THE NUCLEIC ACIDS OF T_2, T_4, AND T_6 BACTERIOPHAGES

By MARGERIS A. JESAITIS, Sc.D.

(From The Rockefeller Institute for Medical Research)

(Received for publication, April 11, 1957)

Some years ago it was shown by Wyatt and Cohen (1) that the nucleic acids of the three coli-dysentery phages, T_2, T_4, and T_6, contain nearly identical quantities of the same purine and pyrimidine bases and that they differ from the deoxyribonucleic acids derived from other sources in that they contained hydroxymethylcytosine instead of cytosine or its 5-methyl derivative. It was subsequently found in this laboratory that a hexose was present in the T_4 phage (2). This sugar was identified as glucose and was shown to be a constituent of the viral nucleic acid (3). Sinsheimer (4) and Volkin (5) have shown that the T_2 and T_6 phages also contain glucose and that this saccharide can, in part, be isolated as a glucoside of hydroxymethylcytidylic acid from enzymatic hydrolysates of T_2 and T_4 nucleic acids. It was recently reported that the nucleic acids of the three even numbered T phages contained varying amounts of glucose (6–8).

In the present communication the chemical properties of the nucleic acids of the wild type strains (r^+) of T_2, T_4, and T_6 phages will be described and the differences in their chemical composition will be discussed. It will be shown that the three nucleic acids differ not only in their content of glucose but also in that they contain chemically different hydroxymethylcytosine mononucleotides.

Materials and Methods

Bacteriophages.—The wild type (r^+) strains of T_2, T_4, and T_6 phages used in this study were originally obtained from Dr. Mark H. Adams of New York University. They were maintained by occasional transfer on *E. coli* B in nutrient broth. Mass cultures of T_2 and T_4 phage were prepared by infecting *E. coli* B grown in a glucose-phosphate buffer medium (9). In order to propagate the T_6 phage successfully it was necessary to add 0.1 per cent Difco nutrient broth shortly before infecting the culture with virus. The phages themselves were isolated and purified as previously described (9).

Analytical Methods.—The nitrogen and phosphorus content of the materials studied was determined colorimetrically by the procedures of Koch and McMeekin (10) and of Allen (11). Protein was determined with Folin reagent (12) using crystalline bovine albumen as a standard. The hexose content of the nucleic acids and of their degradation products was determined colorimetrically by means of the anthrone reaction as follows (13):

1 ml. portions of an aqueous solution of unknown substance were placed in test tubes of 16 mm. diameter. To each was added 0.2 ml. of a 2.5 per cent solution of anthrone in ethyl acetate followed by 2.5 ml. of concentrated sulfuric acid. The reagents were mixed and the

tubes heated for 3 minutes at 100°C. They were then allowed to cool at room temperature for 20 to 30 minutes. Since hexose and deoxyribose both produce colored products which have overlapping absorption bands, the absorbance was measured at 620 and at 500 mμ. The amount of glucose (C_G) was calculated from equation derived from Beer's law (14):

$$C_G = \frac{A_{620} - (K^T_{620}/K^T_{500})\, A_{500}}{K^G_{620} - (K^T_{620}/K^T_{500})\, K^G_{500}} \quad (1)$$

in which A_{500} and A_{620} are the absorbances of the unknown sample at 500 and 620 mμ. The coefficients K^G_{500}, K^G_{620}, K^T_{500}, K^T_{620} are the absorbances of samples containing 100 μg. of glucose (G) and thymus nucleic acid (T) respectively, measured at the two wave lengths. The following values were determined experimentally: $K^T_{500} = 0.082$, $K^T_{620} = 0.026$, $K^G_{500} = 0.49$, and $K^G_{620} = 1.53$.[1] By substituting these values in equation (1) the quantity of glucose in the sample can be calculated from the following: μg. glucose/sample $= 72.8A_{620} - 23.2A_{500}$. In a sample of nucleic acid weighing 500 μg. the precision of the method permits the estimation of glucose within ± 2 to 4 μg. depending upon the hexose content.

Deoxyribose was determined by the method of Dische using L-2-deoxyribose as a standard (15). Colorimetric tests for pentoses and uronic acids were made according to the techniques of Mejbaum (16) and of Dische (17) respectively. Hexosamines were estimated on samples hydrolyzed in 4 N HCl for 16 hours at 100°C. using the method of Sørensen (18).

