Studies on the action of ultraviolet radiation on E. coli K-12 and its derivatives

1. Comparison of lambda sensitive cultures with lambda lysogenic cultures as regards their ultraviolet sensitivity.

A summary comparison may be found in figure 1 where nearly all survival data obtained has been plotted. The data shown was obtained from observations of aerated cultures in Penassay broth. The cultures were 3-4 hours old, contained about 10^9 cells per ml, and had been started from previous Penassay cultures(overnight, unaerated). Samples for irradiation were prepared simply by diluting a portion of the aerated culture with either Weigle-Delbrück(1952) buffer or saline such that one ml of sample contained between 1000-2000 cells. Samples of 10 ml volume were exposed to ultraviolet radiation at a distance 41 cm, from a stirilamp in sterile petri dishes centered below the source on a shaking machine. The samples were gently agitated (machine setting 20) during the exposure. Samples (0.2ml) were removed at appropriate intervals and o.1 ml plated on EMB lactose. Single plate assay was used throughout. The plates were incubated 18-20 hours at 37C before colony counts were made.

Although there is some overlap in the data, the results in figure 1 indicate that lambda lysogenic cultures are more sensitive to ultraviolet than lambda sensitive cultures. A breakdown of some of the data (table 1.) suggests that there may be differences among the lambda sensitive cultures in their ultraviolet sensitivity.however, in view of thelimitations of single plate assay, this may not be real.

Table 1

Comparison of the ultraviolet sensitivity of lambda sensitive cultures with that of K-12

Culture	Fraction surviving after 15 second exposure
K-12	0.30 (approx. mean)
W1655	0.64, 0.62
W1898	0.62
W1436	0.69
W1953(ω)	0.73
W1435	0.39, 0.46, 0.46, 0.43
W1661(382)	0.47
W1503	0.37
W518	0.28

Artificial lysogenics, prepared from some of these sensitive cultures appear in most instances to be more ultraviolet sensitive than their lambda sensitive parents(Table 2). In addition they have an ultraviolet sensitivity approximately that of K-12.

Most of the early data obtained on this subject was from unaerated,overnight cultures in broth. In general, such cultures appear more variable in their response than aerated cultures. They have not been included in the data presented. The effect of growth in different media upon ultraviolet sensitivity may be judged from Figure 1b.

