Glucosylated Nucleotide Sequences from T-even Bacteriophage Deoxyribonucleic Acids

By MARY R. LUNT* and ELIZABETH A. NEWTON
Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford

(*Received* 31 *August* 1964)

1. The frequencies of various pyrimidine nucleotide sequences in phage-T2VG111 DNA and phage-T6 DNA have been measured. The hydroxymethylcytosine nucleotides that bear glucosyl groups in phage-T2VG111 DNA and gentiobiosyl groups in phage-T6 DNA are not randomly distributed. 2. Sequences in which two or three hydroxymethylcytosine nucleotides are terminated at both ends by purine nucleotides bear only one sugar substituent and this is attached to the first hydroxymethylcytosine when the sequences are written in the conventional notation; i.e. with the 5′-phosphate before and the 3′-phosphate after each nucleoside residue. This resembles the distribution of glucosyl residues previously found in the corresponding sequences from phage-T2 DNA. Sequences in which one hydroxymethylcytosine nucleotide is attached through the 3′-position to a purine nucleotide are more fully glucosylated in phage-T2VG111 DNA and phage-T6 DNA than in the DNA of phage T2. 3. Pyrimidine sequences from the DNA of phage T6(S) have been found to contain glucosyl but not gentiobiosyl residues. The relative amounts of the sequences isolated indicate that the distribution of the glucosyl residues in this DNA resembles that of the gentiobiosyl groups in phage-T6 DNA. 4. It is suggested that the distributions of glycosyl substituents in these T-even-phage deoxyribonucleic acids reflect the specificity requirements of the α-glucosyltransferases induced by the different phages. The results obtained with the DNA of phage T6(S) suggest that this phage induces the usual phage-T6 α-glucosyltransferase but not the phage-T6 β-glucosyltransferase.

The deoxyribonucleic acids of coliphages T2, T4 and T6 contain hydroxymethylcytosine nucleotides to some of which are attached α-glucosyl, β-glucosyl or α-gentiobiosyl groups in proportions that are characteristic of each phage (Table 1; Lehman & Pratt, 1960; Kuno & Lehman, 1962). The introduction of the different sugar substituents depends on the action of specific phage-induced enzymes that attach glucose to hydroxymethylcytosine residues after their incorporation into the DNA (Kornberg, Zimmerman & Kornberg, 1961; Zimmerman, Kornberg & Kornberg, 1962; Josse & Kornberg, 1962; Erikson & Szybalski, 1964).

All three phage strains induce the formation of a transferase that substitutes about 70% of the hydroxymethylcytosine in the DNA with α-glucosyl groups. In phage-T4-infected cells a further transferase adds β-glucosyl residues to the remain-

* Present address: Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.

Table 1. *Amounts of hydroxymethylcytosine nucleotides in phage deoxyribonucleic acids*

The data are taken from Lehman & Pratt (1960), Kuno & Lehman (1962) and Pratt, Kuno & Lehman (1963).

	Percentage of total hydroxymethylcytosine in DNA			
Phage	Unsubstituted	α-Glucosyl	β-Glucosyl	α-Gentiobiosyl
T2	25	70	0	5
T4	0	70	30	0
T6	25	3	0	72
T2VG111	16–18	80–83	0	1·3–1·5

ing hydroxymethylcytosine residues; in phage-T6-infected cells a different β-glucosyltransferase converts most of the α-glucosyl residues into α-gentiobiosyl groups by attaching second glucose residues to the monoglucosyl groups already present. The α-glucosyltransferases induced by the three different phages resemble each other in many of their properties; however, the phage-T4 and phage-T6 enzymes differ from the phage-T2 transferase in their ability to introduce additional α-glucosyl residues into phage-T2 DNA (Zimmerman *et al.* 1962; Pratt, Kuno & Lehman, 1963; de Waard, 1964*b*).

