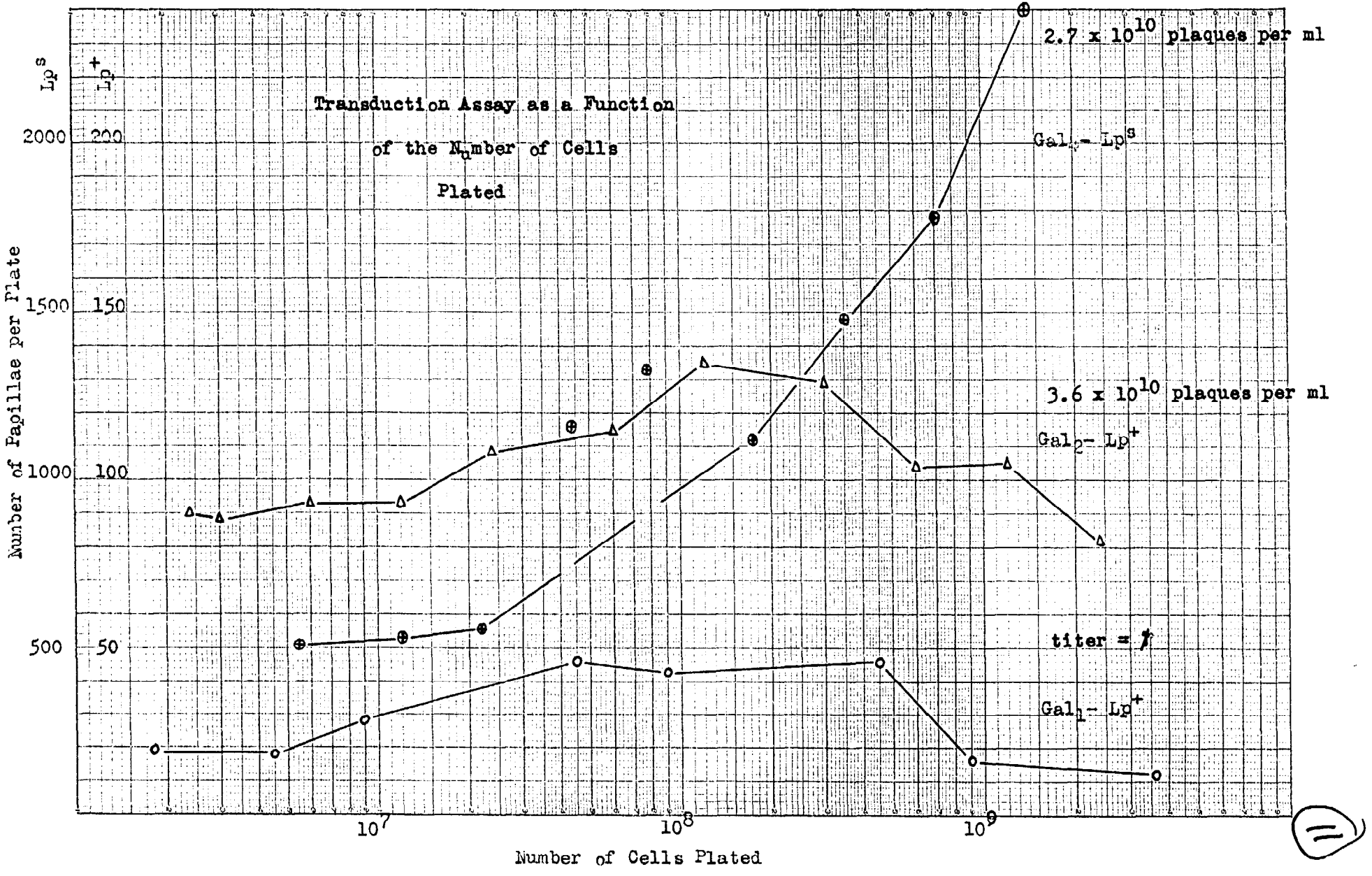


Table 8
Adsorption of the Transducing Activity from Lysates

Adsorbing Cells		Phage Titer X 10^9	Cell Titer X 10^9	Percent Adsorbed 1st Ads. Phage	1st Ads. Trans.	2nd Ads.* Phage	2nd Ads.* Trans.	3rd Ads. Phage	3rd Ads. Trans.
Gal_4- Lp^s	(1)	2.5	0.71	60	79	50	41	16	46
	(2)	3.9	0.55	52	33	-	-	-	-
Gal_4- Lp^+	(1)	14	c. 10	-	79				
	(2)	18	16	-	72	-	56	-	0.0
	(3)	14	c. 10	-	97	-	-	-	-
Gal_2- Lp^+		18	6	-	35	-	33	-	0.0
Gal_1- Lp^+		18	6.5	-	45	-	100	-	0.0

* The supernatant from the first adsorption was decanted and an equivalent volume of fresh lysate added. Similarly for the third adsorption. Assays were made of the amount of material remaining in the supernatants. Titers given are for the phage-lysate adsorbing mixture.
In the experiments involving Gal4- Lp^s assay of the sediment was made in some instances. Total recovery in these cases was more than 100% - presumably due to the fact that total activity was underestimated by the use of too few assay cells.

Some of the papillae evoked by lysate exposure have a property which distinguishes them at once from spontaneous reversions. That is, they are unstable for galactose fermentation and segregate (-) cells over many single colony transfers. The frequency of unstable transductions and the nature of the segregants will be taken up in a later section, it is necessary to mention them now in order to consider the realtionship between the transducing agent and the phage lambda. It is also necessary at this time to mention some special cultures encountered during the analysis of the segregants mentioned above. These special cultures are notable for the fact that they give rise to lysates by the Lwoff technique in which the ratio of transduction activity to lambda plaque forming activity is much closer to unity than is found in the usual cultures of K-12. These cultures will also be considered in a later section and it will suffice to say here that exposure of a population of gal- cells to one of these lysates can result in the transduction of several percent of the cells to gal+.

The data in table 9 indicates that when lamda sensitive cells are transduced the resultant cells and their gal- segregants have for the most part become lysogenized. When Lp_1^r forms are transduced they also may become lysogenized, but much less frequently than sensitives. However, these results may be misleading since the platings involve large quantities of phage and it cannot be certain that lysogenization was not prior or subsequent to transduction. When the transductions are made with the special lysates mentioned above, results such as those shown in table 10 are obtained. Under conditions where one percent of the cells have been transduced to gal+ the transductions have become lysogenized, or Lp_1^r, while the gal- cells in the same environment have remained lambda sensitive.

Table 9
Correlation of Lysogenization with Transduction

Locus transduced and Lp1 genotype	Lysate source	Transductions Number	Transductions Percent Lp_1^+	Segregants Number	Segregants Percent Lp_1^+
Gal_1- Lp_1^s	wild	23	87	1	100
	gal_2-	24	75	7	100
	gal_2-	12	58	0	-
	gal_4-	22	77	9	100
Gal_2- Lp_1^s	wild	13	85	13	85
	gal_1-	20	95	20	95
	gal_4-	23	100	23	100
	wild	18	100	-	-
	wild	-	-	28	50
	gal_1-	-	-	44	86
	gal_4-	-	-	40	83
Gal_4- Lp_1^s	wild	-	-	18	100
	gal_2-	-	-	19	100
	gal_x-	-	-	45	100
Gal_4- Lp_1^r	wild	-	-	29	3.1
	gal_2-	-	-	18	5.5
Totals		154	86	267	89

Table 10
Correlation of Transduction with Lysogenicity Using Lysates Giving a High Frequency of Transduction

Cells Exposed to	Post Exposure Cell Titer	Number of Colonies Observed			
		Gal-	Gal+	Gal- partially lysed	Total
Broth	4.1×10^9	3280	0	0	3280
HFT lysate*	3.5×10^9	2801	31(1.1%)	54	2886

* titer = 1.2×10^9 λ plaques per ml.

Table 10a

Examination of Colonies after HFT Lysate Exposure

Colony Type	Number of Colonies Examined	Numbers of Colonies of Each Class		
		Lp^s	Lp^+	Lp^r
Gal-	31	31	0	0
Gal+	26	0	23	3

The occurrence of stable transductions among the various combinations of transductions possible is indicated by the data shown in table 11. With but six exceptions the difference between expected and observed fequency of stable gal (+) on the transduction plates is sufficiently great not to require statistical treatment. In setting out this data it has been assumed that the only source of stable (+) on the plates is from spontaneous reversions and that the use of a no lysate addition plate as an indicator of the number of spontaneous reversions is adequate in this sense. It is notable that transductions involving gal_1 and gal_4 are nearly all stable and it will be remembered that lysates of these cultures have less papillae promoting activity upon one another than upon other cultures. These two loci are readily distinguishable by crossing test and by use of the HFT lysates mentioned above. In the other combinations of transductions possible stable transductions occur, varying in frequency from less than one percent to more than 50 percent.

The segregants from the unstable transductions can be classified for locus by three separate methods: (1) by the lysate by which they are not transduced (transduction test), (2) by that locus which cannot be transduced to (+) via a lysate (lysate test), (3) by allelism test in crossing (crossing test). In classifying the segregants it will be convenient to refer to the origin of the locus by specific terms. By homotypic will be designated the locus of the cell transduced to (+), by heterotypic will be designated the (-) locus (if any) of the transducing lysate, and by homo-heterotypic will be designated cultures with the loci of both transduced cell and transducing lysate.

Since the order of segregation from a transduced cell can not be specified without micromanipulative means the analysis of segregants from a single transduction in its absence is without great significance. However, the data in table 12 indicate that a single transdubtion can give rise to all three types of segregants, homotypic, heterotypic, and homo-heterotypic,

11

Table

The Occurrence of Stable Transductions

Cell Genotype	Numbers of Stable Transductions Source of Lysate: Wild Type Exp't	Wild Type Obs.	Gal_1- Exp't	Gal_1- Obs.	Gal_2- Exp't	Gal_2- Obs.	Gal_2- Exp't	Gal_2- Obs.	Gal_4- Exp't	Gal_4- Obs.	Stable	total	% Stable
Gal_1- Lp_1^s 2373	1/33	14	-	-	1/11	11	0/56	20	1/30	29	74 (61)	130	53.8 57
750 Lp_1^+	1/46	2	-	-	-	-	1/92	0	-	-	2 (0)	138	1.5 0
2343 Lp_1^+	1/143	42	-	-	1/84	4	-	-	12/27	27	73 (59)	254	23.3 28.7
Gal_2- 2281 Lp_1^s	0/46	15	0/214	27	-	-	-	-	0/98	4	46	358	7.8 12.8
2175 Lp_1^+	17/248	21	14/83	61	-	-	-	-	14/79	52	135 (90)	410	22.0 32.4
1210 Lp_1^+	4/23	6	2/65	0	-	-	-	-	5/56	0	6 (-5)	144	0 4.1
Gal_4- Lp_1^s	19/835	383	29/72	72	11/472	20	4/128	21	-	-	496 (433)	1587	28.7 32.7
Lp_1^+	41/573	133	51/96	96	-	-	-	-	-	-	229 (137)	669	20.5 38.3
Lp_1^r	31/320	127	-	-	31/238	50	-	-	-	-	177 (115)	558	20.6 31.7

Exp't = number of stable expected = $\frac{\text{no. papillae control}}{\text{no. papillae lysate plate}}$

Obs. = number of stable observed = $\frac{\text{No. stable observed}}{\text{no. in sample}} \times \frac{\text{no. papillae Transd.}}{\text{no. pap. in sample}}$

Note: A number of different lysates were employed. In the case of Gal_2- lysates, the first column represents lysates of W902, the second column, W1210. In the case of the Gal_1- Lp_1^+ cells, the first is W750, the second W2343, a prototroph derived from W750

Table 12

Segregants from a Single Transduction, tested by Transduction Test.
The sequential order of the segregants is unknown

Cell Genotype	Lysate Source	Classification of Segregants		
		Homotypic	Heterotypic	Homo-heterotypic
Gal_4-	Gal_2-	17	2	1

but it can not be stated that the segregations occurred in any sequence or if sequential. The analysis of single segregants from a large number of transductions was undertaken to clarify this process. In the initial experiments the transduction tests were performed by mixing a portion of lysate from a culture of specific locus and the culture to be tested upon EMB galactose medium, but after the discovery of the HFT lysates test for allele was by cross brush with lysates of this property upon the same medium.

The results ~~of a large number~~ of tests of segregants by transduction test is given in table 13 and a summary of the cultures in this table which were also tested by lysate test is given in table 14. The agreement between the two tests was complete, that is, a culture classified by the first method as gal_4^- was also classified ~~by~~ as this locus by the second test, and vice versa. A summary of the segregants which were tested by all three methods of determination is given in table 15. Agreement between the crossing test and the other two tests was also complete. Some indication of the distribution of the segregant types, as judged by transduction test, can be obtained from the distribution data given in table 16.

With regard to the crossing data given in table 15 it will be noted that no crossing data for gal_1^- segregants ~~is~~ are reported or crosses of heterotypic segregants from gal_1 transductions by gal_1^- testers. ~~This is~~ because a suitable stock is not yet available. W2373, a $hist^- leuc^- gal_1^-$ made by transducking W1765 to gal_1^- has not been found sufficiently fertile in crosses with $Meth^-$ stocks to warrant its use. A new $T^-L^-B_1^-$ (also Het) gal_1^- also made by transduction to (-) may prove suitable. It should be noted that the number of prototrophic recombinants given in table 15 is probably low by as much as 25 percent since in many instances only the smallest plates with the smallest number of prototrophic recombinants were counted in ~~many~~ experiments involving many replicate plates.

Table 13

Analysis of Segregants by Transduction Assay. Summary.

Nature of Original Transduction	Type of Segregant: Homotypic*	Heterotypic**	Homo-heterotypic***	Total
Wild type on Gal-	169	0	0	169
Gal- on Gal-	240(85.4) ~~80?(91.0)~~ 40?	37(13.2) 37~~(8.2)~~	4(1.4) 4~~(0.88)~~	281 450

* having the Gal- locus of the transinduced cell
** having the Gal- locus of the transducing lysate
*** having the Gal- loci of both transinduced cell and transducing lysate.

Table 14

Analysis of Segregants by Lysate Test. Summary. Agreement between Lysate Tests and Transduction Tests was Complete

Nature of the Original Transduction	Homotypic	Heterotypic	Homo-heterotypic	Total
Wild type on Gal-	21	0	0	21
Gal- on Gal-	39	~~16~~ 19	0	58
	60	~~16~~ 19	0	79

Table 15

Summary of the Analysis of Segregants by Transduction test, Lysate test and by Crossing test.

Original Transduction	Number of Segregants	Transduction test	Lysate test	Classification by Crossing test: Homotypic No. (+)	Homotypic Tot. Prot.	Heterotypic No. (+)	Heterotypic Tot. Prot.
Gal_2- --x Gal_4-Lp^s	5 (1)	Gal_4-	Gal_4-	0	2786	3	3183
	(2)	"	"	0	2675	2	3471
	(3)	"	"	0	3485	23	5342
	(4)	"	"	0	5952	1	1665
	(5)	"	"	0	5000	1	891
	2 (1)	Gal_2-	Gal_2-	7	3102	0	1988
	(2)	"	"	10	4364	0	1187
Gal_2- --x Gal_4-Lp^+	4 (1)	Gal_4-	Gal_4-	0	16104	3	1389
	(2)	"	"	0	5730	1	164
	(3)	"	"	0	3358	0	202
	(4)	"	"	0	12848	1	171
HFT	3 (1)	Gal_2-	Gal_2-	1	11200	0	827
	(2)	"	"	6	10608	0	718
	(3)	"	"	3	5000	0	409
Wild --x Gal_2-Lp^s	4 (1)	Gal_2-	Gal_2-	0	7805		
	(2)	"	"	0	4992		
	(3)	"	"	0	106		
	(4)	"	"	0	4552		
Wild --x Gal_2-Lp^+	4 (1)	Gal_2-	Gal_2-	0	4070		
	(2)	"	"	0	5384		
	(3)	"	"	0	2072		
	(4)	"	"	0	6988		
Wild --x Gal_4-Lp^s	4 (1)	Gal_4-	Gal_4-	0	896		
	(2)	"	"	0	918		
	(3)	"	"	0	1134		
	(4)	"	"	0	863		

Table II

Distribution of the Segregant Types by Transduction Assay

Transinduced cell		Source of Lysate: Wild type	Gal_1-	Gal_2-(W902)	Gal_2-(W1210)	Gal_4-
Gal_1-	Lp_1^+(W2343)	18 Gal_1-	-	18 Gal_1-, 5 Gal_2-	-	no seg. found
	Lp_1^+(W750)	16 Gal_1-	-	18 Gal_1-, 1 Gal_2-	18 Gal_1-, 3 Gal_2-	no seg.
	Lp_1^s	9 Gal_1-	-	1 Gal_1-	6 Gal_1-, 1 Gal_2-	1 Gal_1-
Gal_2-	Lp_1^+(W2175)	20 Gal_2-	14 Gal_2- 3 Gal_1- 2 Gal_1-Gal_2-	-	-	8 Gal_2- 7 Gal_4-
	Lp_1^+(W1210)	15 Gal_2-	19 Gal_2- 2 Gal_1-	-	-	
	Lp_1^s	16 Gal_2-	20 Gal_2-	-	-	21 Gal_2- 1 Gal_4- 1 Gal_2-Gal_4-
Gal_4-	Lp_1^+	20 Gal_4-	nsf	16 Gal_4- 3 Gal_2-	-	-
	Lp_1^s	13 Gal_4-	nsf	18 Gal_4- 3 Gal_2-	17 Gal_4- 2 Gal_2-	-
	Lp_1^r	29 Gal_4-	nsf	15 Gal_4- 3 Gal_2-	-	-

nsf = no segregants found

Cultures giving lysates with the HFT property have been prepared for each of the gal- loci which have been given consideration to date. These cultures have the common property that each is derived from the transduction of a gal- culture by a lysate of gal_2-. It is not known whether the transductions themselves of this type are capable of giving rise to HFT lysates or not, but the HFT stocks thus far obtained have been segregants from such transductions. Whether the transductions of gal_2- by other cultures gives rise to HFT segregants is not known, but one instance in which the transduction of gal_2- by gal_1- resulted in an unstable (+) which had HFT property has been encountered. Some idea of the frequency with which the HFT occur can be obtained from the following. In the case of transductions of gal_1- by gal_2-, out of 28 gal_1- segregants examined 4 had this property and of the heterotypic gal_2-, one out of five examined was HFT. In the case of transductions of gal_4- by gal_2-, of 31 gal_4- segregants tested one was HFT, while of the three heterotypic gal_2- tested one was HFT. In the above tests segregants which had been purified through several single colony isolations were used. Since the HFT cultures segregate NFT lines it is possible that the above estimations are low.

Attempts to obtain (+) cultures with HFT property by reversion of (-) have been unsuccessful in the limited attempts made thus far. This too may be in part due to the fact that the HFT cultures segregate NFT lines and since it was not known at the time of examination that this was the case and the NFT reversions obtained could well have been from NFT components of the culture. The conversions of a HFT culture to NFT is fairly rapid and the HFT cultures are easily lost. On one occasion it was noted that a culture which had been on stock slant only a few weeks had changed such that of ten colonies tested 4 were found to be NFT. The NFT cultures which are derived from HFT lines have not been investigated except in the instances. In addition to NFT property (or possibly no activity at all) the segregants were in the one case ~~the same type as from which they were derived and in the other instance they were~~ of a gal- type which was not transducible except by a lysate of an unstable gal(+).

In one case (and the negative results in the other cases can possibly be explained

by the contamination of the HFT cultures with NFT cells) gal (+) reversions of an HFT culture were found to be unstable for gal(+) and segregated (-) which were of the same (-) type as the reverted locus. The examination of more HFT cultures to to determine the relationship between duplication of certain loci and HFT property and is in progress.

The lysates of the HFT stocks which have been prepared thus far have not had high phage titers although they have been prepared in a manner which gives high titered lysates in NFT stocks. Whether this indicates a lower yield per bacterium of plaque forming particles or different conditions for induction is not known at the present. Preliminary experiments to determine the yield of HFT particles per bacterium are regarded with reservation since the purity of the culture with regard to NFT cells was not known.

The HFT lysates have been used principally for allelism tests. Transductions can also be made via these lysates and the resultants studied. This has not been carried very far. The data in table 1ϕ indicate that transductions by HFT lysates are not appreciably different from those of NFT lysates as regards occurrence of stable tramsductions and distribution of segregants.

The HFT lysates can be used for transduction from gal(+) to gal(-) and have proved of value in creating new stocks. Table 1ϕ lists some of the information available on the stocks transduced to (-). Since the completion of the table gal_1- and gal_2- Lp^s $T^-L^-B_1^-$ Het_A strains have been prepared. The (-) stocks prepared thus far have been made starting with Lp^s cultures. The resultant cultures may be Lp^s, Lp^+ or Lp^r. In general the procedure has been to mix HFT lysate and cells on EMB(0) and incubate for 12-18 hours and then to streak out the growth and search for gal (-). On other occasions examination of single colonies from cell populations exposed to HFT lysate has been used.

Table 1B

Transduction by HFT Lysates. Distribution of the Segregants by Transduction Assay

Transinduced Cell Genotype	HFT Lysate Gal_1-	Gal_2-	Gal_4-
Gal_1- Lp_1^+	-	10* Gal_1- 2 Gal_2- 1 Gal_1- Gal_2-	9 Gal_1-
Gal_2- Lp_1^+	6 Gal_1- 3 Gal_2- 1 Gal_2- Gal_1-	-	8 Gal_2- 4 Gal_4-
Gal_4- Lp_1^+	not done	15 Gal_4-	-

* Out of a total of 18 transductions (or transductions and spontaneous papillae) analyzed. The difference between the number of segregants reported and 18 represents the number of stable papillae observed.

Table 17

Transductions to Inability to Ferment Galactose

Culture Transduced	Lp_1 Genotype	Galactose Locus Transduced	Resultant Lp_1 Genotype	Comment
W1485	Lp^s	Gal_2-	Lp^+ or r	8 distinct (-) obtained from single colonies
			?	2 distinct (-) obtained
		Gal_1-	+ and r	2 distinct (-) obtained
W1673	Lp^s	Gal_2-	+ or r	-
W1765	Lp^s	Gal_1-	s	-
W2252	Lp^s	Gal_1-	r ?	2 distinct (-) obtained
		Gal_2-	r	2 distinct (-) obtained

Separate mention of the cultures that were classified as double (-) by transduction test must be made partially because the results are more incomplete and partially because they may offer some additional information upon the transduction phenomenon. Four such (—) have been obtained, three of the gal_1-gal_2- type and one of the gal_2-gal_4- type. The evidence that such cultures are (--) is that they are transduced not either by homotypic nor heterotypic lysates but are transduced by wild type or some other gal (-).

Lysates of these (--) cultures have been found to have little transducing activity regardless of the gal (-) tester used with but one exception. Whether this implies a failure of the phage particles to pick up a fragment of cell chromosome or whether the resultant thansduction is not phenotypically (+) through some interaction among the genes concerned is not known. The exceptional case resulted in the recovery of each of the (-) making up the (—) individually and not conjunctively. The homotypic locus transduced with this lysate was not recovered among the segregants.

As might be expected the (—) are more stable on galactose medium and have seldom been seen to revert.

Some experiments of interest have been performed with one of the (—) obtained. It was unfortunately a prototroph and the results obtained with it must also be repeated and extended with auxotrophic strains.

Although this (—) was not transduced by either, lysates of wither (-) singly it was transduced to a lesser extent (where a solid layer of papillae with a (-) would have been obtained, less than 100 papillae were found by a mixture of the two HFT lysates). In this case it was taken that the cells transduced to (+) had received two phage particles with the addition of two (+) alleles in separate segments.

The cell that was transduced to (+) may be represented as follows:

$$---2^{-}--1^{-}---*$$

and the resultant transduction as follows:

$$\begin{array}{l} ---2^{-}--1^{-}---* \\ \quad -2^{-}--1^{+}- \\ \quad -2^{+}--1^{-}- \end{array}$$

In this case the extra (-) added in the segments are inferred from the results with transductions of single (-) in which the heterotypic locus is recovered among the segregants. Segregation from this transduction in the absence of crossing over or exchange between chromosome and segments can result in three types of (-) segregants.

(1) $---2^{-}--1^{-}---*$ (2) $\begin{array}{l} ---2^{-}--1^{-}---* \\ \quad -2^{-}--1^{+}- \end{array}$ (3) $\begin{array}{l} ---2^{-}--1^{-}---* \\ \quad -2^{+}--1^{-}- \end{array}$

which would be classified as (—), (2^{-}) and (1^{-}) presumably. With exchange between segments and the chromsome segregants with the (+) alleles would be found in the chromsome and subsequent segregation would yield(in addition to the types 2 and 3 above with the (+) transposed) the following types:

(4) $---2^{-}--1^{+}---*$ (5) $---2^{+}--1^{-}---*$

An additional type can be obtained if there be exchanges between segments. The order of frequency of exchange and segregation of the above types is unknown but on analogy with the simple transductions the first three mentioned would be expected most frequently, that is, loss of a segment is more frequent than exchange and loss of a segment.(This in turn is dependent upon the independence of exchange and loss) Examination of 24 separate segregants from one such transduction gave the following distribution of segregants by transduction test: 13 (—), 6 (1^{-}) and 5 (2^{-}). Since over 50 percent of the segregants were (—) it appears that when loss of a segment occurrs it is more likely to involve loss of both segments. The (1^{-}) and (2^{-}) found could be of two types, 2,4 and 3,5 above respectively. These types can be distinguished by means

of analysis of (+) reversions. In cases 2 and 3 the reversions will be unstable and segregate, and in cases 4 and 5 they will be stable for galactose. Reversions were examined for their stability from each of the (-) obtained. All the (1^-) ~~xxxx~~ gave stable reversions and therefore were presumably of the $---2^+--1^-----*$ type. Of the (2^-) examined all but one gave stable reversions and therfore the two types $---2^---1^+---*$ and $\begin{matrix}---2^-\\-2^-\end{matrix}\!\!-\!\!\begin{matrix}-1^+---*\\-1^+-\end{matrix}$ were indicated with the most frequent being the former.

Examination of the ~~xxx~~ (2^-) culture giving the unstable reversions showed that it ~~xxxxx~~ did segregate (—) cells but as yet it has not been established that it segregates (2^-) of the following type $---2^-----1^+---*$.

The reversions of ~~this~~ the type 2 (2^-) can be of two types and they should (perhaps) be distinguishable in turn by the segregants that they yield. Reversion of the form $\begin{matrix}---2^-\\-2^+\end{matrix}\!\!-\!\!\begin{matrix}-1^-----*\\-1^+---\end{matrix}$ should be expected to segregate (—) predominately and reversions of the form $\begin{matrix}---2^+\\-2^-\end{matrix}\!\!-\!\!\begin{matrix}-1^-----*\\-1^+--\end{matrix}$ should be expected to segregate (1^-) predominately.

Reversions of the type 2 (2^-) appear to be of two types. From one type 33 segregants were obtained, of which 32 were (—), the remaining one a (2^-). The other type gave almost equivalent amounts of (2^-) and (—) and no (1^-) thus far. The failure to recover (1^-) types from the ~~xxx~~ reverted cultures is disturbing but this may be related to elimination of the gal_1 locus in crossbs. Presumably crosses between $\begin{matrix}---2^-\\-2^-\end{matrix}\!\!-\!\!\begin{matrix}-1^-----*\\-1^+---\end{matrix}$ and $---2^+--1^-----*$ should yield a larger number of (+) than crosses between (1^-) and (2^-) of normal constitution when there is sucessful transfer of the segment through the zygote. these (+) in addition would be unstable for galactose. The culture used unfortunately is a prototroph and unless sucessful crosses between it and a Hfr strain can be accomplished the problem can not be attack from this aspect. (Sucessful transmission of the segment through the zygote was observed in some early experiments not related to the above.)

Examination of another (—) has begun. In this case Gal_2^- and Gal_4^- are involved and a crossable stock has been selected. There has been another complication in this case. That is when the culture was first isolated, and also in the case of a repeat test, it was not found to be transduced by either (2^-) or (4^-) lyssates. In several additional tests it has also reactive in this manner. In the instances where it was attempted to obtain transductions by mixtures of the two lysates it was found that the culture was transduced, to a lesser extent, by lysates of (2^-). It was thought to explain this incongruent result by postulating that reversions had occurred during the growth of the culture and that in effect the culture consisted of (—) with (4^-) contaminants. On this assumption the aberrant transductions of the culture would in effect be of the form (2^-) —x (4^-) and the resultant transductions would be expected to segregate (4^-) predominately. This was not the case, of the six segregants examined (from six separate transductions) 3 were (2^-), 2 were (—) and only one was (4^-). This does not rule out the explanation but requires a great frequency of exchange between segment and chromosome for compatibility.

Examination of this culture had progressed to the stage of isolating a (4^-) segregant that gave unstable reversions as well as a type which did not, at the time of writing.

Not all of the Gal- cultures studied have been found transducible although the most frequently occuring (-) after ultraviolet radiation appear to be of this type. Three distinctly different occurrences of non-transducible gal- have been found. Two of these were induced by ultraviolet, and the third by copper exposure (H. Byers). One of the ultraviolet mutants has been examined to some extent. The results are given in table 18. It appears that this (-) is not transduced by any of the lysates and futher that lysates of it in turn transduce all known transducible loci, but Gal_2 with lowered frequency.

Table 18
Analysis of a Non-transducible Galactose Locus in W2312
by Transduction Assay

Experiment		Plate Additions: None	HFT Lysates: Gal_1-	Gal_2-	Gal_4-	NFT Wild Type
206	(1)	0*	0*	0*	0	-
	(2)	0	0	-	-	0
220	(1)	0	0	0	0	-
	(2)	0	0**	0**	0**	0

* number of papillae per plate
** NFT (normal frequency of transduction) lysates used in these cases

Table 14
Activity of Lysates of W2312 on Selected Galactose Loci

Galactose Locus		Plate Addition: None	W2312 Lysate
Gal_1- Lp^+		4*	37*
Gal_2- Lp^+	(220)	8	7
	(221)	19	28**
Gal_4- Lp^+		17	74
Gal_6- Lp^s		3	121

* numbers of papillae per plate
** 12/12 examined were found to be stable Gal+

Table 15
Results of Crosses of W2312 with Selected Galactose Loci

Selected Galactose Locus	Numbers: Gal+	Total Prototrophic Recombinants	Percent Gal+
Gal_2- F^-	1	2112	0.05
Gal_4- F^+	1	198	0.5

For the purpose of collecting new gal- and for observing the occurrence of transducible loci two separate experiments were performed. Gal- mutations were induced in W1673 (glyc or ser)$^-$ pro$^-$ and W1765 hist$^-$ leuc$^-$ by means of ultraviolet. Table 19 gives a summary of these experiments. Reccurrences of both Gal_1- and Gal_2- were found as well as a number of new loci and possibly several (—). No recurrences of Gal_4- were observed.

The effect of ultraviolet radiation on the transducing activity of lysates has been investigated in three experiments. The first two experiments were concerned with NFT lysates, the last with an HFT lysate. The effect of ultraviolet upon NFT lysates is shown in figure 2. With increasing dose of ultraviolet there is a linear increase in the activity of the lysates on Lp^+ or Lp^r assay cells until a survivial of the plaque-forming titter has become reduced about 10^{-3}. Thereafter there is a gradual decease in transduction activity with increasing dose. On Lp^s there is a slight increase in transducing activity and then a gradual decrease. The maximum reached by the lysates on Lp^+ or Lp^r cells is about four times the maximum reached on Lp^s cells. In performing this experiment about 10^8 Lp^s assay cells were used, since figure 1 indicates that this number of cells may indicate only about one-third to one-fourth the number of transductions actually present and the Lp^s assay is probably that much low. This then would suggest that the absolute number of transductions is approximated upon Lp^s cells when a sufficient number of cells are used and that the action of ultraviolet is to increase the assay on Lp^+ or Lp^r cells to the level of the absolute number present. In connection with this it should be noted that survival of the transductions of Lp^s is still about 0.5 even at the extreme doses used. From the above it is suggested that the action of ultraviolet is several fold. First and most rapid is the destruction of plaque forming activity of Lp^s cells. Secondly, to destroy that property of the phage which causes them to be "excluded" by lysogenic cells, as regards transduction, and thirdly to destroy

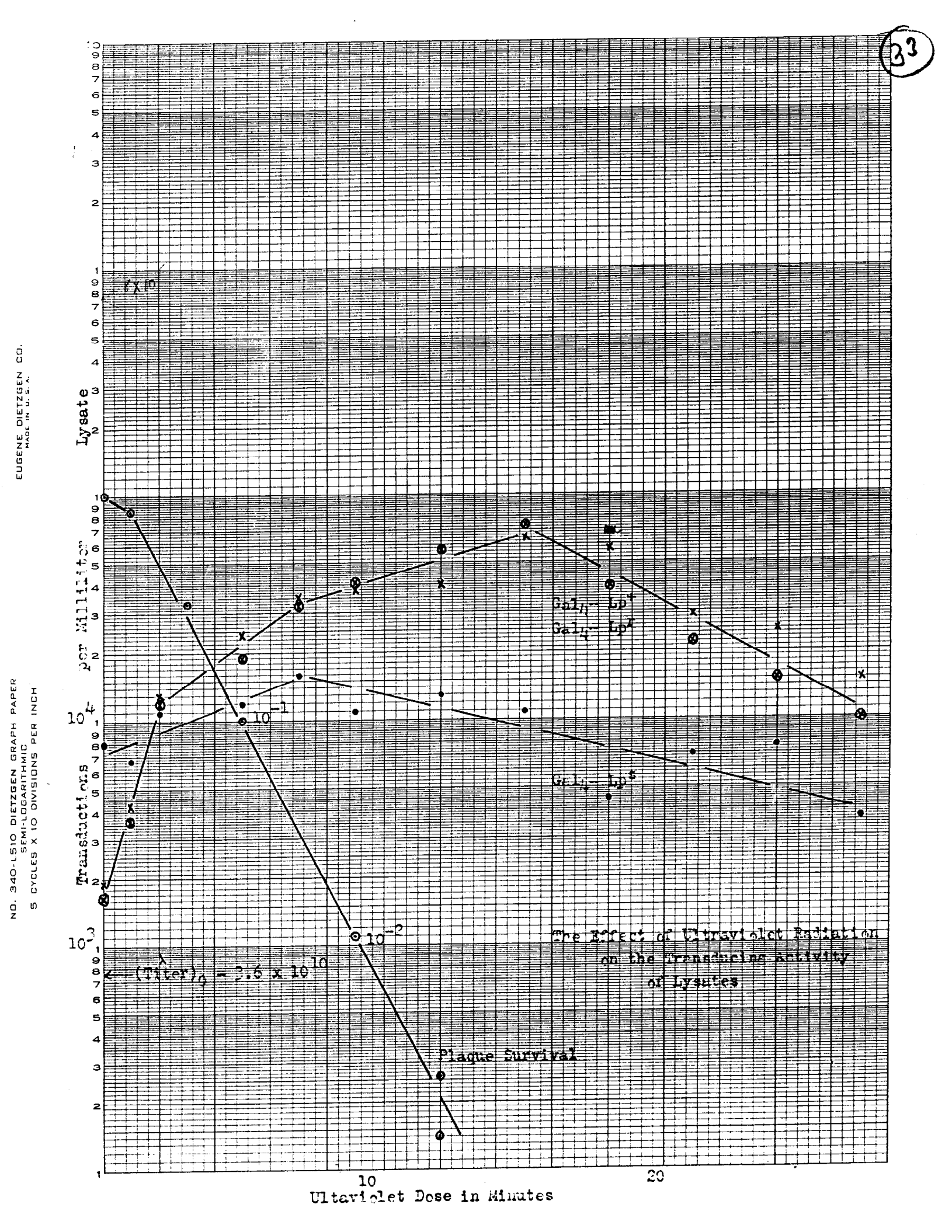
Table

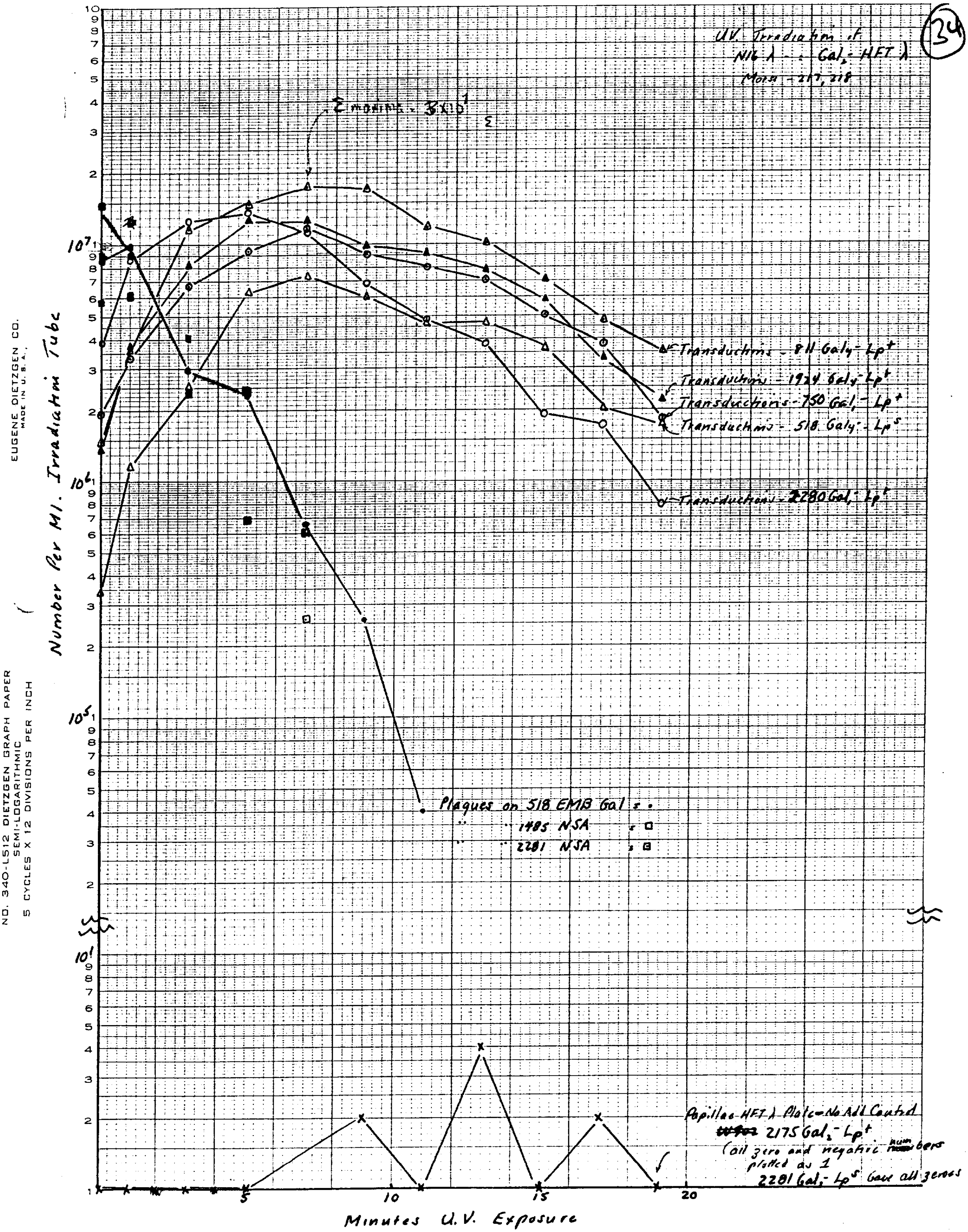
Transduction Assay of Some Galactose Negative Mutants Induced by Means of Ultraviolet

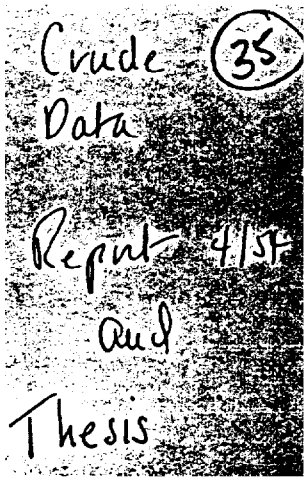
Culture Treated	Mutant Designation	Transduced by HFT Gal_1-	Gal_2-	Gal_4-	Possible Genotype
W1673 Lp^s	W2310	0	+	0	Gal_1-Gal_4-
	W2311	0	+	0	" "
	W2312	0	0	0	nontransducible
	W2313	+	0	+	Gal_2-
	W2314	+	+	+	Gal_x-
	W2315	+	+	+	Gal_x-
	W2316	0	+	+	Gal_1-
	W2317	0	+	0	Gal_1-Gal_4-
	W2318	0	0	0	nontransducible
W1765 Lp^s	238-2	0	0	0	nontransducible
	238-5	+	+	+	Gal_x-
	238-6	0	+	+	Gal_1-
	238-8	+	+	+	Gal_x-
	238-10	+	+	+	Gal_x-
	238-11	0	+	0	Gal_1-Gal_4-
	238-12	+	0	+	Gal_2-
	238-13	+	0	+	Gal_2-

the transducing activity itself, perhaps by destroying the adsorption of the phage particles.

