Genet. Res., Camb. (1967), **9**, *pp.* 149–158
With 2 text-figures
Printed in Great Britain

The bar-properties, in particular glucosylation of deoxyribonucleic acid, in crosses of bacteriophages T2 and T4*

By B. DE GROOT

Department of Radiation Genetics, University of Leiden, and Institute for Radiopathology and Radiation Protection, The Netherlands

(*Received* 14 *May* 1966)

1. INTRODUCTION

Bacteriophage T2 is partially excluded from the progeny of crosses with phage T4. A fraction of approximately 0·1 of the progeny shows the adsorption properties of T2, when plated on mixed indicator *Escherichia coli* B and B/2 (Streisinger & Weigle, 1956). These progeny strains were repeatedly backcrossed with parental T2 and, in this way, they were made isogenic with T2 for most of the loci. However, three properties of T4, called the bar-properties, were transmitted to the last progeny from the backcrosses. The bar-properties are the following: first, partial exclusion of parental T2; second, a high plating efficiency (e.o.p.) with large plaques on certain *E. coli* K strains in contrast to the low e.o.p. and pin-point plaques of T2 on these strains; third, the complete glucosylation of the hydroxymethylcytosine (HMC) residues in the DNA.

Analyses by Lehman & Pratt (1960) have revealed that HMC in T4 DNA is completely glucosylated, 0·7 with α-glucose and 0·3 with β-glucose. In T2 DNA 0·7 of the HMC residues are α-glucosylated and 0·3 are unsubstituted. The incorporation of glucose is brought about by T4 α-, T4β-, and T2 α-glucosyltransferases respectively, which are phage-specific enzymes induced after infection of *E. coli* with T4 and T2 (Kornberg, Zimmerman & Kornberg, 1961).

That the bar-properties were separable was shown by Jesaitis (1961), who studied the recombinants arising in crosses between phages T6 and T2 and found that the characteristic glucosylation patterns of T6 and T2 and the plating properties on *E. coli* K strains were not the expression of one gene with a pleiotropic effect or even necessarily genetically linked.

The relations between T4 genes and exclusion have been investigated with T2-like phage strains which contain genes rescued from ultraviolet-irradiated T4 (De Groot, 1966*a*) and were isolated from plates with indicator bacteria that permit the selection of recombinants between T2 and T4. In this way, T2-like phage strains with T4 adsorption properties were isolated from plates seeded with *E. coli*

* This work was carried out partially within the frame of the association between Euratom and the University of Leiden, contract number 052-64-1 BIAN.

B/2. These strains did not show exclusion of parental T2 and were sensitive to exclusion upon subsequent crossing with the parental T4.

In this paper, other T2-like strains will be described that were isolated from crosses between T2 and ultraviolet-inactivated T4 on plates with *E. coli* K(λ*h*) as the indicator. The segregation of the bar-properties obtained by marker rescue in the progeny will be discussed. One of these strains is partially resistant to exclusion and shows the T4 glucosylation of HMC. A possible relation between glucosylation and exclusion will be discussed together with some additional information obtained from the analysis of the bar-properties in the progeny from a single burst from a cross of phages T4 and T2.

2. MATERIALS AND METHODS

(i) *Phage strains*

Standard type T4 D, standard type T2 H and T2 L, and T2 L*h*, a host range mutant. T6 was used as a source of glucoseless phage DNA. Phage stocks were cultured in liquid medium as the clone of a single particle. After complete lysis, the cultures were shaken with chloroform and the debris was spun down. They were stored at a titre of 6×10^9/ml. after dilution in 0·1 M phosphate buffer with 0·01% gelatin.

The parental origin of a phage gene will be designated following the symbolism of Streisinger (1965*b*) by adding the superscripts +2 or +4 for a standard-type gene and 2 or 4 for a mutated gene. Thus, T2 *exr*$^{+4}$ means a phage strain isogenic with T2 and with a locus for resistance to exclusion originating from T4.

(ii) *Bacterial strains*

Escherichia coli B was used for growth of phage stocks and as a host for phage crosses. *E. coli* B/2 was used as an indicator bacterium to distinguish between adsorption properties of T4 and T2, and *E. coli* KS 112-12 (λ*h*) # 3 to distinguish between plaque types of T4 and T2. *E. coli* U95 was used for the propagation of glucoseless T6 (Shedlovsky & Brenner, 1963). Strains of *E. coli* will hereafter be referred to without the species name.