To investigate whether any structural features might influence the content and arrangement of glucose residues, phage-T2 DNA was degraded with 2% diphenylamine in 67% formic acid, and with pancreatic deoxyribonuclease (Burton, Lunt, Petersen & Siebke, 1963; Lunt, Siebke & Burton, 1964). The results showed that glucose is not randomly distributed among the hydroxymethylcytosine residues, and also suggested that the observed glucosylation pattern in this DNA probably reflects the specificity requirements of the phage-T2 α-glucosyltransferase.

To investigate the distribution of glucose residues in other deoxyribonucleic acids, the diphenylamine degradation procedure has now been applied to phage-T6 DNA and phage-T2VG111 DNA. Phage T2VG111 was isolated from crosses originally involving phages T2 and T4 (Streisinger & Weigle, 1956; Weigle, Streisinger, Hershey & Kim, 1959) and has been shown to induce an α-glucosyltransferase that resembles that induced by phage-T4; the phage does not induce a β-glucosyltransferase (Pratt *et al.* 1963). The DNA of phage T2VG111 therefore provides convenient material for studying the distribution of glucose residues introduced by the phage-T4 α-glucosyltransferase. The DNA of another strain, phage T6(S), has also been examined; this phage is of interest since, although it has the usual phage-T6 host range, the DNA glucose content resembles that of phage T2 (Dr S. Silver, personal communication).

Some of the results described in the present paper have been briefly reported (Burton *et al.* 1963; Lunt & Newton, 1964).

METHODS AND MATERIALS

Bacteriophages. Phage T6r$^+$ with a requirement for L-tryptophan as an adsorption cofactor was provided by Dr K. Burton, Department of Biochemistry, University of Oxford, and is the same strain as that described by Putnam, Kozloff & Neil (1949). Phage T6(S), which also requires L-tryptophan for adsorption, was obtained from Dr S. Silver, and was originally supplied by Dr A. Garen, Division of Biology, University of Pennsylvania, Philadelphia 4, Pa., U.S.A. Phage T2VG111 was supplied by Dr G. Streisinger, Institute of Molecular Biology, Eugene, Oregon, U.S.A.

The phages were prepared by lysis of *Escherichia coli* strain B, which was grown as described by Lunt *et al.* (1964). Before infection with either of the phage-T6 strains, sufficient L-tryptophan was added to the cultures to give a final concentration of 1μg./ml. With both phage-T6 strains the bacteria were grown to a cell density of 2×10^8/ml., and then sufficient phages were added to give multiplicities of infection of 0·5 for phage T6 and of 2–3 particles/cell for phage T6(S). Phage T2VG111 was prepared by infecting bacteria at $1·3 \times 10^8$ cells/ml. with a multiplicity of infection of 3–5. After infection, in all cases aeration was continued for a further 7hr., after which the cultures were kept at room temperature for 12–36hr. The phages were purified by differential centrifuging (Kay, 1959).

Bacteriophage deoxyribonucleic acid. The DNA was prepared by the method of Mandell & Hershey (1960) except that 0·05M-tris–HCl buffer, pH7·1, was used instead of phosphate buffer. Before chemical degradation the DNA was freed from salt either by dialysis against distilled water, or by being precipitated with ethanol, redissolved in water and reprecipitated.

Enzymes. The phosphodiesterase from *Lactobacillus acidophilus* was prepared according to the procedure of Fiers & Khorana (1963), with the omission of the calcium phosphate-gel stage. The remaining enzymes were the same as those used by Lunt *et al.* (1964), except that spleen phosphodiesterase was prepared according to the method of Heppel & Hilmoe (1955).

Two-dimensional paper chromatography. For two-dimensional separations of compounds from phages T2VG111 and T6(S) the procedures and solvent systems described by Burton & Petersen (1960) were used. For compounds from phage T6 the procedures were the same, except that the second solvent in the first dimension was propan-2-ol–aq. 5N-NH_3 (7:3, v/v).

Electrophoresis combined with chromatography on paper. The apparatus and procedures described by Petersen (1963) were used, except that 0·02vol. of 3-methylbutan-1-ol was added to the solvent for chromatography.