The effect of ultraviolet on HFT lysates is similar to that of UV on NFT lysates. The increase in transducing activity with dose in this case is not as great as with NFT lysates. A maximum is reached that is approximately equivalent to the plaque titer of the lysate which suggests that plaque and transducing particles may be the same but that appearance of a particle as a plaque excludes its appearance as a transduction. Platings for plaque formation on EMB galactose have not indicated that one particle can function in both capacities but the appearance of a plaque might be obscured by papillae formation. The sum of the activities (maximal) of the lysate on the two assay loci is 2-3 times the plaque titer, which may be an indication that the activities are confined to a single particle. The occurrence of transductions with Lp^r genotype has been noted with this lysate, and the equivalence of plaque and transduction titer might not be expected on the assumption that in these cases the effect was accomplished by a defective phage particle which would not give rise to plaques as well as to lysogenization. (This would require that Lp^r genotypes were the result of such defective particles rather than of a defective act of lysogenization.)




U.V. Irradiation of
N16 λ - - Gal₂- HFT λ
Morse - 217, 218
Σ moving - 3 x 10¹
Σ
Number Per Ml. Irradiation Tube
10⁷
10⁶
10⁵
10¹
Transductions - 811 Gal₁- Lp⁺
Transductions - 1924 Gal₁- Lp⁺
Transductions - 750 Gal₁- Lp⁺
Transductions - 518 Gal₁- Lp⁵
Transductions - 2280 Gal₁- Lp⁺
Plaques on 518 EMB Gal ; •
" " 1485 NSA ; □
" " 2281 NSA ; ⊡
Papillae HFT λ Plate - No Add Control
2175 Gal₂- Lp⁺
(all zero and negative numbers plotted as 1
2281 Gal₂- Lp⁵ Gave all zeroes
5
10
15
20
Minutes U.V. Exposure
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH


Crude
35
Data
Report
Thesis

Interaction of the Gal- lysate

Numbers of papillae/plate - 0.1 ml lysate plated

Cells	No addition →	Gal$_1^-$ 2.4×10^{10}	Gal$_2^-$ 4.9×10^{10}	Gal$_4^-$ 1.7×10^{10}	Wild 1.4×10^{10}
Gal$_1^-$ Lp$^+$	(1) 2	—	176	43	[illegible]
	(2) 2	2	—	—	405
Gal$_2^-$ Lp$^+$	(1) 14	52	11	43	—
	(2) 20	—	10	—	356
Gal$_4^-$ Lp$^+$	(1) 89	—	202	—	296
	(2) 50	85	—	—	417
	(3) 47	—	—	50	394

$\frac{24\times10^9}{3.2\times10^2} = 8\times10^7$ $\quad \frac{4.9\times10^{10}}{1.7\times10^3} = 3\times10^7$ $\quad \frac{1.7\times10^9}{4.1\times10^2} = 4\times10^7$ $\quad \frac{1.4\times10^9}{4\times10^3} = 3\times10^6$

Data from Arnold p. 92

Lysate

Cells	Gal1 W750		Gal1 W2319	Gal1 W2343	Gal2 W902	Gal2 W2251	Gal2 W2175	Gal2 V1210		Gal4 W811	Gal4 W1821
Gal1 W750	2/2	3/0	4/0	0/0	176/2, 116/2, 83/0	40/0	148/0	92/1		8/4, 43/2, 9/1, 10/3, 8/0, 10/2, 14/2, 33/2, 25/2	31/2
(7300r) W2319	25/21				428/21					128/29	
(part) W2343	0/1				84/1					14/1, 8/5, 12/3	
W2373					11/1			56/0		30/1	
Gal2 W2175	52/14, 71/5, 50/15, 88/14	10/4	16/4		10/20, 11/14, 14/51, 0/0, 0/1			10/18		43/14, 51/5, 39/13, 50/13, 32/15	
W1210	119/2, 6/4									78/14, 56/3	
W2251	364/5, 214/0									98/0	
W1924	25/31, 43/33				235/31						
Gal4 W811	201/16, 85/50, 96/51, 55/45				147/44, 202/89, 100/2	40/2				50/47	51/47
W1736										27/12	13/12
W1402	28/17				61/17					22/17	
W518	40/45, 79/28, 73/29, 73/10	3/2	3/2		113/4, 128/28, 113/29, 473/11		109/2	128/4		4/11, 86/163, 17/41	
W1578										18/10	
W1436	145/24										
811 prototype										18/23	

Not used in calculating

Heated

— see pg 121 also

no acid 37
heated 19
λ 219
} 56C - 30'

λ titer = 2.3×10^{10}
heated λ titer = 2.2×10^{9}

Culture	no acid	boiled (KA)	K12 λ
W1736	17	22	335
	—	13	410
W1662	—	19	311
W811	—	66	535
W1821	—	30	581
W750	0	0	469
	—	2	542
W518	—	4	2112
W1924	—	29	129

Other transduction attempts

good comparison on pg 94
W112 lp, R – lp2 ...

	marker	culture
71 (82)	lac	W112 (W1736 gal+R)
74	serine or glyc	W1678
75, 78	leuc	W1736
82 (83) (85)	methionine (BM-) (BM)	58-161 (W811) (W821)
83 (85) (130)	xylose	W1821 (rpt.) (rpt.)
95	S R	W578
96	proline	W1692, W1920
100	proline	2062 (W1692 x W1402) a galy- pr -
104, 105, 106	proline	" "
113	Leucine	W1436
119	mal x -	~~[illegible]~~ W2071
180	BM - (with HFT)	W518
200	pr (with HFT's)	W2062
227	pr with lytic d)	W2062

VV

Σ

Locus	Attempts	Culture
Lac1	4	W112
(ser or glyc)	1	W1678
Leuc	3	W1736, W143
Methionine	4	58-161, W811, W1821
Xylose	3	W518, W1821
S	1	W518
proline	7	W1692, W1921, W2062
mal x -	1	W2071

for multiple transduction (other factors)

Partial attempts – (indirectly through checks to see if gal only transd.)

pg	markers	culture
84	TMB, L	W1736, W1662
86 (87)	BM lac	W811
	TLB, lac	W1736
87	BM xyl lac	W1821
93	BM lac	W750

P 133, 135

DNA ase effect

Lysate	No. plaques on 0.1 ml		Titer
	Gal4⁻ hp⁵	Gal4⁻ hp⁺	
wild untreated	960	—	—
DNA ase treated	998	—	—
gal4⁻ reversion untreated	—	201, 204	6.1×10^9
DNA ase treated	—	296	6.0×10^9

Effect of hp_2 allele on transd.

Allele	Spont.	Popular $hp_1 + hp_2^S$	$hp_1 + hp_2^R$	Pfu
Gal_1 -	1	426/1	2/1	91, 99
Gal4⁻	420/1 = 17	356/20	14/14	100
Gal_3 -				
Gal4⁻	50	296/89	57/50	92, 99

Action of Lysates of $(+)^{Rev}$

PL	Culture	Lw ods	$+^R$ culture	#
89	W1736	12	W811	191
93	W750	2	W811	144
128a	~~W578~~	~~41~~	~~W811 #1~~	~~27~~ } don't transduce 811 either
		~~41~~	~~" 2~~	~~15~~
133	W578	30	W811 #5	883
134	W811	39	W811#5	204
135	W811	25	W811 #5	201, 296
		25	#8	291
151	811 prototroph	~~23~~	~~W811 #1~~	~~15~~
		~~11~~	~~#2~~	~~22~~
		"	#5	214 461 (another lysate)
		"	#8	389
240	W750	0	W750	648
240	W2175	10	W2175 #1	96
		6	" #2	552
240	W750	0	W811 #5	146
		1	W811 #8	153

Used in paper

Table 4

Restoration by Reverse Mutation of the Ability to Transduce ~~[illegible]~~ loci

Locus	Revertant	Lys on culture	Reversion Rate
Gal_1^- (Lp^+)	Gal_1^+ #1	0	648
Gal_2^- (Lp^+)	Gal_2^+ #1	10	96
	Gal_2^+ #2	6	552
Gal_4^- (Lp^+)	Gal_4^+ #5	39	204
	Gal_4^+ #8	25	291

(41)

Transduction of Lp^v + Lp^+ Lp^+

Expt	Cultures	λ	transd. assay
92	W518	+	2112/4
		2-	1112/4
	W811	+	296/89
		2-	202/89
140	W518	2-	1152/29
	W811	2-	147/44

not needed?

Lyhi λ

227 used λ on 518 WS

228 also no produce deletion 2062 with gal- ... W1485 no transd. c 750, 518, 2175

239 (1) rpt of gal/gal 1485 — " ..

(2) growth of HFT λ lyhi · 518 — no growth

(3) used w/ 518 (above) transd. 1-, 2-, 4-

Expt	Assay cult	no pt	lyhi λ
228	W750	3	2
	W518	9	8
	W2175	7	8
239	W750	2	0
	W518	13	8
	W2175	6	2

254		
W750	—	3
W518	—	6
W2281	—	9
W2373	—	6
W811	—	39

Absorption of λ

cells	rg	λ adsorbed	% transduction adsorbed
~~W518~~	~~127~~	~~61~~	~~52~~
W528	128	52	67.5
	—	~~37~~	7
W811	285 ?		
W250	226 ?		
W2175	" ?		

Relationship of the gal⁻ 199 (210)

Cross	minimum no. progeny	No. (+)	% (+)	Recombinant
Gal_1^- × gal_2^-	>1500	2	<0.13	199
Gal_1^- × gal_3^-	>1600	2	<0.13	~ 200
Gal_1 × gal_4^-	4554		0.13	210
Gal_2^- × gal_4^-	2654		0.22	174, 175
Gal_1^- × gal_2^-	>6517	4	<0.06	214
Gal_1 × gal_2 (2151)	3606	1	0.027	240
Σ gal_1 × gal_2	623 / >11620	7	<0.06	

.06 .13
2 ⟷ 1 ⟷ 4
.24

16/19
0.81
19 | 160
157
30

Correlation of transduction with lysogenicity

Allele transduced	source lysate	No.	% lysogenic	Ref
gal_4^- tp^s W518	wild	18	100	170a
gal_4^- tp^s W518	gal_2^-	19	100	146
gal_4^- tp^s W518	gal_x^- (84₂)	22	100	147
gal_4^- tp^s W518 11	11 (84₁)	23	100	153
gal_4^- tp^r	wild	29	3.	216
gal_4^- tp^r LW1924	gal_2^-	18	5.5	213
gal_2^- tp^s W2281	wild	9 (16)	44 (44)	229A
	gal_1^-	23 (21)	95 (42)	229B
	gal_4^-	19 (21)	81 (81)	229C
	wild	18	69	25
W2373	gal_4^-	22	77	249A
	gal_2^- (1210)	24	88	249B
	gal_2^-	12	58	249C
	wild	23	87	249D

0.87
23 | 20.4
18
160
157
1

0.15
13 | 2.0
1
1.7

2 The Occurrence of Stable Transductants

λ Source

Recipient Cells	K-12		Gal$_1$- W750		Gal$_2$- W902		Gal$_3$- W2238		Gal$_4$- WF11	
	Stable Expected	Observed	Stable Expected	Observed	Stable Expected	Observed	Stable Expected	Observed	Stable Expected	Observed
Gal$_1$- 234^{rr}	1/143	42			1/84	3.5 $X^2 = 6.25$	~~not done~~ 4/24*	10	12/27	27 * all stable
Gal$_2$-	17/248	20.7 $X^2 = 6.87$	14/83	61.1			~~not done~~ 7/48 = .32		14/79	52.1
Gal$_3$-	not done		2/88	88 all stable	5/34	34 all stable			12/56	48.7 (possibly 56)
Gal$_4$- Lp^s	19/835	383	29/72	72 * all stable?	11/472	19.7	not done, doesn't go?			
Gal$_4$- Lp$^+$	41/573	133	51/96	96 * all stable?	47/147	30.6 **	not done doesn't go?			
Gal$_4$- Lp^r	3/320	127	25/31	not done * all stable?	31/238	49.6 $X^2 = 12.7$	not done			

* These may be instances of stable transductions or just estimates of the variation in spontaneous reversions on the plates.

** Estimated from two different experiments.

Explanation:

Stable Expected = $\frac{\text{no. papillae control plate}}{\text{no. papillae transd. plate}} = \frac{\text{spont. reversions}}{\text{transductions + sp. reversions}}$

Observed = $\frac{\text{no. stable observed}}{\text{no. in sample taken}} \times \frac{\text{no. papillae transd plate (= sp. + revers. transd.)}}{\text{no. papillae in sample taken}}$

Stability determined by streaking out single colonies (consecutive) on EMB gal.

Segregant Σ

Expt	Allele Homo	Allele Hetero	Numbers Homo	Numbers Hetero	Homo Hetero	Total
248	1⁻ +	—	√ 16	—	—	16
249D	1⁻ s	—	√ 9	—	—	9
247 A	2⁻ +	—	√ 15	—	—	15
233	2⁻ s	—	√ 16	—	—	16
212	4⁻ +	—	√ 20	—	—	20
205	4⁻ s	—	√ 13	—	—	13
—	4⁻ R	—	—	—	—	— —
196	2⁻ +	—	√ 20	—	—	20
192A	1⁻ +	—	√ 17	—	—	17
247C	1210 2⁻ +√	1	19	2	0	21
236 B	2⁻ s√	1	20	0	0	20
209	2175 2⁻ +√	1	14	3	2	19
230	750 1⁻ +√	2 (902)	18√	1	0	19
243	750 1⁻ +√	2 (1210)	18	3	0	21
249 B	1⁻ s√	2 (1210)	6	1	0	7
249 C	1⁻ s√	2 (902)	1	0	0	1
202	4⁻ +√	2	16	3	0	19
242	4⁻ s√	2 (1210)	17	2	0	19
198	4⁻ s√	2 (902)	18	3	0	21
~~213~~	~~4⁻ R√~~	~~2~~	~~15~~	3	0	18
192B	1⁻ +√	2 (902)	18.	5	0	23
249A	1⁻ s√	4	√ 1	0	0	1
247B	1210 2⁻ +√	4	22	1	0	23
236 C	2⁻ s√	4	21	1	1	23
207	2175 2 +√	4	9	7	0	16
			359 √	35 √	3 √	397 (397)
24			90.5	8.8	0.76	100.06
single transd. → 146	√4⁻ s	2 (902)	17.	2	1	20
single transd. → 145	√4⁻ s	+	15	0	0	15
170a	√4⁻ s	+	18	0	0	18
			409	37	4	450
			(90.9%)	(8.2%)	(0.88%)	

Nat. ~~Segregant~~ from wild type transducts

	Homo	Hetero	Total
169	168	0	168
2	100	0	

409 − 169 = 240; 281 + 169 = 450; 248 + 169 = 417; 409 − 136 = 273

Homo	Hetero	Di	Total
390	35	3	
+ 17	2	1	
240	37	4	281
240	37	4	281

Transd.	Homo	Hetero	Di	Total
Wild type	169	0	0	169
Between gal (?)	240 (.85)	37 (0.132)	4 (0.014)	281
Total	409 (0.91)	37 (0.82)	4 (0.0088)	450

Occurrence of Stable transductions - Reversed Lysates

Cells	Wild 1212 Expected	Observed	Gal1- W750 ~~Expected~~	Found	Gal2- W902 Expected	Found	W1210 Expected	Found	Gal4- W811 Expected	Found
Gal1-										
W2343 λp+	1/143	42	—		1/84	(3.5)	1	—	12/27	27
2373 λp+	1/33	14	—		1/11	11	0/51	19.5	1/30	28.7
W750	1/46	(1.9)	—		—		1/92	0		
Gal2-										
W2175 λp+	17/248	20.7	14/83	61.1	—		—		14/79	52.1
W1210 λp+	4/29	6.3	2/65	(0)	—		—		5/56	(0)
W2281 λp+	0/46	~~15.2~~ 11.6	0/214	26.7	—		—		0/98	3.9
[illegible]-										
W578 λp+	19/835	383	29/72	72	11/472	19.7	4/128	21.4	—	—
W811 λp+	41/573	133	51/96	96	47/147	([illegible])	—	—	—	—
W1924 λpR	31/330	127	—	—	31/238	49.6	—	—	—	—

Stable expected = # pap. cured / # pap. lysate plate = spont / spont + transd.

Observed = # stable obs / # in sample × # pap transd. plate / # pap in sample

Nature of the Segregants

Cells	Wild	Gal_1^- ±210	Gal_2^- W902	Gal_2^- W1210	Gal_4^- W911
W2342 4pt	17 gal_1^-	—	14 gal_1^- 1 gal_1^-	— —	no segregants found
W2373 4pt	9 gal_1^-	—	1 gal_1^-	6 gal_1^- 1 gal_2^-	1 gal_1^-
W1750 4pt	16 gal_1^-	—	16 gal_1^- 1 gal_2^-	18 gal_1^- 3 gal_2^-	no segregants found.
W2175 4pt	20 gal_2^-	14 gal_2^- 3 gal_1^- 2 gal_2^- gal_1^-	—	—	8 gal_2^- 7 gal_4^-
W1210 4pt	15 gal_2^-	19 gal_2^- 2 gal_1^-	—	—	22 gal_2^- 1 gal_4^-
W1281 4pt	16 gal_2^-	20 gal_2^-	—	—	21 gal_2^- 1 gal_4^- 1 gal_2^- gal_4^-
W578 4pt	13 gal_4^-	no seg found	18 gal_4^- 3 gal_2^-	17 gal_4^- 2 gal_2^-	—
W811 4pt	20 gal_4^-	no seg found	16 gal_4^- 3 gal_2^-	—	—
W1924 4pt	29 gal_4^-	no seg found	15 gal_4^- 3 gal_2^-	—	—
—	15 5	—	~~35~~	—	—

Type of Segregant

Transl. Lysate	Homotypic	Heterotypic	Homo-Hetero	Total
Wild	169	0	0	169
gal(-) on gal(-)	240 (0.854)	37 (0.132)	4 (0.014)	281
Total	409 (0.91)	37 (0.08)	4 (0.0088)	450

Summary HFT deletion - Analysis of Segments by Transduction Test

HFT Lysate

Transduced Cell Genotype	Gal_1^-	Gal_2^-	Gal_4^-
Gal_1^- W750	—	18 { 10 gal_1^- ; 2 gal_2^- ; 1 $gal_1^- gal_2^-$	18 { 9 gal_1^-
Gal_2^- W2175	18 { 6 gal_1^- ; 3 gal_2^- ; 1 $gal_2^- gal_1^-$	—	18 { 8 gal_2^- ; 4 gal_1^-
Gal_4^- W565	not done	18 { 15 gal_4^-	—

$2.5 \times 10^{?}$

(49)

# cells	no papillae	1/#cell	$\times 10^{-9}$	1/no papillae
1.4×10^{9}	2504	0.59×10^{-9}	0.59	0.4×10^{-3}
7×10^{8}	1780	0.14×10^{-8}	1.4	0.57×10^{-3}
3.5×10^{8}	1472	0.28×10^{-8}	2.9	0.68×10^{-3}
1.75×10^{8}	1120	0.57×10^{-8}	5.7	0.89×10^{-3}
8.75×10^{7}	1688	0.11×10^{-7}	11.0	1.5×10^{-3}
4.4×10^{7}	[illegible]	0.23×10^{-7}	23	1.72×10^{-3}
2.2×10^{7}	562	0.46×10^{-7}	46	1.78×10^{-3}
1.1×10^{7}	533	0.91×10^{-7}	91	1.87×10^{-3}
5.5×10^{6}	509	0.18×10^{-6}	180	1.97×10^{-3}

Extrapolation to ~~zero~~ infinite no cells gives reciprocal ^ no. of papillae is $0.34 - 0.36 \times 10^{-3}$

$= 2780 - 2980$ pap / 0.1 ml

27800 - 29800 / 1 ml

ave $= 2.88 \times 10^{4}$

From mediation experiment -
Back extrapolation

$\frac{5 \times 10^{5} \text{ transd}}{3.6 \times 10^{10} \lambda} = \frac{1.5 \text{ transd}}{10^{5} \lambda} =$

In this experiment estimated λ titer $= 2.7 \times 10^{10}$

$\frac{2.88 \times 10^{4}}{2.7 \times 10^{10}} = \frac{1 \text{ transd}}{10^{6} \lambda}$

$\frac{1}{x} = 3.6$

$3.6x = 10$

$x =$

$\frac{1}{x} = 0.36$

$1.00 = 0.36x$

2.78×10^{3}

2780

2980

Non transducible Gal$_x$ – W2312

Expt	No Additions	Gal$_1$ –	HFT Lysate Gal$_2$ –	Gal$_4$ –	NFT Wild type lysates
206 (1)	0	0	0	0	–
(2)	0	–	–	–	0
220 (1)	0	0	0	0	0
(2)	0	0 *	0 *	0 *	0 *

* = NFT lysate

Action of Lysate of W2312 on Various Gal– Cultures.

Gal–	No Addition	Numbers of Papillae W2312 lysate
Gal$_1$ – Lp$^+$	4	37
Gal$_2$ – Lp$^+$ (220)	8	7
(221)	19	28 *
Gal$_4$ – Lp$^+$	17	74
Gal$_1$ – Lp^s	3	121

* 12/12 examined found stable +

Crosses of W2312 with known Gal –

Heterozygote –	(+)	Number total	Prototrophic recombinants	% (+)
Gal 2 – P –	1	2112		(0.05) %
Gal 4 – F+	1	198		0.5

Table 5

Absorption of the Transducing Activity

P. Adenine

(52)

Adsorbing Cells	Phage titer	Cell titer	1st Absorption Phage	1st Absorption Transd	2nd Absorption Phage	2nd Absorption Transd	3rd Absorption Phage	3rd Absorption Transd
W84 gal^- bp^+	~~1.8~~ 1.9×10^{10}	1.6×10^{10}	—	72%	—	56%	—	0
W750 gal^- bp^+	~~1.8~~ $\times 10^{8}$	6.5×10^{9}	—	45%	—	110%	—	0
W2175 gal_2^- bp^+	1.8×10^{10}	6.0×10^{9}	—	35%	—	33%	—	0
W518 gal^- bp^3 (1)	2.5×10^{9}	7.1×10^{8}	60	79	50	41	16	46
(2)	3.9×10^{9}	5.5×10^{8}	52	33	—	—	—	—
W811 gal^- bp^+	1.4×10^{10}	c. 10^{10}	—	79	—	—	—	—
W1736 gal^- bp^+	1.4×10^{10}	c. 10^{10}	—	97	—	—	—	—

Table 10

Number Colonies Observed

Expression	Post Expression Cell titer	Gal -	Gal +	Gal - Partially lysed	Total
Broth	1.1×10^9	3280	0	0	3280
HFT lysate	3.5×10^9	2801*	31 (1.1%)	54	2286

Table 11

Examination of Colonies after HFT Expression

Colony	Number	Lysogenic	Sensitive	Resistant
Gal (-)	31	0	31	0
Gal (+)	26	23	0	3

Transductions in Gal-

Culture	trp	Gal⁻ locus transduced	Resistant culture and trp genotype	Comment
W1485	trp⁻	Gal_2^-	—	trp⁺ & R_{trp^r} & destruct (-) obtained from single colonies
		Gal_2^-	— ? [illegible]	2 destruct (-) obtained from separate colonies
		Gal_1^-	— trp⁺ and trp⁻	2 destruct (-) obtained from separate colonies
W1673	trp⁻	Gal_2^-	— trp⁺ or trp^R	? (-) obtained not known whether from same clone
W1765	trp⁻	Gal_1^-	W2373 ⁻	3 (-) obtained not known whether clonal or not
W2252	trp⁻	Gal_1^-	W2345 trp^R?	2 (-) obtained from [illegible] clones
		Gal_2^-	W2341 trp^R	2 (-) [illegible] clones

(55)

Transduction in E. coli K-12
Progress Report August 1953
September

M. L. Morse

1. References

λ Source

Recipient Cells	K-12 Gal +	W750 Gal_1^-	W902 Gal_2^-	W2238 Gal_3^-	W811 Gal_4^-
Gal_1^- prototroph	Experiment M page 190 192A		Experiment N page 190 192B, 192C	not done in progress 221	doesn't go ? page 206
Gal_2^- W2175 (prototroph)	Experiment O page 191 196	Experiment T page 206 209		not done in progress 222	Experiment U page 206 207
Gal_3^- W2297 (prototroph	not done	page 208	page 208		page 208
Gal_4^- bp^s W518	Experiment Q page 200D 205	doesn't go? page 189	Experiment R page 191 198	~~not done~~ doesn't go ? 222	
Gal_4^- bp^t W811	Experiment V page 212	doesn't go? page 195	Experiment S page 202	~~not done~~ doesn't go ? 222	
Gal_4^- bp^r W1924	Experiment X page 216	doesn't go? page 208	Experiment W page 213	not done	

Lysates

	K-12	W750	902 W1210	W811 (1607)
cells	248 + Redone seq anal. complete		by 902 p. 230 seq anal complete + by 1210 p. 243 seq anal complete	Doesn't go? p 206
W750 lp+				
W2373 lp+ W2765 gal-	249 D + Do seq anal complete		by 1210 249B + seq anal complete Do by 902 249C + seq anal complete	249 A + Do seq anal complete
W1210 lp+	247A + seq anal complete	247C + seq anal complete		247B + seq anal complete
W2281 lp+	p. 233 + seq anal complete worse almost complete	pg. 236B + seq anal complete		p 236C + seq anal complete
W811 lp+	Expt V p. 242 + seq. anal complete	doesn't go? 1Sq	by 1210(do) - by 902 p 202 (Expt) + seq anal complete	
W918 lp+	Expt Q + p. 205 seq anal. complete Eyes anal. almost complete	doesn't go? 194	by 1210 p. 242 + seq anal complete by 902 p 198 (Expt R) + seq anal complete	
W1724 lp+	Probably good. seq.	doesn't go? 208	by 902 p 213 (Expt W) + seq anal complete by 1210 (Q4)	
W475	Expt O. 196 + complete	Expt. T. page 208 + seq anal complete	X	Expt U p 207 + seq anal complete
W2342 (W750 prm)	Expt. M 192A + Seq anal complete	X	Expt N 192B + seq anal complete.	not done

3. Status of the Segregant Analysis

Recipient Cells \ Source	K-12	Gal_1^- W750	Gal_2^- W902	Gal_3^- W2238	Gal_4^- W611
Gal_1^-	17 17/17 gal_1^- 4/4 gal_1^- 4/4 gal_1^- (incomplete)	—	23 18 gal_1^-, 5 gal_2^- 5/5 gal_2^-, 4/4 gal_1^- 5/5 gal_2^-, 4/4 gal_1^- (incomplete)	not done in progress	stable (+) ?
Gal_2^-	20 20/20 gal_2^- 4/4 gal_2^- 4/4 gal_2^- (incomplete)	19 14 gal_2^-, (3) gal_1^-, (2) gal_x^- (double ?) incomplete incomplete	—	not done in progress	16 8 gal_2^-, 7 gal_4^- (double ?) incomplete incomplete
Gal_3^-	not done	stable (+)	stable (+)	—	stable (+) ?
Gal_4^- Lp^S	13 13/13 gal_4^- 4/4 gal_4^- 4/4 gal_4^- (incomplete)	stable (+) ?	21 18 gal_4^-, (3) gal_2^- 16/16 gal_4^-, gal_2^- incomplete 4/4 gal_4^- complete gal_2^- incomplete	not done does it go?	—
Gal_4^- Lp^L	20 20/20 gal_4^- incomplete incomplete	stable (+) ?	19 16 gal_4^-, (3) gal_2^- 15/15 gal_4^-, gal_2^- incomplete 4/4 gal_4^- (complete) gal_2^- incomplete	not done does it go?	—
Gal_4^- Lp^r	29 29/29 gal_4^- incomplete incomplete	stable (+) ?	18 15 gal_4^-, 3 gal_2^- incomplete incomplete	not done	—

Explanation

17 = no (-) segregants

17 gal_1^- = "allele" by transduction test

4/4 gal_1^- = "allele" by donor lysate test

4/4 gal_1^- = "allele" by cross with gal_1^- The same segregants as ↑

← what a cell is not transduced by what "allele" cannot be transd. by a lysate of segregant

Segregants from a single transduction - The ordering of the segregants is not specific

Transduced Cell Genotype	Lysate Source	Homotypic	Heterotypic	Homo-Hetero	Total
Gal4-	gal2-	17	2	1	20

P	Gal	% +	No. Prts	Fraction Gal+ in crosses (+) x (-)
190	Gal3- x Gal+ (F-) 2238 x 1655 (F+)	12.4	864	
EML Thesis 49	F(+) Gal1 x Gal+ (F-) 750 Y-10	14.7	-	
169	F+ Gal1- x Gal+ (F-) 750 Y-10	0.66 % (F-)	c.300	
149	(F+) Gal4- x Gal+ (F-) 811 Y10	3.5	480	
143	F- Gal4- Lp^s x F+ Gal+ 518 1678	0	71	Generation phenocopies
	F+ Gal4- Lp^s x F- Gal+ 518 1678	0.52	574	
	F- Gal4- Lp^s x F+ Gal+ (Lp^s)	3.5	29	
	F+ Gal4- Lp^s x F- Gal+ (Lp^s) 518 1673	3.8	427	

Transduction	Number Segregants		Classification by Transduction	Classification by Lysate	Classification by Crossing tetrads Crossed with	Heterotype (+)	Heterotype total progeny	Homotype (+)	Homotype total progeny
$a\ d \times gal_4^-$	4	(1)	gal_4^-	gal_4^-		0	896	—	—
		(2)	gal_4^-	"		0	918	—	—
		(3)	"	"		0	1134	—	—
		(4)	"	"		0	863	—	—
$gal_1^- \times gal_2^-$	4	(1)	gal_2^-	—		0	1037	—	—
		(2)	"	—		0	10512	—	—
		(3)	"	—		0	110	—	—
		(4)	"	—		0	800	—	—
	no gal_1^- segregant found								
$gal_4^- \times gal_2^-$	2	(1)	gal_2^-	—		0	117	—	—
		(2)	gal_2^-	—		0	1096	—	—
		~~(3)~~	~~gal_2^-~~			~~0~~	~~12000~~		
	1	(1)	gal_4^-	gal_4^-		—	—	3+	391
	1	(1)	$gal_2^- gal_4^-$	—		—	—	0	12000

Transduction	Number of Segregants		Classification by Transduction Assay	Classification by Lysate	Classification by Crossing: Homotype (+)	Homotype total prototrophs	Heterotype (+)	Heterotype total prototrophs
$gal_4^- \times gal_4^- lp^s$	5	(1)	gal_4^-	gal_4^-	0	2786	3	3183
		(2)	"	"	0	2675	2	3471
		(3)	"	"	0	3485	23	5342
		(4)	"	"	0	5952	1	1665
		(5)	"	"	0	5000	1	891
	2.	(1)	gal_2^-	gal_2^-	7	3102	0	1988
		(2)	gal_2^-	gal_2^-	10	4364	0	1187
$gal_2^- \times gal_4^- lp^+$	4	(1)	gal_4^-	gal_4^-	0	16,164	3	1359 ~~650~~
		(2)	"	"	0	5730	1	164
		(3)	"	"	0	3358	0	202 ~~142~~
		(4)	"	"	0	12848	1	171
	3	(1)	gal_2^-	gal_2^-	1	11200	0	827 ~~604~~
		(2)	"	"	6	10608 ~~7720~~	0	718 ~~540~~
		(3)	"	"	3	5000 ~~2552~~	0	409 ~~272~~
wild $\times$ gal_1^-	4	(1)	gal_1^-	gal_1^-	0	426	—	—
		(2)	"	"	0	554	—	—
		(3)	"	"	0	529	—	—
		(4)	"	"	0	391	—	—
wild $\times$ gal_2^- (2251)	#4	(1)	gal_2^-	gal_2^-	0	7805	—	—
		(2)	"	"	0	4992	—	—
		(3)	"	"	0	106	—	—
		(4)	"	"	0	4552	—	—
	2175	(1)	gal_2^-	gal_2^-	0	4071	—	—
		(2)	"	"	0	5384	—	—
		(3)	"	"	0	2072	—	—
		(4)	"	"	0	6988	—	—

(d)

Lysate Assay

Nature of Original Transfection	Homotypic	Heterotypic	Homo-hetero	Total
wild type on gal$_1^-$	21/~~21~~	0	0	21
gal$_1^-$ on gal$_1^-$	39/39	16	—	~~55~~
	60	16		76

Lysate E

Page	No. Homotypes	Heterotypes	Total	wild →
205	4 4	0	4	+
196 2175	4 4pt	0	4	+
192A pan gal	4 4pt	0	4	+
✓192 B pan gal	4	5 gal-902	9	→ 9
✓202 W84	15	3 (902)	18	→ 12
✓198 W518	16 (W84)	3 (902)	19	→ 15
				64
✓212 W911 35	4	0	4	+
✓247A W1210	4	0	4	+
✓248 W750	1	—	1	+
Total 56		11	67	
✓207 L2175 39	4	0 911	4	→
Total 60		11	71	
✓247B W1210	—	1 (84)	1	→
Total 60		12	72	
✓236C W2211	●	1 (911)	1	→
Total 60		13	73	
✓242 W518	—	1 (124)	1	→
✓243 W750	—	2 (3)(1210)	2	→
	60	16	76	
1.207 L2175	—	gal- 2 (750)		

19

4. Occurrence of HFT λ-

Recip. Cell	λ Source: K-12	Gal$_1$- W750	Gal$_2$- W2175	Gal$_3$- W2237	Gal$_4$- W811
Gal$_1$-	0/4 HFT	—	1/5 gal$_2$- HFT 2/4 gal$_1$- HFT 2/24 gal$_1$	~~not~~ done	not done Stable(+)?
Gal$_2$-	0/4 HFT	not done 1/1 unstable (+) HFT	—	~~not~~ done	not done
Gal$_3$-	not done	not done stable(+)	not done stable(+)	—	not done stable(+)?
Gal$_4$ Lp^s	0/4 HFT	not done stable(+)?	0/16 gal$_4$- HFT gal$_2$- not done	~~not~~ done	—
Gal$_4$- Lp^r	not done	not done stable(+)?	1/15 gal$_4$- HFT gal$_2$- not done 1/3	~~not~~ done	—
Gal$_4$- Lp^r	not done	not done stable(+)?	not done	not done	—

Previously - unstable (+) from 518 K-12
750 & 1821
1436 & K-12
(gal$_1$- mixed by gal$_4$-) examined and not found HFT

Explanation

0/4 - no. lysates formed HFT / no. lysates examined

Misc.