(iii) *Exclusion test and marker rescue*

Exclusion tests were carried out as phage crosses, described previously (De Groot, 1966*b*). Marker rescue was performed as a cross of ultraviolet-irradiated T4 with unirradiated T2. The techniques of irradiation have been presented elsewhere (De Groot, 1966*a*, *b*). Doses were calculated as multiples of that depressing survival of T2 by e^{-1} (one phage lethal hit) on the exponential survival curves. The selective indicator bacterium was K (λ*h*) and large T4-like plaques were picked from plates of a cross where T4 had received a dose of 90 PLH. The majority of the progeny from the cross was the helper T2 which forms a background of pin-point plaques.

Strains carrying the T4 marker were isolated from a series of plates, using dilutions such that superposition of T2 plaques with large T4-like plaques and, consequently, additional recombination in the lawns of K (λh) was avoided.

(iv) *Media and phage techniques*

The media were tryptone broth with 0·01 M $MgSO_4$ and tryptone agar (Hershey & Rotman, 1949). The general phage techniques employed were those described by Adams (1959).

(v) *Preparation of phage glucosyltransferases*

The glucosyltransferases from T4 and T2 and from the recombinant strains were prepared from 10^{11} cells of B harvested in the cold 16 min. after infection at a multiplicity of four. The glucosyltransferases obtained by the method of Kornberg *et al.* (1961), were not purified beyond the precipitation step with ammonium sulphate and the concentrated samples in glycylglycine buffer were used for assay.

(vi) *Preparation of DNA*

DNA was prepared from purified bacteriophage suspensions (10^{12}/ml.) by shaking with phenol. DNA from glucoseless T6 phage will hereafter be referred to as HMC DNA.

(vi) *Assay of glucosyltransferase*

The techniques employed were those described by Kornberg *et al.* (1961). Glucosyltransferases were assayed *in vitro* by incubation of T-even phage DNA with radio-active uridine diphosphate [^{14}C]glucose (UDPG). Radio-active UDPG with a specific activity of 5 μc/μmole was a generous gift by Dr A. de Waard, Leiden. Incorporations were measured with a Nuclear Chicago gas-flow counter with low background.

The glucosyltransferases induced by the progeny strains from crosses of T4 and T2 were assayed with T2 DNA as a substrate. T2 α-glucosyltransferase does not glucosylate T2 DNA in the standard assay, while the T4 α- and T4 β-glucosyltransferases are, to some extent, capable *in vitro* of glucosylation of free HMC residues present in T2 DNA. Enzymes induced by standard-type T2 or T4 served as references.

3. RESULTS

From a cross between T2 and ultraviolet-irradiated T4 six recombinant phage strains were isolated from plates with K (λh) indicator bacteria showing large T4-like plaques. They possessed the h^{+2} locus for they did not absorb to B/2 and showed the sensitivity to ultraviolet (v^{+2}) of the parental T2. They did not exert exclusion,

when crossed with T2 L h^2 and were, therefore, assumed to possess the homologous site (ex^{+2}) of the exclusion locus in T4 (ex^{+4}) which resides between h^+ and rII (De Groot, 1966*b*). Since, apparently, the six strains show to some extent isogenicity with the parental T2, they were designated as T2 k^{+4} 1–6, where k^{+4} indicates the T4-like efficiency of plating on K (λh).

Table 1. *Excludability of T2* k^{+4} *by T4 D in* E. coli *B. The T2 frequencies presented are the frequencies of turbid plaques in lawns with mixed indicator bacteria* E. coli *B and* E. coli *B/2.*

Strain T2 k^{+4}	Parental ratio T4:T2	Multiplicity of infection	Burst size (90 min.)‡	T_2 frequency
1	2·7:1	10	42	0·05
	1:1·4	8	61	0·05
2*	1·7:1	13	12	0·14
3	1·0:1	6	103	0·08
	2·6:1	11	58	0·04
4	1:1·2	6	56	0·05
	1:1·2	12	59	0·02
5	2·5:1	7	82	0·01
	1·7:1	7	92	0·10
6†	2·0:1 to 1:1·6	6–10	65–98	0·25 to 0·43

* Strain T2 k^{+4} 2 shows a rapidly decreasing titre after dilution. Only one cross meets the standards for a normal cross.