Ion-exchange resins. Dowex 1 and Dowex 50 resins were prepared as described by Burton & Petersen (1960). DEAE-Sephadex A-25 (medium grade) was washed according to the makers' instructions and converted into the formate form by treatment with M-sodium formate. It was washed with water and stored at 4° until used.

Diphenylamine digests. By using the procedures of Burton & Petersen (1960) the samples of phage DNA were degraded with 2% (w/v) diphenylamine in 67% (v/v) formic acid. After diphenylamine digestion, the amine and formic acid were removed by extraction with ether, and the purines by treatment of the digest with Dowex 50 (X8; H^+ form). For electrophoresis or chromatography on paper the nucleotide products were treated with *E. coli* phosphomonoesterase (Lunt *et al.* 1964) to remove terminal phosphate groups. For separations on DEAE-Sephadex the digests were used without monoesterase treatment.

Phosphate, glucose and nucleotide estimations. The procedures used by Lunt *et al.* (1964) were used. In the analyses of diphenylamine digests the total amounts of material on paper chromatograms were usually estimated from the inorganic phosphate eluted from the papers (Burton & Petersen, 1960); alternatively, measured volumes

of digest solutions of known phosphate content were applied to the papers. The latter method was also used when digests were separated by electrophoresis and chromatography on paper.

In estimating the amounts of nucleotides the extinction coefficients given by Lunt *et al.* (1964) were used. The expected extinction ratios E_{280}/E_{260} were calculated from the extinction coefficients of the expected or known components without allowing for any possible effects due to hypochromicity or the presence of glucosyl or gentiobiosyl residues. The E_{280}/E_{260} ratios have been used to determine the proportions of thymidine and hydroxymethyldeoxycytidine in each nucleotide product.

RESULTS

Pyrimidine nucleotide sequence from phage-T6 deoxyribonucleic acid. Diphenylamine digests of phage-T6 DNA were treated with monoesterase and the pyrimidine nucleotide products separated by combining electrophoresis with chromatography on paper, as shown in Fig. 1. The analyses of the compounds that contain hydroxymethylcytosine are given in Table 2. The presence of gentiobiosyl residues in most of the compounds permitted satisfactory resolution of only the shorter sequences. Two-dimensional chromatography was satisfactory for obtaining the compounds of composition $HT_2p_2Glc_2$,* and for resolving the dinucleotide isomers HTpGen, and the nucleosides GenH, GlcH and H. The dinucleotide isomers were assigned sequences on the basis of their chromatographic positions relative to those of the corresponding compounds from phage-T2 DNA (Lunt *et al.* 1964). Oligonucleotides of composition HT_np_n corresponding to those obtained from phage-T2 DNA (Lunt *et al.* 1964) were not detected on two-dimensional paper chromatograms. The amounts of the different compounds are given in Table 3. The nucleotides isolated by these methods account for 66–70% of the total glucosylated hydroxymethylcytosine in the DNA.

Larger amounts of the compounds that contained hydroxymethylcytosine as the only base were obtained by adsorbing the diphenylamine digest from about 330mg. of phage-T6 DNA on to a column (30cm. × 0·9cm. diam.) of DEAE-Sephadex A-25 (formate form). Purines were removed with