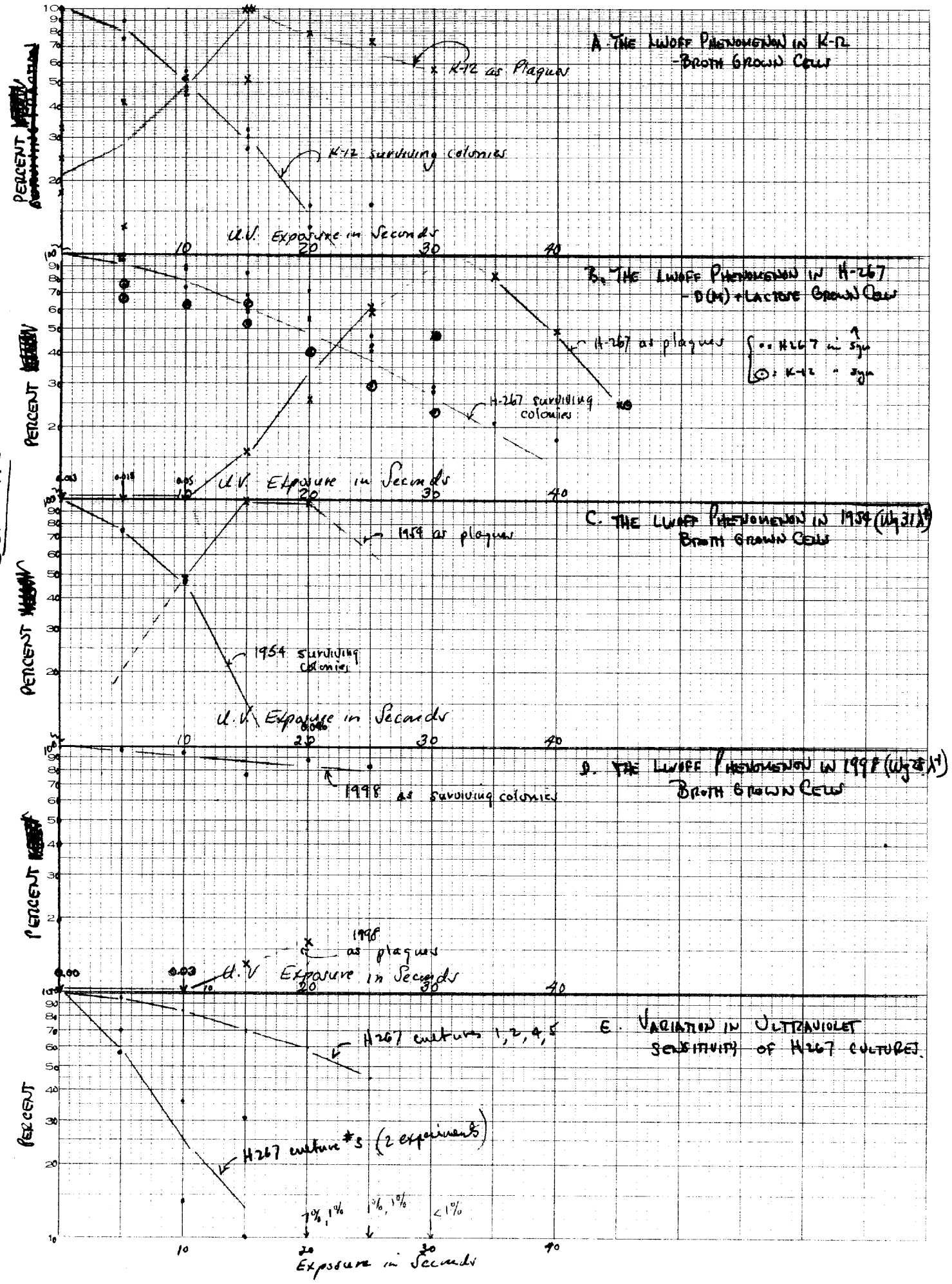
Table 2
The effect of lambda lysogenicity on ultraviolet sensitivity

Lambda sensitive parent	Derived lambda lysogenic	Fraction of cells surviving after 15 second exposure
W1655	-	0.64, 0;62
	W1932(882)	0.31
	W1831	0.27, 0.58, 0.41, 0.26
	W1682	0.37
W518	-	0.28
	W1402	not done yet
	W1821	not done yet
	W811	0.38
W1485	-	0.39, 0.46, 0.46, 0.45
	W1678	0.50
W1953(ω)	-	0.73
	W1972(ω)	0.52

2. The Lwoff effect

The induction of cell lysis by ultraviolet "activation" of the carried phage has been studied in K-12 itself and in several of its derivatives. Data on K-12 is shown in figure 2a. Table 3 lists the derivatives studied. The effect has been "observed" by three criteria; by the occurrence of gross lysis in cultures after exposure to radiation, by the preparation of high titered ($>10^{3}$) phage stocks after exposure of the cells to radiation, and by means of simultaneous phage and cell assay of suspensions receiving graded doses of radiation. The second catagory is included under the first since the occurrence of high titered phage preparations has not been found to occur in the absence of gross lysis. The division has been made so as to include those cases where lysis has been observed but was not followed by phage assay. In the last catagory it was not possible to observe gross lysis because dilute suspensions (about 2000 cells per ml) were employed. In the preparation of phage lysates best results have been obtained by irradiating saline suspensions (10ml, containing about 10^{8} cells per ml) for 35 seconds with the sterilamp at a distance of 41 cm. The saline suspensions were made from 2-3 hour aerated cultures in Penassay broth which had been previously seeded from an overnight unaerated culture in the same medium. After irradiation the saline suspensions were either diluted with an equivalent volume of broth or again centrifuged and the sedimented cells resuspended in broth. If the dose was optimum, lysis was apparent in aerated culture 90-120 minutes after irradiation. There is some variability among cultures but in general the method has been found to give phage preparations of 10^{10} lambda particles per ml.