† The range is presented of the results of nine crosses.

‡ At 90 min. most burst sizes have not reached maximum due to premature lysis.

T2 k^{+4} 1–6 were examined for sensitivity to exclusion when crossed with the parental T4. In crosses with T4, T2 k^{+4} 1–5 appeared to undergo partial exclusion like the parental T2, but T2 k^{+4} 6 revealed progeny with a significantly higher frequency of T2 adsorption properties, when plated on mixed indicator B and B/2 (Table 1). It is assumed that this strain had rescued at least one other gene from T4, called exr^{+4} in addition to that for growing with large plaques on K (λh) and the property will be referred to as partical non-excludability.

Incubation of the glucosyltransferases from T2 k^{+4} 1–6 with T2 DNA and HMC DNA led to their identification (Table 2). All samples tested showed glucosyltransferase activity, when incubated with HMC DNA. The α-glucosyltransferases from strains T2 k^{+4} 1–5 behaved like the T2 control. The activity of the T2 control sample under the conditions for activity of the β-glucosyltransferase can be ascribed to a residual activity of α-glucosyltransferase, usually observed in preparations containing both α- and β-glucosyltransferase (De Waard, personal communication). The α-glucosyltransferases from strains T2 k^{+4} 1–5 are identified as T2 α-glucosyltransferase. The activities of strain T2 k^{+4} 6 are the level of the T4 control under the conditions of both the α- and β-glucosyltransferase; the enzymes induced in the bacterial host are therefore identical to those of T4.

Table 2. *Identification of the glucosyltransferases induced by strains T2* k^{+4} 1–6 *in* E. coli *B by means of incorporated glucose (c.p.m.) from UDPG – ^{14}C*

Substrate:	T2L DNA		HMC DNA	
Test condition:	α	β	α	β
T2 k^{+4} 1–5	74 ± 13*	48 ± 5	1022 ± 142	—
T2 k^{+4} 6	576	512	1741	1327
T2 L	46	48	590	172
T4 D	480	512	1450	1020

* Mean ± standard error ($n = 12$).

The fate of the bar-properties of T4 was further followed by an analysis of all twenty-seven progeny recovered from a single burst of T4 D and T2 H. The following properties were determined: Host range (h^{+4} or h^{+2}), partial exclusion of T2 (ex^{+4} or ex^{+2}), degree of partial excludability by T4 (exr^{+4} or exr^{+2}), ultraviolet sensitivity (v^{+4} or v^{+2}), e.o.p. on K (λh) (k^{+4} or k^{+2}), and glucosyltransferases induced (αg^{+4}, βg^{+4}, αg^{+2}). The properties have been listed as monogenic; as will be shown below, this might be an oversimplification for the e.o.p. on K (λh).

Figure 1 presents the map of that segment of the T2 genome which contains the loci mentioned above whose locations are known. This segment is also known to be the residence of many of the genes for the early functions (Edgar & Epstein, 1963). The genes h^+ and ex^+ are closely linked (De Groot, 1966*b*) and they are found at the beginning of this map segment whereas v^+ is found near the end (Streisinger, 1956*a*). The position of the other loci is not known at present. The types of progeny and their frequency are presented under the map. Three progeny with T2 adsorption

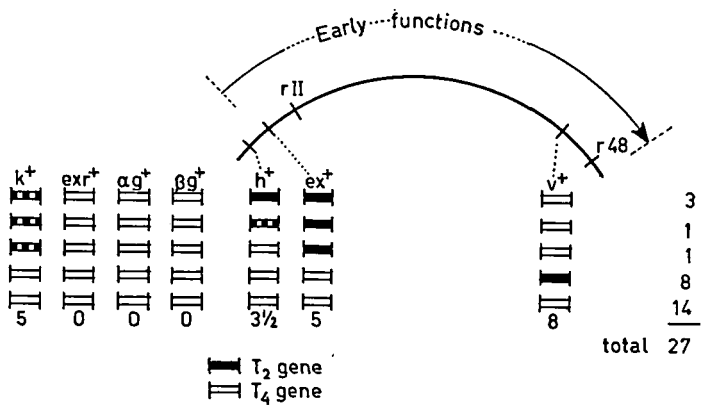

Fig. 1. Genetic segregation in a single burst from a cross of bacteriophages T4 and T2. The map segment for the early functions is presented as an arc with *r*II and *r*48 as reference markers. The location of v^+ at the left-hand side of *r*48 is not quite certain. The genotypes of the twenty-seven progeny are presented with their frequencies given on the right. The genes k^+, exr^+, αg^+ and βg^+ are presented separately, because their location is unknown. The broken symbols of k^+ indicate intermediate phenotype and the broken symbol of h^+ indicates a heterozygote. Under the genes, the T2-gene frequencies are presented; the heterozygote is counted as ½.

