

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<sub>1</sub>-gal<sub>2</sub>- type and one of the gal<sub>2</sub>-gal<sub>4</sub>- type. The evidence that such not cultures are (--) is that they are transduced meither by homotypic mor 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 (--) was reserved individually and not conjunctively. The homotypic locas 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 infortunately a prototroph and the results obtained with

must

it will also be repeated and extended with auxotrphic strains.

Although this (--) was not transduced by sitter, lysates of wither

(-) singly it was transduced to a lesser extent (where a solid layer of papillae by a musture of the by a must be a mu



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

and the resultant transduction as follows:

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. Same 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<sup>-</sup>-1<sup>-</sup>--\* (2) ---2<sup>-</sup>-1<sup>-</sup>--\* (3) ---2<sup>-</sup>-1<sup>-</sup>--\* 
$$-2^+$$
-1<sup>-</sup>--\*

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:

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 anazlogy with the simple transfertions the first these 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 62-). 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<sup>-</sup>) were gave stable reversions and therefore were presumably of the ---2<sup>+</sup>-1<sup>-</sup>-\* type. Of the (2<sup>-</sup>) examined all but one gave stable reversions and therfore the two types---2<sup>-</sup>-1<sup>+</sup>--\* and ---2<sup>-</sup>-1<sup>+</sup>--\* were indicated with the most frequent -2<sup>-</sup>-1<sup>+</sup>-being the former.

Examination of the the (2-) culture giving the unstable reversions showed that it apart did segregate (--) cells but as yet it has not been established that it segregates (2-) of the following type ---2--1+--+.

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 exx reverted cultures is disturbing

but this may be related to elimination of the fall locus in crosses. Presumably

crosses between --2"-1"---\* and ---2\*-1"---\* should yield a larger number of

-2"-1"+==

(+) than crosses between (1") and (2") of normal constitution when there is

successful transfer of the segment through the zygote, these (+) in addition would

be unstable for galactose. The culture used unfortunately is a prototroph and

unless successful crosses between it and a Hfr strain can be accomplished the problem

can not be attack from this aspect. (Successful 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 Gal2 and Gal4are 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. Inseveral 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 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 (--) and (4-) contaminants. On this assumption the Atransductions 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 the explanation and but requires a great week 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 market type which did not, at the time of writing.

though the most frequently occurring (-) after ultraviolet radiation appear to be of this type. Three different occurrence, of non-transducible gal- have been found. Two of these were induced by ultraviolet, and the third by copper exposure (H. Bayers). One of the ultraviolet mutahas 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 Gal2 with lowered frequency.

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

			AMILE IN THE		aduction Assu	ay	
Experim	en <b>t</b>	None	Gal <sub>2</sub> -	Plat	e Additions IT Lysates Galu-	NFT Wild Type	
206	(1)	0*	0*	0*	0	•	
	(2)	0	0	-	-	0	
220	(1)	0	0	0	0	<b>-</b>	
	(2)	0	0**	0**	<b>)**</b>	0	

\* number of papillae per plate

\*\* NFT (normal frequency of transduction) lysates used in these cases

v Table 14

		Activit	y of Lysates of W2312 on Selected	Galactose Loci	
Galactose Locus		None	Plate Addition W2312 Lysate		·
Gall- Lp+		4*	37*	•	
_ 2- Lp+	(220)	8	7	·	•
. =	(221)	19	28**		
Gal4- Lp+		17	74		
Gal6- Lp8		3	121		