FIGURE 2.



EUGENE DIETZGEN CO. MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER SEMI-LOGARITHMIC 5 CYCLES X 12 DIVISIONS PER INCH

Table 3

The Lwoff effect in K-12 and its derivaties- Summary

A. Lamoda lysates prepared in: (not necessarily on hand)

K-12
58-161
W1177
W1736
W511
W1321
W502
W750
W1439
W352
W750transd.K-12
W811gal+ rev.
W750transd.W1321
W1736gal+rev.

B. Gross lysis observed after u.v. irradiation(not listed under A)

W518transd.K-12
W811transd.K-12

C. Studied by simultaneous plaque and cell count

K-12
W1177
W1073
W1352

In addition to the observations in K-12 and its derivatives, the Lwoff effect has been observed in a diploid strain, H267. Summary of these observations may be found in figure 2b. Although H267 appears more resistant to ultraviolet when compared with K-12 in figure 2a, the difference is the result of cultivation in two different media, since K-12 cultured in D(M) plus lactose has about the same sensitivity as H267 (K-12 under these conditions is also shown in figure 2a). Gross lysis of H267 after irradiation has been observed but high tittered stocks of lambda grown in this host have not been prepared as yet. An extreme example of the variation sometimes encountered in examining the ultraviolet sensitivity of cultures was noted in the case of H267 culture #3 which was quite different from the other cultures studied. A comparison of this culture with the others is shown in figure 2e. This culture appeared no different from the others as regards ~~number~~ percent of segregating colonies on EMB lactose. Even should this culture have been contaminated with a large number of prototrophic segregants it is difficult to see why such segregants should be more sensitive than the K-12 cells grown under similar conditions.

The establishment of lambda lysogenicity in other strains of E. coli allows the comparison of these strains with K-12 as regards the Lwoff effect. Culture 1554 (Wg31 lambda lysogenic) has been given preliminary examination for this effect. The results are shown in figure 2c. "Activation" of the phage in this culture by ultraviolet is also possible, and the sensitivity of the culture is similar to that of lysogenic K-12 lines. This may not be the case with culture

1998 (W₂ 28 lambda lysogenic) as figure 2d suggests. Here it is apparent that larger doses of ultraviolet will be required to produce comparable amounts of cell killing and phage liberation.

3. Observations on other phages for the Lwoff effect

Examination of K-12 cultures lysogenic for other phages (882, 1676, ω) for "activation" of these phages by ultraviolet has given negative results. These phages apparently contribute little to the ultraviolet sensitivity of cells lysogenic for them (see Table 4 for results with 882). When strains are multiply lysogenic, as W1736, which is lysogenic for lambda, 882, and 1676, the lambda lysogenicity is apparently not influenced by lysogenicity for the other phages. Plaques formed by ~~singly~~ individual lysing cells induced by u.v. have always been found to ~~be~~ contain only lambda. Lysates of such multiply lysogenic cultures prepared by the Lwoff technique do show the presence of several phages. Lysates of 1736 for example, show the presence of phages forming three plaque types when plated in low dilution on a lamoda sensitive strain. Phages other than lambda were indicated by the appearance of plaques on lambda lysogenic strains. In this instance, the presence of the other phages can be explained on the basis of the number of cells involved in the preparation of a high-titered lysate and does not necessarily indicate the liberation of more than one type of phage by a single cell.

