Year End Report - M.L. Morse January 1953

The Galactose transduction in *E. coli* K-12

1. The frequency of transduction among the various alleles.

The frequency (as of this time) of transduction of the various negative strains is shown in the following table

NUMBER OF LAMBDA PARTICLES X10^6 REQUIRED FOR ONE TRANSDUCTION

	Lysate				
Cells	gal_1^-	gal_2^-	gal_3^-	gal_4^-	K-12
gal_1^-	—	36	8.7	25, 31	2.9, 3.3, 3.3, 3.6, 5.5, 7.6
gal_2^-	6.3, 3.6	—	0.65, 0.26	c. 3.5, 2.2	4.2, 5.8, 9.3
gal_3^-	0.33, 3.3	3.1, 4.9	—	4.0	1.6, 1.7, 2.6
gal_4^-					
W518 λ^S	not observed yet	0.12, 0.40	1.2	—	0.13, 0.13, 0.23, 0.5
W811 λ^t	66	4.3, 4.9	8.5	—	2.0, 3.8, 4.0, 6.6, 9.5, 10, 17, 17
W1924 λ^R	—	—	—	—	11, 14, 25, 26

Although the values are all about 10^6-10^7, there appear to be some differences. For example, among the gal_4^-, the order of transducibility by K-12 appears to be W518 > W811 > W1924. Also gal_1^- - gal_4^- do not readily transduce each other, but gal_1^- appears not to be readily transduced by gal_2^- as well.

2. Further observations on the agent affecting transduction

Some additional evidence has been obtained. Adsorption experiments (2) with W518, and 1C-12 lysates have been performed. Both lambda and the gal agent were followed through several repeated adsorptions. In general there was fair agreement between the amount of lambda remaining in the supernatant after adsorption and the number of gal+ transductions remaining. Total recovery was always too high, sediment transductions + supernatant transductions > total transductions added. This is probably caused by low assay of the number of transductions added. W518 has shown an odd relationship of number of transductions in a unit quantity of lysate as a function of the number of cells added. This is shown in the attached figure. Presumably, the adsorption tube assay method will be necessary for all further quantitative work.

A complete set of bp_2^R forms of the various gal- has been examined for transducibility and found non-transducible. Adsorption experiments with these cultures have not been performed as yet to see at which stage the process is stopped. It is presumed to be at the adsorption of phage stage but this is not known from experimental observation. In connection with adsorption it should be noted that lambda star (λ^*) which is presumably adsorbed by W518 but not able to infect W518, has not shown any transducing ability. on W518

The "transducing agent" and lambda have similar heat sensitivities, both being moderately sensitive. This unfortunately eliminates the likelihood of using pasteurized lysates to avoid filtration losses. A comparison is shown in the following