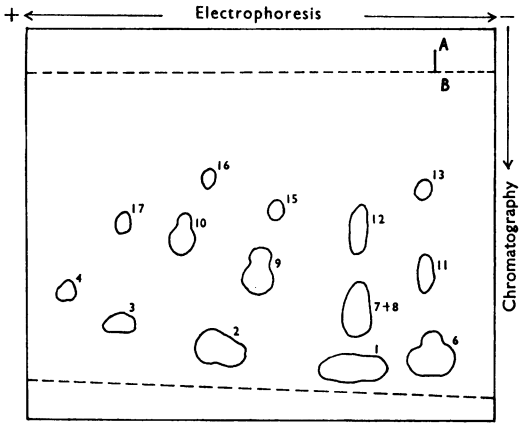

Fig. 1. Separation on paper of nucleotides obtained from phage-T6 DNA by degradation with diphenylamine in formic acid and treatment with phosphomonoesterase. The nucleotides were applied along the line *A–B*, which was 15cm. from the negative end of the sheet. Electrophoresis in ammonium formate buffer, pH 2·7, was followed by two chromatographic developments at right angles (Petersen, 1963). The solvent front is marked *C*. The analytical data for compounds 6–17, which contain hydroxymethylcytosine, are given in Table 2. Compounds 1, 2, 3 and 4 were identified as T, TpT, T_3p_2 and T_4p_3 respectively.

0·01M-formic acid, after which elution of the column with 0·9M-formic acid gave a large peak of ultraviolet-absorbing material (Petersen & Burton, 1964). After removal of water and formic acid on a rotary evaporator, the material in the peak was treated with monoesterase to remove terminal phosphate groups. The components of the mixture were then separated by two-dimensional chromatography or by electrophoresis and chromatography on paper. The compounds obtained in this way had the compositions GenH, GlcH, H, H_2pGlc_2, $H_3p_2Glc_2$ and $H_4p_3Glc_2$ (traces only). The identities of the nucleosides GlcH and H were confirmed by comparison of their chromatographic positions with those of compounds of known composition isolated from phage-T2 DNA. Separation on a Dowex 1 (formate form) column was also used to obtain the nucleotides that contain hydroxymethylcytosine as the only base (Lunt *et al.* 1964). In this experiment, besides a large peak that contained only nucleotides with gentiobiosyl substituents, a second small peak was obtained and found to yield GlcH and some H_2pGlc_2.

To determine the structure of the dinucleotide H_2pGlc_2, samples of the compound were treated either with purified spleen phosphodiesterase or with purified diesterase from *Lb. acidophilus* (Fiers & Khorana, 1963); both enzymes release nucleoside 3′-phosphates. The results, summarized in Table 5,

* The following abbreviations are used, the prefix 'd' being omitted since all nucleosides and their derivatives are of the deoxyribose series: H, 5-hydroxymethyldeoxycytidine; T, thymidine; Py, pyrimidine deoxyriboside; Pu, purine deoxyriboside; Glc, glucosyl residue; GlcH, 5-(α-glucosidomethyl)deoxycytidine; Gen, gentiobiosyl residue; GenH, 5-(α-gentiobiosidomethyl)deoxycytidine; p, phosphate residue. When p is placed on the left of a nucleoside symbol it represents a 5′-phosphate and when on the right a 3′-phosphate. Compounds whose sequences are unknown or which may consist of sequential isomers are designated by empirical formulae of the type $H_3p_2Glc_2$, HTp etc.

Table 2. *Identification of compounds obtained from phage-T6 deoxyribonucleic acid by diphenylamine degradation and treatment with phosphomonoesterase*

Compounds are numbered as in Fig. 1. The calculated E_{280}/E_{260} ratios are those expected from the component nucleotides without allowing for any hypochromic effects. Since the procedures described in Fig. 1 do not effectively separate the nucleotides into glucosylated and non-glucosylated components, the glucose contents are omitted from the formulae given in the last column. Compound 14 is not shown in Fig. 1. It was only separated on paper after prior chromatography on DEAE-Sephadex.