Pg	Obs	Archive
62	transduct. not effected by presence of other phage	W1786
80 (?)	~~spread of λ (1612)~~	W1786
~~89~~	~~adsorption of transd. factor~~	~~W811, W1786~~
99	F- gal+	W1574
121	pasteurization of lysate not possible	W811
141	no. cells vs transd. [illegible]	W518
~~177~~	~~HFT fraction 4.2%~~	~~W518~~
178, 168, 169, 170	instability of D1	
~~193~~ (186)	HFT (-) fraction N16	W1485
(203)	" " " N1, N16	W1485, W1673
	HFT not destroyed by DNAase	W518
197 (199)	anti λ sera on HFT	D1 λ
200A	~~U.V. effect on nat. λ~~	2175, 750, 811, 1924, 518
200 (also 220, 221 et seq)	~~non transducible gal~~	—
218	u.v. on HFT λ	811, 1924, 750, 518, 2280, 2281, 2175
219a	u.v. lysate of 4p^R (~~[illegible]~~)	1924
~~223~~	~~HFT fraction of N16 (3%)~~ also correlation with lysogenicity	W518
224	u.v. lysate of 4p^R also a look at 1'/4' } transd. by adsorpt effect 1X	1027
~~225~~	~~adsorpt of λ~~	811
~~226~~	~~" "~~	750
~~240~~	~~transd. of by gal+R~~	750, 2175

(2)

Misc (2)

(65)

pg	Topic	cites
241	~~(1) HFT (-)~~	~~W1765 — clas cross (X) 750F~~
	~~(2) HFT (+) [illegible]~~	
	correlation with [illegible]	W518
	again	
244	~~HFT (-)~~	~~W2252 [illegible]$_1$~~
		~~[illegible]$_2$~~

HFT (+) ductons

Expt	Relation	# of (+)	Numbers (-)	% (+)
177				
	No hyperon	0	936	0
	HFT hyperon	72	1708	4.0
223				
	No hyperon	[illegible]	[illegible]	—
	HFT hyperon	39	1312	3

Expt	Page	(X)	hyp	Descript
V	212	1, 10, 12, 18 x 1436		FH t K12
Q	205	4, 6, 8, 10 x 1436	complete	518 t K12
247A	—	4, 13, 22, 23 x 902	←	1210t K12
248	-	1, 7, 9, 11 x —?	←	750 t K12
249D	-	4, 11, 8, 12 14 x —?	←	1765 gal, - t K12
233		13 x 902F+	3, 7, 13, 16	221 t K12
247C	-	11, 13, 22, 24 x 902	←	1210 t 750 (2 mol-)
236B	-	4, 6, 21 x 902	4 8, 12	221 t 750
O	196	complete	complete	2175 t K12
M	192A	2, 14, 15, 17 x — ?	complete	2342 (750 pr t1212)
N	192B	2 - complete WO 1 - confused	complete	750 pr t 902 (2342)
T	209	all	all	2175 t 750
230	-	230-5 x 902 3 others	5 +3 others	250 t 902
243	-	3, 6, 7 x 902	3, 6, 7	750 t 1210
249B	—	10, 13, 20, 21 x —?	10, 13, 20, 21	1765 gal, - t 1210
249C	—	12 x —?	12	1765 gal, t 902
S	202	4 Gal4 x 1436 complete 14, 15, 17, 17 x 902 5, 9, 16 x 1436, 902 *	4 complete 5, 9, 16	811 t 902
242	—	5, 8, 9, 20 x 1436, 902 *	5, 8, 9, 20	518 t 1210
R	198	4 Gal4 - x 1436 complete 4 " x 902 " 2 Gal2 x 902 complete 2 gal 2 x 1436 "	complete 1, 4, 17	518 t 902
X	213	1, 4, 14, 17, 18 x 902, 1436 *	←	1924 t 902
249A	—	6 x —?	6	1765 gal, - t 811
247B	—	1, 8, 14, 18 x 902, 1436 *	1, 8, 14, 18	1210 t 811
236C	—	6, 10, 14, 23 x 902, 1436?	6, 10, 14, 23	2281 t 811
U	207	1, 3, 7, 13 x —?	1, 3, 7, 13	2175 t 811

(67)

Gal⁻ mutants induced

Σ = 3 1⁻4⁻
1 1⁻
1 2⁻
2 X⁻
2 nontransd

(68)

Confirm /

pg.	of culture	designation	locus (if known)	
206	W1673	-2 (2310)	transd by 2, not by 1,4 = double?	✓ ✓
	p. 206	-4 (2311)	" " " " " " " " "	✓ ✓
		-5 (2312)	~~not " " " 1,2,4~~ non transducible? (⊗ 811 → (+))	
		-6 (2313)	" " 1,4 " " 2 = 2⁻	✓? ✓
		-7 (2314)	" " 1,2,4 ——— = new locus?	✓ ✓
		-8 (2315)	" " 1,2,4 not by 3 = " " ~ 3⁻?	✓ ✓
		-9 (2316)	" " 2,4 not by 1 = 1⁻?	✓
		-10 (2317)	" " 2 not by 1,4 = double?	✓
		-11 (2318)	not transd by 1, 2, 4 = non transducible?	

2312 x 811 → 1+/84

220 W2312 not transd. by 1, 2, 4 (HFT), 892(λ), K12
" " " " 1, 2, 4 (HFT), K12

made λ⁺ lysate
transd. 1, 4, 6 possibly not gal_2^-

W2318 not transd by 1, 2, 4 (HFT) —

221 W2318 x811 0+/206

W2312 lysate of does not transd 2175 but others.

222 W2312 x 902 1+/2112
x 811 Σ = 1+/198

225 W2312 x 1655 to see if 2 types (-) obtainable
only 17 pure (+) obtained

~~[illegible]~~
W2312 X 1655F- (with proline added) → 3(-)/11

Gal⁻ mutants induced
by 176 nm

Box	orig designation	from:		
238	2	W2645	not + by $4^-, 1^-, 2^-$	unn band. ?
	5	W2646	+ by all	new ? ✓
	6	W2647	+ by all but 1	1^- ? ✓
	8	W2648	+ by all	new ? ✓
	10	W2649	" " " "	new ? ✓
	11	W2650	not + by $1^-, 4^-$	$1^- 4^-$?
	12	W2651	not + by 2^-	2^- ✓
	13	W2652	" " " 2^-	2^- ✓

Cross of #11 × 57♂ → 0+/6050

× 750 → 0+/2683

Σ (69)

2	2^-
3	x^-
1	1^-
1	$1^- 4^-$
1	unt band.

Page 245, 246, 246A

(70)

T18 .. 2175t 750 = lac+ gal2- gal1- by transd. assay

① lac+gal2- transd lac1- gal1-

② transduced by mixture of HFT 1- / HFT 2- but not by the ants singly

③ Proposed

2- 1-

2+ 1-

2- 1+

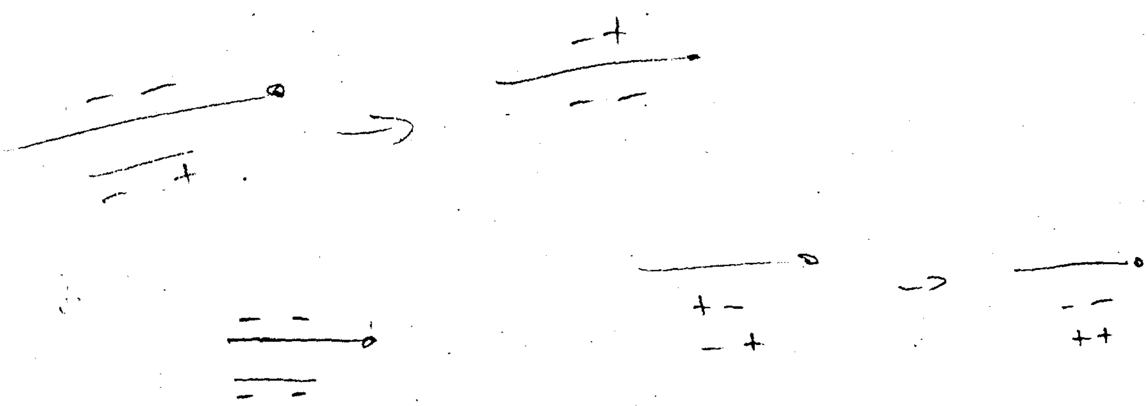
	Segregants	on lac	Assay transducibility	Reversion segregate	Loss
Forward ①	1- 2-	slow	not.	no	—
Forward ②	1- 2+	slow	1-	no	—
Forward ③	1+ 2+	+	2-	no	—
Forward ④	1- 2- / 1+ 2-	+	2-	yes	① 1- 2- / ① 1-
Forward ⑤	1- 2- / 1- 2+	slow	1-	yes	① Forward 1- 2- / ② Forward 2-

③ Update on 2070

7 seg tested

5 1-

2 2-



Dwoble (– –)

237A -10 = 750 t N16

not t by N16, N1 (repeated), possibly by 518

contains Lp_2^s

Lysate apparently did not transduce 2175, 750

Crossed with Φ02 0(+)/1656

209-18 2175 t 750

not t by N1, N16, ~~[illegible]~~ but by 1612
8H

Lysate failed to t 750, 2175, (2297), 2070
possibly 8H, 518 weakly. – these gave all stable

(actual results don't suggest this; see p. 209)

209-19 similarly to 718

236C -W23 50

246
246A

(72)

T78 = 2175t.750

1. The transduction

	Lysate HFT gal_1^-	Lysate HFT gal_2^-	Lysate gal_1^- gal_2^- HFT + HFT	Proposed Genotype
Gal_1 Gal_2 -	0	0	+ (>10)	1 2 - - — — - + — — + -

2. The Segregants. (not possible to distinguish Gal+ from Gal++)
Red. Papillae streaked on Gal and ←, classified by Transduction

	Transduced by Only HFT gal_1^-	Only HFT gal_2^-	Neither	Total
Number	5	10	17	32
Genotype (proposed)	Gal_2^-	Gal_1^-	Gal_1^- Gal_2^-	—
Structurally	1+ 2-	1- 2+	1- 2-	
	OR 1- 2- — — + -	OR 1- 2- — — - +		
Number of Cultures found Giving Gal+ unstable Reversants	0	1	—	
Possible Reversion Structure	—	1+ 2+ — — - + OR 1- 2- — — + +	—	

Correlation of Lp^+ Change with Transduction

Recipient Cells

	Lysate	Transduction:		Total	Segregants		Total
		Lp^s	Lp^+	Lp^R	Lp^s	Lp^+	Lp^R	
Gal_1^-	When Pastes of galactose-negative cultures grown with Lp^+	0	9	0				9
	the various galactose negative cell results similar to those shown							9
	in Table 4 are obtained. With the possible exception of the intersections	1	0					1
	of Gal_1^- and Gal_2^-, each of the pastes is capable of evoking galactose							
Gal_2^-	fermenting papillae upon plated spread with non-homologous negative							
	cells. With the usual lysates plated, only interactions are erratic, some-							
	times 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		(1)					1
Gal_4^-	such interaction does not produce clones that are phenotypically (+).	0						19
	The differentiation by lysate interaction corresponds to the	0						45
	differentiation of these loci by recombinational analysis.							
	Reverse mutation restores the ability of lysates of a galactose							
Lp^R	negative culture to evoke papillae from cells of its own type (table 5).	1	28					29
Lac_4^-	Presumably phenotypic reversions (of both types, reverse mutation	0	4					4
	at the mutated locus, and mutation at a second locus whose action	1	17					18
	mimics the action of the first gene. Reversions of this second class							
	should not be able to evoke papillae from cells of both types. Such reversions							
	as the latter have not as yet been investigated.							

Examination of the other characteristics of the cells transduced to acidity to fermenting galactose by lysate enhance the uniformly shown no changes in any of them with the exception of the induction of lysogenicity in the lambda sensitive forms.

tot	Lp^+
23	20
36	25
22	17
12	11
20	19
24	24
127	116

0.91
127 | 116.0
1143
170

Amended Table on Correlation between Lysog. + Transd.

(74)

Lp^s Gal₂-2373	Lysate (~~Transd~~)	Lysogenized	Transd.	$+^4$	$+^4 \lambda^{Rx}$
249A	Gal₄-	30/1	5S, 17+, (12) 1		1+
249B	(1210)	56/0	3S, 18+, 3R (24) 8		7+, 1
249C	(902)	14/1	3S, 7+, 2R (13) 1		1R
249D	Wild	33/1	3S, 20+, 1		
Gal₂- 22H	wild	46/0	11	9S	7R
229A		2/		5S	16R
229B	Gal₄			4S	17R
229C	Gal₄-				
Gal₄- 518 (1709)	wild	4/0	1	1	18R
146	Gal₂-	115/29			
147, 150	Galₓ-	247/41		4S Lp^+	

Table 9

Lysates of Unstable Galactose (+)

Titer of Transductants

Page	Recipient Cells	Lysate	Gal$_1^-$	Gal$_2^-$	Gal$_4^-$	Plaque Transductants *
284	(1) Gal$_1^-$	wild type	3.8×10^8	1.5×10^8	7.5×10^7	32
284	(4) Gal$_2^-$	Gal$_1^-$	1.5×10^8	5×10^7	1.5×10^6	4
286	(3) Gal$_1^-$	Gal$_2^-$	6.3×10^7	2.5×10^7	2.8×10^5	29
286	(6) Gal$_4^-$	Gal$_2^-$	7.3×10^8	2.5×10^7	2.8×10^5	—
271	(2) Gal$_1^-$	Gal$_2^-$				60

* the highest single assay of transductant activity

Stable (from unit)

Table 10

Lysate

(15)

Recipient cells	Treated	Gal1- Sample	Gal2- Sample	Gal4- Sample
Gal1- Lp^S	33/1 1 — 14		11/1 1 — 11	30/1 1 — 29 (29/30)
Lp^R	46/1 1 — [illegible]		84/1 1 — 4	27/12 12 — 27 (9/27)
Lp^T	143/3 1 — 42 (17/24)		92/1 1 — 0 (23/23)	
Gal2- Lp^S	46/0 0 — 15	214/0 0 — 27		98/0 0 — 4
Lp^R	248/17 17 — 21 (20/22)	83/14 14 — 61		79/14 14 — 52
Lp^T	23/4 4 — 6	65/2 2 — 0		56/5 5 — 0
Gal4- Lp^S	320/19 19 — 383	72/29 29 — 72	472/11 11 — 20	
Lp^R	573/41 41 — 133	96/51 51 — 96		
Lp^T	320/31 31 — 127		231/31 31 — 50	

Gal1- Lp^S

Total	Stable
33	14
11	11
56	20
30	29
130	74

74 St./150

stable transconjugants / sample tubes × transductants / sample tubes

[illegible] × [illegible]

Table 12

Transduction assay

Transd. [illegible]

Segregants

Lg	Recip. cells idiotype	trans lysate allotype		idiotype	allotype	amphitype	total
s	1⁻	[illegible]	±	9	0	0	9
+	1⁻	[illegible]	±	33	0	0 granulares	33
s	2⁻	[illegible]	+	16	0	0	16
+	2⁻	[illegible]	+ × (1) 2175 cult	20	0	0	20
		[illegible]	+ × (2) 1210 cult	15	0	0	15
s	4		+	46	0	0	46
+	4		+	20	0	0	20
s	1		× 3(2) 1210 lysate	6	1	0	7
			× 4(2) 902 lysate	1	0	0	1
s	1		4	1	0	0	1
+	1		2 × 5(4) (902) lysate	36	6	0	42
			× 6 (2) (1210) lysate	18	3	0	21
s	2		1	20	0	0	20
			4	21	1	1	23
+	2		1 × 7(2) 1210 (cult)	19	2	0	21
			× 8 (2) 2175 (cult)	14	3	2	19
+	2		4 × 9 (1) 1210 (cult)	22	1	0	23
			× 10 (2) 2175 (cult)	9	7	0	16
s	4		× 2 11 (1) 1210 lysate	17	2	0	19
			× 12 (2) 902 (lysate)	35	5	1	41
+	4		2 × (13) (902) (lysate)	16	3	0	19
R	4		2 × (14) (902) (lysate)	15	3	0	18
							450

(1), (8), (10) = cult 2175
(2) (7) (9) = cult. 1210

(3) (6) (11) = lysate 1210
(4) (5) (12) (13) (14) = ly 902

Table 13

Segregant Analysis via lysate action on known cultures.

(XX)

Recip. cell	Sp. Qualifier	Transd. lysate	Segregant: Identity	Segregant: Cell type	Segregant: [illegible]	Total
Gal_1^-	+	+	5	0		5
Gal_2^-	+ √(1) 2175	+	4	0		4
	(2) 1210	+	4	0		4
Gal_4^-	+	+	4	0		4
	+	+	4	0		4
Gal_1^-	+	Gal_2^- √(3) 90°	4	5		9
		(4) 1210	0	3		3
Gal_2^-	+	Gal_4^-	0	1		1
	+√(5) 2175	Gal_1^-	0	2		2
	+√(1) 2175	Gal_4^-	4	0		4
	(7) 1210		0	1		1
Gal_4^-	+	Gal_2^- √(8) (W 902)	16	3		19
		(9) 1210	0	1		1
	+	√ (10) 902	15	3		18
						79

Segregants from Table 12 were classified by means of their lysate action on known cultures.

Confirmatory

Segregants were classified by means of their lysate action on known cultures.

Table 14

(78)

Galactose negative cultures giving high frequency of transduction lysates

Culture	Galactose locus	Recip. cells	Transd. lysate	Nature of Gal(+) reversion	NFT segregant	Nature of Gal (+) reversion NFT seg.
230-4	(+) Gal_1^-	Gal_1^-	Gal_2^-	unstable	Gal_1^-	stable
246A-15	(+) Gal_1^-	Gal_1^- Gal_2^-	Gal_1^- (+) Gal_2^-	"	Gal_1^-, Gal_2^-, Gal_1^--Gal_2^-	"
241-14	(+) Gal_2^-	Gal_1^-	Gal_2^-	"	Gal_2^-	stable
241-19	(+) Gal_2^-	Gal_1^-	Gal_2^-	"	Gal_1^--Gal_2^-	none observed
192B-16	(+) Gal_2^-	Gal_1^-	Gal_2^-	"	Gal_1^--Gal_2^-	none observed (2346)
257-2	(+) Gal_2^-	Gal_1^-	Gal_2^-	unstable	Gal_2^-	~~stable~~ stable
257-4	(+) Gal_2^-	Gal_1^-	Gal_2^-	unstable	Gal_2^-	—
153-1	(S) Gal_2^-	Gal_4^-	[illegible] lysate	unstable	Gal_2^-	
153-4	(S) Gal_2^-	Gal_4^-	[illegible] lysate	"	Gal_2^-	stable
202-16	(+)(S) Gal_4^-	Gal_4^-	Gal_2^- Gal^S-	"	Gal^S-	"
202-18	(+) Gal_4^-	Gal_4^-	Gal_2^- Gal^S-	[illegible]	Gal_1^-, Gal^S-, Gal^S-	stable
247B-1	(+) Gal_4^-	Gal_4^- (8)	Gal_4^- Gal^I-	"	Gal^I-	"

See[illegible]

267

270

Basic — Table 16

Gal_1^- Gal_4^- Interaction.

P. 72 (I) The transduction

282 Gal_4^- Lyp^s cells exposed to HFT gal_1^-

	(+) colonies	(−) colonies	Colonies resp. after 2 days
Lysate treated	0	408	2
Control	0	440	0

(II) The purified colonies gave mixed (+), (−) and purified colonies

(A) streaked out. 24 pure (+) colonies picked and streaked out

(B) Of the 24 colonies - 6 were found stable gal(+)

(C) Of the 18 apparently (−) colonies (picked at 24 hours) derived from pure (+) tested against HFT 1$^-$ and HFT 4$^-$

Gal_4^-	Gal_1^-	Gal_1^- Gal_4^-	Parental (−) population	Doubtful reaction
6	5	2	4	+ leave out

all colonies lambda resistant

---

285 I The transduction

Gal_1^- Lyp^s cells treated with HFT gal_4^-

	(+)	(−)	resp. colonies	partially lysed
(A) Control	0	465	6	0
(B) lysate	0	316	2	38

II (A) the purified colonies streaked out - Each gave (+), (−), resp (−)

(B) Colony 1 - 24 gal(+) col streaked out - 11 were stable gal(+)

(−) tested against HFT 1$^-$ and HFT 4$^-$ Are lambda resist.

Gal_4^-	Gal_4^-	Gal_1^- Gal_4^-	parental (−) population
10	2	0	1

(C) Colony 2 - 48 gal(+) purified and streaked out - 23 (?) were stable (+)

(−) tested against HFT 1$^-$ and HFT 4$^-$

Gal_1^-	Gal_4^-	Gal_1^- - Gal_4^-	Parental (−)
4 *	0	0	22

* 1 or 5

Table 16

The gal_1 - gal_4 Interaction

1 The transduction

Recipient cells	Transduced lysate	Numbers of Clones Gal (+)	Gal (−)	Papillating Gal−
gal_4				
Gal_4^-	broth	0	440	0
	HFT Gal_1^-	0	808	2
Gal_1^-	broth	0	465	0
	HFT Gal_4^-	0	316	2

2 Examination of galactose negative segregants from galactose positive clones found in papillating galactose negative colonies

Classification of segregants

Recipient cells	Transduced lysate	Gal_4^-	Gal_-	Gal_1^-	Gal_4^-	Papillating Gal−
Gal_4^-	HFT Gal−	6	5	2		1
Gal_1^-	HFT Gal_4^-	2	0	1	0	

Table 17

~~More markers have not suggested so freely a similar mutation in the~~

Parents from 2A × 22A strains	Prototrophic Recombinants	
	Galactose (+)	Galactose (−)
$Gal_2^{\pm}$ (1)	Gal_4	
Gal_4^-	Gal_2^+ (2) 541**	99

* unstable for galactose, galactose negative segregants

** 25 of 30 examined were unstable for galactose fermentation. Segregants, one from each of the 25, were all Gal_2^-.

(1) control plating showed the ratio 109/57, 115/13

~~E. coli system;~~ the great majority of the prototrophs were Gal- Xyl-: however, a very ~~small~~ good number of the other combinations have been found also.

In one comparison of the Salmonella and E. coli systems, Davis' (1950) filtration experiment was duplicated. A U-tube with a sintered glass sterile filter plate in the cross-limb was filled with broth and the two compartments inoculated with #2A and #22A respectively. From time to time, the liquid was flushed back and forth between the compartments by alternating suction. When the broth was saturated, the cells from each compartment were washed and plated separately on minimal agar. It was repeatedly found that prototrophs appeared from the 22A culture (about 10), but not from the 2A culture. Control experiments in which only one compartment was inoculated verified the integrity of the filter.

This experiment appeared to show that a filtrable agent (FA) was produced by 2A that reacted with 22A to produce prototrophs. However, filtrates prepared directly from 2A were inactive. The paradox was resolved when it was found that the addition of a 22A filtrate, or of a lysate of 2A ~~cultivated~~ from a lysogenic phage secreted by 22A, provoked the formation of FA by 2A. FA, then, is not a normal component of 2A, but is produced under the stimulus of a latent phage. We have not succeeded in extracting significant FA activity from 2A cells heat-killed, dried, or autolysed under conditions which do not destroy FA activity.

Page	Lysate	Assay cells	Remarks	Titer lysate
176 b	D4E750	811, 2050, 750, 2175,	(42/43)(420/16+)(2/2)(18/24) respectively	—
177	842λ	518	4% of cells (+)	—
177 b	lysate sterility			
241	2175E750	811,	solid smear	—

Summ. sheet 214 before – says 518+KK2 E^a not active

(87)

W2070 (= gal₆⁻ ?) derived from W1673 by UV.
tp^s

Page

135a - origin (three gal- obtained, one retained) (#1)

136 - transd. by (+) no add = 7
K12 (25') = 1560

137 - pop. check - 7/7 spontaneous (+) stbl.
7/7 lysate (K12) (+) unstbl.

145 - trsd. test no add 10
750λ 54 — 4/8 (+) unstbl
902λ 1256 — 5/8 (+) unstbl
84λ 175 — 0/8 (+) unstbl.

245 - trsd. by T18, T19λ to recover (- -) of the T.
lysates not active T18: 5/1 → apparent (+) unstbl found.
Segregants tested
5 1⁻
2 2⁻
7

T19 = 7/2
apparently, (+) unstbl segregants found
of 16 examined 15 2⁻
1 1⁻2⁻
16

226 Stocks of 2070λ⁺ made

Stability of transductants by reversion assays.

Exp.	Observation		
134	5 subcultures	7/7 stable	transd/control = 353 2/30
135	8 subcultures	8/8 stable	transductants/Control = 291/25

Correlation of ip change c Transduction

(85)

236A 2281 t K12

Pap	λ^{RX}/2281	Sg	/λ
1.	+	2-	R
2.	+	"	R
3.	+	"	R
4.	+	"	R
5.	+	"	R
6.	+	"	R
7.	+		R
8.	+		
9.	+		
10.			
11.			
12.	R or S	"	S
12			

236 D 2281 t 760

Pap	λ^{RX}/1485	Sg	/λ
19	+	2-	R
1	S or R	2-	S
20			

236 C 2281 t 84

Pap	λ^{RX}/1485	Sg	/λ
22	+	2	R
1	+	4	R
	+	24	R
24			

Crosses of +⁴

(86)

P.	Cross and details		
1-0 at seg.	811+K12 x 1436	73 (+)	14 (−)

↑ at least 20/73 showed mosaic colonies on strk-1, all later shown unstable

	518+K12 x 1436	86 (+)	1840 (−) ✓

↑ 5/20 showed mosaics on strk-1, all " were mixed on strk 2

106	518+K12 x 1436	0 (+)	65 (−) ✓
	811+K12 x 1436	27 (+)	2 (−)

↑ shown unstable (107)

Total

518+K12: 86 (+) 1905 (−)

811+K12: 100 (+) 16 (−)

1924+K12: 27 (+) 1944

112	1924+K12 x 1436	16 (+)	952 (−)
		11 (+)	992
		27 (+)	1944

1924+K12 ↑ shown not to be lysogenic in cross brush.

May be higher medium not good → 27 (+)

Rpt 286(?) 3+/1(−) Ratio with non aerated cult.

1944 ← 26 found non lys on 518 ← was this

15 " " " " " Curated 518 1436?

← 30 pick picked at random found non lys.

279

① 2580+K12 x 1321 ("902 met+ lys", "518 F−") → 1(+)/c. 6000(−) → str lac cult. — +⁴, 6 seg tested, = gal₂⁻
F+ F−

② 2580+K12 x 518 → 541(+)/99(−) → 30 picked: 4 +strs, 25 seg all gal₂⁻
F− F+

Table 17

HFT - historical - Why + " not found HFT ?

(87)

Page	Lysate	Assay Cells	Reaction	Lysate titer	
135	750 E 1821	811	2470 pap/ml	6.5 X 10^9	
date 10/9/52	"	578	9630+/ml	"	$\frac{10^4 + /ml}{10^{10} \lambda /ml} = \frac{1+}{10^6 \lambda}$
142	578 E 892-1	518	no pap. less than control	?	
11/26/52	811 E 892	"	no pap. at all	?	
	518 E 892-1	2050	solid smear	?	lysate found not sterile
	811 E 892-1	"	" "	?	lysate found sterile
143	518 E 892-1	2050	solid smear	?	(1.8 X 10^9 λ/ml) all lysate titers sterile on penassay (none) control 287 (very high) small papillae - spreader contam.?
	518 E 892-2	"	solid "	?	
	811 E 892-1	"	solid "	?	
	811 E 892-2	"	188 pap.	?	
157	518 E 892 → (-) say gal 2-	2050	solid smear	?	
			" "	?	
	518 E 892-1	"		?	
	1436 E 1612-1	"	227/22		
161	beclouded by contamination				
165, a, b	518 E 892-1 (good dilutions)	750, 518, 2050, 2175	solid smear	1.8 X 10^9 (p 166)	
	D1 (578 E 892 → say gal 2-)	518, 750, 2050 (2175)	" " — not found		
168	D1 E 750, D4 E 750	stated as unstable	—	—	
169	D4 E 750	titer > 10^10	—	—	
170 b	D4 E 750 (1-10^3)	518	24/67	↑	

HFT NFT Summary (4+)

	Recipient cells	Lysate source	Test 1⁻	Test 2⁻	Test 4⁻
~~gal2⁻ (NW175) gal4⁻ (Hh~~					
①	Gal_1^- ①	+	+++	++	+++
②	" ②	+	+++	++	+++
③	Gal_4^- (4p⁰)	+	+++	100	+++
④	Gal_1^-	Gal_2^-	+++	+	—
	Others				
⑤	Gal_2^- ①	Gal_4^-	++	200	+++
	②	—○—		—○—	2
⑥	Gal_4^-	Gal_1^-	+++	+++	+++

(?)

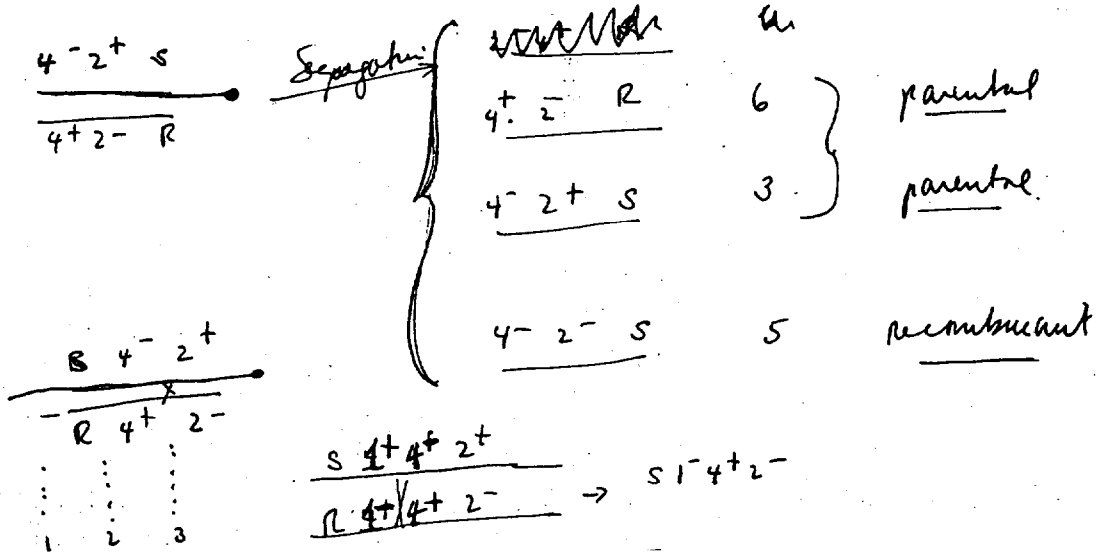
(-) Tetrad

Culture	Recipient cells	lysate	Tetrad Genotype	HFT segregant	HFT unstable segregant	NFT secondary segregant	Presumed structure of HFT
2342	gal_2^-	gal_1^-	$\frac{2^-\,1^+}{2^+\,1^-}$ ○	2^-	12/12	1^-2^-	$\frac{2^-\,1^-}{2^+\,1^+}$
2346	gal_1^-	gal_2^-	$\frac{2^+\,1^-}{2^-\,1^+}$ ○	1^-	4/5	1^- (8/10 unstable)	$\frac{2^+\,1^-}{2^+\,1^-}$
241-14	gal_1^-	gal_2^-	$\frac{2^+\,1^-}{2^-\,1^+}$ ●	2	12/12	2 (12/12 stable)	$\frac{2^-\,1^+}{2^-\,1^?}$
241-19	"	"	"	"	12/12	1^-2^- (-)	$\frac{2^-\,1^-}{2^+\,1^+}$
2464-15	$gal_1\,gal_2^-$	$\{gal_1^-$ + $gal_2^-\}$	$\frac{1^-\,2^-}{1^+\,2^-}$ → $\frac{}{1^-\,2^+}$	2^-	—	1^-2^-	$\frac{2^-\,1^-}{2^-\,1^+}$

Sift N6

P 262

Segregation from $4^- 2^+ Lp^s$ transformed cell



Remaining questions. 4/10/54.

(90)

(1) Relationship of transduction + lysogenization.

× diploids. Lp^+/Lp^+ from $+/s$; s/s. (EML)

Marked λ ; Lp^r.

(2) Hft basis: construct $\frac{s}{s}$ ✓ s. r (R) c UV'd phage. Infect c λ

(3) Position effect. (BW)

(EML) (BW)

(4) Association of fragment + chromosome. × diploids; crossing behavior of various Gal+ transduction types. Size of fragment. Behavior from $\frac{+\ -}{-\ +}$. Crossover + segregation mechanisms.

(5) Other transducible loci; other phages.

(6) Cytology of λ, Lp^+ Hft.

(7) Lytic λ! (Especially when grown on $\frac{s}{s}$ types! How?)

(8) How many Gal⁻ types; mapping (sep. 12)

Morse 4/10/54

Table 8: Study absorption with multiplicity < 1. Heated cells? Hft. of Lp_2^s / Lp_2^r.

Table 9: Any Lp_1^s Clarify headings. Discuss Gal_2^- x Gal_4^- behavior.

Table 11 Explain Obs columns

12 Total: homo/heterotype + test homogeneity.

Hft: inductive behavior! (basis now studied)

Table 18. Again verify Gal⁻ types. p.12 ¶2: meaning?

Double⁻. Papillae i mixed phage $\approx c^2$?

Fig 2. Or UV improves survival. Effect of excess UV'd incomp. H

Variance in output of Hft.

(92)

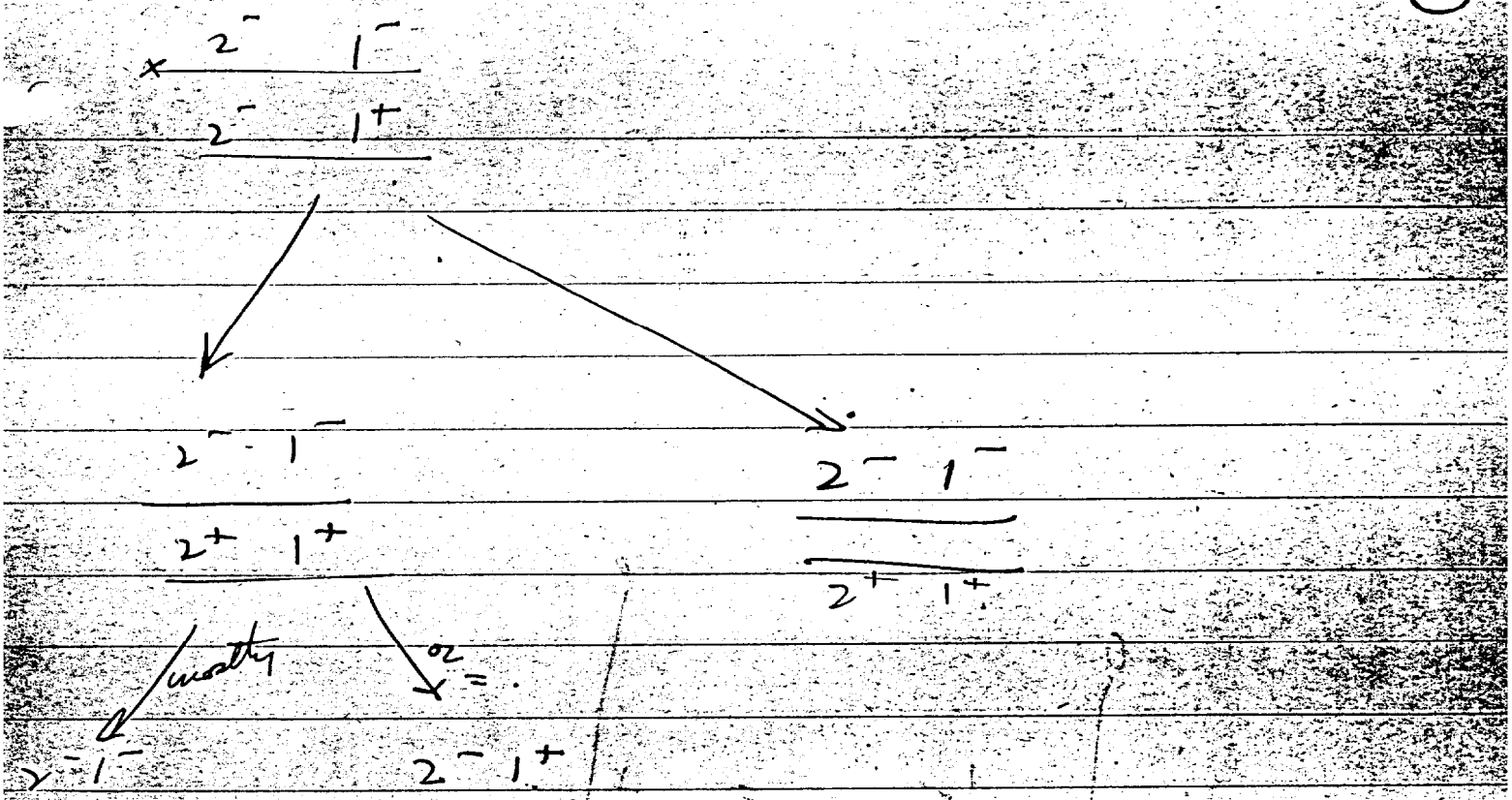
{ 24 → × 1⁻ illoty pu / allotypu
{ 12⁻ → × 4⁻ should fail

12. Behavior of ⁻⁻ → × ⁻. Detail? 14⁻ → × 2 should be OK

13. ? Are exchange + segregation independent? Many "segregations" may be autonucleic.

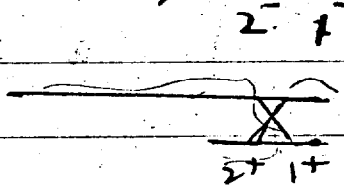
What's up c tal₃⁻?

14. statistical adequacy.