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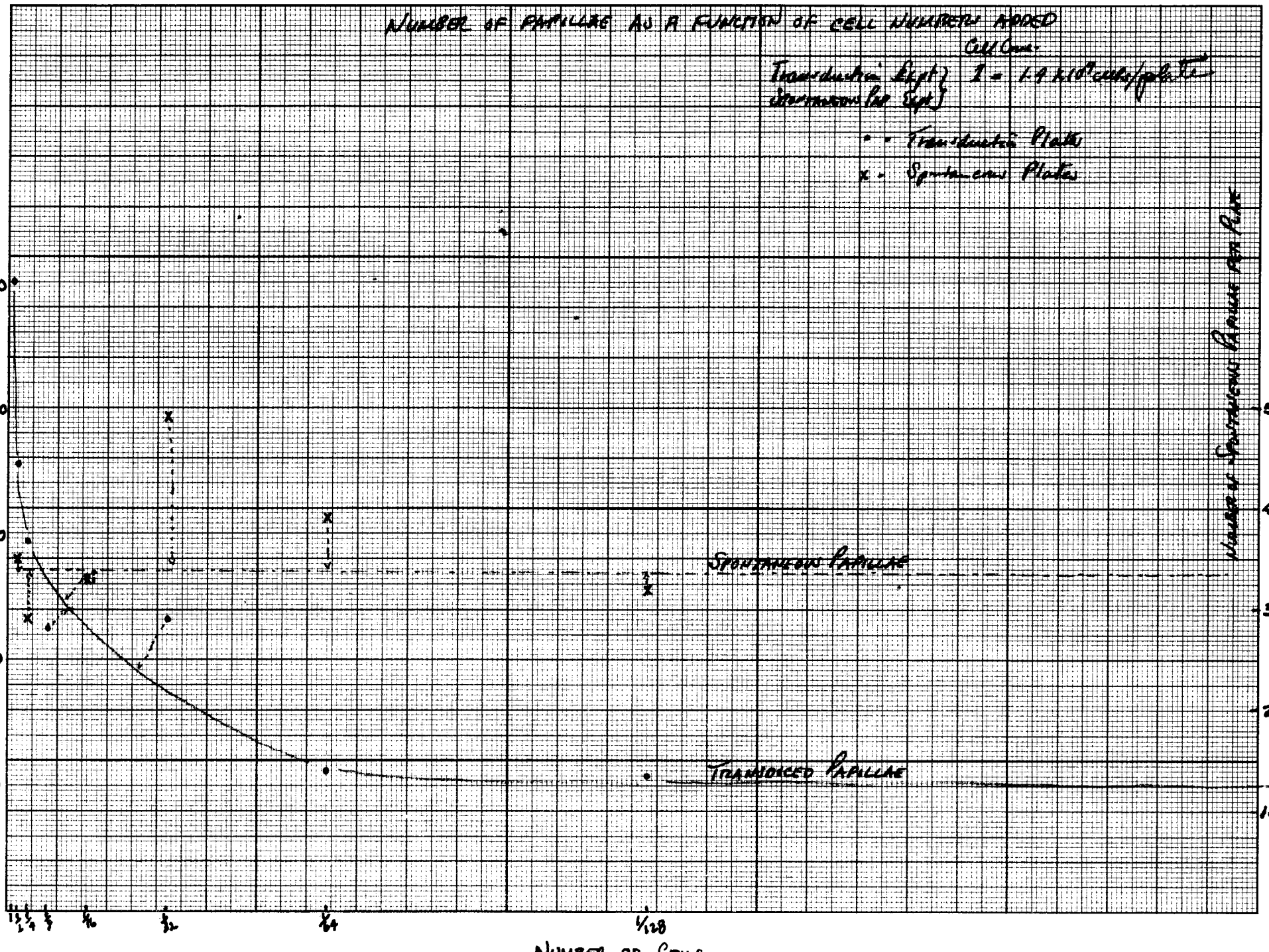

Table of the heat sensitivity of Lambda and the transducing agent (from IL-12)

SURVIVAL AFTER HEAT EXPOSURE

OF 56C FOR 30 MINUTES

	LAMBDA	TRANSDUCING AGENT
FRACT. SURVIVING	0.099	0.086
	0.11	

Phage and transduction titers of lysates appear to be little affected by exposure to DNAase even when there is gross evidence of DNAase action. Many lysates are viscous, but lose their viscosity on standing. The rapid change in viscosity brought about by exposure to DNAase is evidence that the viscosity is largely due to DNA. Slight change in titer of both phage and transduction, (with the direction of higher titers) can be expected because of the increased recovery of viscous lysates from the first dilution. The effect of DNAase (or both thereof) is as follows:

	Lysate	
	IL-12 (25')	811 gal+ #5 -1
Untreated*		
λ/ml	—	6.11×10^9
transd/ml	9.6×10^3	2.0×10^3
Treated**		
λ/ml	—	6.0×10^9
transd/ml	10×10^3	3.0×10^3

* viscous lysate ** non viscous after DNAase treatment

The influence of ultraviolet irradiation has been reported previously. A consideration of the data on the action of ultraviolet irradiated K-12 lysates on W518, (obtained from several experiments performed for different reasons), suggests that the stimulatory effect of u.v. may apply to lysogenic cultures and not to phage sensitive cultures.. Since the amount of information available is small, the whole matter must be reconsidered. See the attached figure.

The use of lysates of galactose + reversions could aid in the understanding of this transduction. The phenotypic reversions to gal+ are divided at least into two groups, one, the true reversing the mutant locus to the wild type form, and the second group of "minor" or "suppressor" mutations affecting genes which can be distant from the original gal- gene. The first group should have the ability to transduce the mutant locus from which it reverted. The second group should not have this ability. The identification of the reversions as to the group in which they fall can be accomplished by either crossing tests or in some instances by reversibility of the cells on E.M.B lactose. Ten spontaneous galactose + mutants of W811 were selected to exploit this possibility. The results are shown in the following table

	FRACTION OF GAL+ PROTOTROPHS IN CROSSES WITH Y-10	NUMBER PAPILLAE / COLONY (APPROX) ON EMB LACTOSE	ABILITY TO TRANSDUCE GAL+
1.	0/1986	<1	no
2.	0/1879	<1	no
3.	–	<1	–
4.	–	c. 5	–
5.	–	5-10	yes
6.	0/976	<1	–
7.	0/615	<1	–
8.	–	5-10	yes
9.	0/762	1	–
10.	0/563	<1	–