Table 4
The effect of lysogenicity for phage 882 on ultraviolet sensitivity

Parent 882 sensitive strain	Derived lysogenic	Fraction of cells surviving after 15 second exposure
W1655	-	0.64, 0.62
	W1931	0.59
	W1681	0.60
W1436	-	0.69
	W1661	0.47

Examination of K-12 lysates for transductive action

First attempts were with K-12 lysates and cultures of W58-161 and W1655 in an attempt to transduce the methionine in each culture. Mixtures of lysates and cells on D(O) failed to give any methionine independent cells. An early attempt to obtain transduction to lactose fermentation of W518 cells was likewise negative.

The first suggestive evidence was obtained with W1736 and K-12 lysates on EMB lactose. Examination of the transduced cells revealed that the transduction was really for galactose fermentation and that there was probably no transduction for lactose at all. The transductive effect is readily observed on EMB galactose by mixing lysate with appropriate gal - cells on this medium. Some of the observations on the gal_4- locus are shown in table 5. Action is shown on both lambda sensitive and lambda lysogenic cells and has been observed with all gal4- cultures except W1439, which is Lp_2^r and is presumed not to adsorb the phage. Attention is called to the fact that the activity in the lysates is destroyed by heating in a boiling water bath.

In order to examine the possibility of selection of preexisting gal+ cells in the large gal- population by the lysates a reconstruction experiment was performed. The data from this experiment is shown in table 6. In addition to the tabular data, it was noted that on plates containing K-12 lysate that the papillae were separated into two groups. One group, forming large papillae apparent by the end of the first day of incubation, was approximately equivalent in numbers to the number of gal+ cells added. The other group, smaller in papillary size and appearing on the second day of incubation, was

[redacted] r[redacted]

Table 5

Some observations on the transduction of the gal_4-locus

Culture	ml heated K-12 lysate**	ml K-12 lysate***	no. of papillae****	Examination of papillae
W1736(a)*	-	0	38	
		0	12	
		0.05	153	none made
		0.10	302	
		0.20	620	
(b)	0.10	-	29	-
	-	0.10	425	34/34 lac-
W1662	0.10	-	19	-
	-	0.10	311	45/45lac-, ng on D(0)
W811	0.10	-	47	14/14 lac-, ng on D(0)
	-	0.10	394	25/25lac-, ng on D(0)
W518	0.10	-	4	
	-	0.10	2112	none made
W1821	0.10	-	30	10/10lac-,xyl-, ng on D(0)
	-	0.10	581	58/58lac-,xyl-, ng on D(0)
W1439	-	-	0	
	0.10	-	0	none made of course
	-	0.10	0	

* except in the case of W1736(a), experiemnts were performed using o.1o ml of 10X(saline) cells, 3-4 hours aerated in Penassay. In W1736(a) 0.10 ml of overnight unaerated culture was used as cell source

** heated in a boiling water bath for 15 minutes. No plaque forming lambda present.

*** 2-3 x 10^{10} lambda particles per ml

**** 1736(a) after 3 days, 1821 after 6days, the remainder after 2 days. incubation at 37C

Table 6.

Reconstruction Experiment

	No. papillae after 2 days	Totals
Expected spont.papillae (from previous expts.)	12-38	-
No. gal+ cells added to c. 10^8 gal- cells plated	71	
Total gal+ cells (expected plus added) expected	-	83-109
Total gal+ observed	111	109
Expected plus added plus 0.10ml heated K-12 lysate	111	111
Expected plus added plus 0.05ml K-12 lysate	217	
Expected plus added plus 0.10ml K-12 lysate	326	

apparently the results of lysate action. The gal+ cells added in this experiment failed to give evidence of inhibition by the large gal- population.

It was of interest (in connection with an explanation based on a selective phenomenon) next to examine the ability of lysates from gal negative cultures for their ability to produce the effect. Lysates were prepared of cultures of W811 and W1821, both gal_4-, and the ability of these lysates to cause the effect examined. Results are shown in table 7.