https://doi.org/10.1017/S0016672300010454 Published online by Cambridge University Press

properties and one heterozygote in a burst of twenty-seven phage is compatible with the usual frequency after exclusion by T4. The results lead to the following conclusions:

(1) The linkage of h^+ and ex^+ is also found after exclusion. Between ex^+ and v^+ no parental T2 combinations, thirteen recombinants and fourteen parental T4 combinations are found. This is in agreement with the expected values calculated from

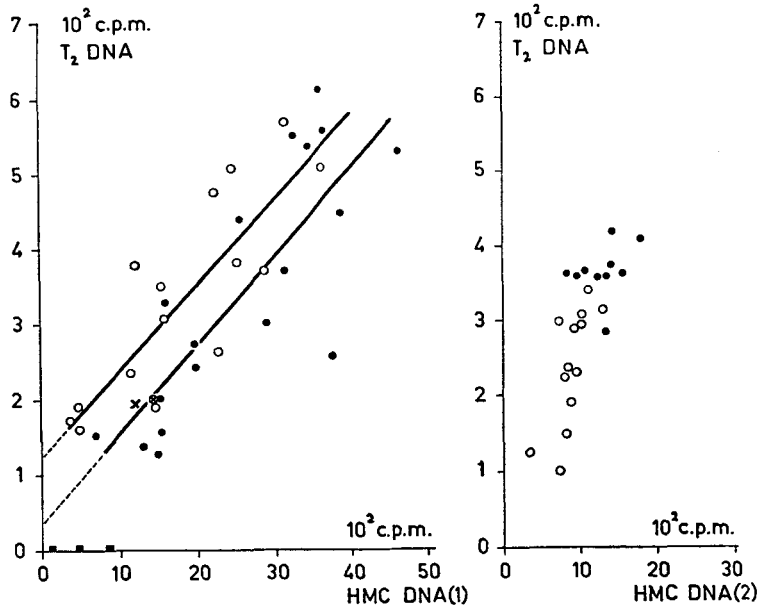

Fig. 2. Activities of glucosyltransferase preparations from the progeny of a cross of bacteriophages T4 and T2 on T2 DNA and HMC DNA. The two HMC DNA preparations were different for unknown reasons. Explanation of the symbols: Filled circles: α-transferase; open circles: β-transferase; cross: T4 α control; cross circle: T4 β control; filled squares on the abscissa from left to right: T2 β control and T2 α control ($2\times$).

the observed gene frequencies with the assumption of non-reciprocity of the recombinational events, although one or two parental T2 combinations might have occurred.

(2) The polarity of increasing frequencies of T2 genes from h^{+2} to v^{+2} along the map segment of the early functions, observed in other material (De Groot, 1966*b*), is also found here for the genes h^{+2}, ex^{+2} and v^{+2}.

(3) The e.o.p. on K (λh) of none of the progeny was like T2. However, the five progeny with h^{+2} and ex^{+2} were intermediate and the plaque size varied from small, but not pin-point like, to large with an e.o.p. of about 0·5. The e.o.p. of the other progeny were like T4 or lower, but no small plaques were observed.

(4) All progeny, when crossed with the parental T4, appeared to be non-excludable; sensitivity to exclusion was not recovered.

(5) The glucosyltransferase preparations from the twenty-seven progeny were capable of introducing glucose in T2 DNA under the assay conditions for α- and

β-glucosyltransferase. This means that all progeny induced T4 α- and T4 β-glucosyltransferase upon infection of the bacterial host. Figure 2 presents the activities of the samples.