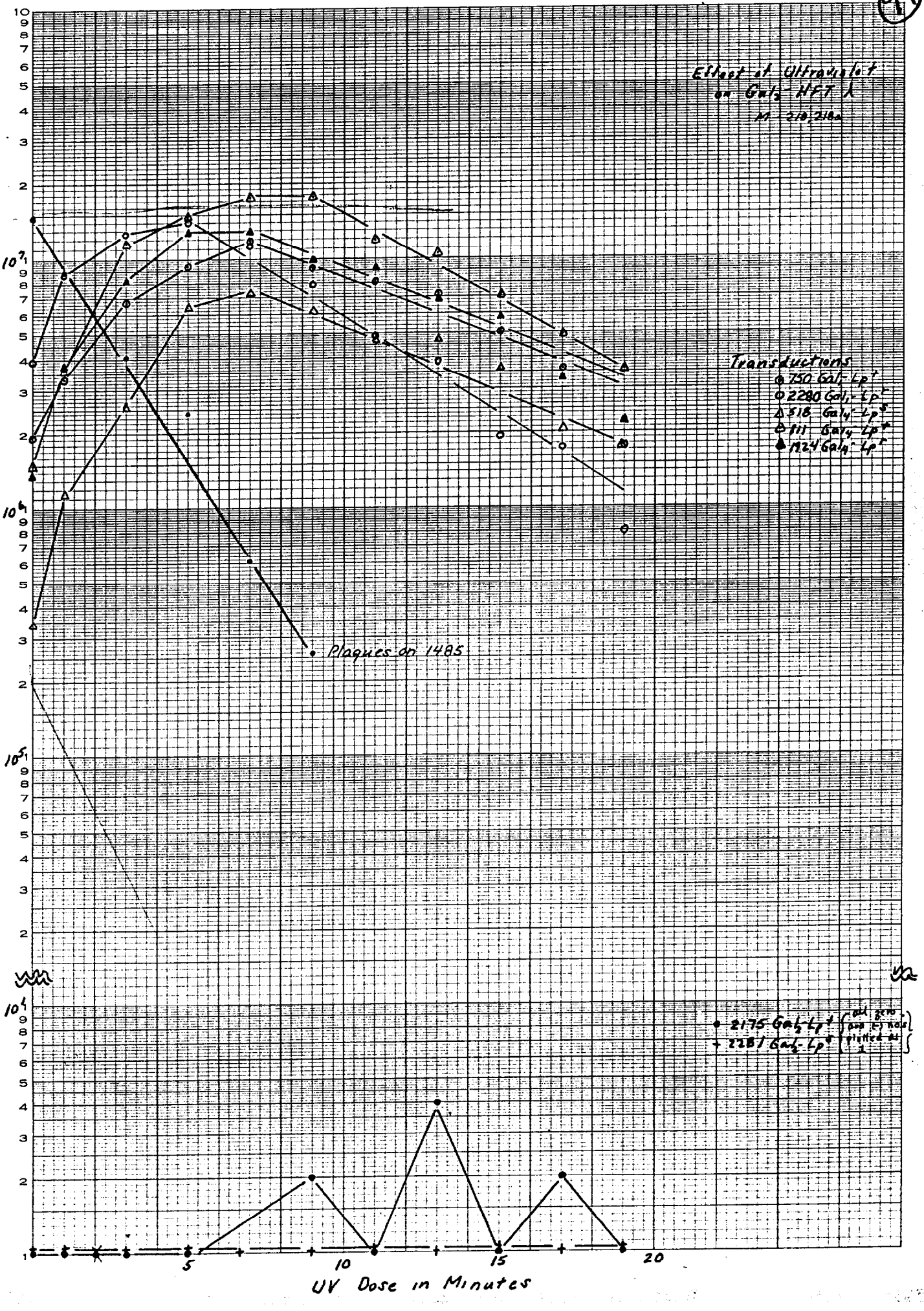


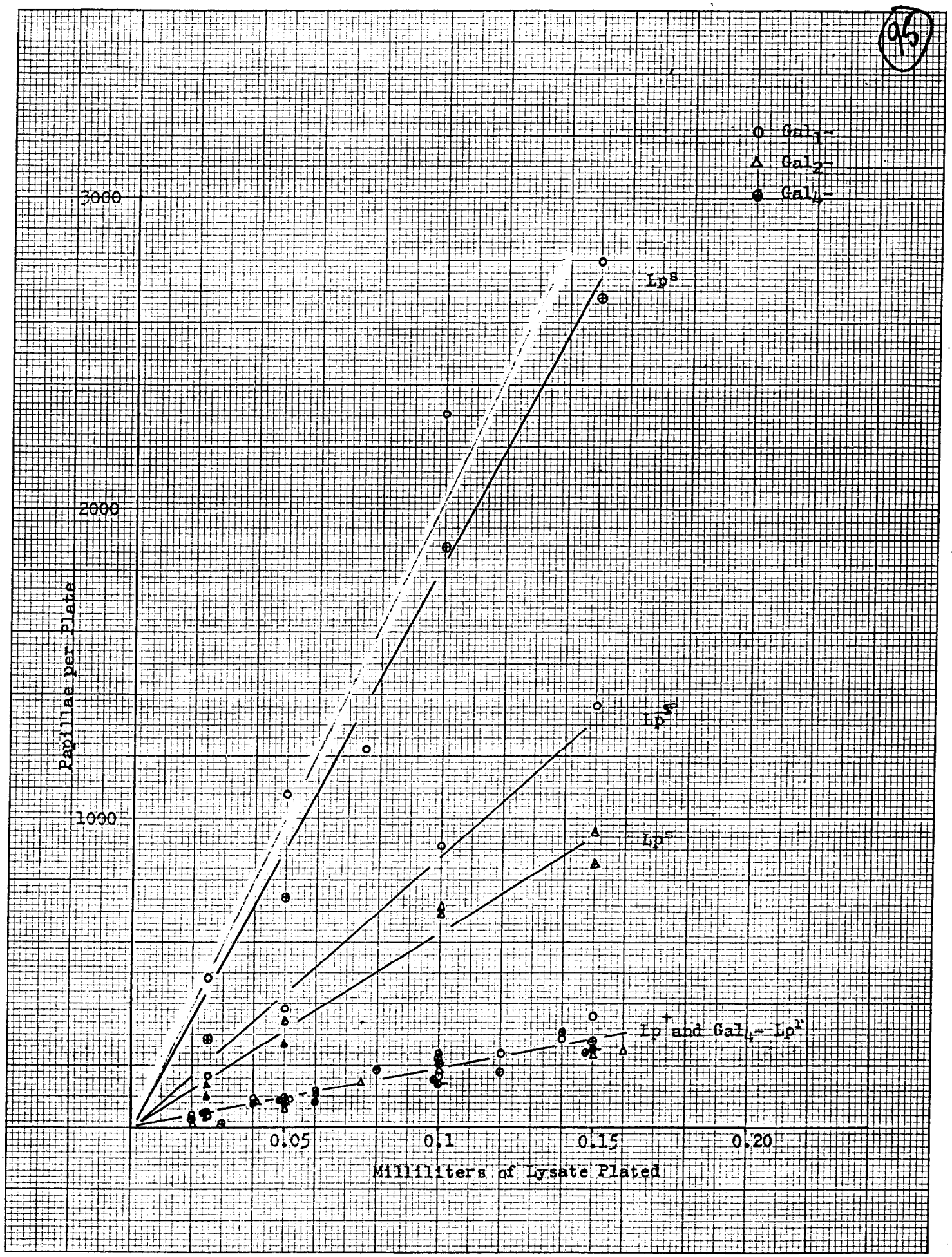
are the 2⁻ segments now ~~pos~~ hemizygous?

Suppose fragment is terminal.



Only 1 crossover type feasible! I.E. $1^- 2^+$ recombinant would be a fragment. Why no recessions of type $\frac{2^+\ 1^-}{2^-\ 1^+}$? These should give mostly the $2^+ 1^-$ type. Were enough tested?





EML - Report 12/1/53

(16)

Action of K rays on transducing and plaque ability in hydatis

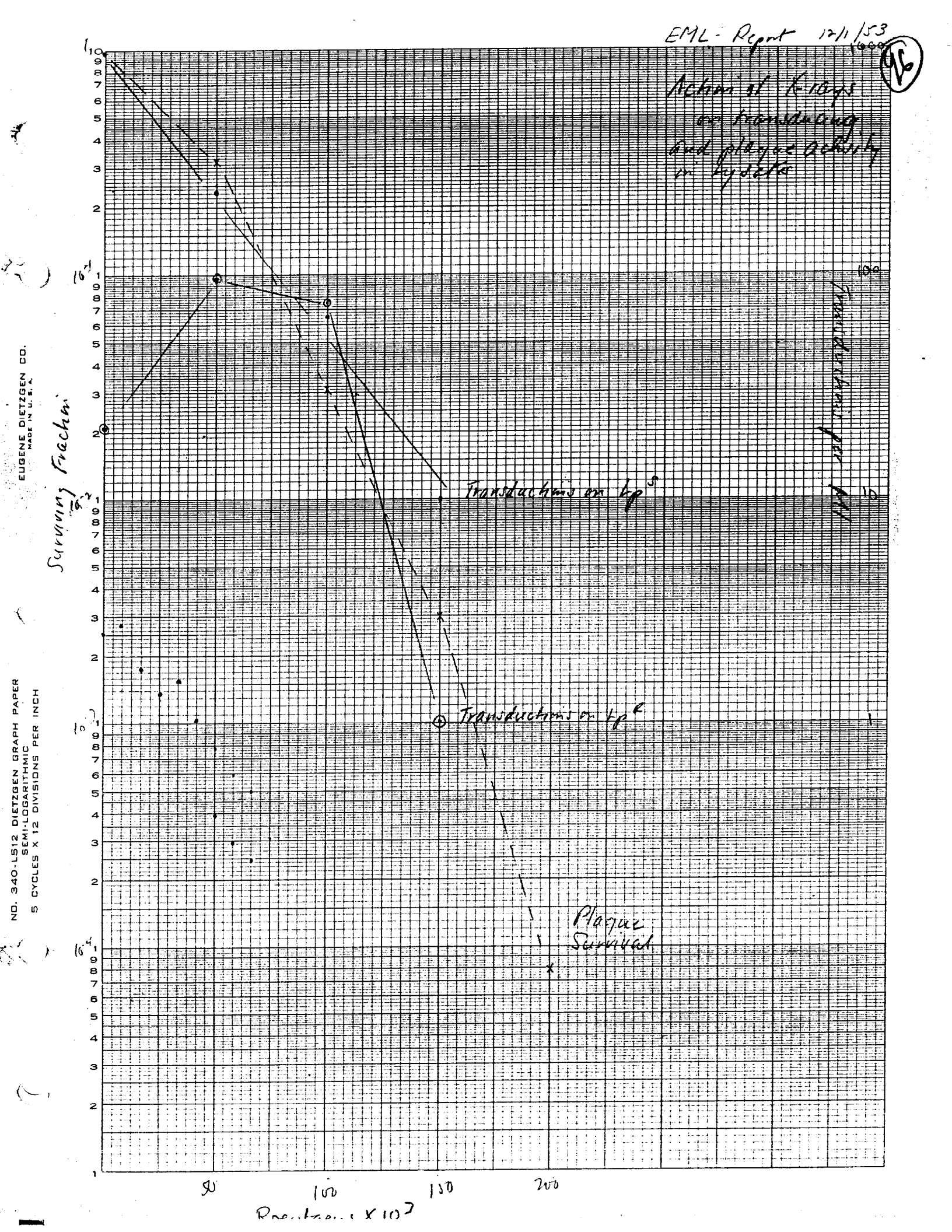


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Table 1

Principal cultures

Wisconsin Stock Number	Genotype
W518	$F^+ M^- Lac_1^- Gal_1^- Lp^S$
W750	$F^+ M^- Lac_1^- Gal_1^- Lp^+$
W811	$F^+ M^- Lac_1^- Gal_1^- Lp^+$
W902	$F^- T^- L^- B_1^- Mal_1^- Gal_2^- Lp^+$
W1210	$F^+ M^- Lac^- Gal_2^- Lp^+$
W1436	$F^+ T^- L^- B_1^- Lac_1^- Gal_1^- Lp^S S^R$
W1924	$F^+ M^- Lac_1^- Gal_1^- Lp^R$
W2175	$F^+ Gal_2^- Lp^+$
W2279	$F^+ M^- Lac_1^- Gal_1^- Lp^S$
W2281	$F^+ M^- Gal_2^- Lp^S$

Genotypic symbols refer to the following characters:

F, Compatibility status, F;
M, T, L, B, nutritional requirements for methionine,

Compatibility status, F;
Nutritional requirements; M, methionine; T, threonine; L, Leucine; B_1, thiamin;
Fermentation Reaction; Lac-, lactose negative; Gal-, galactose negative; Mal-, maltose negative;
Phage status; Lp^S, lambda sensitive; Lp^+, lambda lysogenic; Lp^R, lambda resistant, but not overtly lysogenic
Drug Resistance, S^R, streptomycin resistant.

Table 3 (Expansion)

Failures to transduce

(98)

Marker	Recip Culture	Donor	Pg	
lac	W112 (Lp_2^R)	K12	71	
	" (Lp_2^R)	"	85	
	" (Lp_2^R)	"	94	}
	" (Lp_2^s)	"	94	}
Semi or flag	W1678	"	76	
Leuc.	W1736	"	75	
	W1736	"	78	
	W 1476	(W928) W2046, (W931) W1954	113	
Methionine	58-161	K12 (UV irradiated)	82	
	W811	K12 (UV mod.) (lytic λ)	83 (280)	(M added also to get B^+)
	W1821	K12	85	
	W518	HFT 892(mix)	180	(on B gal, replica to D(O))
Xylose	W1821	K12 (UV mod.)	83	
	W1821	K12	85	
		W811	85	
	W1821	K12	130	
Streptomycin	W518	W1821	95	
Proline	W2062	K12	104 (?)	
	W2062	K12	105	
	W2062	K12	106	
	W1692	K12	96	
	W1920	K12	96	
	W2062	(prototroph) HFT 1 (2). (prototroph) HFT 2. (M-) HFT 4. (prototroph) Heterogenote.	220	
	W2062	lytic λ (from M-)	227	

Table 3 (cont)

Marker	Recip Cult	Donor	Page
Mal$_X$ −	W2071	K12(?)	119
Mal$_1$ −	W2347, W2331	HFT2	295, 275
Ara −	W2307	HFT2	298
F+	1321	HFT2	294

Frequency of Unstable Transductants - Hysals

(100)

Neig Ces		(+)	1⁻	2⁻	4⁻
1⁻	Lp^s	9/22 ~~(41%)~~	—	0/11 0	0/29 (0)
	$Lp^+(1)$	23/24 (96)	-	23/24 (96)	0/27 (0)
	$Lp^{(+)(2)}$	17/24 (71)	—	24/24 (100)	—
2⁻	Lp^s (2281)	~~20/40~~ (58)	~~63/72~~ (88)	—	~~64/72~~ (89)
	$Lp^+(1)(2.75)$	22/24 (92)	19/24 (79)	—	16/24 (67)
	$Lp^+(2)(12.10)$	16/24 (67)	21/24 (88)	—	22/24 (92)
4⁻	Gs	13/24 (54)	~~0~~/72 (0)	21/24 (88)	—
	Lp^+	20/24 (83)	~~0~~/96 (0)	19/24 (79)	—
	Lp^R	29/48 (60)		~~16/24~~ (67)	—

Total
Total
484 / 609 total

Gal_6 Lp^s 6/8

Gal_8 : 1210, 2281 above

Lp^s (2273)

$S = 56/102 + (47/60) = 59\%$

$+ = 98/120 = 81\%$

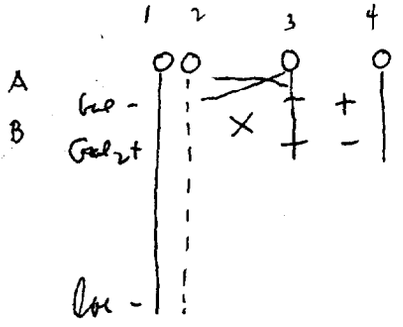
$R = 29/48 = 60\%$

$$609 \overline{)484.0} \quad 0.7$$

Cavalli - Expt

(101)

Shared segregation



	1,3	1,4	2,3	2,4
Crossover				
A	- -	- +	+ -	+ +
	+ +	+ -	- +	- -
	- -	-	-	
				lethal?
B	- +	- +	— +	- +
	+ +	+ -	— +	- -
	- -	-	-	
				lethal?

A 1,3 is only homozygous (-) possible in single crossover. Identifying gal- must be gal_2^- in this model to detect defect for loc by reversion test

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

To obtain allotypic 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 − −

Nuclés

①

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

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⁺ → W2657

↓ ?

W2656 Hfr Gal₄⁻ S^R lac⁺ Lp⁺ M⁻

↓ ? sel Bloc

W2662 Hfr Gal₄⁻ lac⁺ Lp^S

W1503 T2B₁ Het Lp^S lac⁺ V₁^R

HFT 1⁻ → W2430 Het Gal₁⁻ Lp^S

↓ mut.

W2593 Het Gal₁⁻ Lp^S F⁻

↓ λ₂

W2606 Het Gal₂⁻ F⁻ Lp⁺ Mal⁻ Lp₂^R

λ₂ → W2607 Malt 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 Malt seq sel. λ^R

Idiotype S = 1⁻

Case	① 2 3 4	Crossover ①	Strands	1, 3	2, 3	2, 4
①	+ + − −			+ −	+ −	+ −
②	S S R R			S S	R S	R R
	− − + +			− −	+ −	+ +
			Phenotype	(−)	(+)	(+)
			Segregant	−	both probably mistaken for stable (+) because of selective action of gal.	
			Comment	not from (+)	—	
		Crossover ②		+ −	+ −	+ −
				S R	S R	S R
				− −	+ −	+ +
			Phenotype	(−)	(+)	(+)
			Segregant	—	As above	
			Comment	not from (+)	—	—

C.o outside the region

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

Two disjunctions	strands	1, 3, 4	2, 3, 4
	Crossover ①	+ − −	+ − −
		S S R	R S R
		− − +	+ − +
	Phenotype	≠ (+) ?	(+)
	Segregant	—	probably mistaken for stable (+)

Given

(−) Closed Region ① — ϕ Seg

	① ②	③ ④	
Lig	S S	R R	idio R
	4⁻ 4⁻	4⁺ 4⁺	
	2⁺ 2⁺	2⁻ 2⁻	

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

allo R

Region ②

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

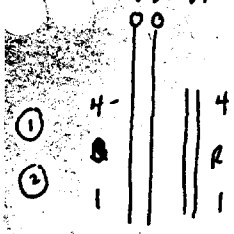
allo R

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	dis S	ampli 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	dis S	ampli 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	dis S	ampli R

Crossover ②

	− + +	− + +
	S R R	S R R
	+ + −	− + −
Segregants	dis S	ampli S

General Comment.

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

4⁻	4⁻	4+	4+
S	S	R	R
2+	2+	2⁻	2⁻

①

	1, 3	1, 4	2, 3	2, 4
	+ −	− +	− +	− +
	S R	S R	R S	R R
	+ −	+ −	− +	− −
		①	②	
ϕ	(−)(4⁻R)	(+)	(+)	(−)(2⁻R)
sa	idis S	idis S	amphi R	amphi R

②

	− +	− +	− +	− +
	S R	S R	S R	S R
	+ +	+ −	− +	− −
	③	④	⑤	
ϕ	(+)	(+)	(+)	(−) R⁻R
	idis S	idis S	amphi S	amphi S

tris anomer

tris di:

−	+	+
S	R	R
+	−	−

tris di

①

	1, 3, 4	2, 3, 4
	− + +	− + +
	S S R	R S R
	+ + −	− + −
ϕ	(+)	(+)
sa	idis S	amphi R

Extremis

②

	− + +	− + +
	S R R	S R R
	+ + −	− + −
ϕ	(+)	(+)
sa	idis S	amphi S

1 2 3 4

(1) S S R R
4⁻ 4⁻ 4⁺ 4⁺

(2) 1⁺ 1⁺ 1⁻ 1⁻

(1) — ∅ sey

		S	R		
SR 1⁻1⁻ 4⁺4⁺	1, 3	4⁻	4⁻	(−)	4⁻ S
		1⁺	1⁺		
SR 1⁻4⁺ 4⁺4⁻	1, 4	4⁻	4⁺	(±) parental	4⁻ S
		1⁺	1⁻		
SR 1⁺1⁻ 4⁻4⁺	2, 3	4⁺	4⁻	(±) parental	1 S
		1⁻	1⁺		
SR 1⁻1⁻ 4⁺4⁺	2, 4	4⁺	4⁺	(−)	1⁻ S
		1⁻	1⁻		

(2)

		S	R		
SR 1⁻1⁺ 4⁺4⁺	1, 3	4⁻	4⁺	(−)	4⁻ S
		1⁺	1⁺		
S,R, 1⁻4⁺ 4⁺1⁻	1, 4	4⁻	4⁺	(−) parental	4⁻ S
		1⁺	1⁻		
S R 1⁻1⁺ 4⁻4⁺	2, 3	4[illegible]	4⁺	(+) [illegible]	[illegible]
		1⁻	1⁺		
	2, 4	4⁺	4⁺	(−)	1⁻ S
		1⁻	1⁻		

1 3

S S R R
− − + +
(1) X
+ + − −
↓ (2)

1, 3
S R
− +
+ +

Two (+) by single cross over

Triple interactions

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

	1,2	3,4	1,3	2,4	1,4	2,3
①	+ +	− −	+ −	− +	− −	+ +
	+ +	− −	− +	+ −	+ +	− −
	− −	+ +	− +	+ −	− −	+ +
ϕ	−		(+)		(+)	
Allele	2		−		−	
Seq	2 −		2 −		(−)	

Two disjunctions

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

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

③	+ − −		+ − −	
	+ − −		+ − −	
	− − +		+ − +	
ϕ	(−)		(+)	(+)
Allele	2 −		−	
Seq	2 −		2 −	

apparently stable ⊕

(110)

GENETIC TRANSDUCTION IN *ESCHERICHIA COLI*

By

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(iii)

Page TABLE OF CONTENTS

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 the pneumococcus [redacted] have been observed in Hemophilus influenzae

(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 Atchley, 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) [redacted] 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, or 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 [redacted] on the cells, as [redacted] for [redacted] 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 X 10^7 and 5 X 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 tpr 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.

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(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 for this purpose is available.

[redacted] is, a culture classified by the first method was Gal_4- was also classified as this [redacted] 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 [redacted] 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 [redacted] 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

negative alleles

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

(double ~~[illegible]~~ mutant)

Although the amphitypic cultures are not transformed to wild type by the action of a ~~[illegible]~~ single pure lysate, galactose positive clones are obtained by the action of a mixture from the two single mutants genotyped ~~[illegible]~~ the ~~[illegible]~~. 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 ~~[illegible]~~ 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 ~~[illegible]~~ 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 ~~[illegible]~~ plague titer was ~~[illegible]~~ REMARKABLY high, ~~[illegible]~~. In fact, these cultures were not the ~~[illegible]~~ that were discovered first ~~found~~ to give HFT lysates. In the course of examining nonfermenting segregants from transductions by means of lysates of them, several exceptional ~~[illegible]~~ lysates were encountered, ~~[illegible]~~ which gave a high frequency

(15)
(125)

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].

[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

of the transduction clone. The galactose positive reversions of these segregants are stable.

A characteristic HFT culture has been obtained for each galactose negative as well as for wild type. These cultures were isolated initially by making lysates of random segregants from heterogenic transductions and assaying the lysates on the appropriate cells. This method is laborious and inefficient. To assist in the isolation a more rapid method was devised. Random segregant colonies were picked to small volumes of water or broth and samples of each suspension were then spotted on an EMB galactose plate spread with cells suitable for the detection of the HFT culture desired. The plate was given a small dose of UV (about 10-20 seconds at 50 cm from a Sterilamp) and incubated for 24 hours. At the end of this time HFT cultures were usually detected by the raised welt of galactose positive growth where lambda produced by the induction and lysis of the HFT culture had transformed bacteria of the background film of growth.

The incidence of HFT galactose negative cultures is not high. Of 67 segregants tested, 7 were found to be capable of HFT lysates. The true frequency might be higher than this, since purified segregants were examined and there was opportunity to pick HFT segregants from originally HFT clones.

Cultures giving HFT lysates that are pure for a particular galactose negative allele are suitable for allelism tests of unknown galactose negative cultures by the cross brush method.

Experiments with lysates giving a high frequency of transduction

Although the HFT lysates have not yet been obtained with phage titers comparable to LFT lysates the titers have been sufficient for transforming a large fraction of a cell population exposed to them. The largest fraction of transformation observed thus far has been 12.5 percent of exposed cells, but in most experiments the fraction has been between 1 and 5 percent.

The use of HFT lysates has permitted the study of several problems not attackable with LFT lysates. One of these is the relationship of transduction to lysogenization with the phage lambda. Another problem is that of the interaction of Gal_1 and Gal_4. Both of these problems will be dealt with in the next sections. With LFT lysates, transduction was experimentally feasible with X only when a galactose phenotype is generated that can be selected from a galactose negative background. HFT lysates, permit the detection of galactose negative segregants from transductions clones derived from galactose positive recipient cells. Transductions in this sense have facilitated further studies of the interaction of the galactose loci with the Lp locus.

The relationship of lysogenization to transduction

By exposing cultures of Lp^s cells to HFT lysates, diluting, and then plating on galactose medium to obtain isolated colonies it is possible to study the behavior of individual cells with regard to ~~their~~ transduction and lysogenization activities. Table 1~~4~~ shows the results of an experiment in which 1.1 percent of a cell population was transformed after exposure to a HFT lysate. The second portion of table 1~~4~~ gives the phage reactions of the galactose positive (transductions) and galactose negative colonies derived from cells exposed to the HFT lysate. All of the transductions were lysogenized or converted to the Lp^r state while the non-transformed colonies were either phage sensitive or contaminated with phage.

These results suggest that lambda carries the transducing activity. However, under the experimental conditions employed it could be argued that the transductions are the results of the action of two entities. The first, which would act upon the cells and makes them "potential" transductions, and the second, lambda, which in the process of lysogenizing the cells, would sometimes convert them to actual transductions. In order for so many phage contacts to result in transductions (1/3 of 3%) to be present in about the order of ten-fold over excess of lambda. ~~It might be argued that because (in this the experiment recorded in table 1~~4~~) only about one-third of the lambda-cell contacts became transductions that the ratio of the "potentiating" agent to lambda was not high. This would not necessarily be so, since this ratio (transductions/ total lambda contacts) could merely be an indication of the efficiency of lambda's conversion of potentiality to actuality.~~ The observed proportionality of number of transductions to amount of HFT lysate at high dilutions (10^{-5}-10^{-6}) would imply such an overwhelming excess of any accessory factor in addition to lambda as to make this hypothesis untenable.

At these ~~dilut~~ ~~dilt~~ dilutions the probability that a single cell would encounter both activities would be (approximately the square of the dilution (10^{10}-10^{12}). Lambda most certainly must be the vector of transductio-

The interaction of Gal_1 and Gal_4 (Positive effect).

With the use of HFT lysates it has been possible to study the interaction of Gal_1-and Gal_4- cells with HFT Gal_4- and Gal_1- lysates respectively. The results from one set of interactions is shown in table 16. After a preliminary period for the adsorption of the transducing activities the cultures were centrifuged, the supernatant lysate discarded and the cells resuspended in broth. The cells were then diluted and plated on EMB ~~gluc~~ galactose medium. No galactose positive colonies were observed on the ~~control unexposed plates~~ plates made from control unexposed cells or from lysate treated cells. After 24 hours incubation at 37C two raised, slightly orange-pink colonies were observed in each experiment on the plates from cells exposed to lysate. These colonies were slightly larger than the other ~~galactose negative colonies~~ and after 24 hours develope

a roughened papillate surface. On streaking out they gave rise to positive colonies, negative colonies that remained negative, and to papillating galactose negative colonies.

In each experiment a number of galactose positive colonies derived from the papillating negative colonies were picked and streaked out twice for purification purposes. From the second streaking galactose negative segregants were obtained and classified with regard to negative allele. In each experiment idiotypic and allotypic segregants were observed and in one experiment amphitypic segregants were found. The amphitypic (Gal_1- Gal_4-) segregants were transformed to a wild type phenotype by lysates of wild type cells, and a lysate of the amphitype formed galactose positive transduction clones when applied to Gal_2- recipient cells.

The failure to realize a wild type phenotype when the positive alleles are in a trans-position, and its realization in the cis-position constitutes a positional effect for these loci.

The action of HFT lysates on lambda-2 resistant cultures

In the previous discussion (table 7) HFT lysates were stated not to transduce lambda-2 resistant recipient cells. HFT lysates, on the other hand, do transduce lambda-2 resistant cells, but at a low frequency (one per 16^6 transducing particles). This is presumably caused by the owing to the potency of HFT lysates, which helps to uncover any residual interaction of transducing phage and lambda-2 resistant bacteria, regardless of which element had varied. Such variation might then be either phenotypic (expressivity) or genotypic (mutation) either in virus or bacterium.

The interaction of HFT lysates with lambda-2 resistant cells is illustrated by the following observations. Some mutations to lambda-2 resistance are accompanied by a coincident change to inability to ferment maltose (E. Lederberg, unpublished). Reversions to ability to ferment maltose is accompanied by reversion to lambda-2 sensitivity and vice versa. These two phenotypic effects have never been separated in crosses and it is presumed that they are the result of a single mutation.

The transductions of a galactose negative, maltose negative lambda-2 resistant, lambda sensitive culture obtained by the action of an HFT lysate are of two types. Maltose positive and lambda-2 sensitive, and maltose negative and lambda-2 resistant. The first of these types represents the detection by the HFT lysate of reverse mutation of the locus in the recipient cells controlling lambda-2 resistance.

The second type of transduction in about 95 percent of the cases is stable for galactose fermentation. Study of the transformability of galactose negative segregants from the unstable transductions clones found showed them not to be susceptible

to a higher frequency of transduction than the parental maltose negative lambda-2 resistant culture. In these cases, at least, there has not been a mutational change in the recipient cell to a greater aptitude for transformation.

About 95 percent of the maltose negative lambda-2 resistant transductions have been found Lp^s, the remainder Lp^r. The Lp^r forms may be stable or segregating for galactose, but all segregating clones are Lp^r. Segregation for galactose fermentation is usually accompanied by segregation at Lp. Presumably in these cases there has been variation in the transducing particles, although it is possible that in the transductions ending in Lp^s clones that an agent distinct from lambda is opergting.

Crossing behavior of the transduction clones

In previous sections it was noted that transduction clones gave HFT lysates after UV induction. Since spontaneously produced phage is similar tp phage produced by the induction technic it might be expected that in crosses between transduction clones and galactose negative cultures, or between HFT galactose negative cultures and non-allelic galactose

There are several observations which suggest that transduction does not play an important part in such crosses. The first observation, which minimises the possibility, is that in a cross between an HFT allotypic segregant and an idiotypic tester, 11,200 prototrophs were examined before a galactose positive recombinant was encountered. A second observation is from the comparison of a cross of a lysogenic unstable transduction (capable of giving HFT lysates) with a Lp^s galactose negative culture, and a cross of an Lp^r transduction (incapable of giving HFT lysates) with the same Lp^s galactose negative culture. A comparison of these crosses showed no significant increase in the number of galactose positive colonies in the cross between the lysogenic transduction and the sensitive culture. Apparently transduction does not confuse the results of crosses.

The transmission of galactose heterogenicity in crosses is greatly influenced by the F polarity of the cross (table 1). When an unstable heterogenic F^+ culture is crossed with a non-allelic galactose negative Lp^s F^- culture, unstable galactose positive prototrophs are rare. When the unstable culture is F^-, and crossed with a non-allelic galactose negative Lp^s F^+ culture, most of the prototrophs are galactose positive and unstable. Some of the galactose negative prototrophs in these crosses can be explained by galactose negative segregants in the unstable galactose positive parent clone.

Galactose negative cultures that are not transformed by lysates.

Seventeen galactose negative mutants have been induced by means of ultraviolet light in auxotrpphic stocks suitable for crossing with the Gal_1-, Gal_2- and Gal_4- stocks used above. Of these 17, 14 were found to be transformed by lysates to wild type. Five of the 14 were recurrences of Gal_1- OR Gal_2-, four were apparently double mutations at Gal_1 and Gal_4, and the remaining five were new loci transduced by lysates. One of the three cultures not transformed by any lysate was examined further. Lysates of it (W23.12) transduced Gal_1^+, Gal_4^+, and Gal_6^+, but APPARENTLY NOT Gal_2. In recombination tests this culture has given galactose positive recombinants with both Gal_2- and Gal_4-. For explanation of these results it is necessary to postulate a double change, one OUTSIDE SEGMENT THAT CAN BE TRANSDUCED the region of transduced loci, and one within the region, non-allelic to any of the known loci, which in addition gives an interaction with Gal_2- such that the heterozygous combination is not phenotypically galactose positive. AN unresolved technical difficulty seems a more likely explanation.

The study of the galactose negative cultures not transformed by lysates has been partly hindered by difficulty in discerning which cultures were truly "negative" and which cultures were merely "slow positive", so that galactose positive transductions are not readily selected.

DISCUSSION

The results presented above can be placed in an orderly fashion by the following scheme. When lysogenic cells are exposed to ultraviolet radiation and the prophage is induced to form mature phage, on rare occasions a fragment of the bacterial chromosome is included within a phage particle. When this particle injects its genetic material into another bacterial cell, the fragment is also injected and if the recipient bacterial cell has the proper genetic constitution the presence of this extra genic material is made obvious. ~~The fragment remains within the bacterial cells that survive, and its multiplication and distribution among the daughter cells closely but not completely parallels the multiplication and distribution of the other genetic material in the cell.~~

The allotypic fragment usually persists at cell division, so that segregating clones can be maintained indefinitely in mass culture. At least two additional events are inferred: (1) diploid crossing over leading to reorganized digenotes. Since these may be heterogenic or homogenic, a four strand (or more) stage is implied. (2) segregation occurs leading to stable haplogenotes, the state typical of E. coli. The fate of the fragment is unknown. Crossover haplogenotes (amphitypes) have also been isolated and may represent either a third process, or the first two in sequence (cf Pontecorvo, 1954). Since heterogenotes give HFT lysates, the fragment or a replica of it, is assumed to have a high probability of incorporation in the phage obtained by UV induction. The low yields suggest a burst of one phage particle, a reversal of transduction.

From this description it is evident that the genetic transfer is intimately associated with the process of lysogenization and lysogenicity. Concerning the process of lysogenization in K-12 little is known beyond the fact that cell and phage interact, there is a period of indecision, and the

infected bacterium either dies or generates a clone containing lysogenized cells. Once lysogenicity is established the capacity to produce phage behaves as a nulcear gene that is closely linked with a number of loci controlling galactose fermentation.

The first step in the scheme is the inclusion of a fragment within a phage particle. In Salmonella the fragment is a random section of the cell's genetic material, but in E. coli K-12, it is quite specific, for only a restricted group of loci are transduced by lambda. Again in contrast to Salmonella, "lytic" lambda is incompetent in transduction. This may reflect an inherent difference between lytic and UV induced phage.

In the establishment of lysogenicity the genetic material of lambda enters the cell and associates itself in some way with a specific region of the bacterial genome. In the induction process it is presumably emerges from its place and starts to multiply. Transduction could be accounted for by some latitude in the separation of the galactose loci from the prophage linked to them, and their common inclusion in some mature phage particles. The close genetic proximity of the galactose loci would suggest their increased liklihood of inclusion, but there is no necessity that closely linked genes be also spatially close to one another.

There are two types (HFT and NFT) of culture in which transducing particles are formed and it is legitimate to ask whether the two are different phenomena or merely quantitaively different aspects of a single phenomenon. The evidence for a unitary process is negative in nature. That is, no difference have been noted between HFT lysates and NFT lysates, except possibly the higher incidence of transductions that culminate in a Lp^r phenotype with the former. This exception, if it be one, could itself be explained on the basis of quantitative differences between the two lysates.

The study of the production of transducing particles in cultures giving HFT lysates has not passed beyond the preliminary stage. The evidence thus far suggests that most of the cells yield transducing particles and that the yield per cell is not large. With regard to the determination of the frequency of cells emitting transducing activity it should be noted that cultures started from a single colony with HFT property may contain as much as 30 percent of cells with HFT property by virtue of segregation.

The specific activity of the HFT lysates of segregating heterozygous galactose positive clones indicates that the fragment is preferentially included within the phage particles. Presumably exchange between fragment and intact chromosome can also occur so that instead of giving lysates predominately allotypic in character, idiotypic lysates are obtained. The exchange is sufficinetly rare, however, that observation remains objective in nature.

The nature of the association of the fragment with the infective phage particle is not known. Presumably the material is within the phage membrane since it is not attacked by desoxyribonuclease. The availability of lysates in which most of the phage particles have activity (HFT lysates) or have no activity (NFT lysates) suggests that morphological comparisons might possibly be made via electron micrsocopy of intact or disrupted phage particles.

The fragment enters the bacterial cell in company with the prophage, by analogy with T2, probably by the injection process (Hershey and Chase, 1952).

The association of the fragment with the prophage in transduction to lysogenic cells cannot be stated in the absence of phage markers, since it is not possible to distinguish between the previously carried and the newly entered prophage. The carriage of more than a single prophage by cells of E. coli K-12 has been reported by Appleyard (1954) and it is likely that the transductions of lysogenic recipient cells are also carrying more than a single prophage.

In only one instance, from more than 250 segregations studied, has segregation from a transduction of lysogenic cell resulted in a change at Lp. In this case an idiotypic segregant became Lp^s, and this might have been a spontaneous "mutation".

In the transductions to Lp^s recipient cells the association between transducing prophage and the fragment is possibly better seen. These transductions are of two kinds, Lp^+ and Lp^r. All segregants from Lp^+ clones have been lysogenic. On the other hand, Lp^r transduction clones segregate Lp^r/Lp^s as well as Gal+/Gal-. The incidence of Lp^s Gal- idiotypes supports the notion that these loci are linked.

In considering the relationship of the fragment to the rest of the genome no specific statements can be made with regard to its perpetuity in the heterogenic clone. One would depend upon its possession of a functional centromere, so that it would behave as a small autonomous chromosome, or the fragment would be attached to the homologous chromosome segment, either intersitially or terminally. Either attachment position presents difficulties for crossing over, and the fragment as a separate chromosome seems more plausible.

In the above sections the results have been treated and discussed in a general way. It is obvious that the study of this transduction system has only begun and that many experiments and interesting observations will be made before the problem is completely understood. It is proposed to investigate lambda transduction further along the following lines.

1. Whether the production of transducing activity in HFT cultures is related to the interaction of radiation and cells, or is the result of a mutational like event in the cell population.

2. The production of transducing particles in HFT lysates.

3. The action of radiation on transducing particles and the possibility of inducing mutations.

6. Further studies on crossing over between fragment and idiotypic loci using additional markers.

7. The relationship between lysogenization and transduction, and between lysogenization and crossing over.

8. Estimation of the gene order of the transduced loci and their relationship to other mapped loci.

9. Study of the biochemical steps controlled by the various loci and the fermentation of galactose.

4. The detection of other loci within the transduced region.

5. The behavior of the fragment transduced during syngamy and meiosis.

SUMMARY

A cluster of loci in Escherichia coli K-12 was found previously to control the fermentation of galactose. Lysogenicity for the temperate bacteriophage, lambda, was also found to be closely linked to these loci in crosses. The phage lambda now has been found to transduce these loci, as can be readily demonstrated by mixing lysates of galactose positive cultures with galactose negative cells on a selective medium, EMB galactose agar. The transductions result in clones that are heterogenic, that is, they are diploid for a small region of chromosome. The small fragment of chromosome transduced appears to have a functional centromere, and is perpetuated within the clone even after many single colony isolations, but it may on some occasions be lost. While in the clone it has been found to crossover with its homologous region, on some occasions at least, at a four strand stage. Each of the new phage particles formed in lysates of heterogenotes has a high probability of containing ~~not only a fragment, but~~ the fragment carried in the heterogenic clone. A position effect on the expression of two of the transduced loci has been observed. Diheterogenotes of Gal_1 and Gal_4 are not phenotypically galactose positive in the *trans* positions, but are so in the *cis*.

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Table 16

The transmission of heterogenicity in crosses

Parental cells F^+	Parental cells F^-	Prototrophic recombinants Galactose (+)	Prototrophic recombinants Galactose (-)
$Gal_2^{\pm}$(1)	Gal_4- Lp^s	1*	about 6000
Gal_4- Lp^s	$Gal_2^{\pm}$(2)	541**	99

* unstable for galactose fermentation, 6 galactose negative segregants tested were Gal_2-

** 25 of 30 examined were unstable for galactose fermentation. One segregant from each of the 25 was tested, all were Gal_2-

(1) control platings showed the ratiox of (+)/(-) in this culture was 109/57

(2) control platings showed the ratio of (+)/(-) in this culture was 115/13

Gal_2^- Lp^s	Gal+ Lp^+	329	25
Gal+ Lp^s	Gal_2^- Lp^+	107 (approx.)	757 (approx.)

Table 1

Principal cultures

Wisconsin Stock Number	Genotype*
W518	F^+ M^- Lac_1- Gal_4- Lp^s
W750	F^+ M- Lac_1- Gal_1- Lp^+
W811	F^+ M- Lac_1- Gal_4- Lp^+
W902	F- T-L-B_1- Mal_1- Gal_2- Lp^+
W1210	F^+ M- Lac_1- Gal_2- Lp^+
W1436	F^+ T-L-B_1- Lac_1- Gal_4- Lp^s S^r
W1924	F^+ M- Lac_1- Gal_4- Lp^r
W2175	F^+ Gal_2- Lp^+
W2279	F^+ M-Lac_1- Gal_1- Lp^s
W2281	F^+ M- Lac_1- Gal_2- Lp^s

* Genotypic symbols refer to the following characters.

(1) Compatibility status, F

(2) Nutritional requirements; M, methionine; T, threonine; L, leucine; B_1, thiamin

(3) Fermentation reactions; Lac-, lactose negative; Gal-, galactose negative; Mal-, maltose negative

(4) Phage reaction; Lp^s, lambda sensitive; Lp^+, lambda lysogenic; Lp^r, lambda resistant, but not overtly lysogenic.