Table 7
The effect of gal- lysates

Culture*	heated K-12 lysate**	K-12 lysate***	W811 lys. ****	W1821 lys. *****	No. pap. 2 days	Examination of papillae
W1736	0.10	-	-	-	12	5/5lac-, ng D(0)
	-	0.10	-	-	276	47/47lac-, ng D(0)
	-	-	0.10	-	27	-
	-	-	-	0.10	13	-
W811	0.10	-	-	-	47	see table 5
	-	0.10	-	-	394	" "
	-	-	0.10	-	50	-
	-	-	-	0.10	51	-
W1821	0.10	-	-	-	30	see table 5
	-	0.10	-	-	581	" "
	-	-	0.10	-	36	-

* c. 10^9 cells plated, from 10X conc., aerated culture
** as in previous cases with heated lambde
*** 2-3 x 10^{10} lambda particles per ml
**** 1.7 x 10^{10} " " " "
***** 1.0 x 10^{10} " " " "

From the data it appears that lysates of gal_4- cultures have little or no activity, with the possible exception of W811 lysate on W1821 cells. Spontaneous reversion of gal_4- restores (partially?) the ability of the lysates to cause papillation in gal_4- cultures not the source of the reversion. This latter point requires confirmation. See table 8.

Table 8

Action of lysates of gal_4- reversion

Culture*	heated K-12 lysate **	K-12 lysate ***	W811gal+f lysate ****	No. of papillae after 2 days
W1736	0.10	-	-	12
	-	0.10	-	321
	-	-	0.10	191
W811	0.10	-	-	29
	-	-	0.10	31

* c. 10^9 cells from a 10X conc. aerated culture
** as in previous cases
*** 2-3 x 10^{10} lambda per ml
**** 4.0 x 10^{10} " " "

From the fact that gal_4- cultures show the effect, provided that they do not contain the Lp_2^r locus, it may be inferred that the effect is mediated via lambda. The association of lysate activity with lambda is perhaps strengthened by the observation that filtrates of the culture medium of W1485 also show no activity. See table 9.

Table 9

Action of W1485 culture filtrate

Culture	heated K-12 lysate	K-12 lysate	W1485 filtrate	No. of papillae after two days
W1736	0.10	-	-	12
	-	0.10	-	321
	-	-	0.10	6

The activity of K-12 lysates is removed by exposure to either W811 or W1736 cells for 15 minutes at 37C (see table 10).

Table 10

Removal of ~~filtrate~~ lysate activity with W811 and W1736 cells

Treatment	No. of papillae after 2 days	
	W811	W1736
c. 10^9 cells* susp. in 1.5ml K-12 lysate 15 min., centrifged, resusp. in 1.5 ml broth. --0.1 ml plated	341	281
c. 10^9 cells* susp. in supernat above 0.1ml plated	65**	33***

* equivalent nos. of cells ** usual no. pap. with heat. lys 30-80
*** " " " " " " 12-38

The results thus far have been concerned with effects upon the gal_4- locus. In addition, a similar effect has been observed in a culture with the gal_1- locus(table 11)

Table 11
Action of K-12 lysate on the gal_1- locus

Culture	heated K-12 lysate	K-12 lysate	No. of papillae after 2 days
W750	0.10	-	0
	-	0.10	409

Procedure and lysates as used in previous expts.

The gal_2- locus of W902 which originally did not show the effect of lysate action because of the presence of the Lp_2^r has been found to be transductible when the two loci are separated (from a cross with 1655).

With the availability of several different gal- loci it was of interest to examine the effect of lysates of the several gal- cultures upon one another(table 12)

Table 12
Action of gal- lysates

Culture	Lysates of K-12(heata) ①	K-12 ②	811 ③	1821 ④	902 ⑤	1485(filt.) ⑥	No. of papillae after 2 days
W750	0.10	-	-	-	-	-	2
	-	0.10	-	-	-	-	542
	-	-	0.10	0	-	-	48
	-	-	-	0.20	-	-	31
	-	-	-	-	0.10	-	176
	-	-	-	-	-	0.10	3
W518 (a)	0.10	-	-	-	-	-	4
	-	0.10	-	-	-	-	2112
	-	-	-	-	0.10	-	1112
(b)	0.10	-	-	-	-	-	163
	-	0.10	-	-	-	-	2248
	-	-	0.10	-	-	-	86
W811	0.10	-	-	-	-	-	39
	-	0.10	-	-	-	-	256(poor plate)
	-	-	-	-	0.10	-	202

Procedure as in previous expts.
① no plaque forming lambda present
② $2\text{-}3 \times 10^{10}$ λ/ml
③ 1.7×10^{10} λ/ml
④ 1.0×10^{10} λ/ml
⑤ 4.9×10^{10} λ/ml
⑥ no plaque forming lambda present.