Two HMC DNA preparations were used of which HMC DNA (2) was inferior to HMC DNA (1), but served the purpose of indicating any activity. The activities of the samples on T2 DNA and HMC DNA are correlated. From the data with HMC DNA (1) the following regression formulae were calculated with the least-squares method:

$$\text{for } \alpha\text{-glucosyltransferase: } y = 38 + 0{\cdot}119\,x, \text{ and}$$
$$\text{for } \beta\text{-glucosyltransferase: } y = 118 + 0{\cdot}116\,x,$$

where y and x stand for the incorporations in T2 DNA and HMC DNA respectively. The results for each condition can be understood as the variations of one variable factor, i.e. the concentration of one glucosyltransferase. The regression coefficients are the proportions of glucosylation *in vitro* in both DNA's. Kornberg *et al.* (1961) demonstrated that 0·28 of the free HMC groups in T2 DNA could be glucosylated rapidly by T4 α-glucosyltransferase. This fraction is approximately 0·09 of the sum of the substituted and the free HMC in T2 DNA. This value is in agreement with the regression coefficients and indicates that the proportions of glucosylation of T2 DNA and HMC DNA correspond with the amounts of free HMC available for incorporation. The fact that the ordinate was positively intercepted by the regression lines was not investigated any further.

4. DISCUSSION

The properties of strains T2 k^{+4} 1–5 demonstrate that T4-like e.o.p. on K (λh) can occur segregated from the other bar-properties: the exclusion property and T4 glucosylation. These results agree with those obtained by Jesaitis (1961) with progeny from crosses of T6 and T2, among which recombinants were found with an e.o.p. on K like T6 and T2 α-glucosylation of HMC. Apparently, the extension of glucosylation of DNA by T4 enzymes is not required for raising the limitations for growth of T2 on K (λh).

The results of the single-burst experiment (Fig. 1) confirm this conclusion; the five progeny with an intermediate e.o.p. on K (λh) have T4 glucosylation of the HMC residues in their DNA. This is the reverse situation: limitation of growth on K (λh) can occur, at least in part, when the DNA is completely glucosylated. The intermediate e.o.p. is found in the five progeny with ex^{+2}. This suggests that at least one factor for sensitivity to limited growth on K (λh) is closely linked to, or identical with, ex^{+2}. The nature of the factors is not yet known.

The T4 gene for exclusion (ex^{+4}) has not been transmitted to all progeny from the single burst and the frequency of ex^{+2} follows polarity according to its position on the map between h^+ and v^+. However, all five progeny show T4 glucosylation of their DNA and so again this segregation of exclusion and glucosylation does not agree with the concept of the bar-properties. Only partial resistance to exclusion (exr^{+4}) and T4 glucosylation are transmitted to all progeny of the single burst, while

some factor for increased e.o.p. on K (λh) may also be present in all progeny. Both strain T2 k^{+4} 6 and the progeny of the single burst, show coincidence of resistance to exclusion and T4 glycosylation.

In the case of T2 k^{+4} 6 the new properties are considered to have been rescued from T4 independently of k^{+4}. Rescue of more than one gene from ultraviolet-irradiated T4 has also been observed in a system where the unirradiated helper phage was a mutant T4 (Stahl, Edgar & Steinberg, 1964). Although the dose applied was very high, rescue of more than one gene from T4 is favoured in the system with T2 as a helper phage. T2 is excluded by ultraviolet-irradiated T4 (unpublished observations) and the burst size of such crosses is small, which probably increases the chance for rescue of more than one T4 gene per T2 progeny. The e.o.p. of strain T2 k^{+4} 6 on K (λh) is not different from that of the other strains T2 k^{+4} 1–5. It is therefore assumed that the gene or genes for the new properties in T2 k^{+4} 6 have no relation with the e.o.p. on K (λh) and have not been rescued by selection, but by chance.

If the genes for partial non-excludability (exr^{+4}) and T4 glucosylation (αg^{+4} and βg^{+4}) are not related, their coincidence might reflect close linkage so that the genes appear in the same recombinants. If the genes are identical, the question arises how additional glucosylation by T4 enzymes can enhance resistance to partial exclusion. There are some examples that glucosylation can protect DNA from the effect of enzymes. Analyses of nucleotide sequences in T-phage DNA by De Waard (1964) have shown that α-glucosyltransferases have low affinities for HMC residues which are the neighbours of HMC residues that have already been glucosylated. Recently, Richardson (1966) found a protective effect of glucosylation on DNA when incubated with exonuclease III from *E. coli*; glucoseless T2 and T6 DNA were degraded twenty times more rapidly than DNA with the normal glucose content. Therefore, additional glucosylation might render the T2 DNA capable of reducing the affinity of the exclusion factor of T4. The nature of the exclusion factor is not known. Crosses of an 'early *amber*' of T4 with T2 revealed progeny from which T2 was nearly completely excluded, while breakdown of DNA was observed in these crosses (De Groot, 1966*b*). The exclusion factor might therefore be related to some nuclease activity.