\* mumbers of papillae per plate

\*\* 12/12 examined were found to be stable Gal+

Table 15

R	esults of Cross	ses of W2312 with Selected Gal	actose Loci
Selected Galactose Locus	Gal+	Numbers Total Prototrophic Rechm	
Gal <sub>2</sub> - F	1	2112	0.05
Gal <sub>4</sub> - F <sup>+</sup>	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) prol and W1765 hist leuc by means of ultraviolet. Table 19 gives a summary of these experiments. Reccurrences of both Gal<sub>1</sub>- and Gal<sub>2</sub>- were found as well as a number of new loci and possibly several (-). No recurrences of Gal<sub>4</sub>- 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 MFT lyeates, the last with an HFT lysate. The effect of ultraviolat 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 Lpr assay cells until a survivial of the plaque-forming titter has become reduced about 103. Thereafter there is a gradual decease in transduction activity with increasing dose. On Lp. there is a slight increase in transducing activity and then a gradual decrease. The maximum reached by the lysates on Lp or Lp cells is about four times the maximum reached on Lp8 cells. In performing this experiment about 168 Lp8 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 the Lps assay is probably that much low. This then would suggest that the absolute number of transductions is approximated upontp8 cells when a sufficient number of cells are used and that the action of ultraviolet is to increase the assay on Lpt or Lpr cells to the level of the absolute number present. In connection with this it should be noted that survival of the transductions were a Lps is still about 0.5 even at the extreme doses used. From the above it is suggested that the action of treates of ultraviolet is neveral fold. First and most rapid is the destruction of plaque forming activity of Lp cells. Secondly, to destroy that property of the phage which causes them to be excluded by lysogenic cells, and thirdly to destroy

most date...

Table

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

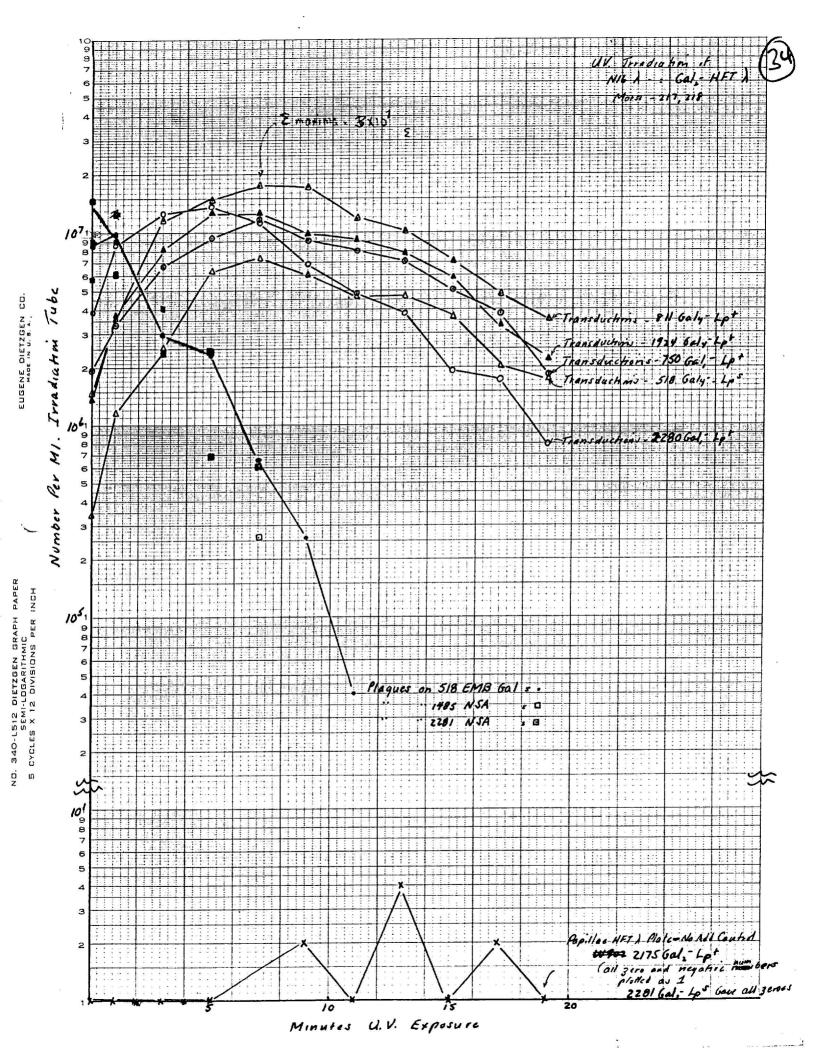
Culture	Mutant		Means of U	ced by HFT	Possible Prantia
Treated	Designation	Gal <sub>l</sub> -	GET <sub>2</sub> -	Gal <sub>H</sub> -	Genotype
W1673 Lp <sup>s</sup>	W2310	0	+	0	Gal <sub>l</sub> -Gal <sub>4</sub> -
	W2311	0	+	o	н #
	W2322	0	O	0	nontransducible
	W2313	+	0	+	Gal <sub>2</sub> -
	W2314	<b>+</b>	. +	+	Gal_x-
	W2315	+	+	+	Gal <sub>x</sub> -
	W2316	0	+	<b>+</b>	Gal <sub>l</sub> -
	W2317	o	• +	0	Gal <sub>l</sub> -Gal <sub>4</sub> -
	W2318	0	0	0	nontransducible
W1765 Lp <sup>8</sup>	238-2	0	0	0	nontransducible
	28845	+	+	+	Gal <sub>x</sub> -
	238-6	0	+	+	Gal <sub>l</sub> -
	238-8	+	+	+	Gal <sub>x</sub> -
	238-10	+	+	+	Gal_x-
	238-11	0	+	0	Gal <sub>1</sub> -Gal <sub>4</sub> -
	238-12	+	0	+	Gal <sub>2</sub> -
	23 <b>9–</b> 13	+	0	+	Gal <sub>2</sub> -