In general the results indicate some activity of lysates of gal- cultures on gal- cultures as long as the recipient culture is a different gal- locus from the donor(lysate culture). Previously it has been shown that gal_4- lysates have no activity upon gal_4- cells. Similarly, gal_1- lysate has no activity on gal_1- cultures. See table 13.

Table 13
Action of gal_1- lysate on gal_1-

Culture	heated K-12 lysate	K-12 lysate	W750 lysate	no. of papillae after 2 days
W750	0.10	-	-	2
	-	0.10	-	405
	-	-	0.10	2

procedure and K-12 lysates as previously, W750 lysate contains more than 2.4×10^{10} lambda particles per ml.

The activity of K-12 lysate is increased by exposure to ultraviolet radiation (table 14).

Table 14
Effect of ultraviolet on the activity of K-12 lysate

[redacted]	U.V dose in seconds	No. of papillae after 2 days W1730	W811
	0	275*	585**
	60	503†	-
	120	1984	-
	180	2952	-
	240***	3188	c.4170

* usual spontaneous 12-32
** spontaneous papillae this expt. 66
*** surviving fraction of lambda after this dose, 4.4×10^{-1}

Whether the effect of ultraviolet is non-specific or not is not known, or whether the increased lysate effect is caused by the formation of peroxides in the broth portion of the lysate is also not known. Portion of the lysate exposed to ultraviolet for 240 seconds appear to have no activity in altering the met iodine requirement of 58-161 and W811, the lac- character of W112 and W1730 gal+ lac,

the gal_1- locus of 1430, and possibly not the xyl- locus of W1821. The observation that the gal_1- locus is acted upon by irradiated lysate in Lp_2^s cultures and not in Lp_2^r suggests that the irradiation effect is mediated via lambda. The lack of effect on other loci also suggests some specificity.

Attempts to transduce other characters in some of the cultures mentioned above have been unsucessful. In general the transduction has been attempted by mixing K-12 lysate with cells on agar medium. See table 15

Table 15
Unsucessful transductions

Culture	Character not transduced	Medium
W1780	leucine	D(O) plus B^1_1
W811	methionine	D(O)
W1078	(serine or glycine)	D(O) plus proline
W1821	xylose ferm.	EMB xylose
W12Lp_2^s	lactose ferm.	EMB lactose
W511	streptomycin	NSA (strep. added after some incubation)
W518	"	" "

APPENDIX

Additional information avialable since the initial preparation of this report.

1. Making transducible cultures Lp_2^r by selection of lambda-2 resistant destroys transducibility. Believed previously and known specifically for W611-W1439. Now demonstrated for W750 and W1736.

2. Stability of the transduced cells- for the most part unstable, in some instances segregating [redacted] gal- cells after 8 single colony isolations. Appears to be dependent upon the particular gal-locus.

3. Gal_3- is also transducible. This makes all gal-loci transducible except gal_5- which has not been isolated yet [redacted] free of [redacted] the Lp_2^R locus.

4. W1944gal_4-Lp^r is apparently transducible for galactose fermentation, some of the transduced cells are unstable, none appeared lysogenic for lambda

5. Transduced cells are crossable with other strains(gal_4-). The gal+ prototrophs from the cross are also unstable.

6. Attempts at transducing a lambda sensitive culture with irradiated lambda and obtaining a non-lysogenic transduced cell may have sucessful in one instance.

7. A gal- mutant of W1673 has been isolated which is not transducible.