(5) Drug resistance; S, streptomycin

Table 2

Recombination between the various Galactose loci

Cross		Minimum Number of Prototrophic Recombinants		Percent Galactose Fermenting Recombinants
F_9^+ Gal_1^-	X F^- Gal_2^-	(1)	1500	0.13 ✓
		(2)	6517	0.06 ✓
		(3)	3603	0.03 ✓
			11620	0.06 –
F^+ Gal_4^-	X F^- Gal_1^-		4588	0.13 ✓
F^+ Gal_4^-	X F^- Gal_2^-		2654	0.23

F^+ Gal_1^- : W750
F^- Gal_1^- : W750 (aeration phenocopy)
F^- Gal_2^- W902
F^+ Gal_4^- : W811, W518, W1436

Table 3

Observations on lambda lysate transductions

Locus	Number of experiments	Cultures involved[1]
1. Loci not transduced		
Lac_1	1	W112
(serine or glycine)	1	W1678
Leucine	3	W1736, W1436[a]
Methionine	4	58-161, W811, W1821, W518[b]
Xylose	3	W1821[c]
Streptomycin	1	W518[d]
Proline	9	W1692, W1920, W2062[e]
	1(lytic lambda)	W2062[f]
Mal_1	2	W2331, W2347[g]
Mal_x	1	W2071
Ara	1	W2307[h]
2. Loci transduced		
Gal_1	-	W750, W2279, W2280, W2373
Gal_2	-	W1210, W2175, W2281
Gal_3	-	W2297
Gal_4	-	W518, W811, W1821, W1436, W1924
Gal_6	-	W2070

(Footnotes table 3 continued)

f- lytic lambda grown on M- culture
g- lysate of prototrophic HFT Gal_2- culture
h- lysate of prototrophic HFT Gal_2- culture

Table 4

The interaction of lysates and cells of galactose negative cultures

Recipient Cells (Lp^+)		Plaque titer (x 10^{10})	None Lysate	Lysates Gal_1-	Gal_2-	Gal_4-	Wild type
			-	2.4	4.9	1.7	1.4
Gal_1-	(1)		2*	-	176	43	-
	(2)		2	2	-	-	405
Gal_2-	(1)		14	52	11	43	-
	(2)		20	-	10	-	356
Gal_4-	(1)		89	-	202	-	-
	(2)		50	85	-	-	417
	(3)		47	-	-	50	394

* The no added lysate plate which represents the number of spontaneous reversions occuring on the plate. The remaining figures are the numbers of papillae occuring on the plates per 0.1 ml of lysate added.

Table 5

Restoration by reverse mutation of the ability to transduce previously non-transducible loci

Recipient cells (Lp^+)	Reversion	Lysate: None	Lysate: Reversion
Gal_1-	Gal_1^+ (1)	0	648*
Gal_2-	Gal_2^+ (1)	10	96
	Gal_2^+ (2)	6	552
Gal_4-	Gal_4^+ (5)	39	204
	Gal_4^+ (8)	25	291

*number of papillae per plate, 0.1 ml of lysate plated.

The necessity of lambda adsorption for transduction

Table 6

The necessity of lambda adsorption for transduction

Recipient Cells (Lp^+)	Lambda-2 phenotype** Lp Allele	Plate, Adsorbed None	Wild type lysate
Gal_1-	s	1	426*
	r	1	2
Gal_2-	s	20	356
	r	14	14
Gal_4-	s	89	296
	r	50	57

*Number of papillae per plate, 0.1 ml of lysate plated

** s = lambda-2 sensitive, r = lambda-2 resistant. r forms do not adsorb either lambda or lambda-2

Table #7

The activity of lytically grown lambda

Experiment	Recipient cells	Lp Allele	Plate addition: None	Lytic lambda	Plaque titer
228	Gal_1-	+	3	2*	2.4×10^{10}
	Gal_2-	+	9	8	
	Gal_4-	s	9	8	
239	Gal_1-	+	2	0	2.4×10^{10}
	Gal_2-	+	6	2	
	Gal_4-	s	13	8	
254	Gal_1-	s	-	6**	2.4×10^{10}
	Gal_1-	+	-	3**	
	Gal_2-	s	-	9**	
	Gal_4-	s	-	6**	
	Gal_4-	+	-	39**	
280	Gal_1-	+	0	2**	8.6×10^{9}
	Gal_2-	+	1	2**	
	Gal_4-	+	14	10**	

*Papillae per plate, 0.1 ml lysate plated. Lysate prepared by growing Gal_4- lambda (UV induction) on a galactose fermenting culture.

**These papillae picked and streaked on EMB galactose medium and found stable for galactose fermentation.

Table 8

The specific activity of lysates of the transduction clones

Recipient Cell	Transducing lysate	Titers Plaques	Transductions on Lp^+ assay cells Gal_1-	Gal_2-	Gal_4-	P/T*
Gal_1-	wild type	5.8×10^8	2.4×10^6	1.8×10^7	1.3×10^7	32
Gal_1-	Gal_2-	7.2×10^9	1.2×10^8	1.0×10^6	-	60
Gal_1-	Gal_2- **	? $\times 10^6$	1.8×10^6	6.3×10^4	-	
Gal_2-	Gal_1-	6.2×10^8	4.3×10^7	1.5×10^8	-	4
Gal_4-	Gal_1-	1.5×10^8	5.0×10^7	7.5×10^7	7.4×10^7	2
Gal_4-	Gal_2-	7.3×10^8	2.5×10^7	2.8×10^5	-	29

* Ratio of plaques to transductions; the maximum transduction titer observed is used for this estimate. Usual ratio P/T is about 10^6.

** A second isolation.

9

Table

The occurrence of stable transductions

Recipient cells		Lysates											
		Wild type			Gal_1-			Gal_2-			Gal_4-		
		T/C*	C	T**	T/C	C	T	T/C	C	T	T/C	C	T
Gal_1-	Lp^s	38/1	1	14	-	-	-	11/1	1	11	30/1	1	29
	Lp^+	46/1	1	2	-	-	-	84/1	1	4	27/12	12	27
	Lp^+	143/1	1	42	-	-	-	92/1	1	0	-	-	-
Gal_2-	Lp^s	46/0	0	15	214/0	0	27	-	-	-	98/0	0	4
	Lp^+	248/17	17	21	83/14	14	61	-	-	-	79/14	14	52
	Lp^+	23/4	4	6	65/2	2	0	-	-	-	56/5	5	0
Gal_4-	Lp^s	835/19	19	383	72/29	29	72	472/11	11	20	-	-	-
	Lp^+	573/41	41	133	96/51	51	96	-	-	-	-	-	-
	Lp^r	320/31	31	127	-	-	-	238/31	31	50	-	-	-

* Papillae transduction plate/ papillae control plate. T = transduction plate, C = control plate

** Corrected for sample taken, $\frac{\text{stable obs.}}{\text{sample size}} \times \text{Papillae transd. plate}$

With the exception of the T/C column, numbers given are number of stable galactose fermenting papillae.

Table 10

Summary of the analysis of segregants by transduction test, lysate test and by crossing test

Recip. Culture	Trnsd. Lysate	1 Trnsd.	Classification of segregant by Lysate 2	Cross 3 Idiotypic (+)	Idiotypic Tot. Prot.	Allotypic (+)	Allotypic Tot. Prot.
Gal_2^- Lp^s	wild	(1) Gal_2^-	Gal_2^-	0	7805	-	-
		(2) "	"	0	4992	-	-
		(3) "	"	0	106	-	-
		(4) "	"	0	4552	-	-
Gal_2^- Lp^+	wild	(1) Gal_2^-	Gal_2^-	0	4070	-	-
		(2) "	"	0	5384	-	-
		(3) "	"	0	2072	-	-
		(4) "	"	0	6988	-	-
Gal_4^- Lp^s	wild	(1) Gal_4^-	Gal_4^-	0	896	-	-
		(2) "	"	0	918	-	-
		(3) "	"	0	1134	-	-
		(4) "	"	0	863	-	-
Gal_4^- Lp^s	Gal_2^-	(1) Gal_4^-	Gal_4^-	0	2786	3	3183
		(2) "	"	0	2675	2	3471
		(3) "	"	0	3485	23	5342
		(4) "	"	0	5952	1	1665
		(5) "	"	0	5000	1	891
		(1) Gal_2^-	Gal_2^-	7	3102	0	1988
		(2) "	"	10	4364	0	1187
Gal_4^- Lp^+	Gal_2^-	(1) Gal_4^-	Gal_4^-	0	16104	3	1389
		(2) "	"	0	5730	1	164
		(3) "	"	0	3358	0	202
		(4) "	"	0	12848	1	171
		(1) Gal_2^-	Gal_2^-	1	11200	0	827
		(2) "	"	6	10608	0	718
		(3) "	"	3	5000	0	409

1. Test of the segregant against the lysates of a known cultures
2. Test of lysate of the segregant against known cultures
3. Test crossing with know cultures

Table 12

The examination of segregants by testing with lysates of known cultures

Recipient cells	Lp genotype	Trnsd. lysate	Segregants idiotypic	allotypic	amphitypic	total
Gal_1-	s	wild	9	0	0	9
	+		33	0	0	33
Gal_2-	s		16	0	0	16
	+ (1)		20	0	0	20
	+ (2)		15	0	0	15
Gal_4-	s		~~46~~ 31	0	0	~~46~~ 31
	+		20	0	0	20
Gal_1-	s	Gal_2- (3)	6	1	0	7
		(4)	1	0	0	1
		Gal_4-	1	0	0	1
	+	Gal_2- (5)	36	6	0	42
		(6)	18	3	0	21
Gal_2-	s	Gal_1-	20	0	0	20
		Gal_4-	21	1	1	23
	+ (7)	Gal_1-	19	2	0	21
	(8)		14	3	2	19
	(9)	Gal_4-	22	1	0	23
	(10)		9	7	0	16
Gal_4-	~~s~~	Gal_2- (11)	17	2	0	19
		(12)	~~35~~ 18	~~5~~ 3	~~2~~ 0	~~43~~ 21
	+	(13)	16	3	0	19
	r	(14)	15	3	0	18

(1),(8),(10), cultures of W2175. (2),(7),(9), cultures of W1210 (3),(6),(11), lysates of W1210. (4),(5),(12),(13),(14) lysates of W902. W902 is the Lp_2^r parent of W2175.

Table 12

Segregants in table 11 whose classification was confirmed by the action of their lysates on known cultures

Recipient cells	Lp genotype	Trnsd. lysate	Segregants idiotypic	allotypic	total
Gal_1-	+	wild	5	0	5
Gal_2-	+ (1)		4	0	4
	(2)		4	0	4
Gal_4-	s		4	0	4
	+		4	0	4
Gal_1	+	Gal_2- (3)	4	5	9
		(4)	0	3	3
Gal_2-	s	Gal_4-	0	1	1
	+ (5)	Gal_1-	0	2	2
	(6)	Gal_4-	4	0	4
	(7)		0	1	1
Gal_4-	s	Gal_2- (8)	16	3	19
		(9)	0	1	1
	+	(10)	15	3	18
			60	19	79

(1),(5),(6), cultures of W2175. (2),(7), cultures of W1210
(3),(8),(10), lysates of W902. (4),(9), lysates of W1210

Table 18.

Galactose negative cultures giving HFT lysates

HFT culture	Recipient cell	Trnsd. lysate	Nature of Gal^+ reversions	HFT segregant	Nature of Gal+ reversion HFT seg.
Gal_1^-	Gal_1^-	Gal_2^-	unstable	Gal_1^-	stable
	Gal_1^- Gal_2^-,	Gal_1^- + Gal_2^-*	unstable	Gal_1^-, Gal_2^-, Gal_1^- Gal_2^-	stable
Gal_2^-	Gal_2^-	Gal_1^-	unstable	Gal_2^-	stable
	Gal_1^-	Gal_2^-	unstable	$Gal_1^-Gal_2^-$	none observed
	Gal_2^-	Gal_2^-	unstable	$Gal_1^-Gal_2^-$	none observed
	Gal_1^-	Gal_2^-	unstable	Gal_2^-	stable
	Gal_1^-	Gal_2^-	unstable	Gal_2^-	stable
	Gal_2^-	~~Gal_2^-~~*	unstable	Gal_2^-	-
	Gal_4^-	**	unstable	Gal_2^-	stable
	~~Gal_4^-~~	~~Gal_2^-~~			
Gal_4^-	~~Gal_4^-~~	~~Gal_2^-~~	——	——	——
	Gal_2^-	Gal_4^-	not done	Gal_4^-	stable

* Transduction made with a mixture of HFT Gal_1^- and Gal_2^- lysates.
** These lysates were from a mixture of cultures.

Table 14

Correlation of lysogenicity with transduction using lysates giving a high frequency of transduction

1. The transductions

Cells Exposed to	Post Exposure cell titer	Number of colonies observed		
		Gal(-)	Gal(+)	Gal(-)partially lysed
Broth	4.1×10^9	3280	0	0
HFTlysate*	3.5×10^9	2801	31	54

2. Examination of the colonies after HFT lysate exposure

Colony type	Number of colonies examined	Number of colonies		
		Lp^s	Lp^+	Lp^r
Gal(-)	31	31	0	0
Gal(+)	26	0	23	3

* Lambda plaque titer was 1.2×10^9. One ml of cell suspension was added to one ml of lysate and the mixture incubated at 37C for 10 minutes. The cells were then centrifuged down, the supernatant discarded and the cells resuspended in one ml broth. The suspension was then diluted and plated on EMB galactose medium.

Table 16

The [redacted] interaction between Gal_1- and Gal_4-

1. The transductions

Recipient Lp^s cells	Trnsd. HFT lysate	Number of colonies Gal(+)	Gal(-)	Gal(-) papillating
Gal_1-	broth	0	465	0
	Gal_4-	0	316	2
Gal_4-	broth	0	440	0
	Gal_1-	0	408	2

2. Examination of galactose negative segregants derived from galactose positive clones found in papillating galactose negative colonies

Recipient Lp^s cells	Trnsd. HFT lysate	Classification of segregants Gal_1-	Gal_4-	Gal_1-Gal_4-	Gal(-) papillating
Gal_1-	Gal_4-	10	2	0	1
Gal_4-	Gal_1-	5	6	2	4

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151

(156)

283-1 x 1476 demarche
HFJ6, 7⁻ → x 518

Position Effect

I Gal₁ - Gal₄

	Genotype	Ref.	Seg.
(A)	$\frac{1^- 4^+}{1^+ 4^-}$	Thesis	–
(B)	$\frac{1^+ 4^-}{1^- 4^+}$	Thesis	—
(C)	$\frac{1^- 4^-}{+ +}$	283, 305, 312	
(D)	$\frac{1^+ 4^+ 2^-}{- - 2^+}$	295	135 - adiv 14 allo 3 amphi (1⁻)

II Gal₁ - Gal₇

	Genotype	Ref.	Seg.
(A)	$\frac{7^+ 1^+}{7^+ 1^-}$	302, 307	7 (7⁻) 4 (1⁻) 3 (1⁻ 7⁻)
(B)	$\frac{7^+ 1^-}{7^- 1^+}$	(323)	
(C)	$\frac{1^- 7^-}{+ +}$	(320)	
(D)	$\frac{1^+ 7^+ 2^-}{- - +}$	(315A)	

11 Genotype Ref Seq

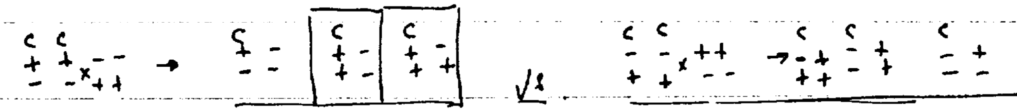
(A) $\frac{5-1+}{6+1-}$ 308

(B) $\frac{6+1-}{6-1+}$ 323

(C) $\frac{6-1-}{+1}$ 320

(D) $\frac{6+1+2-}{6-1-2+}$ 324

Position Effects.

1. Theoretical 

2. Evidence - Segregants from single round.

Idio	Allotype	Stbl.	Unstbl	Idio	Allo	Amphi	P.E. (-)		
				Segregants					
7⁻	1⁻	4	17	7	4	3	3	c 7 1	tentative order
1⁻	7⁻	19	4	1	3	0	0	c 7 1	c / 4,6,7, / 1
6⁻	1⁻	5	19	8	2	3	6	c 6 1	
1⁻	6⁻	16	6	2	4	0	0	c 6 1	
4⁻	1⁻	[illegible]	18	10	1	2	[illegible]	c 4 1 (2 not here)	
1⁻	4⁻	13	9	6	1	0	2	c 4 1 (reservations because Lp^x)	

Completion of 7⁻ x 1⁻

			I	A	Amphi
1.	1⁻7⁻ — x 8⁻	33/0	29	1	0
2	1⁻7⁻ x — (+)	294/0	24	0	0

Completion 6⁻ x 1⁻

			I	A	Amphi	
1.	1⁻6⁻ — x 8⁻	50/0	12	4	0	
2.	1⁻6⁻ x — (+)	278/0	20	0	0	(3 stable?)

Completion 4⁻ x 1⁻

			I	A	Amphi
1.	1⁻4⁻ — x 8⁻	81/6 71/3	135	14	3
2.	(+) — x 1⁻4⁻	c 1000/0	24	0	0

Order c 6 7 4 1

(159)

Interactions between 4, 6, 7,

307A 84 x 7⁻ from cross streak

7 pap → 3(7⁻), 4 (4⁻)

rpt. 3 (7⁻) 3 (4⁻)

310 HFT 6⁻ → x 7⁻ 30/7 (old 4 scale) 27 pRl +

		Sdbl	Unstbl	Idio	Allo	Ampli	TE.	Total	
343	HFT 6⁻ → x 7⁻	17	4	0	3 (4)	1(2)	0	4	
344	HFT 7⁻ → x 6⁻	7	13	5	0	2	4	11	(2 lost)
	+ → x 6⁻ 7⁻	–	–	15	0	0	0	15	
	6 7⁻ → x 2⁻	–	–	18	2	1(?)	0	21	

c 6 7

345	HFT 4⁻ → x 6⁻	3	14	14	0	0	0	14	
	HFT 6⁻ → x 4⁻	17	6	3 (8)	1(5)	1(3)	0	5	(1 lost)
	+ → x 4⁻ 6⁻	–	–	16	0	0	0	16	
	4⁻ 6⁻ → x 2⁻								

c 7 4

1⁻2⁻ ⟵ 4⁻, Positive effect between 1, 4⁻

Order	C	C	C	C	C	C
	4+	2-	1	1 ①	4	2
	2-	1-	4	2	1	4
	1-	4+ ①	2	4 ②	2	1
	90% (2-)	50% (1-2-)	100% (1-4-)	30% (1-2-) ③	50% (---)	90% (2-)
	5% (---)	50% (---)		30% (---) ②	50% (4-)	5% (1-4-)
	5% (4-)			30% (1-4-) ①		5% (4-)
				10% (2-) ①		

↑ ↑

C 2⁻4⁻ ⟵ 1⁻

30% (1-4-)	90% (2-)	50% (---)	90% (2-) ②	90% (1-4-)	50% (2-4-)
30% (2-4-)	5% (1-4-)	50% (1-)	5% (1-) ①,②	10% (2-)	50% (---)
30% (---)	5% (1-)		2.5% (---) ①		
10% (2-)			2.5% (1-4-) ②		

↑ ↑

Recent studies of recombination in E. coli (17) have led to the discovery of a compatibility mechanism (15), a lysogenic system subject to genetic control (10), and a system of limited transduction by temperate phage (22) comparable to that of Salmonella (28). These three phenomena involve transfer of heritable factors by infection in contrast to bacterial mating which involves the entire genotype. The clarification, differentiation, and interrelationships of these mechanisms were emphasized in this investigation.

I The LYSOGENIC SYSTEM IN E. COLI K-12

The relationship of a temperate phage, λ, to a specific locus, Lp_1 (latent phage) has already been reported (10). In summary, the principal reaction types of bacterial strains are: sensitive (Lp^S), lysogenic (Lp^+), and the non-lysogenic resistant type, Immune-I (Lp^r). In crosses they behave as a system of multiple alleles, linked most closely with Gal_4. This linkage has been confirmed in a Gal^+ Lp^+ x Gal^- Lp^S cross in another laboratory (27). In addition, the two factors segregated out of heterozygous diploids in the parental coupling. This evidence points, therefore, to a genic determinant regulating the maintenance of λ provirus.

From a number of direct and indirect experiments it is known that all these types adsorb λ. A second locus, Lp_2, controls resistance or sensitivity to λ-2, a virulent λ mutant, and is situated in the Mal_1--S region of the chromosome. As Lp_2^r strains cannot adsorb λ, they are therefore not subject to any consequences whose initial reaction requires adsorption; Lp_2 does not interfere with the maintenance of λ previously established in Lp^+ strains. The genotype $Lp^sLp_2^r$ is consequently indistinguishable from $Lp^rLp_2^s$ types with respect to lytic effect of λ. Cross-reactions of λ with λ-2 antiserum have been observed.

New Data on Immune-1: The status of the various isolates of immune-1 strains has been reported, and the interpretation of their constitution with respect to prophage had been reserved pending evidence of a "cryptolysogenic" phage that normally fails to mature to give rise to lytic virus. The segregation pattern of $Gal^+Lp^+/Gal_4^-Lp^r$ diploids, also heterozygous for Mtl and Mal_1 (table 7) is identical with similar Lp^+/Lp^s results. The hypothesis that Lp^r types may carry a non-reproducing prophage is supported by experiments in which a low titer of λ was recovered by U-V induction of at least one (22). Lp^r types are also subject to transduction, and the results of these studies will be deferred to that section.

Incidental Variant Types: No new evidence bearing on the problem on the "semilysogenic" strain (10) can be presented. Tests to determine whether host-modified λ was carried (section III) were negative.

An intermediate host reaction, semiresistant to both λ and λ-2, comparable to the one in Shigella paradysenteriae (26) and the V_1^p allele of K-12 (11) has been clarified. Standard λ suspensions have a reduced efficiency of plating (eop) on this mutant such that the plaques produced are reduced in size and number, and also show a reduced efficiency of transduction. The mutants have been successfully lysogenized, but are still semiresistant to λ-2. The protocols for crosses which establish a mutation at a new Lp_3 locus not linked to Lp_2-Mal or Lp_1 - Gal, and conferring partial resistance to λ, are presented in table 13.

Mechanism of infection; Mutation and Selection vs. Induction: Breeding experiments and diploid segregations reveal only the chromosomal determinant of lysogenicity. The facility of the change Lp^s to Lp^+ encourages the possibility that λ directly induces (rather than selects) Lp^+ among the numerous survivors of exposure to phage. The following types of evidence would be useful in elucidating the primary infection process: (1) identification of a "prelysogenic" genotype in the absence of phage

would encourage the mutation hypothesis. It would be characterized as an apparent immune-1 that would be converted to a stable lysogenic after treatment with λ. (2) a careful study of the dynamics of infection, including the isolation of clonal pedigrees of single cells exposed to λ which engender lysogenics. A pure lysogenic pedigree would favor the induction hypothesis.

Attempts to identify the prelysogenic genotype in K-12, and hybrids of K-12 and other crossable lines have been unsuccessful. Preliminary experiments of the infection process (10) have disclosed lysogenic colonies contaminated with sensitive cells and free phage long after initial contact with λ. These mixed clones have since been confirmed in K-12 (18) and Salmonella (14,21,23B). The possibility that spontaneous alteration of the bacteria predisposing to a lysogenic decision plays some role in the recovery of lysogenics is thus not yet excluded. However, the simplest conception remains that the genetic elements of the phage are directly incorporated in, or attached to the bacterial chromosome as we have been able to find no indication of an extra-nuclear inheritance of lysogenicity.

The Effect of λ and F on Crossing Behavior: The presence of λ in one, both, or neither of the parents of a cross does not influence the yield of recombinants. As noted earlier (8) sensitives were not eliminated

as lethal phenotypes, but the progeny of lysogenic x sensitive included both parental types, and no others, in ratios dependent on the selected auxotroph markers. On the other hand, the compatibility factor (F) determines not only the yield but also the segregation pattern of many overtly unselected markers. Prototrophs are recovered only when at least one parent is F^+; F also seems to direct the elimination of certain chromosomal segments after the formation of the hybrid zygote (15,23). The important distinctions of F and λ are summarized in table 1. These are emphasized to mitigate any confusion that might arise from the suggestions that have been recorded elsewhere that λ may play a direct role in sexual recombination as well as to emphasize the distinction between the λ controlled transduction of restricted genetic factors and the F-controlled sexual recombination. The independent transmission of these factors was demonstrated by the recovery of (1) F^+Lp^S cells on the one hand, and F^-Lp^+ on the other, from mixtures of genetically labelled F^-Lp^S and F^+Lp^+, and similarly, (2) Lp^+F^- (but no Lp^SF^+ or Lp^+F^+) as survivors from F^-Lp^S exposed to λ-containing filtrates from F^+Lp^+ cultures.

II TRANSDUCTION

Cell-free filtrates derived from suitable Salmonella strains were capable of transferring unit genetic factors to a competent recipient (28). A wide range of independent markers has been equally subject to transduction. Additional analysis has shown that the temperate phage of the donor strain is the vector of the genetic material (16,25). Attempts to detect transduction in K-12 among the survivors in the turbid centers of λ plaques were negative (10); but by using high-titer lysates obtained by U-V induction (20), a successful transduction was achieved (22). Two striking contrasts with the Salmonella system were demonstrated: (1) the restriction to a single genetic character, galactose fermentation, and (2) a striking instability manifested by mosaic Gal^+/Gal^- colonies after transduction despite repeated single colony purification on EMB galactose agar. The incidence of persistent instability, rarely if ever encountered in Salmonella (14), varies with the recipient strain.

Confounding of Transduction with Recombination ?: The conditions required for transduction are generally precluded in crossing experiments. Moreover, the unstable mosaic Gal^+/Gal^- colony characteristic of transduction has not been so far recovered among recombinant progeny. A

more careful inquiry into the effect of λ and Gal segregation was necessary, however, in view of the transduction phenomenon, since it may provide an alternative interpretation of the Gal-Lp cosegregation ratios currently satisfied by a linkage explanation. Crosses of genetically related parents differing only in the presence or absence of λ were therefore studied. Table 2 demonstrates no significant deviation in the yield of Gal^+ recombinants where parents vary only for the Lp marker.

Is Transduction a Selection Artefact?: Interaction of genetic factors on reverse mutation of entirely independent loci have been reported before (15). An analysis of the Gal- segregation from the unstable transduction, the allelic transduction, reported below, as well as many other types of evidence (22) rule out the interpretation that the transduction is a selection artefact. The most convincing evidence, however, has been the development of specific Gal^- transductions in Gal^+ recipient strains by means of λ with extraordinary high frequency of transduction (22), when the λ donor was Gal^-.

Transduction and F-transfer: Just as lysogenization is independent of the conversion of F^- into F^+ strains, the transduction mediated by λ is unrelated to the F status of either the recipient or the donor cells.

Crosses of F^- x F^- by standard techniques are completely sterile. However, recombination of two nonallelic Gal^- mutants can be indirectly demonstrated by transduction. Lysates from $Lp^+Gal^+F^-$ were completely functional in introducing the Gal^+ factor to Gal^-F^- cells. Similarly, nonallelism of two Gal^-F^- strains can be established by the formation of Gal^+ in transduction experiments whereas the sexual sterility of the cross would block cell recombination in toto.

Crosses of a strain characterized by its enhanced fertility, Hfr, (15) displayed a linkage of the Hfr trait to Gal (12). These data were verified (table 3) for Gal^-_2. Despite this linkage, efforts to transport the Hfr and Gal^+ factors simultaneously into $Gal^-F^-Lp^S$ recipient cells via λ prepared from Hfr bacteria were unsuccessful. The conversion of F^- to F^+ by λ filtrates from F^+ strains was examined by crossing the Gal^+ transduction with F^- tester strains and was likewise unsuccessful. The competence of λ in transduction therefore continues to be confined to the Gal cluster.

The Concurrence of Transduction and Lysogenization: Observations on the E. coli system, as in Salmonella, are consistent with the hypothesis that the vector of transduction consists of temperate phage. As a rule,

(169)

the transductions isolated from Gal⁻Lp^S bacteria exposed to λ are consistently pure, stable lysogenics, despite the persistent instability of the Gal$^+$ trait; the ensuing Gal⁻ segregants are also lysogenic. Lysogenization occurs very much more frequently than transduction, but the correlation of the two remained to be explored as evidence bearing on the hypothesis. In the first experiment (table 4 , part A) transductions were picked as Gal$^+$ papillae and streaked out on EMB galactose agar. A single Gal⁻ (representing non-transinduced cells) and a single Gal$^+$ (the successful transduction) were each tested for lysogenicity on an appropriate Lp^S indicator. In experiment B, marked Gal$^+$Lp^S cells in the approximate proportions expected from transduction were introduced with the Gal⁻ and the mixed culture on EMB galactose plates. With the assumption that both Lp^S strains would adsorb and be equally affected by λ, a disparity in lysogenizations of the two ensuing Gal$^+$ classes was looked for. Whereas all of the transduction Gal$^+$ were lysogenized, only up to 70% of the artifically inserted Gal$^+$ or of the original Gal⁻ had been infected. Both parts of the experiment show a distinct correlation of lysogenization with transduction; the incidence of lysogenization is almost higher in these than in the control bacteria on the same plates.

Segregation of lysogenic sensitive has not so far been observed (up to 500 tests) from these simultaneously transduced and lysogenized recipients. This evidence argues that λ is the passive vector of genetic material from its source strain. This material is injected to the bacterium by the phage. In Salmonella the transduced genetic factors seem to undergo an immediate substitution for the homologues in the recipient bacterium, if they are successful at all. In E. coli K-12, however, an intermediate stage is perceived where one can detect simultaneously the presence of the original recipient and the new transduced genetic factors in the same cells by virtue of their subsequent segregation. The relationship between this replacement of genetic material and the conversion of virulent λ into its prophage stage ("reduction" 6) has not yet been completely worked out. As will be described below, however, these processes have been separated and are therefore not mutually dependent.

Lysogenization of Immune-1 in Transduction Experiments: When immune-1 strains such as W-1027 and W-1924 are exposed to λ, no evidence of their lysogenization is ordinarily perceived. However, under conditions where transductions can be selectively isolated about 5% of these altered bacteria

(171)

are also found to have been lysogenized. Repeated serial segregation of the resulting transductions showed that in some cases, lysogenicity failed to segregate. In others, lysogenicity and Gal segregate together, while in a single instance a lysogenic Gal^- segregant was found which continued to segregate Lp^r colonies. Sometimes a very weak lysogenicity is observed ("one-plaque types" in cross-brush tests), which is completely lost after a few transfers. Some of these atypical cases are presented in table 5, and suggest the following alternative interpretations: (1) Lp^r cells are genetically lysogenic but carry a modified prophage. These cells are generally resistant to infection with λ. However, λ may be exceptionally introduced simultaneously with the Gal^+ fragment and there may displace the avirulent form of the prophage, or when Lp segregation is observed, both prophages persist together for the time being. (2) The Lp^r is a "null" allele. In transduction, Lp^+ and Gal^+ factors are introduced, but the lysogenic/immune segregation occurs when Gal segregates. This hypothesis can not account easily for the Gal^-Lp^+/r types except by devising a complicated scheme involving crossingover. (3) Immunes may or may not be genetically lysogenic. The production of Lp^+ signifies the occurrence of a double transduction at two loci, Gal and Lp. (a) ordinarily these linked factors would tend

(72)

to be lost as a block in the ensuing segregation, or (b) a linked transduction does not operate. By a two-step process, two effective particles have penetrated; one fragment carries Gal^+, the other Lp^+. Independent segregation is permitted and a mechanism requiring the breakage of a 2-factor linked fragment as in (2) is not called for.

In any event, special assumptions must be made on the avidity of the Lp^s locus for pro-λ to account for the failure of transductions to Lp^s to segregate Lp^+/Lp^s along with Gal^+/Gal^-. However, the Lp^r may only block the propagation of λ or its reduction to pro-λ.

Hypothesis (1) accounts for the occurrence of immunes which can be induced by U-V (22). The recovery of unstable Lp^+ transductions in non-transinduced Gal- would tend to support hypothesis 3. The most decisive elucidation of whether transduction displaces a mutant phage particle with a wild type λ or whether a normal Lp^+ allele is substituted for a mutant or null host Lp^r gene would be provided by experiments with genetically distinguishable λ preparations. Lp^r/Lp^s transductions were prominent with irradiated λ, tending to support hypothesis 2.

Irradiation effects: Quantitative assays of transducing potentiality of phage preparation are necessarily based on plaque counts. The survival

after various treatments of plaque-producing particles and transducing particles are not identical either in Salmonella (28) or K-12 (22). In fact, it is known from both studies that transducing power may be increased at some intermediate dosages. A comparison of the effects of U-V and X-radiation is given in table 6. A U-V dose reducing plaque assay from $1/2 \times 10^{10}$ to 16.9×10^{5} per ml yielded 170 transductions from an initial titer of 10^{3} / ml. A comparable X-ray dose was found to be between 150,000 and 200,000 r. No recognizable transductions were recovered at the latter exposure. Two viewpoints are indicated: (1) the lytic and transducing principles in λ are separable by their independent survival, and (2) avirulent λ particles are produced but they are damaged only to the extent of virulence for the host cell. Conclusive evidence favoring one or the other views of Lp^{r}, however, is not yet at hand. A decisive chemical and genetic separation of the transducing material from the virus particle has not yet been experimentally achieved, whether or not it is at all theoretically possible.

GENETIC DEFINITION OF THE GAL LOCI

Recombination: Attention was focused on galactose nonfermenting mutants because of the coincidence of the first recognized λ-sensitive

mutant in Gal^-_4 (W-518), and the subsequent observation of linked segregation of Lp and Gal_4 (10). Gal^- mutants have been isolated directly by inspection of surviving colonies after U-V treatment on EMB galactose agar and also as non-papillating variants of Lac^- mutabile recovered on EMB lactose agar plates. Interaction of Gal^- and Gal^+ on the phenotypic expression and reverse mutation of Lac_1 and Lac_7 alleles have been described (9). Recombination analysis provided the evidence for a cluster of four linked Gal loci (7). Gal_1 and Gal_4 show a very low order of crossovers. Preliminary data could only differentiate them on the basis of behavior in Het crosses; Lp and Gal_1 are both hemizygous, while Gal_4^+/Gal_4^- heterozygous diploids are readily obtained (table 7).

Transduction: Transduction tests reinforce standard allelism tests (table 8), and in fact have tentatively identified several new loci, now awaiting confirmation by recombination analysis. Whether the relative yield of Gal^+ transductions is proportional to the map distance between Lp and the Gal locus is in question. The results of large-scale allelism tests made available to date by new techniques to facilitate crossing are summarized in table 9.

(75)

The instability characteristic of the Gal^+ transduction results in the mosaic colony already noted and deserves further comment. Despite passage through a large number of serial single colonies, Gal- segregants are almost always thrown off. In transductions from Gal^+, i.e. $Gal^+ \rightarrow Gal^-$, these Gal^- segregants have been identified as alleles of the locus of the original recipient strain, both by crossing and further transduction tests. No other kinds of Gal^- have been recovered. On the other hand, if the donor is a non-allelic Gal^-, both donor and recipient Gal^- appear among the segregants from the Gal^+ transduction (22). For example, $Gal_2^- \rightarrow Gal_4^-$ gives galactose-fermenting intermediates, presumably of the constitution $Gal_2^- Gal_4^+ / Gal_2^+ Gal_4^-$. The segregants in all these tests are identified by (1) crossing experiments with Gal_2^- and Gal_4^- testers, (2) deriving λ and subjecting the testers to its action, and (3) applying λ from Gal^+, Gal_2^-, Gal_4^-, etc. The $Gal_2^- Gal_4^-$, a crossover type, has not been conclusively and consistently established. This double mutant would be identified as one which is subject to transduction by λ from Gal^+ and from any Gal^- other than Gal_2^- or Gal_4^-, and would yield no Gal^+ recombinants in crosses with Gal_2^- and Gal_4^- testers.

176

Diploid studies: The preceding evidence points to a chromosomal localization of the Lp lysogenicity determinant closely linked to a series of Gal loci. Evidence for the segregation of a prophage linked to the Gal_4 locus ruled out the possibility of a random distribution of cytoplasmic particles in cells carrying λ(10). These observations have since been extended to Gal_2 and Gal_4 hybrids (all heterozygous Lp^+/s), and also $Gal_4^+Lp^+/Gal_4^-Lp^r$ diploids (table 10). A study of such diploids segregating out distinguishable λ types is in preparation. Preliminary evidence also has been obtained elsewhere from crosses with lysogenic parents, one carrying a mutant λ (or one "doubly lysogenic") the other doubly sensitive, which yielded Gal/Lp progeny in parental couplings (1).

The mutational independence of Gal and Lp was also examined in the doubly homozygous diploid. Comparable experiments with the closely-Lac_1 and V_6 loci have already been reported. Lac^+ reversions were selected in $Lac^-V_6^r/Lac^-V_6^s$ diploids. The resulting doubly heterozygous diploids were of two types: $Lac^+V_6^r/Lac^-V_6^s$ and $Lac^-V_6^r/Lac^+V_6^s$, and with equal frequency (11).

A double homozygote $Gal_2^+Lp^s/Gal_2^-Lp^s$, also segregating a few other markers, (and unfortunately also Lp_2) was prepared by stepwise exposure of

(177)

the double heterozygote to U-V (14) and the isolation of suitable "reorganized" diploids. The resulting diploid, H-331 was infected with λ. Several $Gal_2^-Lp^+/Gal_2^-Lps$ isolations, A to G, were then allowed to papillate on EMS galactose agar. Independently occurring Gal^+ were selected, and the segregation pattern of Lp and Gal_2 of the resulting double heterozygotes was tested. The incidence of mutation to Gal^+ on the Lp^+ chromosome (coupling phase, or cis configuration) was compared with that on the Lp^s chromosome (repulsion phase, or trans-configuration). The analysis included a single Gal^+ and a single Gal^- segregant from a large number of diploids, (pair analysis) and the examination of many segregants from a single mass diploid culture (random analysis). From diploid B, 5 cis configurations and 6 trans configurations were scored. The conclusion from this evidence (table 11) is that the condition of the Lp locus, whether lysogenic or sensitive, has no significant bearing on which one of the 2 Gal^- alleles will mutate to Gal^+. (These preliminary data will be expanded, and also extended to a corresponding study of diploids first made heterozygous $Gal_2^-Lp^s/Gal_2^+Lp^s$, and then infected with λ.)

The above studies provide two kinds of Lp^+/Lp^3; Gal^+/Gal^- diploids: λ coupled on the one hand with Gal^+ (cis) and on the other, with Gal_2^- (trans) If the activity of from "trans" bacteria is confined to non Gal_2^- recipient cells, a chromosomal but not nuclear limitation to λ specificity is indicated. All Gal^- including Gal_2^- is expected to respond to cis λ. A difference in λ from these diploids which are phenotypically identical, and genetically identical except for the arrangement of component parts established a "position effect." So far, only λ from the trans-type diploid has been prepared. Table shows that while Gal_4^- ($Gal_2^+Gal_4^-$) cells are subject to transduction, only rare Gal_2^+ transductions were recovered. The development of an adequate diploid culture to satisfy the nutritional prerequisites for U-V induction in K-12 (3,5) and an intermediate growth period necessarily permits some selection for haploid segregants. The yield of λ obtained very probably includes a limited portion derived from $Gal_2^-Lp^+$ and $Gal_2^+Lp^+$ haploids. The latter crossover types may account for those transductions which were found. The data so far allow the tentative conclusion of a position effect hypothesis and strengthen the concept of an intimate relationship of λ and Gal at a specific action site on the chromosome. Transductions of the double homozygote H-331 and lysogenic

derivatives has apparently been obtained. The analysis is complicated by the fact that diploid-haploid instability can be confounded with transduction instability.