Compound no.	E_{280}/E_{260} ratio		P/Py ratio		Glc/H ratio	Identity (omitting Glc content)
	Found	Calc.	Found	Calc.		
6	2·56	2·55	0	0	1·5–2·0	H
7+8	1·36	1·34	0·50	0·50	1·99	HTp
9	1·10	1·08	0·68	0·67	1·50–2·0	HT_2p_2
10	0·97	0·98	—	—	—	HT_3p_3
11	2·54	2·55	0·50	0·50	0·92	H_2p
12	1·67	1·65	0·73	0·67	0·95	H_2Tp_2
13	2·53	2·55	0·61	0·67	0·68	H_3p_2
14	2·34	2·55	0·84	0·75	0·58	H_4p_3
15	1·33	1·34	—	—	—	$H_2T_2p_3$
16	1·19	1·18	—	—	—	$H_2T_3p_4$
17	0·91	0·91	—	—	—	HT_4p_4

Table 3. *Nucleotides that contain hydroxymethylcytosine obtained from phages T2 VG111, T6(S) and T6*

Amounts were calculated from substances eluted from two-dimensional paper chromatograms, except where indicated.

Compound	Amount isolated (moles of pyrimidine/100 g.atoms of DNA P)		
	Phage T2 VG111	Phage T6(S)	Phage T6
GenH	0	0	3·13
GlcH	3·65	3·45	0·14
H	0·14	0·20	0·24
Tp(GlcH)*	2·87	2·47	2·64
(GlcH)pT*	2·79	2·33	2·84
(GlcH)T_2p_2*	2·65	2·34	3·02
(GlcH)T_3p_3*	2·45	2·29	2·27†
(GlcH)pH*	1·89	1·67	1·75†
(GlcH)pHpH*	—	—	0·39†

* Compounds from phage T6 contain GenH and not GlcH.

† Estimated from material isolated by electrophoresis and chromatography.

Table 4. *Phage-T2 VG111 deoxyribonucleic acid: analytical data for nucleotides that contain hydroxymethylcytosine as the only base*

Compounds are numbered to correspond to those in Fig. 1. The calculated E_{280}/E_{260} ratios are those expected from the component nucleotides without allowing for any hypochromic effects.

Compound no.	E_{280}/E_{260} ratio		P/H ratio	Glc/H ratio	Composition
	Found	Calc.			
11	2·54	2·55	0·54	0·56	H_2pGlc
13	2·85	2·55	0·67	0·28	H_3p_2Glc

indicated that the dinucleotide consists of one sequential isomer of structure (GenH)pH.

The trinucleotide $H_3p_2Glc_2$ was hydrolysed with snake-venom diesterase, which releases nucleoside 5′-phosphates. The products GenH and hydroxymethyldeoxycytidine 5′-phosphate were released in the proportions 1:2·2, indicating that the original compound had the structure (GenH)pHpH (Table 5).

Pyrimidine nucleotide products from phage-T2 VG111 deoxyribonucleic acid. After removal of terminal phosphate groups the diphenylamine digestion products were separated by two-dimensional paper chromatography. The separation of the compounds listed in Table 3 was the same as that described by Lunt *et al.* (1964). Oligonucleotides of composition HT_np_n were not detected. Larger amounts of compounds that contained hydroxymethylcytosine as the only base were isolated after fractionation of diphenylamine digests from 300–400 mg. of DNA on DEAE-Sephadex as described for phage-T6 DNA. After removal of terminal phosphate groups the material obtained in this way was separated by two-dimensional chromatography into components of composition GlcH, H, H_2pGlc and H_3p_2Glc. The analyses for the di- and tri-nucleotide are summarized in Table 4.

Degradation of the dinucleotide H_2pGlc with spleen phosphodiesterase, and the trinucleotide

Table 5. *Amounts of products formed by action of phosphodiesterases on di- and tri-nucleotides from diphenylamine digests*

Compounds are numbered to correspond to those in Fig. 1. For the experiment with the diesterase from *Lactobacillus acidophilus*, 25 units of enzyme were incubated with about 0·1 μmole of dinucleotide in 0·05 M-tris–HCl buffer, pH 7·8, containing NaCl (0·05 M) (Fiers & Khorana, 1963); the total volume was 0·9 ml. After 2 hr. incubation at 37° the reaction was stopped by heating at 100° for 2 min. The products were desalted on a charcoal–Celite column (Petersen, 1963) and separated by two-dimensional paper chromatography. For experiments with the spleen and venom diesterases the procedures of Lunt *et al.* (1964) were followed. In all cases the reaction products were identified by comparison of their chromatographic positions with those of compounds of known composition or by measurement of their phosphate and glucose contents.