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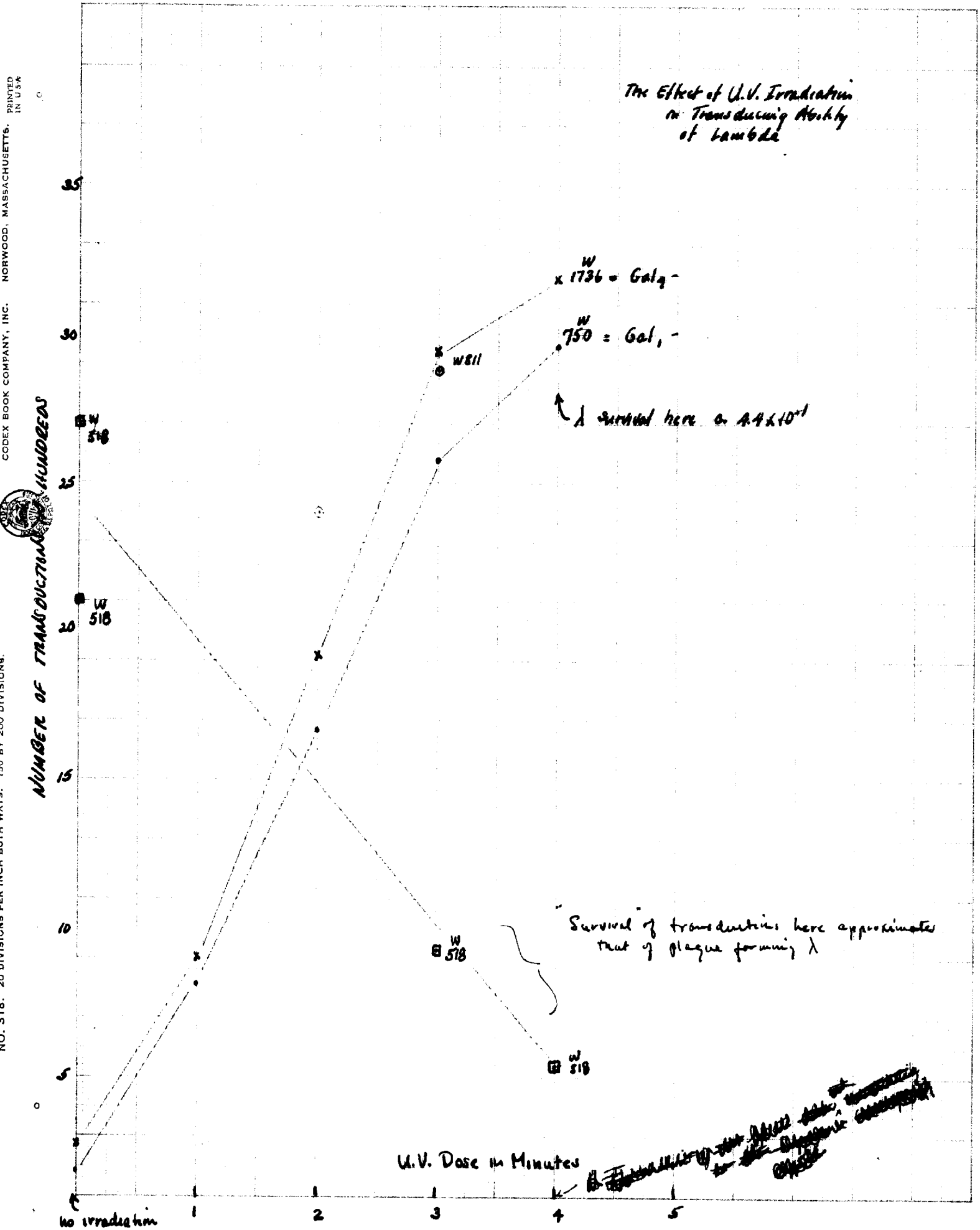

Although the reversions are divided into at least two groups as far as their ability to transduce gal$_4$- is concerned, and the strains with transductive power are the strains which papillate on EMB lactose, indicating that they are true reversions, it has not been possible to show by crossing tests that the non-papillating and non-transducing strains are not true reversants.

In connection with the transduction of gal$_4$- by reversion strains it should be noted that in the instances where the transduced cells were examined it was found that the majority (7/8, 8/8) of transductions had resulted in stable gal+ cells. The stability of other loci transduced by these reversions has not been examined.