COMPARATIVE GENETICS OF Lp AND Gal IN OTHER LINES

Among the independently isolated crossable strains of E. coli (12) the wild type of three lines (28,47, and 51) were sensitive to λ carried by line 1. A fourth, line 31, threw off rough variants which were all λ sensitive. These strains occurred in nature as F^- but could be altered to F^+ by growth with K-12 or suitable derivatives. So far, at least one Gal^- mutant is subject to transduction. Preliminary intra-line-47 crosses established an Lp locus like that of K-12, and a Gal-Lp linkage. Very little mapping work has been completed among these strain, and the emphasis so far in these studies has been the genetic behavior of λ in outcrosses with K-12.

Sensitives of each line are readily lysogenized by K-12 λ but these lysogenics show a reduction of eop on K-12 sensitive indicators. This system is entirely analagous to host modification demonstrated for T2 (19) and λ produced by strain C (2). The terminology established for these systems will be used to describe the properties of our strains.

180

Thus lines 28,31, and 47 can be designated as λ* lysogenic or λ* sensitive. Line 1 sensitives are more resistant to λ* than to type λ. λ* can be introduced at low rates into λ sensitive hosts, but normal rather than λ* is recovered. Similarly, normal λ is converted to λ* after a single passage in λ* sensitive hosts. The four phenotypes are readily distinguishable in cross-brush tests as follows:

Example	Type	Reaction with: λ-sens. C bacteria	λ*-sens. B bacteria	λ	λ*
line 1 lysogenic	A	+	+	R	R
line 47 sensitive	B	-	-	S	S
line 1 sensitive	C	-	-	S	R
line 47 lysogenic	D	-	+	R	R

+/- = lysogenic or not; R/S = resistant or sensitive

Two major hypotheses can be tested by intercrossing these types:

I Lp controls all reactions: the types A-D are determined at a single locus.

II Lp controls lysogenicity/ sensitivity; another locus, Mp, controls resistance or sensitivity to λ*.

(a) Both λ and λ* are fixed at Lp in phenotypes A and D.

(b) λ is fixed at Lp in type A; λ* is fixed at Mp in type D.

The consequences of these hypotheses are shown in table 12. The critical crosses for I and II are A x B and C x D. The only decisive cross for II a vs. II b is A x D. II b would be favored by the recovery of sensitive recombinants as well as a novel genotype whose phenotypic effects are unpredictable. Since there is a possibility that Lp and Mp are closely linked a large sample of progeny many be required. One must bear in mind, in reviewing these intercross data that the prototrophs represent recombination of as yet unmapped nutritional factors. In addition, chromosome and other irregularities correlated with interstrain hybrids have not been analysed.

Effective transductions have been achieved in these strains. Gal- in lines 47 and 31 have been used as recipients, for λ produced by line 1, 28, 31, and 47. A reduction in the effectiveness of transduction to line 1 recipients is parallel with the reduced effectiveness of lysogenization. In general no important differences with the K-12 mechanism have been demonstrated. Hypothesis II b is doubtful so far. The differentiation of the λ* of different lines is still to be tested. A single intercross shows no genetic difference so far.

In preparing this report, it has been necessary to make numerous references to the unpublished work carried on in this laboratory by Professor J. Lederberg, Mr. M. L. Morse, and others, under other auspices. These are cited by number to the bibliography.

Table 1

Characteristics of F (compatibility factor) and λ (virus)

Criterion	F status	λ (effects)
(1) Yield of recombinants	Decisive	None
(2) Type of recombinants	Decisive	None
(3) Transmission to recombinants	100%	Segregated according to linkage with selected nutritional markers; behaves as a genetic locus.
(4) Transmission by infection	Rapid and fixed	Results in mixed clones (3).
(5) Cell-free preparations	Not yet accomplished	Easily filtered.
(6) Effect of antiserum	Slight if any	Blocks adsorption
(7) Role in Gal^+ transduction	None	Decisive

Table 2

The Effect of λ on % Gal^- Progeny

M^-Gal^- parent x	$T^-L^-Th^-Gal^+$ parent: lysogenic	$T^-L^-Th^-Gal^+$ parent: immune
lysogenic	8.0	7.1
immune	6.3	6.3
sensitive	6.7	10.1

Table 3

Linkage of Gal, Lp, and Hfr

W-1895 x W-2308 $^{T L B^{S}}$

BM Hfr Xyl^{-} Gal^{-} Lac^{-} ara^{-} Mal^{+} Lp^{s} Lp$_{2}^{+}$ V$_{6}^{R}$

Part A:

Genotypes recovered[1]			Total
Gal	Lp	F	
+	+	+	14 *
−	s	−	29 *
+	s	+	5
−	+	−	0
+	s	−	4
−	+	+	0

Part B: 2 x 2 contingencies

	Gal^{+}	Gal^{-}	Total	F^{+}	F^{-}	Total
F^{+}	20*	0	20			
F^{-}	9	31*	40			
Lp^{s}	15*	0	15	13*	5	18
Lp^{s}	11	29*	40	6	33*	39
Lac^{+}	26*	5	31	22*	9	31
Lac^{-}	4	26*	30	7	27*	34
V$_{1}^{r}$	1*	9	10	1*	9	10
V$_{1}^{s}$	28	21*	49	23	20*	43
Xyl2^{+}	9*	1	10	7*	2	9
Xyl2^{-}	20	30*	50	16	7*	23

* Parental combination

1 Selected as Gal^{+} and Gal^{-} prototrophs.

Table 4

Lysogenization in Transduced and Nontransduced Lp^s

Part A: Gal^+ and Gal^- from single papillae

Gal^+/Gal^- Pair type	Number
Lp^+/Lp^+	13
Lp^+/Lp^s	15
Lp^s/Lp^+	3
Lp^s/Lp^s	2
Lp^r/Lp^s	2
% Gal^+ sensitive	15.2
% Gal- sensitive	47.2

	Gal^- Lp^s	Gal^- Lp^+
Gal^+ Lp^s	2	3
Gal^+ Lp^+	17	13

Part B: Lysogenization of transduced and inserted Gal^+

Lp^s strains	Av. No. Gal^+ recovered		Types in mixture	No. tested	% lysogenic
	Control	Treated*			
Gal^+Lac^+	109	92	Gal^+Lac^+ (inserts)	46	68.5
Gal^-Lac^-	11**	432	Gal^-Lac^- (original)	40	72.5
Mixture***	106.5	419	Gal^+Lac^- (transductions)	103	100.

* 10^9

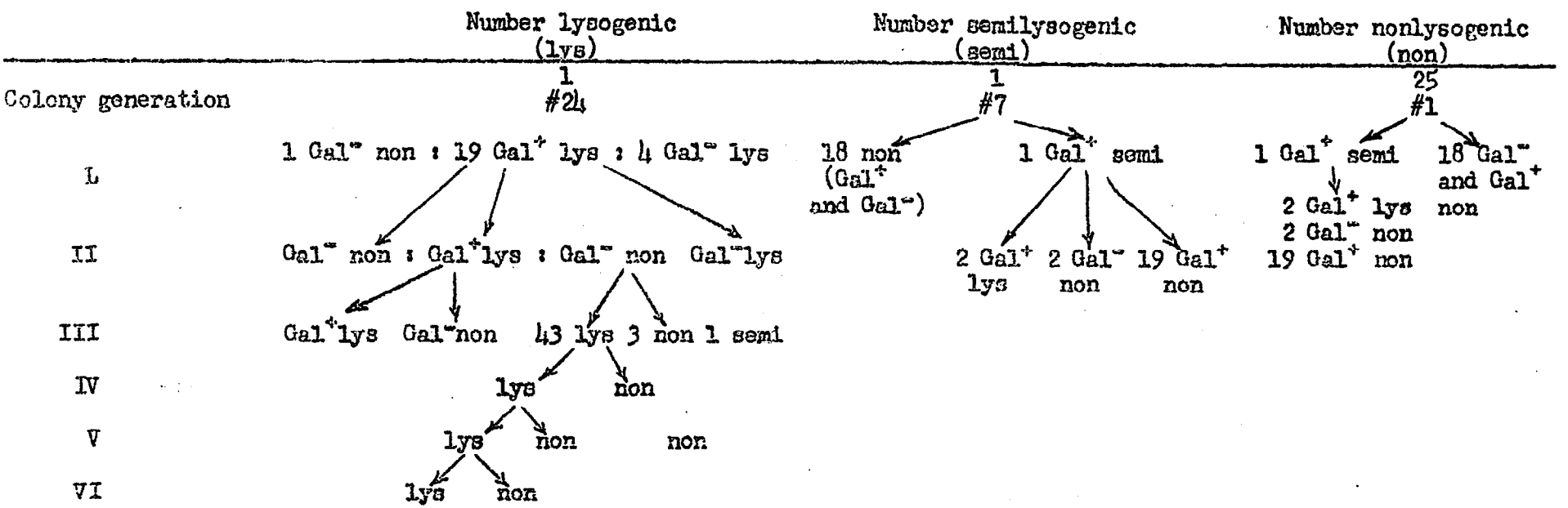
** Spontaneous reversions per 10^8 inoculum

*** 10^8 Gal-Lac- and 109 Gal^+Lac^+.

Table 5

Transductions to Gal_4^- Immune-I: Segregation Patterns

Exp. 385: Strain 1924: 27 Gal^+



Exp. 431: Strain 2110: 38 Gal^+: 28 non, 1 semi (#23), and 9 lys

Segregation patterns of lys	
	all Gal^+ lys, all Gal^- non: 2
	all Gal^+ lys, all Gal^- lys: 5
	all Gal^+ lys, Gal^- lys and non 2
	both Gal^+ and Gal^- non: #23

Table 6

Survival and Transduction with Irradiated λ

				X-ray[2] (x 10^3 r)			
	No phage	Untreated phage	U-V[1]	50	100	150	200
Av. plaques/ml x 10^5	0	127,000	16.9	41,667	3,975	377	100
% survival	-	100	0.013	32.8	3.13	0.297	0.008
Lp^S bacteria							
No. Gal^+ papillae	20	1,000	170	250	85	30	30
% " "	0.5	100	34	25	17	6	6
Lp^r bacteria							
No. Gal^+ papillae	39	60	-	135	115	31	20
% " "	65	100		225	191.7	5.2	3.3

1 20 minutes, sterilamp

2 10^3 r/min. at 250 K.V., courtesy A. Novick, Radiobiology Inst., U. of Chicago.

Table 7

Segregation of Gal, Lp,... diploids

A. H-324 Segregation of Lp_2, B_1, not tabulated.							B. H-325 Segregation of V_6, Mtl, Lp_2, B_1 not tabulated.	
Gal_2^-	Gal_2^+	Lp	Mal	Xyl	M	T,L	Gal_1^-	Gal_1^+
1	47	+	+	+	-	-	1	49
0	1	+	+	-	-	-	0	0
0	1	+	+	+	-	+	2	0
0	0	+	+	+	+	-	0	1
1	0	+	+	+	+	+	0	0
2	0	+	+	-	+	+	0	0
25	0	s	-	-	+	+	13	0
9	1	s	+	+	-	-	13	1
3	0	s	-	-	-	-	0	0
6	0	s	+	+	-	+	7	0
1	0	s	-	+	+	+	0	0
2	0	s	+	+	+	+	3	0
0	0	s	-	-	-	+	12	0
50	50	Total tested					51	51

Table 8

Allelic Specificity of the Gal - λ Transduction at the Gal 1, Gal 2, and Gal 4 loci.

λ- donor bacteria			Recipient cells		
Gal 1	Gal 2	Gal 4	1-2+4+	1+2-4+	1+2+4-
+	+	+	+	+	+
-	+	+	-	+	+
+	-	+	+	-	+
+	+	-	+	+	-
diploids:					
+ / +	- / +	+ Lp$^+$ / + Lp^s (trans)	No data	± (21)*	+ (300)*
+ / +	+ / -	+ Lp$^+$ / + Lp^s (cis)		No data	

* Gal + papillae per 10^9 λ

Table 9

Summary of Current Allelism Tests

Exp. No.	Gal$^-$ type	F$^-$ parent	F$^+$ parent	Total** progeny	No. Gal$^+$	Maxim. % Gal+
535*	1 x 4	W-750 Lp$^+$	W-2234 Lp^S	5000	17	0.3
563*				2000	15	0.75
534*	2 x 4	W-1210 Lp$^+$	W-2234 Lp^S	6000	25	0.4
563*				1600	11	0.68
580*				2400	8	0.3
535	4 x 3	W-518 Lp^S	W-2315 Lp$^+$	807	6	0.74
582	4 x ?3	W-518 Lp^S	W-2315 Lp^S	5000	0	0
				6700	5	0.06
583	1 x ?	W-2291 Lp^S	W-583 Lp$^+$	7603	2	0.026

* All Gal$^+$ recombinants in these experiments are Lp^S.

**Estimated total.

Table 10

Behavior of Gal and Lp in Lac +/- Diploids

Type of cross		Parents F	(T L Th)	M	Lac_1	Lac_4	Gal_1	Gal_4	Lp	Diploid progeny Gal	Lp
1. Het diploids	(a)(Het)	+	-	+	+	+	+	+	+	+/-	+/• or -/• 1/ 5/
		+	+	-	-	+	+	-	s		
	(b)(Het)	+	-	+	+	+	+	+	+	+/• or -/•	not segregating
		+	+	-	-	+	-	+	+		
2. $Lacl^-$ x Lac_4^-	(a)	-	-	+	+	-	+	+	+	Mostly +/•	Mostly +/• 2/
		+	+	-	-	+	+	-	s		
	(b)	+	-	+	+	-	+	+	+	Mostly -/•	Mostly s/• 2/
		-	+	-	-	+	+	-	s		
3. Haploid x auxotrophic diploid	(a)	- 4/	-/•	+/-	+/-	-/+	+	+/•	+/•	Gal^+ Lp^+ / Gal-Lp^s (linked) 3/	
		+	+	-	-	+	+	-	s		
	(b)	same, except M- parent is Lp^r								Gal^+ Lp^+ / Gal-Lp^r (linked)	

1/ In Het crosses, Lp does not segregate. Gal 1 and Gal 4, two closely linked loci also differ: Gal 4 segregates, but Gal 1 does not.

2/ Diploids resulting from delayed disjunction revealed by heterozygotes of two Lac pseudoalleles show no segregation of Gal or Lp. Reversal of F status reverses the polarity of the Gal, Lp segregation.

3/ The only successful demonstration of heterozygosity of Gal and Lp.

4/ Aeration phenocopy.

5/ +/• indicates purity for +, whether hemizygous or homozygous.

Table 11

Segregation Patterns of Gal^+ Reversions in $Gal_2^-Lp^s/Gal_2^-Lp^+$ Diploids

Diploid number	Total segregants	Gal^+		Gal^-		Gal^+		Gal^-		Gal^+		Gal^-		Inferred type of diploid
		Lp^+	Lp^s	Lp^+	Lp^s	Lp_2^r	Lp_2^s	Lp_2^r	Lp_2^s	Mal^+	Mal^-	Mal^+	Mal^-	
A 1	161	76	6	3	76	45	0	39	0	1	53	17	36	cis
B 1	121	2	58	60	1	52	6	60	1	38	22	61	0	trans
B 2	73	0	40	41	0	32	7	31	0	33	7	33	0	trans
B 3	76	61	4	1	10	65	0	57	5	65	0	44	18	cis
C 1	48	1	23	24	0	23	1	24	0	9	15	24	0	trans
E 1	60	30	0	3	27	26	4	24	6	30	0	16	14	cis
E 2	24	0	12	12	0	12	0	12	0	6	6	12	0	trans
E 3	23	12	0	0	11	12	0	11	0	12	0	3	8	cis
F 1	66	32	1	2	31	31	2	30	3	32	1	21	12	cis
F 2	40	20	0	1	19	20	0	20	0	20	0	7	13	cis
F 3	23	12	0	0	11	12	0	10	1	12	0	3	8	cis
F 4	18	11	0	1	6	10	1	0	7	11	0	7	0	cis

Table 12

Genetic Determination of Host Modification: line 1 lines 28, 31, 47

		Genotypes Under				
		Hypothesis I Lp locus with alleles	Hypothesis IIa fixed at Lp, modified by Mp		Hypothesis IIb fixed at Lp in line 1, at Mp in other lines	
Phenotypes	Symbol	Lp	Lp	Mp	Lp	Mp
lysogenic	A	+	+	r	+	r
sensitive*	B	s*	s	s	s	s
sensitive	C	s	s	r	s	r
lysogenic*	D	+*	+	s	s	+
A X B		None	C, D		C, D	
B X C		None	None		None	
C X D		None	A, B		A, B	
A X D		None	None		B and Lp^+Mp^+	

EXPTL. RESULTS:	Lines crossed	Type	A	B	C	D	Gal char.
Expt. No. 419	1 x 28	A Gal^- x B	0	46	1	0	+
			18	0	0	0	-
		C Gal^- x D	0	0	0	34	+
			2	8	18	3	-
418	1 x 31	A Gal^- x B	3	43	26	1	No record
420		A Gal^- x B	4	22	28	12	Gal^+ only
423		A Gal^- x B	8	2	1	37	+
			0	1	0	0	-
423		C x D Gal^-	28	1	3	0	(and 28 Lp_2^r) B or C
444		C Gal^- x D	2	2	19	0	mostly Gal^-
502		B Gal^- x C	0	15	13	0	+
			0	13	68	0	-
443	31 x 31	B x A	0	26	0	1	
468	1 x 47	A x B Gal^-	51	0	0	6	+
			0	2	2	3	-
527		A Gal^- x B	4	7	1	9	+
			41	0	0	2	-
528		B x C Gal^-	0	13	17	0	+
			0	8	24	0	-
529		C Gal^- x D	3	2	2	21	+
			2	2	28	0	-
523		A Gal^- x D	8	0	0	52	+
			37	0	0	19	-

F^- parent underlined.

Table 13

Genetic Control of the Semiresistant Phenotypes:

Nonlysogenic (W-2147) and Lysogenic (W-2172)

Part I

Phenotype symbol	Hypothesis I: A new allele at Lp_2: Lp_1	Lp_2	Example	Hypothesis II: A 3rd locus, Lp_3, is involved: Lp_1	Lp_2	Lp_3
A	+	s	Type lysogenic	+	s	s
B	+	r	Immune-2 lysogenic	+	r	s
C	+	p	W-2172 mutant	+	s	p
D	s	s	Type sensitive	s	s	s
E	s	r	Immune-2	s	r	s
F	s	p	W-2147 mutant	s	s	p

B x F Yields: B, F, E, C progeny Yields B, F, E, C, A, D
C x E

Results:

	No. of Progeny: B x F						C x E					
	A	B	C	D	E	F	A	B	C	D	E	F
Mal^+	55	1	1	1	0	1	22	2	1	26	0	1
Mal^-	0	58	0	0	1	0	0	0	0	0	59	0

Part II Linkage of Lp_3 to Lp_1--Gal and Lp_2--Mal ?

Parents	No. of Progeny: $Mal^+ Lp_1^s$	$Mal^+ Lp_1^+$	$Mal^- Lp_1^s$	$Mal^- Lp_1^+$
F Mal^+ x B Mal^-	4	56	1	58
C Mal^+ x E Mal^-	27	25	59	0
	$Mal^+ Lp_2^s$	$Mal^+ Lp_2^r$	$Mal^- Lp_2^s$	$Mal^- Lp_2^a$
F Mal^+ x B Mal^-	59	1	0	59
C Mal^+ x E Mal^-	51	2	0	59
	$Mal^+ Lp_3^s$	$Mal^+ Lp_3^p$	$Mal^- Lp_3^s$	$Mal^- Lp_3^p$
F Mal^+ x B Mal^-	57	3	59	0
C Mal^+ x E Mal^-	50	2	50	0
C Gal^+ x D Gal^-	$Gal^+ Lp_1^+$	$Gal^+ Lp_1^s$	$Gal^- Lp_1^+$	$Gal^- Lp^s$
	60	0	0	28
	$Gal^+ Lp_3^s$	$Gal^+ Lp_3^p$	$Gal^- Lp_3^s$	$Gal^- Lp_3^p$
	37	23	37	26

The above data are consistent with the hypothesis that an Lp_3 locus separable from Lp_1 and Lp_2 modifies the reaction to λ-1 and λ-2. This locus is not linked to Lp_1--Gal or Lp_2--Mal.

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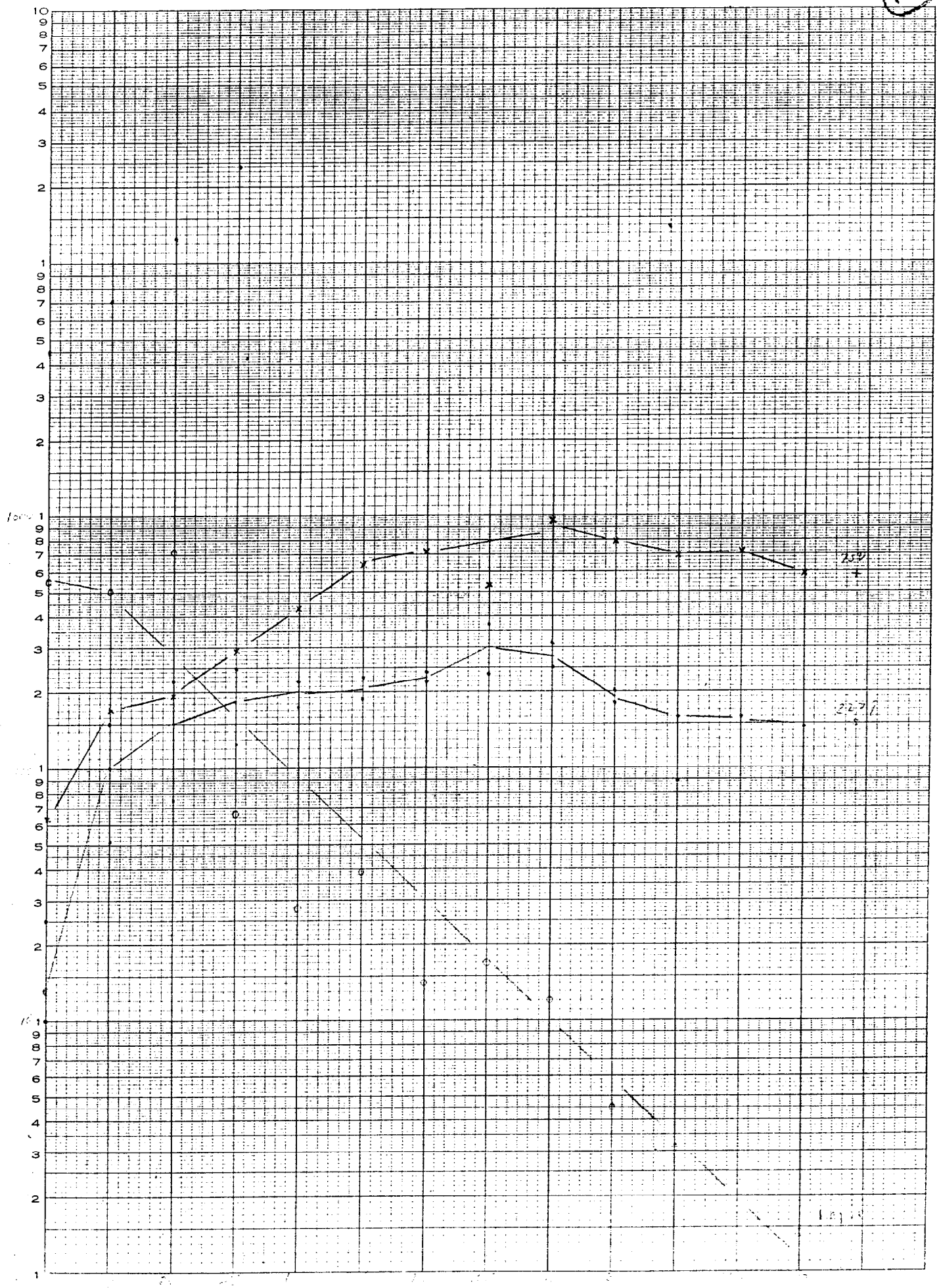

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Keysort cards carry 68 bits. The following scheme is tentatively suggested for organizing the stockbook. Further suggestions urgently requested.

	bits
Stock number and series (3 digits only)	11
Line: (1; 2-10; 11-20; 21-40; 41-...;) and E. coli not wg; Not E. coli; .....;	3
Event and agency:	3

"Mutation" spontaneous, sporad.
spont; selected
UV
X-rays or other mutagen

Not "mutation": new isolate or receipt
segreg. orrecombinant (sex or --x)
Infection (F or lambda...)
"Cure" " " "

Kind of locus changed:	3

Not indicated
Lp_1 or_x
Gal (by transduction)
F

auxotroph
fermentation
Sm
Other resistance

20

Genotype: 1 bit each for:

Gal 1, 2, 4, x	4	S (incl S^d)	2
Lac 1, x	2	Other resist. (lv fut)	2
Mal 1, x	2	Fla	
Xyl, Mtl, Stl, Ara, Stl, Glu, Suc, Cell, Rh, X	10		
M, (T,L)	2	misc.	5
Other aux: AA, Vit, Pur, etc.	3		64
Hfr; F	2		
Het	1	lyophil	1
Heterogenote or heterozygote	1		
any suppressor	1	who entered	3
any temp.-sens.	1		68
Lp or other phage	3		
V_1 V_6 V_x Lp_2 Lp_x	5		
	35		

Incidence of Homozygotes

Age	Ends	Exs	N. Tested		
341	2⁻	4⁻	17	(3 seg segs per one each)	2 HFT 2⁻ / 3×17 = 52 ✓
	4⁻	2⁻	5	(about 9 segs from each)	0 HFT / 9×5 = 45⁻
311	2⁻	6⁻	30	(single seg)	1 HFT 6⁻ / 30 (no tests of 2⁻) ✓
309	7⁻	2⁻	5	(6 segs from each)	1 HFT 7 / 30 (no tests of 2⁻)
295	2⁻	1-4⁻	152	(single seg) only 1-4 tested	1 HFT 1⁴/₁₄ (no tests of others)
293	2⁻	4⁻	5	(sep. segs)	1HFT 2⁻/₁₁ | 2HFT 2⁻/₁₁ | 0/11 | 1HFT 2⁻/₁₁ | 7HFT 4⁻/₁₂ out | 0/10 |
291	8⁻	4⁻	3	(sep. segs)	0/20 | 0/22 | 0/8 |
248	1⁻	+	4	single	0/4
247B	8	4	1	single	1/1 HFT 4⁻
247A	8	+	4	single	0/4
243	1	8	3	single	0/3
242	4	8	1	single	0/1
241	1⁻	2	24	single - done against 1⁻, 2⁻	2 HFT 2⁻/24
236c	8	4	1	single	0/1
212	4	+	4	single	0/4
209	2	1	2	"	0/2
205	4	+	3	"	0/3
202	4	2	18	"	1 HFT 2⁻ / 1 HFT 4⁻ / 18
198	4	2	16	"	0/16
196	2	+	4	"	0/4
192B	1	2	9	"	2 HFT 1⁻ / 1 HFT 2⁻ / 9
192A	1	+	4	"	0/4

24/385

0/300 24/ca 200

Corrected Estimations of Homozygote Frequency

page	2⁻	1⁻	4⁻	6⁻	7⁻	8⁻
341	2/47 (I)		0/5 (A)			
	0/4 (A)		0/41 (I)			
311				1/3 (A)		
309					1/27 (I)	
293	1/10 (I)		0/1 (A)			
	2/10 (I)		0/1 (A)			
	0/10 (I)		0/1 (A)			
	1/10 (I)		0/1 (A)			
	0/9 (I)		0/1 (A)			
291						0/46 (I)
[illegible]		0/4 (I)				
247B			1/1 (A)			
247A						0/4 (I)
243						0/3 (A)
242						0/1 (A)
241	2/2 (A)	0/22 (I)				
236c		0/1 (A)				
212		0/4 (I)				
209		0/2 (A)				
205		0/3 (I)				
202	1/3 (A)	1/14 (I)				
196		0/16 (I)				
196	0/43 (I)					
194B	1/5 (A)	2/4 (I)				
194A	[illegible]	[illegible] (I)				

I	4/14	2/34	1/78
A	0/81	0/2	1/12 A

General

2⁻	1⁻	4⁻	6⁻	7⁻	8⁻
10/114	2/36	2/96	1/3	1/27	0/54
0.09	0.06	0.02	0.33	0.03	0.00

6/11 0.33
2⁻ 0.09
1⁻ 0.06
7⁻ 0.03
4⁻ 0.02
8⁻ 0.00

Obtained from

Exodigenetic (0.04) 10/240

Exogenetic (0.19) 6/31

10 HFT/240

6/31 1⁻

−/+ 0/23

	Page		F+	F−	(+)	Total	% +
1X3	200		M− GaI_1^- Hp^+ (710)	TLB_1^- GaI_3^- Hp^+ (2238)	2	ca. 1600	0.13 ✓
1X2	214	(1)	M− GaI_1^- Hp^+ (713)	$TLB_1 \equiv GaI_2^-$ Hp^+ (2251)	4	1957	0.2 ✓
1X2		(2)	"	"	4	ca. 6517	0.06 ✓
1X2			M− GaI_4^- Hp^+ (711)	$TLB_1 \equiv GaI_2^-$ Hp^+ (2251)	0	1039	0 ✓
8X2		(1)	M− GaI_4^- Hp^+ (2214)	$TLB_1 \equiv GaI_2^-$ Hp^+ (2251)	3	ca 6840	0.04 ✓
8X2		(2)	"	"	0	ca 9640	0. ✓
8X2			"	"	0	ca. 1872	0. ✓
1X2	199		M− GaI_1^- Hp^+ (750)	$TLB_1 \equiv GaI_2^-$ Hp^+	2	ca 1500	0.13 ✓
1X4	210		M− GaI_1^- Hp^+ (750)	TLB_1^- GaI_4^- Hp^+ F+ (1426)	6	4588	0.13 ✓
1X2	240		M− GaI_1^- Hp^+ (750)	TLB_1 GaI_4^- Hp^+ (2251)	1	3606	0.03 ✓
5X4	171		M− GaI_4^- Hp^+ (570)	TLB_1 GaI_2^- Hp^+ (962)	5	1289	0.38 ✓
2X4	171a		" Hp^+ (711)	"	0	706	0.0 ✓
2X4	174		"	"	0	200	0.0 ✓
2X4	175		"	"	1	358	0.28 ✓
Followed 2X4	337		M− GaI_4^- Hp^+ (1402) F^-	TLB_1^- GaI_2^- Hp^+ F+	7	3771	0.18

		(+)	Total	% +
1X2	(1)	1	1957	0.2
	(2)	4	ca 6517	0.06
	(3)	2	ca 1500	0.13
	(4)	1	3606	0.03
			Ave 5/13680	0.06 %
1X3	(1)	2	ca 1600	0.13 %
1X4	(1)	6	4588	0.13 %

2X4	(+)	Total	% +
	0	1039	0.
	5	1289	0.38
	0	706	0.
	0	200	0.
	1	358	0.28
	6	3592	0.17
4X2	7	3771	0.18

Ave F+ ↗

	+	Total	% +
8X2 (1)	3	ca 6840	0.04
(2)	0	ca 9640	0
(3)	0	ca 1872	0

MATRIX TO TEST TRANSDUCTION MAP SEQUENCES

(201)

Sequence operators	Codes for multiple exchange types (Operators on donor genotype)				
3-point test					
123	b				
132	c				
213	a				
4-point test					
1234	b	c	ac	bc	bd
1243	b	d	ad	bd	bc
1324	c	b	ab	bc	cd
1342	c	d	ad	cd	bc
1423	d	b	ab	bd	cd
1432	d	c	ac	cd	bd
2134	a	c	bc	ac	ad
2143	a	d	bd	ad	ac
2314	c	a	ab	ac	cd
2413	d	a	ab	ad	cd
3124	a	b	bc	ab	ad
3214	b	a	ac	ab	bd

The complete table can be generated as the permutations of (a'b, cd') where a'b=bb, bc, bd, and bb=b.

Instructions:

1. Write down the donor genotype (differential markers only) in any arbitrary sequence, e.g., W- X+ Y+ Z-.
2. Group the experimental results into the rare and frequent classes.
3. Code these classes as transformations of the donor genotype. The code "a" means "reverse the sign of the first locus written", "b" the same for the second, etc. Thus, (ad)(W-X+Y+Z-) would be W+X+Y+Z+.
4. The table gives the codes for the multiple exchange classes (mec) corresponding to each sequence. Those models are excluded where frequently found types are included in the mec codes, and vice versa.
5. The sequence codes can be translated into maps by writing the donor genotype as W X Y Z and transposing accordingly. Thus, 2314 would be the map XYWZ.
   1 2 3 4
6. For the reciprocal transduction, superimpose the operation abcd, so that, e.g., ac becomes bd; c becomes abd in the mec codes.

J. Lederberg

Index to Some Topics in Vol. II

Subject	Page
Crossing	
Gal⁻ × Gal₂⁻ (Strykm)	219a
Gal$_x$⁻ × Gal$_y$⁻	221
W2312 Unstransf. Gal⁻ × Gal$_y$⁻	206, 222
" × Gal₂⁻	222
" × Gal+	225, 226
Gal₂ × Gal₁⁻	240
EML642 Gal₈⁻ × Gal$_y$⁻	250A, 250B
Gal₁⁻ × Gal₁⁻	255
Gal₈⁻ × Gal+	253
Gal₂⁻ × Gal+	253
Het Gal⁻ strains	264
Heterogenote × Gal⁻	279, 318
Hfr Gal$_y$⁻ × Het Gal₁⁻	284
1924EML2X1436	287 CONTINUED
Gal$_3$⁻	221, 222, 232, 239, 289
Lytic λ	227, 228, 239, 254, 280,
Hfr λ - lytic growth	239
Linearity	224, 227, 231, 250C, 252, 253 286, 337, 338
UV. Hfr	316
Do mutations in Gal- ferment Gal ?	349
Effect of cell density	227
Multiplicity effects	316, 322, 323, 328
Segregation Rate	351, 351A, 356

Crossing Cont.

	Page
Cross Ups to obtain Up+	294
$Gal_4-F^+ \times Gal_2^- F^-$ and resp F	333, 337
$Gal_4-Up' \times Gal+Up^+$	333

Cont.

Segregations 7- —x 1-6-	319
4- —x 1-6-	319
+ —x 3-7-	320
+ —x 1-6-	320
1-6- —x 2-	321
6- x 7-	323, 343, 344
6- x 1-	323
7- x 1-	327
4- x 1-7-, 6- x 1-7-	326, 336
4-x 1-6-, 7 -x 1-6-	327
1- x 1-7-	332
+ —x 1-7-, 6-7- x 2-	344
4- xx 6-	345, 346
4-6- x 2-	346,

Position Effect

Summary

Syngenotes Endo	Syngenotes Exo	Stble	Segregants from Gal+ Endo	Exo	Amphi	P.E.	Page
1⁻	4⁻	12/24	12	0	0	0	
4⁻	1⁻	7/24 [illegible]	10 (6)	1 (1)	2 (1)	2 (2)	F-X
14⁻	+	0/24	24	0	0	0	
8⁻	14⁻	?	135	14	3	0	
1⁻	6⁻	16/22	2	4	0	0	
6⁻	1⁻	5/24	8	2	3	6	
16⁻	+	3/23	20	0	0	0	
8⁻	16⁻	?	12	4	0	0	
1⁻	7⁻	19/21 32/42	~~1~~ (4)	~~3~~ 4	~~0~~ 5	~~0~~ 1	
7⁻	1⁻	4/21	7	4	3	3	
17⁻	+	0/24	24	0	0	0	
8⁻	17⁻	0/30	29	1	0	0	
6⁻	4⁻	3/17	14	0	0	0	
4⁻	6⁻	17/23	3	1	1	0	
46⁻	+	0/16	16	0	0	0	
2⁻	46⁻	?	52	0	2	0	
6⁻	7⁻	15/21	0	4	2	0	
7⁻	6⁻	7/13	5	0	2	4	
67⁻	+	?	15	0	0	0	
2⁻	67⁻	3/24	18	2	1	0	

Revision summary

Page	Equilo	Exo	Total	Comment	
360	4	4	8	extent genes	360-2 (Col1B, Col5
201	— 4 —		4		W2868
363B	HH=4 6 HHH		10	10 different heterogenate	
364	5	1	26	(-add 20 = 26)	total = 77 W2869
358	— 2 —		2	2 different	358-1, 358-2
291	— 8 —				
	— 5 —				
	— 8 —				
	— 4 —				3 different heterogenate
	— 8 —				
	— 4 —		37		
			77		18 different heterogenate

	373-1	373-2	373-3	373-4	375-1	375-2	375-3	375-4	375-5	375-6
Lp^+ s	0	25	15	21	34	9	10	0	0	15
ns	0	1	0	0	1	2	2	24	0	2
Lp^L s	9	3	2	3	1	4	6	0	0	5
ns	8	1	0	0	0	2	2	0	0	2
Lp^S s	0	0	3	0	0	0	0	0	0	0
ns	4	[illegible]	0	0	0	0	2	0	24	0
	21	[illegible]	20	24	36	17	22	24	24	24
		36				5wt				

Condensed summary of above ↓

Gwen	100	6	20	60	20	75	60	125	1000	100	120
	373-1	373-2	373-3	373-4	375-1	375-2	375-3	375-4	375-5	375-6	375-7
Get	Lp^+ s/Lp^L s/Lp^S s	seq Lp^+	Lp^+	Lp^+	seq Lp^+	seq Lp^+	seq Lp^+	unseq Lp^+	unseq Lp^S	seq Lp^+	seq Lp^+
Lp^+	0	26	15	21	35	11	12 [4]	24	0	17	22
Lp^L	17	4	2	3	1	6 [2]	8	0	0	7	2
Lp^S	4	6	5	0	0	0	2 [4]	0	24	0	0
	21	36	22	24	36	19	24	24	24	24	24
	4/4	–	–	–	2/2	–	1/2	–	–	12/3	11/3

Segregation from single heterozygotes

(Pg)

No. Segregants

Segregate	Endo	Exo.	Endo	Exo	Duplic	Total
365B	1-4-	2-	11	2	0	13
364	4-	2-	13*	7*	0	20
		* 3/7 Exo homozygotes 4/13 Endo "	29	8	0	37
			42	15	0	57
362 (W2566) see 361, 298, 2924	4- S	2- R	S 3 / R 0	S 13 / R 34	S 1 / R 0	51
359B (W.λ)	2-	7-	7♂ → 7	0	0	7
			4♂ → 6	0	0	6
			2♂ 5	0	0	5
			3	4	0	7
			6	1	0	7
			5	1	0	6
			2♂ 6	0	1	7
			♀ 1	0	0	1

P.E.