	Substrate			Products				
Source of DNA	Compound no.	Composition	Percentage hydrolysed	H (μmole)	Hp (μmole)	GlcH* (μmole)	(GlcH)p* (μmole)	Identity of substrate
Lactobacillus acidophilus diesterase								
Phage T6	11	H_2pGlc_2	100	0·102	—	0·017	0·072	(GenH)pH
Spleen phosphodiesterase								
Phage T6	11	H_2pGlc_2	89	0·272	—	—	0·304	(GenH)pH
Phage T2VG111	11	H_2pGlc	100	0·271	—	—	0·259	(GlcH)pH
Snake-venom diesterase								
Phage T6	13	$H_3p_2Glc_2$	84	—	0·172†	0·078	—	(GenH)pHpH
Phage T2VG111	13	H_3p_2Glc	100	—	0·076†	0·042	—	(GlcH)pHpH
			93	—	0·800†	0·341	—	—

* Corresponding gentiobiosyl derivatives for phage-T6 nucleotide products.
† Assumed to be the 5′-phosphate.

H_3p_2Glc with venom diesterase, showed that these compounds have the structures (GlcH)pH and (GlcH)pHpH respectively (Table 5).

Pyrimidine nucleotides from phage-T6(S) deoxyribonucleic acid. A diphenylamine digest of phage-T6(S) DNA was treated with monoesterase and the nucleotide products were separated by two-dimensional paper chromatography. All the compounds separated in the same way as those obtained from the DNAs of phage-T2 DNA and phage-T2VG111 DNA; the amounts of those that contain hydroxymethylcytosine are given in Table 3. No compounds moving in the positions expected for diglucosylated derivatives were detected. Detailed investigations of the sequences in the di- and tri-nucleotides containing hydroxymethylcytosine as the only base were not undertaken.

DISCUSSION

The above results show that gentiobiosyl and glucosyl residues are not randomly distributed among the hydroxymethylcytosine residues in phage-T6 DNA and phage-T2VG111 DNA respectively. Substitution is favoured in certain sequences and restricted in others, suggesting that the ability of the α-glucosyltransferases to glucosylate a given site is influenced by the nature of the neighbouring nucleotides. Those sequences in which two or three hydroxymethylcytosine nucleotides are terminated at both ends by purine nucleotides contain only one sugar substituent, and this is attached to the first of the hydroxymethylcytosines when the sequences are written in the conventional notation. The glucosylation pattern in these sequences thus resembles that found in phage-T2 DNA (see Table 6).

However, phage-T2VG111 DNA and phage-T6 DNA are more fully glucosylated than phage-T2 DNA in other types of sequences, and in particular those in which a hydroxymethylcytosine nucleotide is linked through its 3′-position to a purine nucleotide. For example, in diphenylamine digests of phage-T2VG111 DNA and phage-T6 DNA we have been unable to detect the unglucosylated dinucleotide TpH and longer sequences of general formula HT_np_n, although the corresponding glucosylated compounds are present in amounts similar to those found in digests of phage-T2 DNA. In addition, although the sequence purine–hydroxymethylcytosine–purine is incompletely glucosylated in all three deoxyribonucleic acids, the extent of its substitution in phage-T2VG111 DNA and phage-T6 DNA is much greater than that observed in phage-T2 DNA, where only 67% of this sequence occurs in the glucosylated form (Table 6).

Since phage-T2 DNA and phage-T6 DNA con-

Table 6. *Extents of glucosylation detected in sequences from different phage deoxynucleic acids*

Compounds are numbered to correspond to those in Fig. 1. The values for phage-T2 DNA are from Lunt *et al.* (1964); the remainder are from the values in Table 3. The H residues marked * represent GlcH in compounds from phage-T2 DNA and phage-T2VG111 DNA, and GenH in compounds from phage-T6 DNA.