3. The stability of transduced cells.

It is difficult to estimate the stability of various transductions because of the number of variables involved. In cases of almost qualitative differences it is possible, however. Some observations on the stability of the various transductions are presented in the following table.

Cells	gal$_1$-	gal$_2$- Lysate	gal$_3$-	gal$_4$-	K-12
gal$_1$-	—	some unstable after 4 isol.	?	some unstable after 4 isol.	7/10 unstable after 4 isol
gal$_2$-	3/8 unstable after 3 isol	—	1/8 unstable after 3 isol.	5/8 unstable after 3 isol	4/32 unstable after 3 isol.
gal$_3$-	8/8 pure + after 3 isol.	8/8 pure + after 3 isol.	—	2/8 unstable after 3 isol.	10/10 pure + after 3 isol
gal$_4$-				—	
W1436	1/6 ? weak + after 2 isol	6/8 unstable after 3 isol.	3/6 unstable after 3 isol.	—	6/10 unstable after 4 isol.
W518	6/6 unstable after 1	8/8 unstable after 3 isol.	3/6 unstable after 3 isol.	—	6/10 unstable after 4 isol.
W811	?	?	?	—	18/32 unstable after 3 isol.
W1124					

The most noteworthy point is that in three of four cases

of transduction of gal_3^- there is a high incidence of stable gal+ in the gal+ cells induced. It should be noted in this connection that the ratio of papillae on transduction plates to papillae on control plates was sufficiently high to make it unlikely that the sample examined contained a large fraction of spontaneous gal+ cells. The reason for the stability is not known, but gal_3^- is presumably the most distant from Lp of the gal loci. If stabilization in the gal + condition is dependent upon crossing over between Lp and gal there would be a greater likelihood of this occurring with gal_3 than any of the other loci. However, a converse situation might be expected, that is, that gal_3^- lysates might also have a more "stabilizing" effect on the other loci. There is some suggestion of this but it is not as strong as the "suggestion" for gal_3 stability.

In discussing the stability of transinduced cells some mention should be made of the observations on various artificial lysogenies derived from W518 (gal_4^-). There are three readily available W1402, W811, and W874. These cultures do not appear to be identical in their transduction behavior. W 874 and W811 are similar, but W1402 is different in the sense that it gives approximately 1/10 as many transductions (with the same lysate) as either W811 and W874. A xyl- derivative of W1402 behaves similarly. Although it is possible that these two cultures have become partly lambda-2 resistant it appears unlikely that it should occur simultaneously in both cultures to the same degree. Mention should also be made of W1436, a TLB, gal_4^- λ^s derivative of H217. Transduction of this strain by either K-R or 750 lysates results in stable, weakly (+) cells. The reason for

this is not known.

4. The nature of the gal- segregants from transduced cells

Thus far only a limited number of segregants have been examined and these have been with but one exception from transduced galq- cells. The exception, a gal$_2$- transduced with galq- lysate was studied because of the results obtained from the examination of a galq- transduced by gal$_2$-

(A.) W518 transduced by K-12 (W518 tK-12)

The results of the examination of the segregants of this transduction are shown in the following table.

No. of Seg.	Lysogenic/1485	Number transduced by GALq- lysate	Number transduced by GAL$_2$- lysate	Probable locus
17	17	0	17	17 galq-

In addition these 17 were also transduced with K-12 lysate (all were) and 5 of the resultant transductions were examined for their stability. There appeared to be no difference in stability between these transduced cells and those of W811 transduced by K-12 lysates. In the tabular results above it appears that all the segregants were galq-. However it remains to run representatives with galq- to obtain confirmatory evidence.

(B.) The results of the examination of W518 transduced with W402 (gal$_2$-) lysate are shown in the following table.

No. of Seg.	Lysogenic/1485	Number of transduced by Gal$_2$- lysate	GALq- lysate	Probable Loci
19	19	17+ 2-	2+ 17-	17 galq-, 2 gal$_2$-

The two gal$_2$- segregants were subjected to reexamination

since they appeared to represent an exceptional occurrence. Both were transduced by all lysates except gal_2-. Both were then crossed with W902 (gal_2-) as a means of confirmation. The results are shown in the following table.

	Cross I		Cross II	
Segregant-	B7	B8	B7	B8
Fraction of prototrophs gal^+	13/905	5/363	26/1078	14/399
%	1.4	1.3	2.5	3.5

In addition B8 was crossed one additional time with W902 (The plates were discarded accidentally) but it was noted that they contained between 1 and 5% (+) prototrophs. Thus these two segregants from W518t902 appear to be gal_2- by transducibility criteria, but not gal_2- by back cross test.