1 ~~x~~ x 4

	Ref.	F	Endo	Hp	Hp Ø	Stbl	Endo	Exo	Amphi	P.E.
(285-1)	348	+	~~1~~ 4	S	~~+~~	12/24	12	0	0	0
	368-2	-	~~1~~ 4	S	R/S	18/22	3	0	0	1
(285-2)	331	+	~~1~~ 4	S	R/S	13/22	6	1	0	1
	368-1	-	1	S	+	9/15	3	2	0	1
						52/83 (0.63)	24	3	0	3
	366-1	F-	4-	S	+	11/24	6	1	1	2
	329	F+	4-	S	+	7/24	10	1	2	2
	360-2	F+	4-	R	R/S	12/24	4	4	3	1
	366-2	F-	4-	S	R/S	6/20	3	4	2	2
	360-3	F+	4-	R	R/S	9/19	1	3	3	3
						45/111 (0.41)	24	13	11	10

Hp- Hp+

A A x A A

b b B B

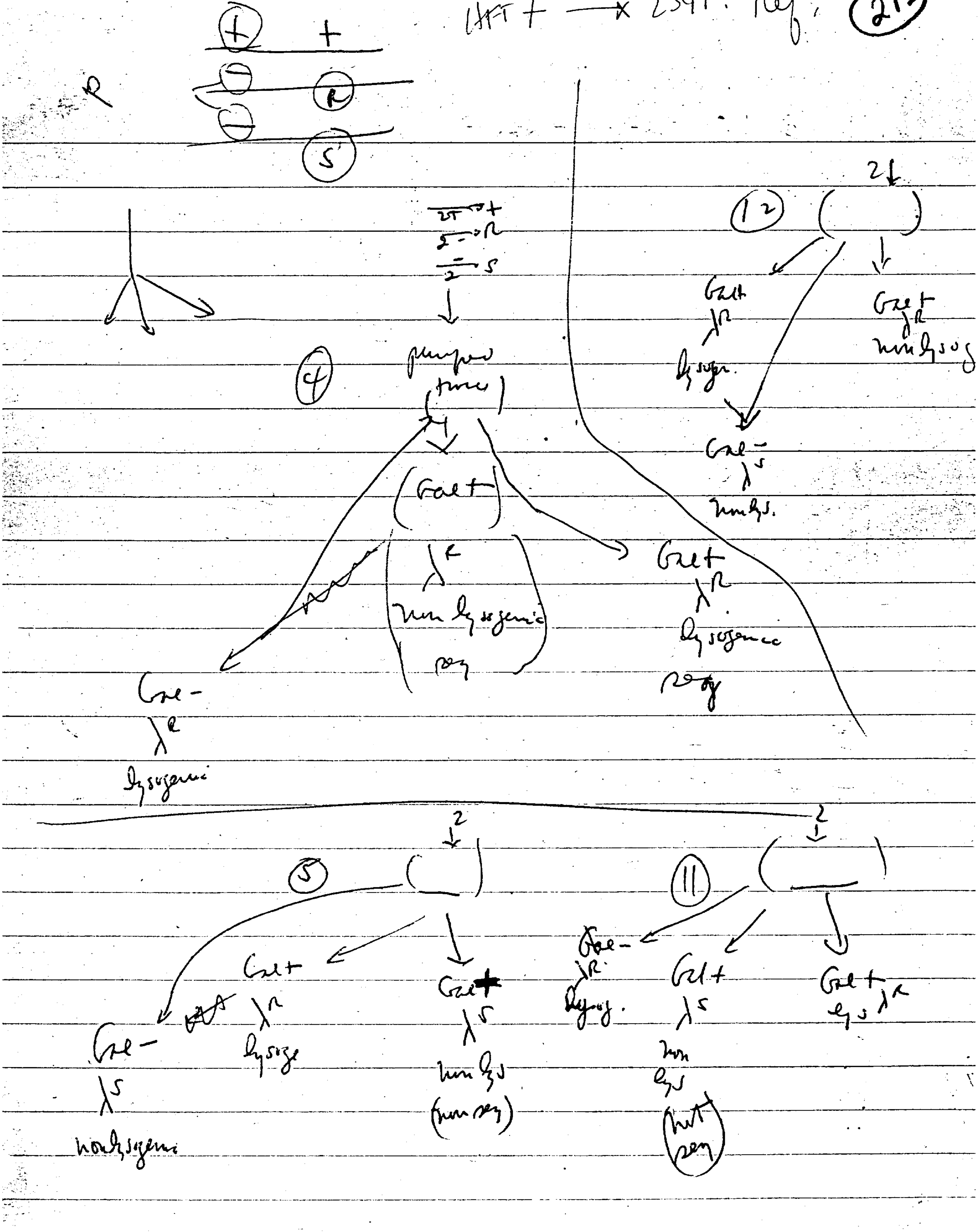
a	A
b	B

A A / b b — Hp-/Hp-

A A / B b — x

A A / B B

HFT + → x 2341. Ref: (212)



Reversion Study

Lp^S Gal- → Gal+ ~~295~~

2341 Lp^R/Lp^S → $Lp^S + Lp^R$ 287

1 Lp^R/Lp^S 8/8 reversion seg

1 Lp^S 6/6 reversions not seg

257C-6 $4^- 2^+ Lp^S // 4^+ 2^- Lp^R$ 292

3 2^- seg Lp^S (single reverted) - (1) non seg.

1 4^- seg Lp^S (") - (1) non seg.

12 $2^-/2^-$ seg Lp^R/Lp^S (single ...) - ~~all~~ 11/12 seg.

257C-6 5 2^- seg Lp^R (five reversions) - 5 clones of 2/2 reversion seg 298

257C-6 { 1 $2^- Lp^R$ (2/2 reversion unstable) 292A

292-1

{ 3 $2^- Lp^R$ (3/3 reversion unstable)

292-22 → 5 $2^- Lp^S$ 5/5 reversion stable)

285-2 $1^+ 4^- Lp^S // 1^- 4^+ Lp^R$ 303

↘ (4) $\frac{++}{--}$ $Lp^?_?$ - of 12 Gal+ obtained

9 λ^R were also seg

3 λ^S were not seg.

2279 × — HFr C- 323

Gal-

↘ Lp^R/Lp^S $6^-/6^+$ $1^+/1^-$ → 1 Gal_6 - Lp^S → 2/2 Gal+ reversion stable

↘ 1 Gal_6 - Lp^R → 1/1 Gal+ reversion stable

Reversions - Other loci - Diploidy

2341 hp^R/hp^S $2^-/2^-$ 288

to see if diploidy for V_1 has occurred: V_1^S/V_1^R would be sensitive. Other V_1^R from 2341, if diploidy for V_1, all V_1^R should be λ^S

21 V_1^R obtained, 20 were hp^R, 1 hp^S

202-16, 241-14, 241-19 } Lac$^-$ Gal_2^-/Gal_2^-, Lac^R were found stable. 241

241-14, 202-16H } λ_2^R should not be possible. Argument similar to 2341 V_1^R above. Selecting 300

2 mal- HFT 2^- obtained 241-14

1 " " " 202-16H

2307X — HFT 2^- 309

Xyl-ara$^-$ Gal_2^- → Xyl-ara$^-$ Gal_2^-/Gal_2^- no value

2350X — Gal_2^- (NFT) 341

Gal-Lac$^-$Xyl-Ara$^-$ → 1 HFT 2^- obtained - reversion blocked 4/6 Gal$^+$ sig

6/6 Lac^R did not sig

6/6 ^{R}Xyl$^+$ " " "

6/6 Ara$^+$ " " "

0.2c
566 ⟌117.0
113 2
3800

Lp^R/Lp^S trans.

Endo	Exo	+/total	Lp^R	Lp^+	Page
4-	2-	39/1312 }	1	7	223
		7/256 }			
4-	2-	—	1	0	241
4-	2-	36/2801	3	23	254
4-	2-	2/142	1	1	257
..	..	26/1870 } high mult.			259
		108/1279 }	——		
		117/566 }	——		
		Lp^S 8/140, Lp^+ 1/426	——		268
		18/199	——		276
		10/215	——		278
4-	1-	2/52	1	1.	274
		2/408	——		282
1-	4-	2/356	1	1	285
6-	1-	3/267	——		308
2-	1-	18/428	——		330
7-	6-	9/423	1	1	342
4-	6-	3/295	1	1	342
4-	2-	4/1331 (37c)	——		}
		9/150 (31c)	——		}
2-	1-	~~[illegible]~~ 3/1254 (37c)	——		}
		3/817 (30c)	——		}
1-	2-	~~1?/478~~ (37c)			}
		5/161 (30c)			} 350

(206)

Exon	Exo	+/total	Lyp^A	Lyp^+
1⁻	2⁻	1/311	1	0
+	2⁻	?	1 ← Reconstructed	1
+	1⁻	?	1 reconstructed from Lyp^v	
+	4⁻	?	0	1
~~2~~	+	9/590⁻	0	8
			13	45

58) 13.0 = 0.22
11 6
1 40

~~[illegible]~~

Exo 4⁻/4⁻

*4⁻ hap 20/2⁻ 2⁻ ← 2/90 cdiv

2/2⁻ synr } (2/24) (10/114 HFT)

2⁻ hap }

4-2⁻ hap 1/24

4⁻5			3
2⁻ R/S	6	~~2~~ ~~16~~	19
2⁻5	2	4	6
4⁻2⁻5	1		1

Exo

pages in red

(217)

Homogenote Summary

Homogenote	Observation	Ref.
[2] (D1)	518 × 892 mix (153)	
	D1 × 2050 → solid smear (157) D1 × 518, 902, 2050 → solid smear (161) D1 titer (164)	
	D1 × 518 → duration solid (165) D1 × 750, D1 × 2050, D1 × 2175 (165b) D1 ⊗ 902, 892, 1436 (166) 2050 × D1, 750 × D1 (167)	
	D1 {clear Gal₂⁻ Galt^R unstable (168) 7/10 D1 Galt^u → (−) neg dom.² (169, 170) D1 × 902 (172, 174, 175) D1 galt^u 3/p (179)	
	D1 × 1673 D1 × 1485 → Gal₂⁻ (178, 183) [struck through] (185) D1 × 1485 (186)	
	D1 one step, single burst (188); 4 P5 ED1 → Gal₂⁻ seq (190) D1 × 1924 (2.0×10⁸/ml) (193) D1 vs anti-serum (197)(199)	
[2] (D4)	Galt^R stable (168) D4 × 902 (172, 174, 175) D4 Galt^S (179) D4 × 518 HFT (181)	
[1] Gal₁⁻ prototroph 2343 902 × (N1)	Gal₁⁻ (192B) N1 & sat HFT (192B) N1 × 912 Gal₂⁻ (5^R P) (192B) N1 × 2251 5^R P m A) (192C) 2 Galt^R non HFT × 811 (192C)	
	N1 × 1485 → Gal₁⁻ (203) 2 Galt^R stable (203) LOST (219)	
[1] Gal₁ prototroph 2343 912 × (N7)	Gal₁⁻ (192B) N7 & sat HFT (192B) N7 × Gal₁⁻ Gal₂ 5^R P (192B) N7 × 2251 5^R P m A) (192C) 2 Galt^R non HFT × 811 (192C)	
	3 Galt^R stable (203)	
[2] ALSO KNOWN AS [2342] Gal₂ prototroph 2343 902 × (N16)	Gal₂⁻ (192B) N16 × 902 (5^R P) (192B) N16 → LFT seq & sat (192C) N16 LFT seq "L"? (192C) N16 HFT (192C)	
	N16 × 1924 (3×10⁸/ml) (201) N16 × 1673 → Gal₁⁻ Gal₂⁻ (W2432) (203) 9/10 colonies HFT (214) N16 × 2252 (2341) (244)	
	12/p Galt^R unstable (227)	

ALSO KNOWN AS [202-16]

(2⁻) (S16) 902-×811, Gal₂⁻ (202) × 1436, 902 (202) Lac^R stbl (290) tested HFT (299) Mal- λ₂^R (300) one step Mal- λ₂^R deriv. (306)

(4⁻) (S18) 902-×811 Gal₄⁻ (202) HFT / Gal₁⁻, Gal₂⁻ Gal₃⁻, wrt F11, 1924 (216)

ALSO KNOWN AS [2346]

(1⁻) (NA-4) 902-×750 Gal₁⁻ (230) NA-4-× 1765 (241) -× 2252 (2345) (244) 4/5 Gal+^R UV stbl (267) LFT eq Gal₁⁻ 8/8 Gal+^R stbl (270)

[2⁻] (241-14) 902-×750, Gal₂⁻ (241) 8/10 all tested HFT (270) 12/12 Gal+^R unstbl (270) LFT eq Gal₂⁻, 12/12 Gal+^R stbl (270) ↙ (298) Lac^R stbl Spontaneous λ HFT (299) tested HFT (299) one step (300)

[2⁻] Mal- λ₂^R (300) UV mod. Mal- λ₂^R deriv. λ (316) spont λ Mal- λ₂^R HFT (340) HFT yield/cell (352) HFT λ yield (368)

(241-19) 902-×750, Gal₂⁻ (241) 8/10 all tested HFT (270) 12/12 Gal+^R unstbl (270) LFT eq 1-2⁻ (270)

(291) Lac^R stbl

one step (376)

[4⁻] (247B-1) 811 → x 1210, Gal₃⁻ (247B-1). 9/10 LFT seg found Gal₃⁻ Gal⁺ᴿ seg found stable (247B-1) → x 2252 (276)

[2⁻] (257-2) 902 → x 750, Gal₂⁻ (257) LFT seg Gal₂⁻, Gal⁺ᴿ stable (257)

[2⁻] (257-4) 902 → x 750, Gal₂⁻ (257) LFT seg Gal₂⁻, Gal⁺ᴿ stable (257).

Reexamine

[4⁻] (293-12 etc) 811 → x 2175 (293) Lyse HFT (349) tested (365) 5/6 Gal⁺ᴿ unstable (365) 1/6 Gal⁺ᴿ unstable (293-12 mel-) (365) 2/4 Gal⁺ᴿ unstable (366) 1/1 Gal⁺ᴿ unstable (366) 7/4 Gal⁺ᴿ unstable (366) tested (366)

[annotations above: "and other HFT seg from same transd. clone"; "Mel 2 / T2"; "(different from) ↓"; "a different"; "← different →"; "← different →"; "← different →"]

[2⁻] (293-1A) 811 → x 2175 (293) tested, (339) HFT 4/4 Gal⁺ᴿ unstab. (339) LFT seg 2⁻ (339)

[2⁻] (293-2A) 811 → x 2175 (293) tested (339) 2/3 Gal⁺ᴿ unstab. (339) LFT seg 2⁻ (339)

[2⁻] (293-2B) 811 → x 2175 (293) tested (339) 2/3 Gal⁺ᴿ unstab (339) LFT seg 2⁻ (339)

[2⁻] (293-11A) 811 → x 2175 (293) tested (339) 3/4 Gal⁺ᴿ unstab (339) LFT seg 2⁻ (339)

[1-4⁻] (295-1) 283-1 → x 1210 (295A) (ALSO 295A-1,2,3,4)

4/6 Gal+R unstbl

HFT [7] (309-1) 2342 → x 2307 (302) obtained ↑ (309) UV mut of gxx (359) (369A) (319B)
7/8 Gal+R unstbl (363B) UV mut (364) LFT seg 7⁻ (390) 0/7 Gal+R LFT seg (390)

[6⁻] (311-2) 2070λ+ → x 2175 (311) 2/2 Gal+R seg (363B) LFT seg 6⁻ (363B) 0/2 seg (390)

[2⁻] (341-9) 811 → x 2580 (335) 4/6 Gal+R unstbl (341)

[2⁻] (341-12) 811 → x 2580 (335)

[2⁻] (364A1) 2342 → x 518 (364) 2/2 Gal+R unstbl (364)

[2⁻] (364B2) 2342 → x 518 (364) 1/2 Gal+R unstbl (364) 4/6 Gal- seg = Gal- (364)

Observations on Homogenotic cultures.

Table 8

	Homogenote			LFT Segregant		
Phenotype	Derived from:		Fraction Gal+ Reversions Segregating	Phenotype	Fraction of Gal+ Reversions Segregating	
Gal_1^-	Wt	$1^- 2^+ / 1^+ 2^-$	—	−	0/4	
	Wt		—	−	0/6	
	2146		4/5	Gal_1^-	0/8	(1)
Gal_2^-	293-1A	$2^- 4^+ / 2^+ 4^-$	4/4	Gal_2^-	−	
	293-2A		2/3	Gal_2^-	−	
	293-2B		2/3	Gal_2^-	−	
	293-11A		3/4	Gal_2^-	−	
	341-9		4/6	−	−	
	3882	$1^- 2^+ / 1^+ 2^-$	12/12	$Gal_1^- Gal_2^-$	none obtained	(3)
	341-14		12/12	Gal_2^-	0/12	(3)
	341-19		12/12	$Gal_1^- Gal_2^-$	none obtained	
	257-2		—	Gal_2^-	0/1 (minimum)	
	257-4		—	Gal_2^-	0/1 (minimum)	
	D1	$2^+ 4^+ / 2^- 4^+$	10/18	Gal_2^- (mm 4^+)	−	
	D4		−	−	0/2 (minimum)	
	302-16		−	−	−	
	341-12		−	−	−	
	3641A1		2/2	−	−	
	3643A2		1/2	Gal_2^-	−	
Gal_4^-	S18	$4^- 2^+ / 4^+ 2^-$	−	−	−	
	2478-1	$8^- 4^+ / 4^- 8^+$	−	Gal_4^-	0/1 (minimum)	
	343-12B	$2^- 4^+ / 2^+ 4^-$	−	−	−	
			(1) 1/6	−	−	
			(2) 1/6	−	−	

223

	(3) 2/4			
	(4) 1/1	—	—	
	(5) 3/4	—	—	
GaI_6^- 311	$2^-6^+/2^+6^-$	2/2	GaI_6^-	$-0/3$
GaI_7^- 309-1	$2^+7^-/2^-7^+$	2/8	GaI_7^-	$-0/7$
$GaI_1^-GaI_4^-$	$8^-1^+4^+/8^+1^-4^-$	—	—	—

Table 5

The frequency of transductions unstable for galactose fermentation

Recipient cells		Lysates Gal (+)	Gal_1-	Gal_2-	Gal_4-
Gal_1-	Lp^s	9/22(41)	-	0/11(0)	0/29(0)
	Lp^+(1)	23/24(96)	-	23/24(96)	0/27(0)
	Lp^+(2)	17/24(71)	-	24/24(100)	-
Gal_2-	Lp^s	28/48(58)	63/72(88)	-	64/72(89)
	Lp^+(1)	22/24(92)	19/24(79)	-	16/24(67)
	Lp^+(2)	16/24(67)	21/24(88)	-	22/24(92)
Gal_4-	Lp^s	13/24(54)	0/72(0)	21/24(88)	-
	Lp^+	20/24(83)	0/96(0)	19/24(79)	-
	Lp^r	29/48(60)	-	18/24(67)	-

The figures shown are the fraction of cultures unstable for galactose fermentation. Percentages are shown in parenthesis.

487 unstable
613 total

Locus

Ashburner and Mathew – The position occupied by a gene on a chromosome, with regard to its linear order.

· Woodruff (31) ..... a series of allelomorphic factors (the positions they occupy is their "locus"); .....

· Sinnott, D, + D (217) .... the term locus is used both to indicate the location of a gene on a chromosome map and also to designate the unit, variants of which are alleles."

· Calkins (11) "The name of a mutant and its symbol represent the locus name and the locus symbol respectively."

(15) "The chromosome theory of heredity states that the genes are situated at definite loci in linear order on the chromosomes."

– Knight (90) "The fixed position of a gene on its chromosome."

· Cole (367) "the position on a chromosome occupied by a gene or any of its alleles"

· Riley (17) In other words, on each homologous chromosome there is a gene at a particular place or locus .....

· Kalmus (166) position occupied by a gene on a chromosome..

· Strickberger + Beadle (94) ..... every gene occupies a fixed position on a chromosome .... such a position is known as a locus ... "

· Jennings .. (166) The position of a gene on the map or on the chromosome is known as its locus..."

Table –

Segregational Behavior of Reversions of Hp^S and Hp^R Segregants

Expt.	Segregant Phenotype	Segregant Number	No. Reversions Per Segregant	Total Number Found Segregating
287	$Gal2^-$ Hp^R	1	8	8
	Gal_2^- Hp^S	1	6	0
292	Gal_2^- Hp^R	12	1	11
	Gal_2^- Hp^S	3	1	0
	Gal_4^- Hp^S	1	1	0
292A	Gal_2^- Hp^R	1	2	2
	Gal_2^- Hp^R	3	1	3
	Gal_2^- Hp^S	5	1	0
298	Gal_2^- Hp^R	5	2	10
323	Gal_6^- Hp^R	1	6	1
	Gal_6^- Hp^S	1	2	0

Heterogenote, 287, Gal_2^- Hp^S // Gal_2^+ Hp^R

292, 292A, 298 Gal_2^+ Gal_4^- Hp^S // Gal_2^- Gal_4^+ Hp^R

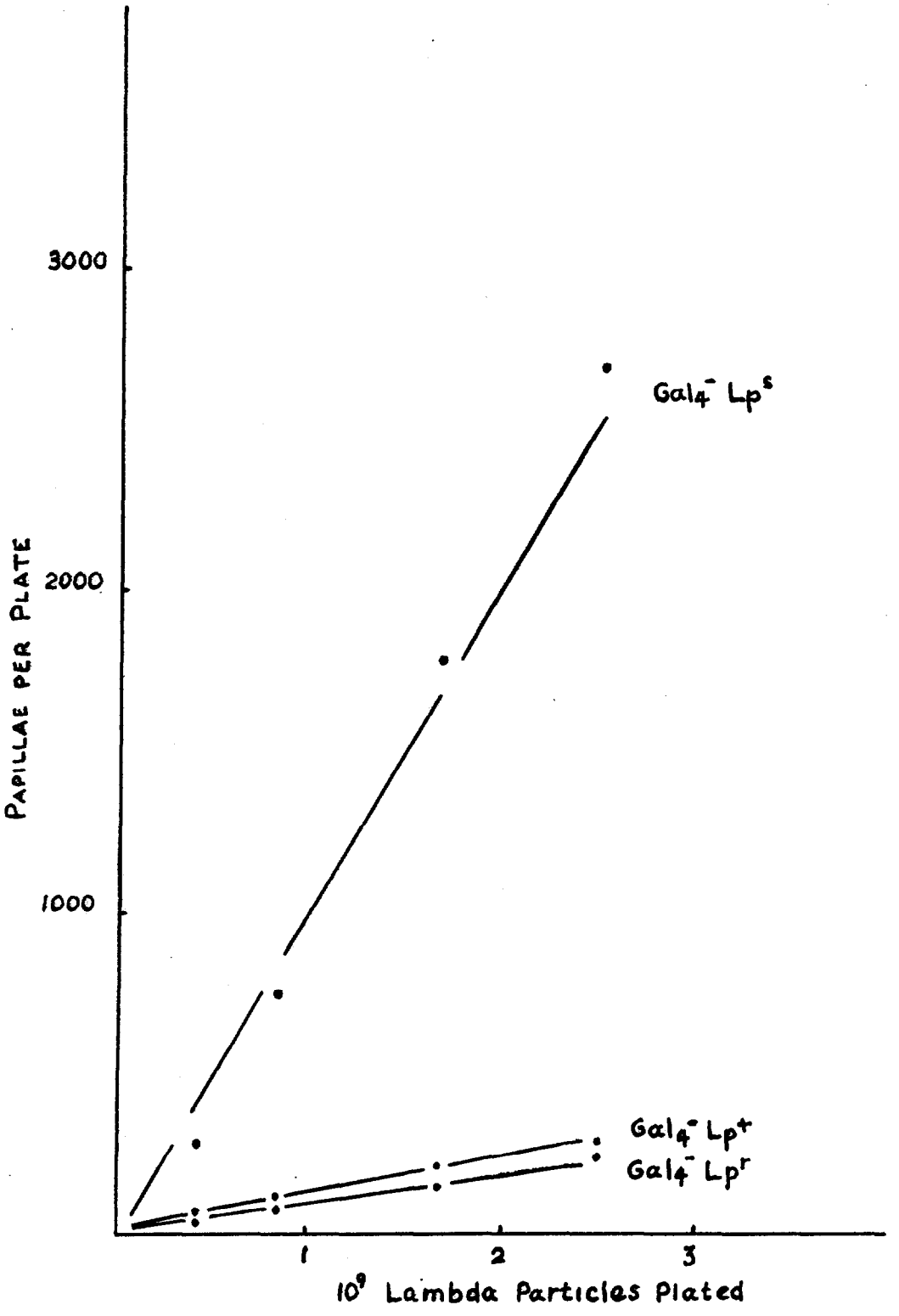
323, Gal_6^+ Gal_1^- Hp^S // Gal_6^- Gal_1^+ Hp^R

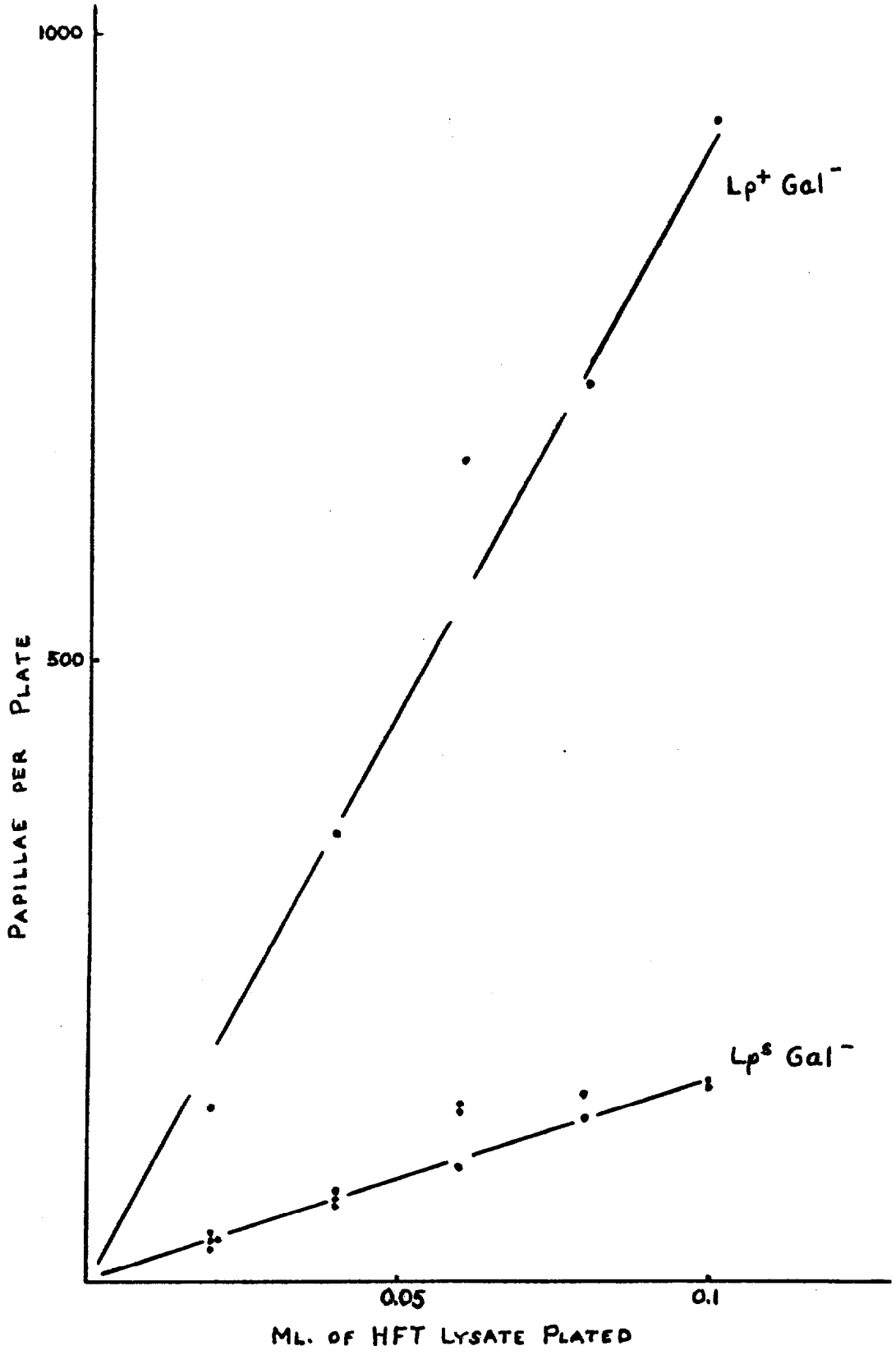
Mtgs. Genetics Soc. Pacific States coll. Sept. 1955

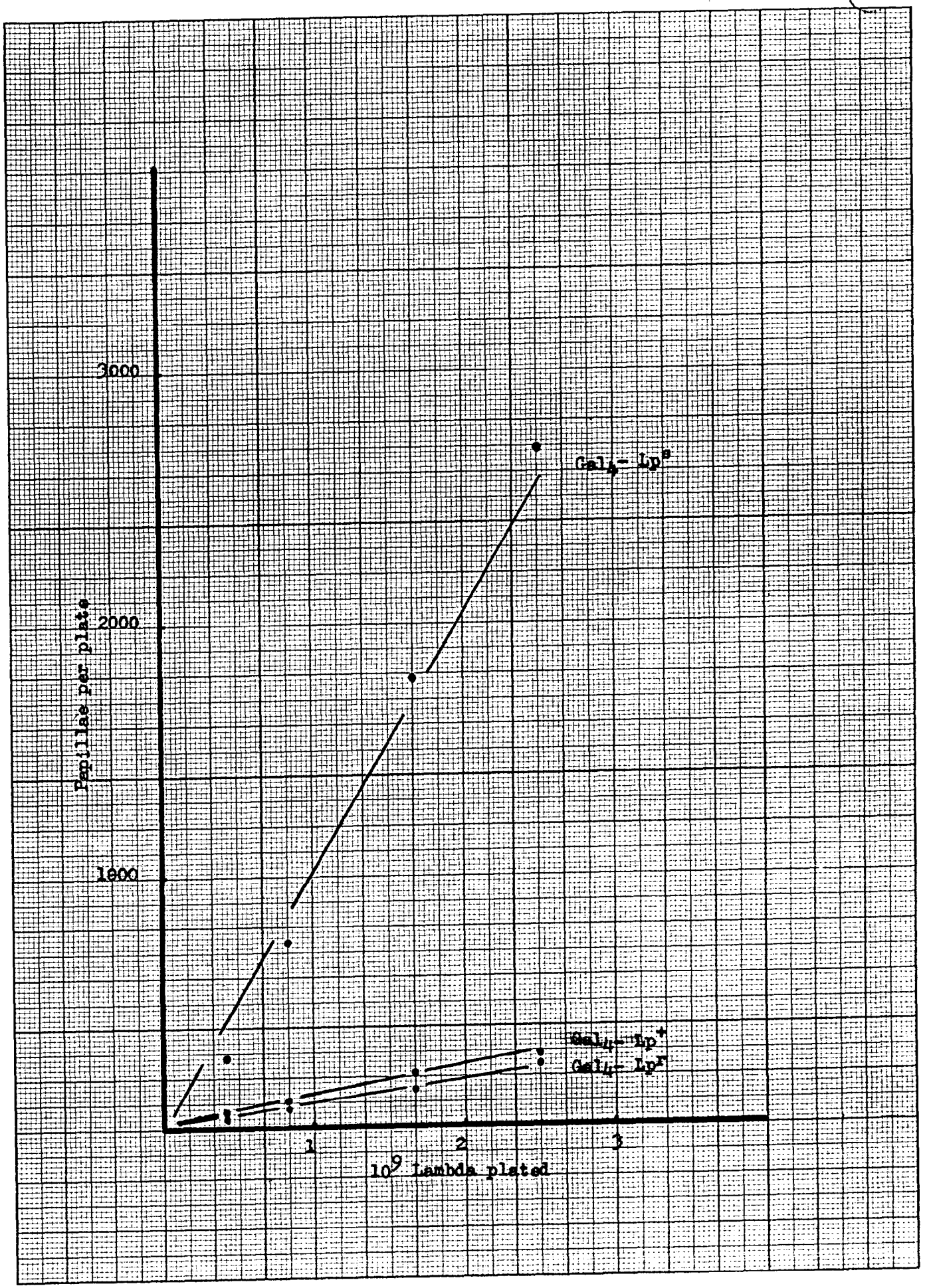
(227)

Cis-trans position effects in transduction heterogenotes of Escherichia coli

—— The phage lambda can transduce a fragment which includes a cluster of genes for galactose fermentation. Most of the transformed clones are "diploid" or heterogenotic for the transduced genes. Many combinations of non-allelic Gal- mutants give galactose positive heterogenotes as readily as Gal+/Gal-. However, some combinations of Gal- gave smaller and delayed yields of positive clones. Further analysis disclosed a cis-trans position effect between certain loci. For example, while the cis ++/— heterogenotes formed by transduction from Gal_1^+ Gal_4^+ to $Gal_1^-Gal_4^-$ are positive, the trans +-/-+ heterogenotes from the transduction from $Gal_1^-Gal_4^+$ to $Gal_1^+Gal_4^-$ are phenotypically galactose negative. In the negative clones, positive heterogenotes are later formed by crossing over in occasional cells. Further segregation results in all possible haploid combinations, +-, -+, ++, and —. The delayed yields that were observed initially are based on these secondary events. Reciprocal transductions have given identical phenotypes, so that in heterogenotes the genes in the fragment are functionally equivalent to the homologous genes in the chromosome. The galactose positive phenotype thus requires that + alleles be in adjacent positions either in the fragment or the chromosome.


3000
2000
1000
PAPILLAE PER PLATE
Gal4⁻ Lp^s
Gal4⁻ Lp^t
Gal4⁻ Lp^r
1
2
3
10⁹ Lambda Particles Plated


1000
500
PAPILLAE PER PLATE
Lp+ Gal-
Lp- Gal-
0.05
0.1
ML. OF HFT LYSATE PLATED



SMG (231)

4. Transduction to Lp^s recipients

It has been stated previously that transductions to Lp^s recipients cells with LFT lambda result in lysogeny of the clone. Nearly all of these lysogenizations are Lp^+, but rarely a clone with Lp^r phenotype results. With HFT lambda there is a higher frequency of the Lp^r type, a result which may only be owing to the lower chances of secondary infection with HFT lysates. Of 58 syngenotes isolated as single colonies, 13 (22 percent) were of Lp^r phenotype. These syngenotes were made with different lysate preparations, and are derived from different homogenotes, and there is no indication, as yet, of an association of Lp^r clone formation with either a locus or a lysate preparation.

The Lp^r clones described previously in this report are carriers of a "defective" prophage (Appleyard, 1954), but plaque-forming lambda, in small quantities, may be obtained from them after irradiation with ultraviolet. The Lp^r clones obtained with HFT lambda have not given lambda after UV treatment, and differ from previously described Lp^r cultures in segregating for Lp, yielding Lp^s. Thus they appear to be syngenotes of the form Gal^- Lp^s //Gal^+ Lp^r. Segregation yields Gal-Lp^s, or Gal^+Lp^s haploid segregants. No non-segregating Lp^r clones have been observed. This last observation suggests that the lambda "defect" in these cases is with lysogenization as well as with production of plaque-forming particles.

SMC

Obvious segregation at Lp was not observed when Gal+ segregated from Lp^s recipients, and it was not possible with these syngenotes to relate the function of the prophage to the genetic material. Lp^r _//Lp^s heterogenotes permit study of this relationship. If the chromosomal fragment is independent of the Lp genotype, Lp^s segregant cultures may be homogenotic. Gal+ reversions of segre ants from Lp^r_//Lp^s syngenotes were examined for their segregational behavior. Under conditions where the reversions test indicated 23/23 Lp^r Gal− segregants to have been homogenotes, 10/11 Lp^s Gal− segregants were found haploid(table 10). Although it is not possible to determine the adequacy of the data, the indication is that the Lp^r allele has a centromeric function, that Lp^+ probably does not, and that the Lp^s allele cannot so function. Failure to obtain segregation of the Lp^+ allele in transductions to Lp^s recipients may only be an indication that the heterogenotes studied are not the primary product of lambda-sensitive cell interaction.