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 titter, which may be an indication that the activities are confined to a single particle. The occurrence of transductions with Lpr 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 as well as to rise to plaques ax lysogenization. (This would require that Lpr genetypes were the result of such defective particles rather than of a defective act of lysogenization.)

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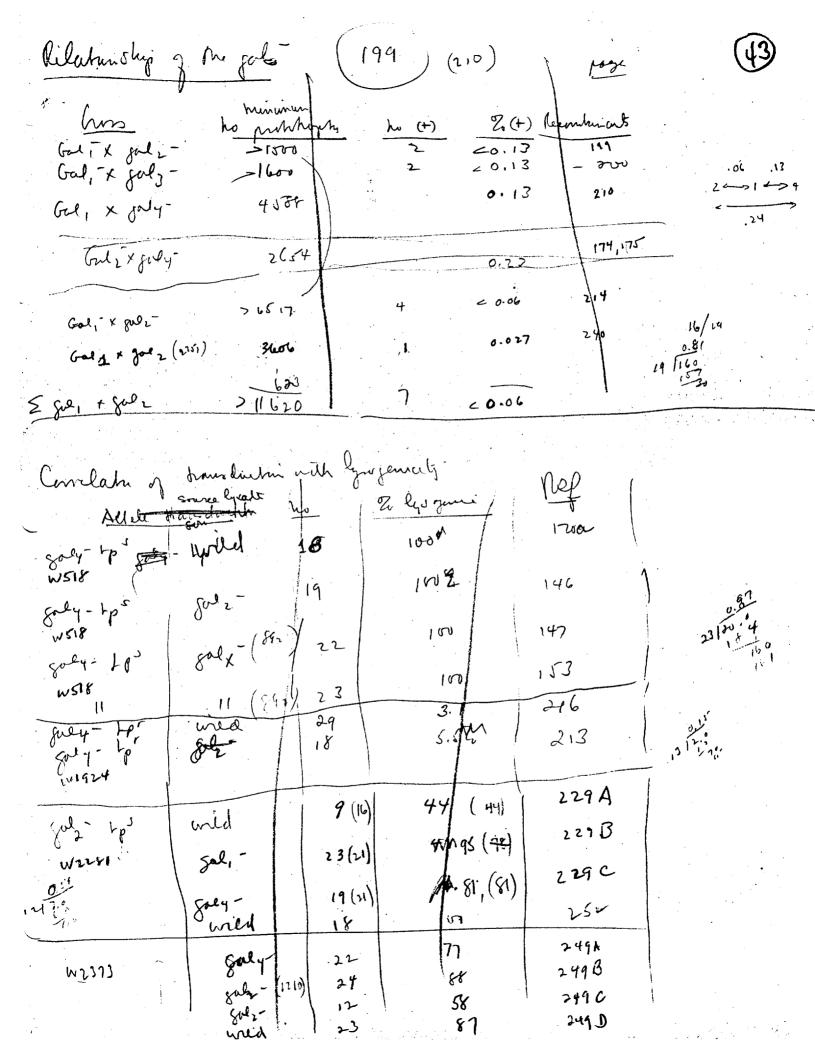
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