(c.) The results of the examination of segregants from W518 transduced by W892 (gal_3-) are shown in the following table.

No. of Seg.	Lysogenic/1485	Number transduced by: GAL_3- alone	GAL_4- lysate alone	Both GAL_3- + GAL_4-	Probable loci involved
22	22	11	0	11	11 gal_4-, 11 unknown

Two representatives of the unknown gal- locus were subjected to further examination. Each was transduced by gal_1-, gal_3-, and gal_4- lysates, but not gal_2-, which indicated that they were gal_2-. Each was then crossed with W902(gal_2-), W892 (gal_3-) and W1436 (gal_4-). The results were as follows.

Segregant	C4	C9
$\times^{W}$ 902	5/374 = 1.5%+	1/298 = 0.33%
$\times^{W}$ 892	2/18 = 11.1%+	0/5 = 0 (+)
$\times^{W}$ 1436	0/284 = 0 (+)	0/225 = 0 (+)

As in the case of W518t902 the segregants were gal_2- by transduction

test and not gal_2^- by crossing test. In view of these results a completely new examination of segregants of W518t892 was made with the following results.

No. of Seg.	Lysogenic/1485	Number transduced by gal_3^- lysate only	Number transduced by gal_4^- lysate only	Both 3 + 4	Probable loci
23	23	18	0	5	18 gal_4^-, 5 unknown

Two of the unknown loci were examined and each found to be transduced by gal_1^-, gal_3^-, and gal_4^-, but not by gal_2^-, again indicating gal_2^- as the unknown locus. One of these two unknowns was crossed with W902 (gal_2^-) with the following result:

7/430 (+) = 1.6% (+)

The second cgt with W518t892 checks the 1st with the exception that the percentage of the unknown locus in the sample of segregants was lower.

D. W2050t811 (gal_3^- transduced by gal_4^-)

~~Transduction of gal_3^- by lysates of~~ Reciprocal of the above transductions. Examination of the segregants as follows:

No. Seg.	Lysogenic/1485	Number transduced by GAL_3^- alone	GAL_4^- lysate alone	Both 3 + 4	Probable locus
20	20	0	11	9	11 gal_3^-, 9 unknown

These results are subject to larger error than the reciprocal case mentioned above since the segregants gave rise to large numbers of small papillae (and confusing the transduction tests thereby) spontaneously. It has not been possible as yet to analyze the segregants further.

5. W1924 - gal^{-} Lp^{R}

The examination of W1924 transduced by K-12 (1924cK12) for lysogenicity gave results as follows

Occasion	I	II	III
Fraction non lysogenic	21/21	8*/8	35/36**

* these were segregating colonies

** one with one plaque. It was subsequently tested and found non lysogenic. A culture was then made and plated out. Ten gal(+) and 10 gal(−) colonies were then examined.

10/10 (+) colonies were non lyp.

9/10 (−) non lysogenic. 1 gave a questionable plaque. The questionable streak was restreaked ~~out~~. and 14 colonies retested. 14/14 were non lysogenic

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4. Under Occasion II above, two of the non lysogenic cultures (segregating) were crossed with W1436 λ^{s} to see if there was a case of masked lysogenicity.

#1 gave c. 16/952 prototrophs gal+

of these prototrophs ~~15~~(+) tested for lysogenicity - ~~all~~ 15/15 non lys.

26(−) prototrophs tested 25/26 non lysogenic,

1 possible (1 plaque) lysogenic

#2 gave c. 11/992 prototrophs gal+

30 prototrophs picked at random and tested for lysogenicity. 30/30 non lysogenic

The remaining 6/8 of Occasion II were retested for lysogenicity

6/6 non lysogenic.

8. Under Occasion III above, 10/36 colonies examined were segregating gal(+) and gal(−) cells after three purifications.

From one of these unstables a stable gal (+) was isolated and carried through 4 additional single colony purifications. It was then plated out and examined.

0/5021 colonies were gal (−)

It was then replated and there were 0/792 colonies gal (−). It was subsequently crossed with Y-10. There were no gal(−) ~~colonies~~ in 750 prototrophic colonies.

Also under Occasion III. Another segregating colony was crossed with W1436 1ˢ. Of 28 prototrophic gal(+) colonies examined, 28/28 were non lysogenic.

During this interval the parent W1924 culture was streaked out on two occasions.

I 9/24 streaks showed many plaques

II 12/12 streaks " no plaques

The "many" plaques suggests gross contamination but it is difficult to decide from the above whether W1924 is a weakly (or rarely) lysogenic culture or not.