Streisinger & Weigle (1956) explained partial exclusion by assuming the elimination by T4 of a sensitive fragment of T2 DNA. They also suggested that T2 glucosylation might be related to sensitivity to exclusion. For the results, reported here, a variant of this explanation is proposed. Among the progeny of the single burst, no parental combinations of the genes ex^{+2} and v^{+2} were found, these genes were only recovered in recombinants. If the gene for T2 α-glucosylation (αg^{+2}) resides in the neighbourhood of v^{+2}, which is not an unlikley residence for a gene coding for an enzyme modifying synthesized DNA, αg^{+2} will predominantly be found in recombinants with ex^{+4}. This recombinant type, however, is not present in the progeny. The reason might be, that these recombinants are suicidal. When such recombinants arise in a cross of T4 with T2, they will be T4 glucosylated due to phenotypic mixing. Upon infection of a new host, this genotype of progeny will produce DNA with T2 α-glucosylated HMC which will be sensitive to its own T4

exclusion factor and undergo suicide within one or a few growth cycles, if for example the exclusion factor is a T4 nuclease or is associated with nuclease activity, as suggested above. The occurrence in the progeny from the single burst of the genes for both the T4 α- and T4 β-glucosyltransferases does not necessarily mean that glucosylation by both enzymes is required for resistance to exclusion. Pratt, Kuno & Lehman (1963) have shown one of the T2 bar-strains isolated by Streisinger & Weigle (1956) to possess α-glucosylation up to 0·85 of the HMC due to the induction of T4 α-glucosyltransferase only. This partial modification of DNA is, if this explanation is correct, apparently sufficient to increase resistance to the exclusion factor. The induction of T4 β-glucosyltransferase by all twenty-seven progeny phages might therefore be no more than the reflexion of a very close linkage of the two genes.

The difference from the explanation of Streisinger & Weigle (1956) is the assumption that the whole T2 DNA molecule is concerned with sensitivity to exclusion, and that there is no direct elimination of a part of the T2 DNA by T4, but elimination of a part of recombinant progeny in the next generations. The role of differential glucosylation might be a particular case of modification known to occur in micro-organisms and leading to resistance to restriction. One of the best-known cases in phage is that of phage lambda propagated on *E. coli* C, which is restricted upon infection of *E. coli* K (Arber, 1965). A small fraction of the phage survives restriction and appears to be modified in such a way as to be accepted without restriction in a further growth cycle. Certain methionine-requiring strains deprived of methionine during phage development, failed to modify the progeny phage, which suggests a role for methylation of bases in the modification of phage lambda.

Partial resistance to exclusion and T4 glucosylation appear to be the only properties behaving in agreement with the bar-concept. The above explanation is based on the assumption that the bar-concept is correct and that the properties are identical. This assumption, however, needs further proof.

SUMMARY

Analysis of the inheritance of the three bar-properties of bacteriophage T4: exclusion of T2 from the progeny of crosses, glucosylation of the hydroxymethylcytosine (HMC)moiety of the DNA according to T4, and plating with large plaques on *E. coli* K strains, was carried out by means of marker rescue from T4 by T2 on *E. coli* K (λh) as a selective indicator. Five of the strains isolated plated with large plaques on K (λh), but did not exclude T2 and showed T2 glucosylation; plating on *E. coli* K (λh) was found to segregate from the other two bar-properties. The sixth isolate showed, in addition to plating with large plaques on K, partial non-excludability by the parental T4 and T4 glucosylation of HMC. If partial non-excludability is the result of T4 glucosylation, the role of the additional glucose substitutions might be a protective effect on the DNA against the exclusion factor of T4. This proposal is supported by the analysis of the progeny from a single burst from

a cross of T4 and T2. The following T2 genes were partially excluded: host-range, no exclusion of parental T2, sensitivity to ultraviolet, and limited plating efficiency on *E. coli* K (λh). The exclusion factor of T4 is not transmitted to all progeny and does not behave like a bar-property. Only resistance to exclusion and T4 glucosylation were transmitted to all twenty-seven progeny of the single burst. The elimination of sensitivity to exclusion and T2 glucosylation is explained by assuming that the recombinant class with the exclusion factor of T4 and T2 α-glucosylation will exclude itself and be suicidal upon infection of a new host. Exclusion and differential glucosylation are discussed with regard to restriction and modification, respectively.