Table 10

Segregational behavior of Gal+ reversions of Lp^s and Lp^r segregants

Experiment	Segregant Phenotype	Segregant Number	Number of Reversions per segregant	Number reversions found segregating
287	Gal_2^- Lp^r	1	8	8
	Gal_2^- Lp^s	1	6	0
292	Gal_2^- Lp^r	12	1	11
	Gal_2^- Lp^s	3	1	0
	Gal_4^- Lp^s	1	1	0
292A	Gal_2^- Lp^r	1	2	2
	Gal_2^- Lp^r	3	1	3
	Gal_2^- Lp^s	5	1	0
298	Gal_2^- Lp^r	5	2	10
323	Gal_6^- Lp^r	1	6	1
	Gal_6^- Lp^s	1	2	0

Heterogenotes. 287; Gal_2^- Lp^s _// Gal_2^+ Lp^r

292,292A,298; $Gal_2^+ Gal_4^-$ Lp^s _// $Gal_2^- Gal_4^+$ Lp^r

323; $Gal_6^+ Gal_1^-$ Lp^s _// $Gal_6^- Gal_1^+$ Lp^r

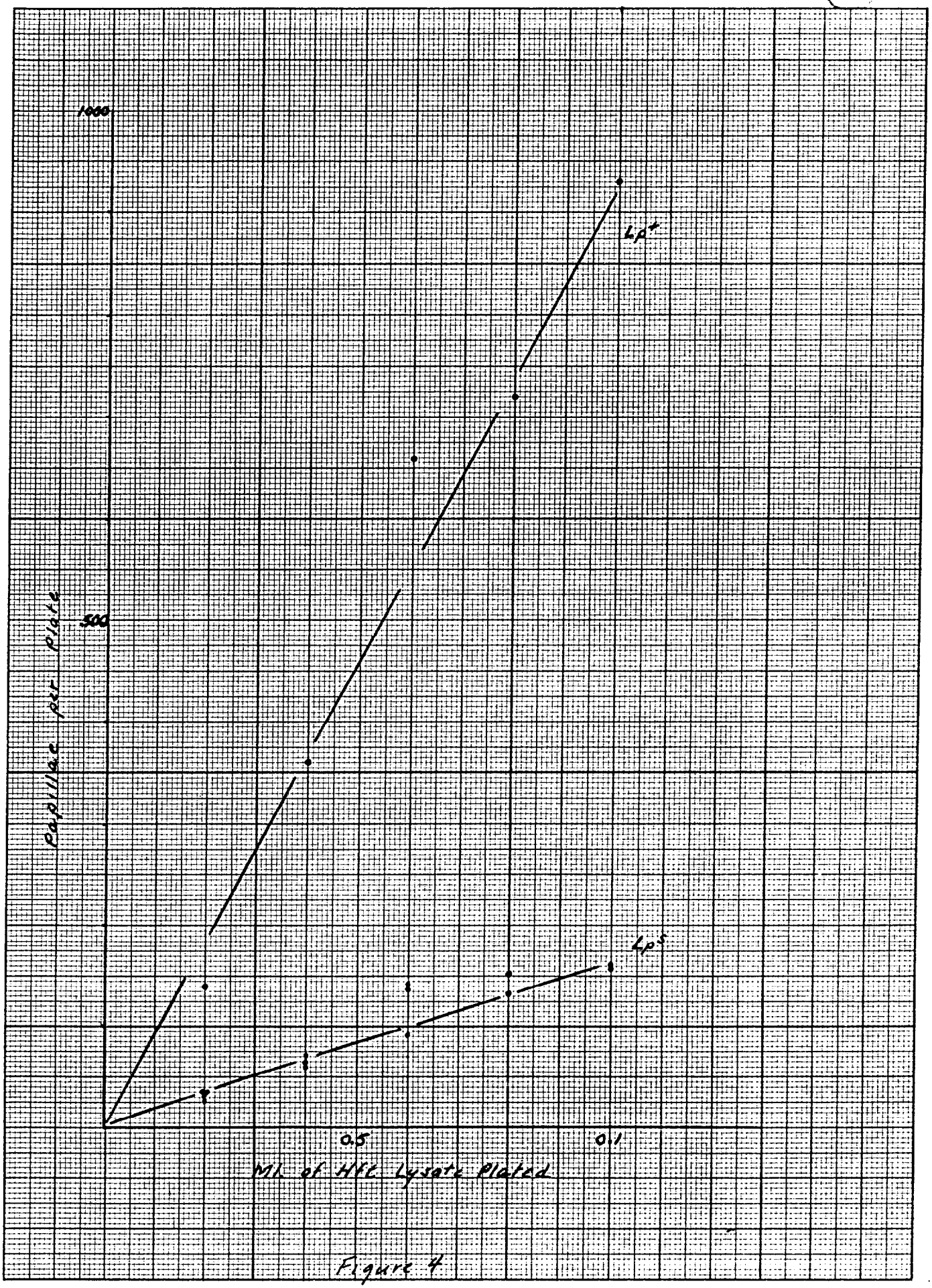
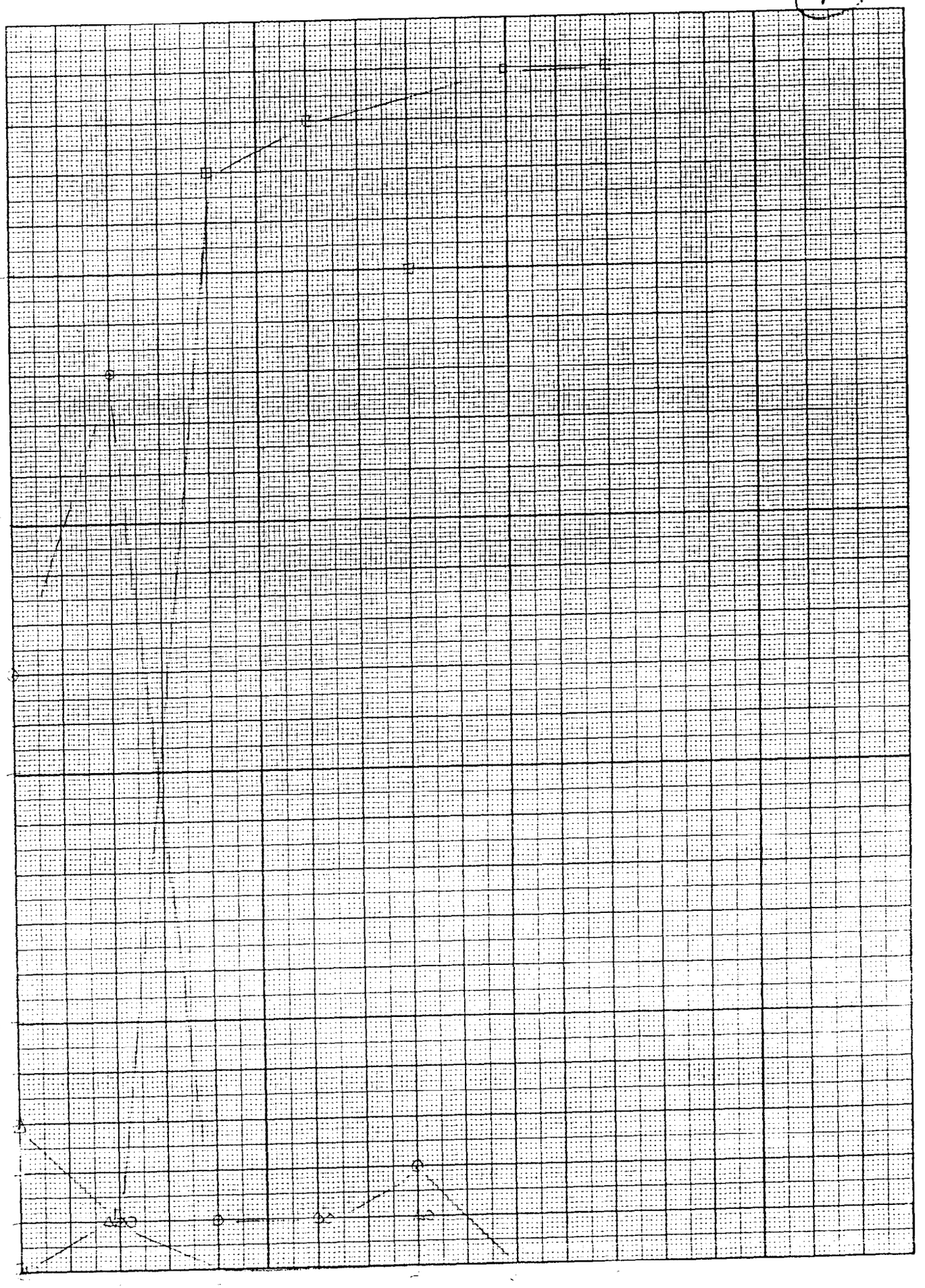
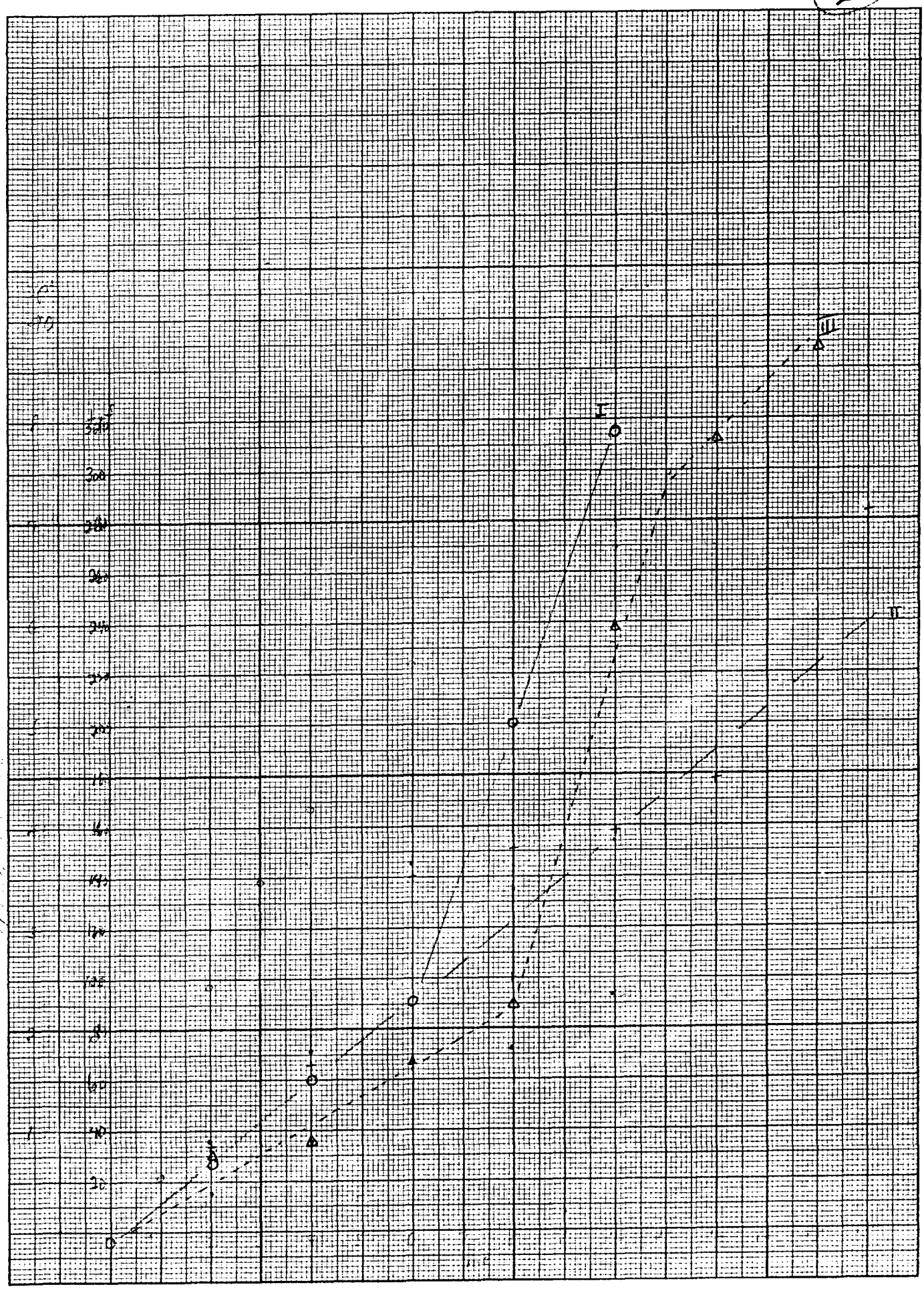


Figure 4





392

285
288
433
327
471

1804/5 = 361

361 / 739 = 2.0
722
170

829
649

1802/2 - 40 = 739

412

103
117
85
113
108
74
94
70
77

9 | 843 = 93

93 / 177 = 1.9
93
840
837

170
183
177

399

27
47
135
100 OD .38
115 % survival = 5.5
176
191
123
96

1018/9 = 112

400

45
34
37
39
40 OD = 0.38 % survival = 39
42
46

283/7 = 40

135
140

112 / 138 / 1.2
112
260

27
24
25
20

96/4 = 24

40 / 24 = 0.6

410

16
14
20
26
5

5 | 81 = 16

10
4
18
28

108/5 = 21

16 / 21 = 1.3
16
50

397

84
101
92
104
121
87

589/6 = 98

66
78
106
78
117

443/5 = 89

OD = 0.35 % survival = 15%

98 / 89.0 = 0.9
882

(1)

238

determ. from B.S. plotting only ↓

Expt	Begin Rise	B. S	Transl. particles Transl. center	Effect length	% Cell destruction
412	100'	ca. 25	1.9	180'	<1.0
410	90'	ca. 17	1.3	180'	<1.0
399	90'	ca. 20	1.2	120'	5%
397	85'	ca. 30	0.9	125'	15%
392	ca. 85'	—	2.0	180'	—
HFT 400	90'	ca 13	0.6	150'	40.

(Time = 5 hours) from Page 354

Crossing over in P.E. heterozygotes.

Recipocal on next page

Het.	Dom	Recip
342E2	6⁻	4⁻

Multiplicity / 0.1 ml = 0.71

Culture with:	No growth	Gal+	Gal+
	3	10	6

Clone size: 7000, — 34

Non-diff. Clones	Opposite clones			
total	(+)	total	Est. rec. prob.	
1406	ca. 1000	ca 3000	—	
ca 3000	4	5144	1.3×10^{-4}	
1544	2	6728	5.3×10^{-5}	
3088	5	2266	4.0×10^{-4}	
ca 150	1	5925	3.0×10^{-5}	
5144	ca 1000	ca 3000	—	
ca 4000				
8728				
2266				
ca 7000				
2245				
748				
34				

clone size 5900.

Using null method $\frac{2\text{-}3}{5900}$ log $\frac{1}{\frac{12}{11}} = 7.0 \times 10^{-5}$

See 354 for recomputation using new clone size

(240)

original see page 354

Crossing over (Time (3.25 hrs.))

Het Donor Recip

4- 6-

Mult. 5.8/0.1 ml

Cultures used.	No. grown	No. Het	Het
	0	17	3

Von dey. clones	Dey. Clones	(+)	Total	Estimate Freq. Het.
2000				
660		2	1004	4×10^{-4}
688		1	185	
640		1	1249	1.4×10^{-4}
392				
640	Using null method, clone size 1200			
728	$a = \frac{2.3}{1200} \log \frac{1}{\frac{17}{20}} = 2.6 \times 10^{-4}$			
644				
1192				
496	Total cells = $\frac{16778}{20} = 838$/clone			
664				
1096	$a = \frac{2.3}{838} \log \frac{1}{\frac{17}{20}} = \frac{(2.3) \log 1.2}{8.38 \times 10^2} = \frac{(2.3)(0.08)}{8.38 \times 10^2}$			
592				
720				
1216	$= \frac{0.18}{8.38 \times 10^2} = \frac{1.8 \times 10^{-1}}{8.38 \times 10^2}$			
680				
496	(2.2×10^{-4})			

$a = 0.602r/N \log N$

241

These experiments indicate washing technique not adequate

368-1 4⁻ −x 1−

multiplicity = 0.45/sample of 0.01⁻ Elapsed time = 4.66 hours.

Samples used: No growth | No. Gal+ 3 | Gal+ 1

Dipiricic cultures. Ratio +/− / total = 1 / 7 / 265

	Gal+	Gal−	Total
(washed)	1	345	858
	0	ca 300	ca 600
	0	ca 300	ca 600
	0	549	549.

Prob left 376 or 1/

$a = 0.602(1)/858 \log 858$

$= 0.602/294(858)$

$= 0.602/2520$

$\frac{6.02\times10^{-1}}{2.52\times10^{-3}}$

$= 2.4\times10^{-4}$

$a = \frac{2.3}{600} \log \frac{1}{3/4}$

$a = 0.38 \log 1.33$ 4.7×10^{-2}

$a = 0.38\,(0.124) = 0.047$

368-2 mult. = 0.34 ratio 10⁺ / 3 / 201

No growth	No Gal+	Gal+	Gal+	Gal−	Total
4	5	1	346	0	ca 500
			0	8	794
			0	1	379
			0	ca 300	ca 600
			0	0	208

per 376

$a = \frac{2.3}{600} \log \frac{1}{5/6}$

$a = 0.38 \log 1.2$

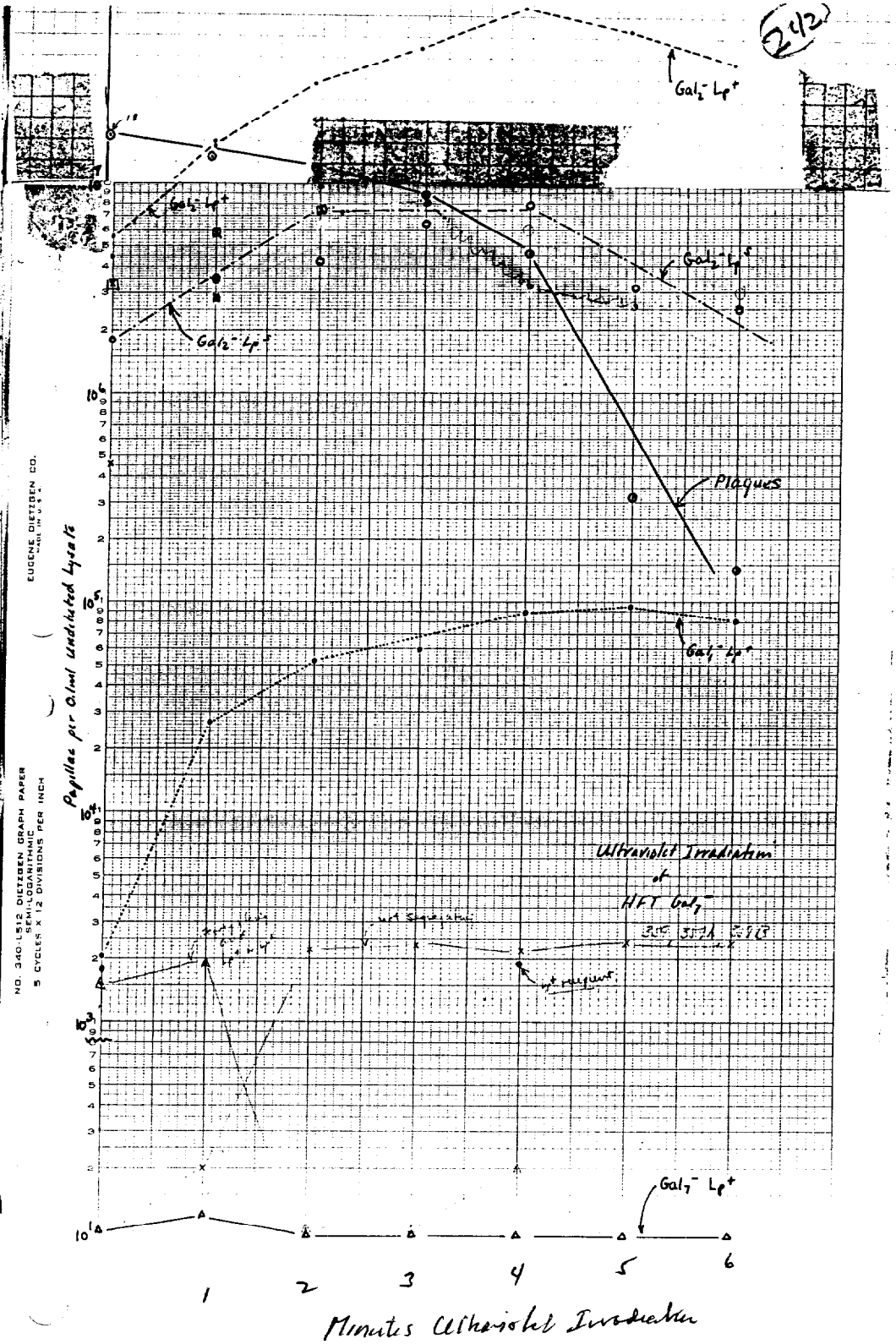
$= 0.38\,(0.079) = 0.030$

$= 3.0\times10^{-2}$

Because of the failure of the washing method in the previous experiments, incubation on Bgal attempted, with respreading. Inc. 5.5 hours.

368-1 mult.= ratio +/− / total = 0/27/173

No growth	No Gal+	Gal+	Gal+	Gal−	Total
1	6	0	0	6	42
			6	5	314
			0	2	71
			0	226	229
			0	0	2
			0	0	9
			0	0	51


Gal2- Lp+
Gal2- Lp+
Gal2- Lp+
Gal2- Lp+
Plaques
Gal7- Lp+
Ultraviolet Irradiation of HFT Gal7-
Gal7- Lp+
Minutes Ultraviolet Irradiation
Papillae per 0.1ml Undiluted Lysate
EUGENE DIETZGEN CO.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH

Defective Heterogenote Number	Gal⁻ Segregants	
	Lambda Sensitive	Lambda Defective
292	24	36
323	4	2
331	6	0
336	12	0
343	5	1
346	5	1
365	20	1
368	3	0
374	9	0
382	14	1
415A	16	1
420	16	2
420A	9	4
Totals	143	49

Table 1

Expt. 316 2/1/54

Procedure: Ultraviolet irradiation of HFT 2⁻, lysate diluted 1-100 in D(M), 0.1 ml. sample removed and added to 10 ml. Penassay. HFT 2⁻ stock = 241-14, mql- derivative. Distance from lamp, 50 cm.

UV Dose in Seconds

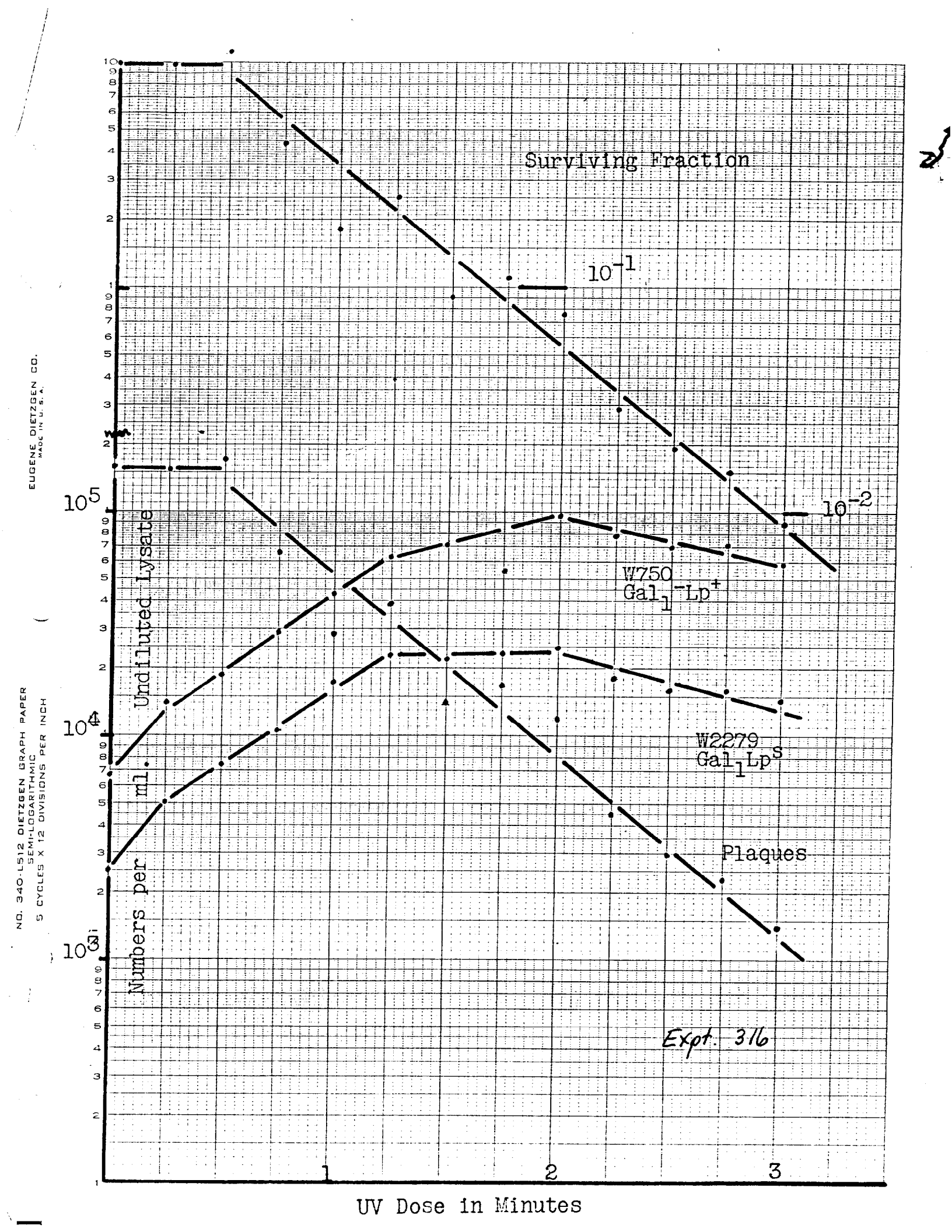
Trnsd. x 10^3 on:	0	15	30	45	60	75	90	105	120	135	150	165	180
$Gal_1^-lp^S$ W2279	2.5	5.1	7.5	10.5	17.3	22.7	21.8	23.4	24.9	18.1	16.0	16.0	14.5
$Gal_1^-lp^+$ W750	6.7	14.0	18.4	29.3	43.4	63.8	71.1	53.6	95.8	79.1	69.9	71.7	58.9
Plaques x 10^3	155	151	172	66	28	39	14	17	12	4.5	3.0	2.3	1.4
Fraction Surviving	1.0	0.97	1.1	0.43	0.18	0.25	0.09	0.11	0.077	0.029	0.019	0.015	0.009

Table 2

Analysis of Transduction on $Gal_1^-Lp^S$ Recipient

UV Dose in Seconds

	0	15	30	45	60	75	90	120 to 180
No. of Trnsd. Tested	1	18	18	18	18	18	18	18
No. Seg.	0	6	1	0	I	1	1	0
% Seg.	0	33	6.0	0	6	6	6	0
Lp Gene Types of Segregating Gal+								
Lp^S	-	-	-	-	-	-	-	-
Lp^+	-	-	-	-	-	-	-	-
Lp^R	-	6	1	-	R	R	R	-
Lp Gene Types of Non-Segregating Gal+								
Lp^S	-	7	14	17	16	16	17	-
Lp^+	-	2	0	0	0	1	0	-
Lp^R	-	3	2	1	1	0	0	-
% Lp^S	-	58	88	94	94	94	100	-


Surviving Fraction
10^{-1}
10^{-2}
W750
$Gal_1^-Lp^+$
W2279
Gal_1Lp^s
Plaques
Expt. 316
Numbers per ml. Undiluted Lysate
10^5
10^4
10^3
1
2
3
UV Dose in Minutes
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH

?

Lysate diluted D(M) 1-100 → 0.1 ml samples to broth
~~10 ml samples~~

246

(229) (750)

Page 316 - Diluted HFT lysate (241-14 mal-) → x Gal⁻ Lp^S, Gal⁻ Lp^+

Spot test	Dose	0	15	30	45	60	75	90	105	120	135	150	165	180
0.1 ml of 1-10 dilution →	Lp^S	25	51	75	105	173	227	218	234	249	181	160	160	145
	x10³ titer/ml UV irr.	25	5.1	7.5	10.5	17.3	22.7	22.8	23.4	24.9	18.1	16.0	16.0	14.5
	Recovery		2.6	5.0	8.0	14.8	20.2	19.2	20.9	22.8	15.6	13.8	15.5	12.0
0.1 ml of 1-100 dil →	x10³ (Lp⁻)	1	15	22	25	22	19	24	37	31	2.0	—	—	—
			14	21	24	21	18	23	32	30	1.0			
	Plaques x10³	155	151	172	66	28	39	14	17	12	4.5	3.0	2.3	1.4
		1.5x10⁵ · 100 · 100 = 1.5x10⁹ plaques/ml												
		6.9x10⁸ · 15% = 6.7x10⁷ Bound Lp⁻												
	No. papillae 40 pick off spot	1	18	18	18	18	18	18	18	18	18	18	18	18
	Exp =	—	33%	6.0%	0	6.0	6.0	6.0	0	0	0	0	0	0
	No. stable	1	12	17	18	17	17	17	18	17	18	18	18	18
	Gal stable prototype	R	24 75 3R	145 2R	175 1R	165 1R	165	175						
	% = 0		0.58	0.875	0.94	0.94	94	100						
Lp^S														
~ x10³ Lp^+	6.7	14.0	18.4	29.3	43.4	63.8	71.1	53.6	95.8	79.1	69.9	71.7	58.9	
	0	7.3	11.7	22.6	36.7	57.1	64.4	46.9	89.1	72.4	63.2	66.0	52.2	

Ignore?

0 15 30 45 60 75 90 105 120

247

Raw data

HFT 7^s undiluted lysate.

Technic. ~~dilute 1-10 with D(?), UV and 0.1 ml added to 10 ml~~

min UV

Bact.	Dilution	0	1	2	3	4	5	6
Transd. Assay /2580	×10⁷ 2×10⁵	4.7 235	14.0 707	—	—	—	—	—
/2790	×10⁷ 2×10⁵	0.46 23	3.5 176	4.2 213	4.5 227	—	3.2 161	2.7 134
purified cult. → /2790 (prewarmed plates)	×10⁷ 1×10⁵	1.8 189	2.8 280	9.8 977	—	—	—	—
/2915	×10⁷ 1×10⁵	3.3 330	5.9 588	6.5 653	—	—	—	—
/750	×10⁵ 1×10³	0.2 21	2.7 273 ~~252~~	5.2 517 ~~496~~	5.4 573 ~~572~~	~~8.8~~ ~~883~~ 864	9.5 950 ~~9~~	8.1 809
/2307	1-20	5	6	-2	-2	2	3	3
Assay /2580	×10⁷ 1×10⁶	4.4 44	18.3 183	34.7 347	54.5 545	68.9 689	53.2 532	38.1 381
/2341	×10⁷ 1×10⁶	3.5 35	—	—	—	25.3 253	—	—

Ave 4p: 5.6/3 = 1.8   12.2/3 = 4.1   20.5/3 = 6.8

		0	1	2	3	4	5	6
Plaques /2790	×10⁷ 2×10⁵	500+	500+	11.7 584	8.7 436	4.7 234	0.32 16	0.14 7
prewarmed plates, purif. cult. /2790		—	—	ca 1000				
/2915	×10⁷ 10⁵	16.5 1650	11.8 1176	6.5 653				

Testing ~~Segregation~~

	0	1	2	3	4	5	6
No. sampled	24	24	23	24	24	24	24
/2790	9 Sens.-ns	3s ns	22 all ns	22s 22 ns	21s-21ns	24s-ns	24s-ns
	3+ s	2+ <1 ns, 1 s	0	0	1 - ns	0	0
	12r s	19r < 1ns, 18s	1r-seg	2 < 1ns, 1s	2 < 1ns, 1seg	0	0
Spont. present	9.6 s	1.7	1.4	—	—	—	—

The streaks of the last two doses / λ were restreak on B gal. No evidence of seg suggesting that addition of λ does not restore seg. behavior

/2915	0	1	2	
No.	36	34	47	Richech (?) this lost by add. Streaks stunned
	0 s	0	38s 38 ns	38 ns
	8+ < 2 ns, 6 s	2 - 5 seg	0	—
	28r < 0 ns, 28 s	32r < 31s, 1 ns	9r 9s	9 < seg 8, ns 1

Total over

Testing Transd	0'	4'
Picked	24	24
/2580 Lp+	24	24
Seg	18	19
NS	6	5
Sp. reversions	9.8	1

on retest not weak
10 single colonies of it
tested found none Lp organism

4' dose 2580

5a = 7endo
5b = 6endo
2c = 6endo 1amphi

	Nature of Segregants	Endo Lp+	Endo LpR	Exo Lp+	Exo LpR	Amphi Lp+	Amphi LpR
→ x	Transd. 1	3	0	4 1wk	0	0	0
c	2	6	0	0	0	1	0
→ x	3.	5 1wk	0	0	0	0	0
a	4.	7	0	0	0	0	0
a	5.	7	0	0	0	0	0
b	6.	6	0	0	0	0	0
b	7.	6	0	0	0	0	0
a	8.	7	0	0	0	0	0
b	9.	6	0	0	0	0	0
x	10.	6	1	0	0	0	0
→ x	11	5 1wk	0	1	0	0	0
→ b	12	6 1wk	0	0	0	0	0
c	13.	6	0	1	0	0	0
a	14.	7	0	0	0	0	0
d	15.	5	0	0	0	0	0
b	16.	6	0	0	0	0	0
c	17.	6	0	0	0	1	0
a	18.	7	0	0	0	0	0

Exo 10f

Summary - Analysis of Transductions

Dose	0	1	2	3	4	5	6
$CaCl_2$ – Lp^s Recip. %							
Lp^+	22 ~~216~~	7 ~~4~~	0	0	4	0	0
Lp^R	78 ~~784~~	93 ~~88~~	14	8	8	0	0
Lp^s	0	2 ~~4~~	86	92	88	100	100
$Lp^+ + Lp^R$	100	98	14	8	12	0	0
% seq.	94 ~~49~~	93 ~~52~~	14 ~~42~~	4	9	0	0
% not seq.	6 ~~51~~	7 ~~46~~	86 ~~60~~	96	91	100	100

249

Table 1

Expt. 316 2/1/54

Procedure: Ultraviolet irradiation of HFT 2⁻, lysate diluted 1-100 in D(M), 0.1ml samples removed and added to 10ml Penassay. HFT 2⁻ stock = 241-14, mal- derivative. Distance from lamp, 50 cm.

Trnsd. Dose x 10³ on:	UV Dose in Seconds 0	15	30	45	60	75	90	105	120	135	150	165	180
Gal⁻ Lp⁵ W2274	2.5	5.1	7.5	10.5	17.3	22.7	21.8	23.4	24.9	18.1	16.0	16.0	14.5
Gal⁻ Lp⁺ W750	6.7	14.0	18.4	29.3	43.4	63.8	71.1	53.6	95.8	79.1	69.9	71.7	58.9
Plaques x10³	155	181	172	66	28	39	14	17	12	4.5	3.0	2.3	1.4
Fraction Surviving	1.0	0.97	1.1	0.43	0.18	0.25	0.09	0.11	0.077	0.029	0.019	0.015	0.009

Table 2

Analysis of Transductions on Gal⁻ Lp⁵ Recipient.

No. of Trnsd. tested.	UV Dose in Seconds 0	15	30	45	60	75	90	180 to 180
	1	18	18	18	18	18	18	18
No. Seg.*	0	6	1	0	1	1	1	0
% Seg	0	33	6.0	0	6	6	6	0
Lp Genotypes of Segregating Gal+								
Lp⁵	—	—	—	—	—	—	—	—
Lp⁺	—	—	—	—	—	—	—	—
Lp^R	—	6	1	—	1	1	1	—
Lp Genotypes of Non Segregating Gal+								
Lp⁵	—	7	14	17	16	16	17	—
Lp⁺	—	2	0	0	0	1	0	—
Lp^R	—	3	2	1	1	0	0	—
% Lp⁵	—	58	88	94	94	94	100	—

Table 1

Expt. 359-359A-359B | 8/17/55

Procedure: Ultraviolet irradiation of HFT 7⁻, undiluted lysate in Penassay. Distance = 50 cm. Irradiation in petri dish, 15 ml volume, 1.0 ml samples removed at varying times. HFT 7 stock = W3067

Assays ① Plaques on B gel on W2915, W2790

Dose →	0	1	2	3	4	5	6
10^7 plaques /ml lysate	16.5	11.8	9.1	8.7	5.7	0.32	0.14
Fraction surviving	1.0	0.72	0.55	0.53	0.35	0.019	0.009

② Transduction on B gal vs. the following cultures

Recipient Culture		Dilution	0	1	2	3	4	5	6
$Gal_2^-Lp^+$ W2580	(1)	10^7	4.7	14.0	—	—	—	—	—
	(2)	10^7	4.4	18.3	34.7	54.5	68.9	53.2	38.1
$Gal_2^-Lp^s$ W2915		10^7	3.3	5.9	6.5	—	—	—	—
W2790	(1)	10^7	1.8	2.8	9.8	—	—	—	—
	(2)	10^7	0.46	3.5	4.2	4.5	—	3.2	2.7
$Gal_2^-Lp^{R/S}$ W2341		10^7	3.5	—	—	—	25.3	—	—
$Gal_1^-Lp^+$ W780		10^5	0.2	2.7	5.2	5.9	8.9	9.5	8.1
$Gal_7^-Lp^+$ W2307		1-20	5	6	-2Ⓐ	-2	2	3	3

Ⓐ All values given have been corrected for spontaneous reversions of the indicator culture. In the assays on W2307 figures given are papillae on lysate addition plate − spontaneous reversions papillae. None of these papillae were checked for Galactose stability.

Table 3

(B) Transduction to Gal_2^- Lp^s Recipients. Total

Dose →	0	1	2	3	4	5	6
% Lp^+	22	7	0	0	4	0	6
% Lp^R	78	91	14	8	8	0	0
% Lp^S	0	2	86	92	88	100	100
% $Lp^+ + Lp^R$	100	98	14	8	12	0	0

Table 4

(A) Transduction to Gal_2^- Lp^+ Recipient. W2580

	UV. Dose (min) 0	4
Number tested	24	24
No. Lp^+	24	24
No. seg.	18	19
No. Not. Seg	6	5
No. Sp. Rec. in Sample	9.8	1

(B) Analysis of the transductions produced with lambda irradiated 4 minutes, survival = 2.0×10^{-2}. 18 different transductions analyzed, about 7 segregants from each tested for Lp genotype and Gal allele.

Number of transductions with the following seg. pattern	Endogenotic Lp^+	Endogenotic Lp^R	Exogenotic Lp^+	Exogenotic Lp^R	Amphitypic Lp^+	Amphitypic Lp^R
~~5~~	7	0	0	0	0	0
~~4~~	6	0	0	0	0	0
2	6	0	0	0	1	0
1	5	0	0	0	0	0
1	6	0	1	0	0	0
1	3	0	1 *	0	0	0
1	5 W	0	0	0	0	0
1	6	1 **	0	0	0	0
1	5 W	0	1	0	0	0
1	6 W	0	0	0	0	0
18						

W = one of these segregants gave slight lysis of Lp^s tester

* This seg. gave slight lysis of Lp^s tester. Streaked out and 10 colonies retested. All found Lp^R

were performed on a pure colony from the 1st streak away from the transduction plate, which was also streaked on B gal to observe segregation for galactose fermentation.

Table 2

Expt 359 - 359A - 359B | 5/17/55

25

Analysis of the transductions formed with UV'd lysate. At this time a number of spontaneous reversions of the indicators were examined and found stable for galactose fermentation and unchanged for lambda reaction. Lp genotypes were determined from tests against both lambda + a lambda sensitive culture. Those tests

(A) Transductions to Gal_2^- Lp^s W2790 . (s = segregating, ns = not segregating)

UV Dose →	0	1	2	3	4	5	6
No. transd. examined →	24	24	23	24	24	24	24
No: Lp^s →	9 ns	3 ns	22 ns	22 ns	21 ns	24 ns	24 ns
" Lp^+ →	(1) 3 s	1 ns 1 s	0	0	1 ns	0	0
" Lp^R →	12 s	18 s 1 ns	1 s	1 s 1 ns	1 s 1 ns	0	0
Spontaneous reversions present in sample	9.6	1.7	1.4	negligible →			

(2) Transductions to Gal_2^- Lp^s W2915

UV Dose	0	1	2	3	4	5	6
No. examined →	36	34	47				
No. Lp^s	0	38 ns	38 ns.				
" Lp^+	6 s 2 ns	2 s	0				
" Lp^R	28 s	31 s 1 ns	8 s 1 ns.				

Table 3

SEPARATE PAGE

(A) Totals for Gal_2^- Lp^s Recipients

UV Dose	0	1	2	3	4	5	6
Segregating	49	52	10	1	2	0	0
not segregating	2	4	60	23	22	24	24
% Seg	94	93	14	4	9	0	0
% not seg.	6	7	86	96	91	100	100

M.L. Morse

Progress Report
4/54

253

Introduction

The transfer of genetic material between bacterial cells by temperate bacteriophages has been shown for certain Salmonella and for Corynebacterium diphtheriae. In each of these cases the transduction of genetic factors singly has been demonstrated. This mechanism of genetic recombination is in contrast with the complete sexual mechanism of recombination in which the whole genetic material of the cell participates at one time. The study of these two mechanisms and their interrelationship is difficult in biological systems in which only one has been found to operate. The present report summarizes a study of E. coli K-12 where the independent occurrence of sexual recombination (Tatum and Lederberg, 1947) and transductive recombination has been demonstrated.