THE GLUCOSE CONTENT OF THE DEOXYRIBONUCLEIC ACIDS OF CERTAIN BACTERIOPHAGES*

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The presence of glucose in the deoxyribonucleic acid (DNA) of the even-numbered bacteriophages of the T series was discovered by Jesaitis[1] and independently by Sinsheimer.[2] This glucose was shown to be associated with the hydroxymethylcytosine (HMC) in these nucleic acids as a glycoside substituent to the hydroxymethyl group (Sinsheimer;[2] Volkin[3]).

Further studies have indicated that the proportion of glucose is not the same in T2L and T4 bacteriophages and that the proportion of glucose seems to be connected with other recognizable traits of the bacteriophages (Streisinger and Weigle[4]). However, the mode of inheritance of these properties is unusual.

Methods.—All phage stocks were grown on *Escherichia coli* "B" according to the usual methods (Adams[5]) in the glycerol-casamino acid medium described by Fraser and Jerrel.[6] The stocks were purified by treatment with pancreatic deoxyribonuclease, followed by two cycles of differential centrifugation. DNA was obtained from the purified viruses by osmotic shock (Anderson *et al.*[7]), followed by high-speed centrifugation to remove "ghosts," deproteinization according to the method of Sevag *et al.*,[8] and alcoholic precipitation.

The molar proportion of glucose was determined by the ratio of the glucose content, as determined by the use of the anthrone reagent for glucose (Morris[9]), to the phosphorus content (Allen[10]). A correction for the color produced by the deoxyribose in the anthrone test was made by subtraction of the color produced by a calf thymus DNA solution (Gary and Klausmeier[11]) of the same phosphorus content. Recovery experiments with added glucose gave quantitative results.

Enzymatic Degradation of Bacteriophage DNA.—If DNA from any of these bacteriophage strains is degraded successively with pancreatic deoxyribonuclease and purified venom phosphodiesterase, about 65 per cent of the phosphorus of the digest can be recovered as mononucleotides.[2] The remainder is present as di-, tri-, and larger polynucleotides, all of which appear to contain HMC, as indicated by their ultraviolet absorption spectra.

If the DNA is that of a wild-type T2L, five mononucleotides are obtained: thymidylic acid, deoxyadenylic acid, deoxyguanylic acid, and two nucleotides of HMC, one with and one without a glucose substituent. The molar proportions of these mononucleotides are presented in Table 1, together with the proportion of each nucleotide in the T2L DNA which is obtained as a mononucleotide. It is clear

TABLE 1

MONONUCLEOTIDES FROM ENZYMATIC DIGEST OF T2L DNA

	Thymidylic	Adenylic	Guanylic	HMC	HMC-Glucose
Per cent of P of digest in mononucleotide	22.8	22.2	12.5	2.2	3.0
Per cent of nucleotide in digest recovered as mononucleotide	70	68	69	54	24

that the proportion of the glucose-substituted HMC mononucleotide found among the 65 per cent mononucleotide fraction is much lower than that of the other nucleotides, and thus, conversely, the enzyme-resistant polynucleotides must be enriched in this component. It seems likely that the presence of the glucose substituent, appropriately situated, confers resistance to phosphodiesterase action upon the polynucleotide.

The presence of the HMC nucleotide lacking glucose in such digests is clear evidence for incomplete glucose substitution of the HMC.

Analysis.—The nucleic acids of T2L and T4 bacteriophage and of the progeny of various crosses of these viruses have been analyzed for glucose content and have been enzymatically degraded to ascertain the possible presence of hydroxymethylcytosine lacking glucose substitution. The results are presented in Table 2.

TABLE 2

Virus Strain	Moles Glucose/ 100 Moles P	Presence of HMC Nucleotide, Not Glucose-substituted	Presence of Glucose-substituted HMC Nucleotide
T2L $r^+h^+u^+$	12.6	+	+
T4 r^+u	16.7	−	+
B×9 T2 $r^+h^+u^+$	17.1	−	+
B×9 T2 r^+h^+u	16.5	−	+
V-G T2 rhu^+	12.2	+	+
V-G T2 rh^+u^+	16.4	−	+

T4 differs from T2, among other traits, by the presence of a locus u conferring to its carrier a high resistance to inactivation by ultraviolet light (Luria;[12] Streisinger[13]). The strains B × 9 T2 are strains of T2 which, after one mating with T4, have been backcrossed to T2 nine times to make them as isogenic as possible with T2.[4, 13] It can be seen that they have acquired the "glucose property" of T4 and that it is not the u locus which is responsible for the glucose content. The V-G T2 stocks resulted from a single burst of a cross of $T2rh^+$ with B × 9 T2rh with delayed input of the second phage (Visconti and Garen[14]). These experiments are described in more detail in the next paper.[4] Under the conditions of delayed input, it can be seen that not all the progeny particles of the cross acquire the glucose property of T4.

Discussion.—In the wild-type T2L bacteriophage some (23 per cent) of the hydroxymethylcytosine is not substituted with glucose, while in T4 all the hydroxymethylcytosine is glucose-substituted. The inheritance of this "glucose property" is unusual in that it does not seem to be linked with any of the markers r, h, or u but does seem to be linked with the "bar" markers, which, in a cross T2 × T4 with simultaneous infection, are transmitted to the vast majority of the progeny.[4]

It is possible that the bipartite nature of the DNA of T2, suggested by Brown and Martin[15] and by Levinthal,[16] may be responsible for this unusual inheritance pattern.

Cohen (*Science*, **123**, 653–656 [1956]) has recently presented data suggesting a relationship between the glucose content of bacteriophage DNA and the *r* property. The data presented here do not support this relationship.

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*PROPERTIES OF BACTERIOPHAGES T2 AND T4 WITH UNUSUAL INHERITANCE**

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The first experiments on infecting bacteria mixedly with two different phages (Delbrück and Luria;[1] Delbrück[2]) were done with pairs of unrelated phages. They led to the discovery of exclusion, i.e., the finding that any one bacterial cell will yield mature particles of only one or the other of the parental types, never both. The nature of the exclusion mechanism is not understood.

Later it was found that exclusion is a function of the degree of relatedness of the two infecting phages. Phages which differ by only a few mutational steps are perfectly compatible (see Hershey[3]); not only do both phages multiply, but they also undergo extensive genetic recombination. Phages somewhat more distantly related show an intermediate behavior (Delbrück and Bailey[4]), the details of which have never been worked out.

The phages T2 and T4 belong to a very large group of related phages (Adams[5]). They are the phages whose genetic maps are better known (Hershey and Rotman;[6] Doermann and Hill[7]) than those of any other phage. They are also the pair for which genetic recombination and partial exclusion[4] were first observed. In the present paper we will describe the partial exclusion of T2 by T4 and will show that the "excludability" of T2 is itself a genetic trait of T2 which is completely excluded from the progeny. The excluding power of T4 is thus inherited in a novel way: it is imparted to the overwhelming majority of the progeny particles, irrespective of what other markers of either of the parents these particles inherit. Moreover, this excluding power of T4 is associated with two other characteristics. One of these is the characteristic that the particles carrying it, in contrast to those which do not carry it, plate with high efficiency on certain bacterial indicator strains.