Sequence in DNA	Compounds derived from sequence	Ratio of amount of glucosylated sequence/total amount of sequence in DNA		
		Phage T2	Phage T2VG111	Phage T6
Pup$\overset{*}{H}$pPu	6	0·67	0·96	0·93†
PupTp$\overset{*}{H}$pPu	7	0·85	1·00	1·00
Pup$\overset{*}{H}$pTpPu	8	0·96	1·00	1·00
Pup$\overset{*}{H}$pHpPu	11	1·00	1·00	1·00
Pup$\overset{*}{H}$pHpHpPu	13	1·00	1·00	1·00

† Represents fraction of GlcH + GenH in the sequence.

tain approximately the same amounts of unglucosylated hydroxymethylcytosine (Lehman & Pratt, 1960), it is surprising to find such a marked difference in the extents of substitution in certain sequences where hydroxymethyldeoxycytidine residues are linked to purine nucleotides. It therefore appears likely that further unglucosylated hydroxymethylcytosine residues occur in phage-T6 DNA in sequences that have remained undetected in our experiments. Such sequences could occur in trace amounts among the shorter pyrimidine tracts as well as being distributed among the longer sequences that are not resolved by the chromatographic procedures used in the present work.

In phage-T6 DNA monoglucosyl- as well as gentiobiosyl-substituted hydroxymethylcytosine has been detected in the sequence purine–hydroxymethylcytosine–purine. Lehman & Pratt (1960) found that in phage-T6 DNA 3% of the hydroxymethylcytosine nucleotides bear monoglucosyl residues. If the same amount of the monoglucosyl derivative is present in our strain of phage T6, then 66% of this is present as the sequence Pup(GlcH)pPu.

Diphenylamine digests of the DNA from phage T6(S) have also been examined. The chromatographic behaviour of the pyrimidine nucleotides obtained indicates that this phage contains monoglucosylated hydroxymethylcytosine nucleotides; no compounds substituted with gentiobiosyl groups were detected. The relative amounts of the different sequences isolated suggest that the distribution of the glucosyl groups in this DNA resembles that found for the gentiobiosyl residues in phage-T6 DNA. These results are consistent with the possibility that phage T6(S) may induce the usual phage-T6 α-glucosyltransferase but not the phage-T6 β-glucosyltransferase.

In the DNA of phage T2VG111 it is possible that the non-glucosylated hydroxymethylcytosine residues may be almost entirely present in sequences containing adjacent hydroxymethylcytosine nucleotides. If each of these sequences contained only one glucosylated nucleotide they would contribute a total of 3·4 unglucosylated hydroxymethylcytosine residues/100 DNA nucleotides (Lunt *et al.* 1964), assuming that this DNA and that of phage T2 have similar frequencies of nearest neighbour pairs. This would correspond to about 20% of the total hydroxymethylcytosine, and is reasonably close to the values obtained by Pratt *et al.* (1963), who found 16–18% of the hydroxymethylcytosine was unsubstituted in phage-T2VG111 DNA.

Even though the α-glucosyltransferase induced by phage T2VG111 resembles that induced by phage T4 (Pratt *et al.* 1963), the distribution of α-glucosyl groups in the two deoxyribonucleic acids cannot be identical, since in phage-T2VG111 DNA 83% of the hydroxymethylcytosine residues bear α-glucosyl groups, whereas in phage-T4 DNA 70% of the hydroxymethylcytosine residues have α-glucosyl and 30% β-glucosyl substituents. The factors that determine the proportions of α- and β-glucosyl groups in phage-T4 DNA are not understood. However, our results with phage-T2VG111 DNA suggest that in phage-T4 DNA a high proportion of the β-glucosyl groups can only occur in sites where a hydroxymethyldeoxycytidine residue is linked through its 5′-position to another hydroxymethyldeoxycytidine residue.