The author wishes to thank Professor F. H. Sobels for his stimulating interest and Dr A. de Waard for advice in the application of the enzyme techniques. I am obliged to Professor J. A. Cohen for technical facilities in the Dept. of Applied Enzymology and Radiobiology, University of Leiden. Miss Th. A. M. Verkley and Miss W. Veerman rendered capable technical assistance.

REFERENCES

ADAMS, M. H. (1959). *Bacteriophages*. New York: Interscience Publ.

ARBER, W. (1965). Host specificity of DNA produced by *Escherichia coli*. V. The role of methionine in the production of host specificity. *J. molec. Biol.* **11**, 247–256.

EDGAR, R. S. & EPSTEIN, R. H. (1963). Conditional lethal mutations in bacteriophage T4. 'Genetics Today'. *Proc. XI. Int. Congr. Genet.* (S. J. Geerts, ed.), **2**, 1–16. The Hague.

DE GROOT, B. (1966*a*). Marker rescue of the adsorption properties of bacteriophage T2. *Antonie van Leeuwenhoek*, **32**, 17–24.

DE GROOT, B. (1966*b*). Partial exclusion or bacteriophage T2 by T4: An early function in the second linkage group. *Genetica*, **37**, 37–51.

HERSHEY, A. D. & ROTMAN, R. (1949). Genetic recombination between host range and plaque-type mutants of bacteriophage in single bacterial cells. *Genetics*, **34**, 44–71.

JESAITIS, M. A. (1961). The inheritance of the glucose component of the phage nucleic acids. *J. gen. Physiol.* **44**, 585–609.

KORNBERG, S. R., ZIMMERMAN, S. B. & KORNBERG, A. (1961). Glucosylation of deoxyribonucleic acid by enzymes from bacteriophage-infected *Escherichia coli*. *J. biol. Chem.* **236**, 1487–1493.

LEHMAN, I. R. & PRATT, E. A. (1960). On the structure of the glucosylated hydroxymethylcytosine nucleotides of coliphages T2, T4 and T6. *J. biol. Chem.* **235**, 3254–3259.

PRATT, E. A., KUNO, S. & LEHMAN, I. R. (1963). Glucosylation of the deoxyribonucleic acid in hybrids of coliphages T2 and T4. *Biochim. biophys. Acta*, **68**, 108–111.

RICHARDSON, C. C. (1966). Influence of glucosylation of deoxyribonucleic acid on hydrolysis by deoxyribonucleases of *Escherichia coli*. *J. biol. Chem.* **241**, 2084–2092.

SHEDLOVSKY, A. & BRENNER, S. (1963). A chemical basis for the host-induced modification of T-even bacteriophages. *Proc. natn. Acad. Sci. U.S.A.* **50**, 300–305.

STAHL, F. W., EDGAR, R. S. & STEINBERG, J. (1964). The linkage map of bacteriophage T4. *Genetics*, **50**, 539–552.

STREISINGER, G. (1956*a*). The genetic control of ultraviolet sensitivity levels in bacteriophages T2 and T4. *Virology*, **2**, 1–12.

STREISINGER, G. (1956*b*). The genetic control of host range and serological specificity in bacteriophages T2 and T4. *Virology*, **2**, 377–387.

STREISINGER, G. & WEIGLE, J. (1956). Properties of bacteriophages T2 and T4 with unusual inheritance. *Proc. natn. Acad. Sci. U.S.A.* **42**, 504–510.

DE WAARD, A. (1964). On the specificity of bacteriophage induced hydroxymethylcytosine glucosyltransferases. I. Specificity differences between the hydroxymethylcytosine–glucosyltransferases induced by bacteriophages T2, T4 and T6. *Biochim. biophys. Acta*, **92** 286–304.