Kornberg and co-workers found that phage-T4 and phage-T6 α-glucosyltransferases can introduce

additional glucose residues into phage-T2 DNA *in vitro* (Kornberg *et al.* 1961; Zimmerman *et al.* 1962; Josse & Kornberg, 1962; Pratt *et al.* 1963). More recently de Waard (1964*a*,*b*) has shown that the initial action of these transferases is to introduce glucose rapidly and preferentially into vacant sites where a hydroxymethyldeoxycytidine residue is situated between two purine nucleotides and in certain other positions where a hydroxymethyldeoxycytidine residue is linked through its 3′-position to a purine nucleotide. Under similar conditions the phage-T2-induced α-glucosyltransferase introduces glucose into phage-T2 DNA at about one-twelfth the rate obtained with the other two enzymes.

The observations on glucosylation by the enzymes *in vitro* provide further evidence that the phage-T4 and phage-T6 α-glucosyltransferases differ from the phage-T2 enzyme in their ability to glucosylate certain sites in deoxynucleotide polymers that contain hydroxymethylcytosine. The results support the view that the non-random distributions of glucose in the deoxyribonucleic acids of phages T2, T2VG111 and T6 reflect the specificity requirements of the α-glucosyltransferases induced by the different phages.

We are grateful to Dr K. Burton for many helpful and stimulating discussions and to Mrs E. Peskett for her valuable technical assistance. This work was aided by grants from the U.S. Public Health Service and the Rockefeller Foundation.

REFERENCES

Burton, K., Lunt, M. R., Petersen, G. B. & Siebke, J.-C. (1963). *Cold Spr. Harb. Symp. quant. Biol.* **28**, 27.

Burton, K. & Petersen, G. B. (1960). *Biochem. J.* **75**, 17.

de Waard, A. (1964*a*). *Biochim. biophys. Acta*, **87**, 169.

de Waard, A. (1964*b*). *Biochim. biophys. Acta*, **92**, 286.

Erikson, R. L. & Szybalski, W. (1964). *Virology*, **22**, 111.

Fiers, W. & Khorana, H. G. (1963). *J. biol. Chem.* **238**, 2780.

Heppel, L. A. & Hilmoe, R. J. (1955). In *Methods in Enzymology*, vol. 2, p. 565. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Josse, J. & Kornberg, A. (1962). *J. biol. Chem.* **237**, 1968.

Kay, D. (1959). *Biochem. J.* **73**, 149.

Kornberg, S. R., Zimmerman, S. B. & Kornberg, A. (1961). *J. biol. Chem.* **236**, 1487.

Kuno, S. & Lehman, I. R. (1962). *J. biol. Chem.* **237**, 1266.

Lehman, I. R. & Pratt, E. A. (1960). *J. biol. Chem.* **235**, 3254.

Lunt, M. R. & Newton, E. A. (1964). *Biochem. J.* **92**, 59P.

Lunt, M. R., Siebke, J.-C. & Burton, K. (1964). *Biochem. J.* **92**, 27.

Mandell, J. D. & Hershey, A. D. (1960). *Analyt. Biochem.* **1**, 66.

Petersen, G. B. (1963). *Biochem. J.* **87**, 495.

Petersen, G. B. & Burton, K. (1964). *Biochem. J.* **92**, 666.

Pratt, E. A., Kuno, S. & Lehman, I. R. (1963). *Biochim. biophys. Acta*, **68**, 108.

Putnam, F. W., Kozloff, L. M. & Neil, J. C. (1949). *J. biol. Chem.* **179**, 303.

Streisinger, G. & Weigle, J. J. (1956). *Proc. nat. Acad. Sci., Wash.*, **42**, 504.

Weigle, J. J., Streisinger, G., Hershey, A. D. & Kim, S. W. (1959). *Yearb. Carneg. Instn*, **58**, 432.

Zimmerman, S. B., Kornberg, S. R. & Kornberg, A. (1962). *J. biol. Chem.* **237**, 512.