Paper chromatography of sugars was carried out by the ascending technique. The materials to be analyzed were hydrolyzed with 1 N H_2SO_4 at 100°C. for 4 hours. After removing the acid with barium hydroxide the hydrolysates were evaporated to dryness and the residues were dissolved in a small volume of water. Aliquots of each solution were placed on sheets of Whatman paper No. 1 together with samples of known sugars. The chromatograms were developed for 24 hours with butanol-pyridine-water (6:4:3) mixture and sprayed with aniline hydrogen phthalate.

In order to determine the purine and pyrimidine content of the apurinic acids, the materials under study were hydrolyzed and the bases isolated by ion exchange chromatography (19). The amount of each base was then determined spectroscopically in the eluates. The samples used for the determination of pyrimidines were hydrolyzed in 6 N HCl for 3 hours at 100°C. and then fractionated on dowex 50 × 12 (H^+) resin. Those employed for the determination of purines were hydrolyzed in N H_2SO_4 for 4 hours at 100°C. and fractionated on dowex 1 × 10 formate resin.

Absorption spectra were measured in a Beckmann spectrophotometer (model DU), and electrophoretic analyses were made in the Tiselius apparatus using a 2 ml. cell (20).

EXPERIMENTAL

Preparation of Phage Nucleic Acids.—The nucleic acids of T_2, T_4, and T_6 phages were obtained by the following procedure:—

100 to 200 ml. portions of an aqueous phage suspension containing 5×10^{12} particles/ml. were frozen in a carbon dioxide—ethanol mixture, and thawed at 30 to 40°C. After repeating the operation 5 to 10 times the infectivity titer of the virus had decreased 3 to 6 log units. The viscous solution was now made 1 molar by the addition of 5 molar NaCl, and was deproteinized by stirring with chloroform-octanol (9:1) in a high speed mixer, followed by

[1] It was observed that the value of these coeficients varied slightly when different batches of sulfuric acid or of ethyl acetate were employed. This variation is presumably due to the presence of traces of oxydizing substances in the reagents.

centrifugation. The nucleic acid was precipitated from the supernate with 2 volumes of cold 95 per cent ethanol. After dialysis and lyophylization some 80 to 90 per cent of nucleic acid was recovered.

Properties of the Viral Nucleic Acids.—

The nucleic acids of the three phages form viscous solutions. Their absorption spectra show characteristic minima at 230 mμ and maxima at 260 mμ. Their molar

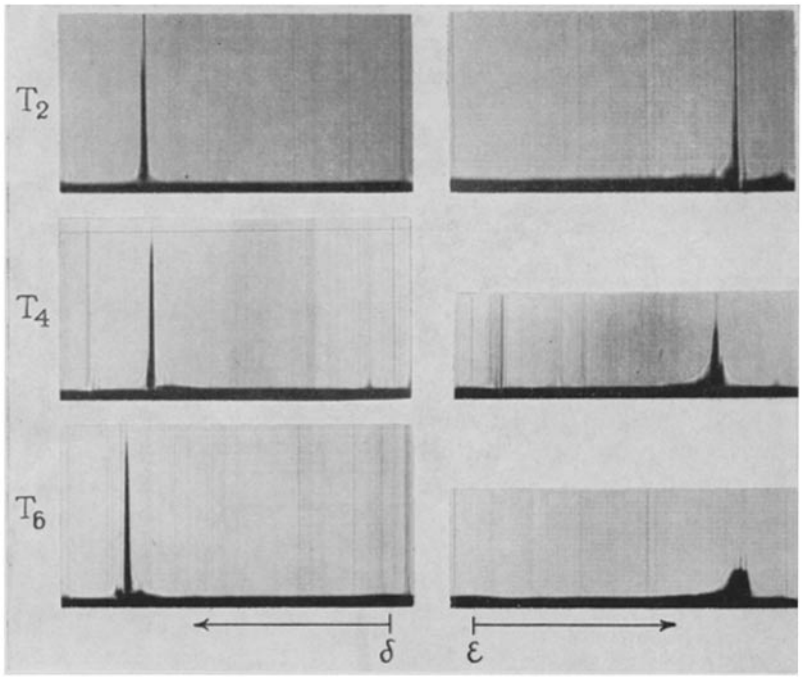

FIG. 1. Electrophoretic patterns of 0.2 per cent solutions of the T_2, T_4, and T_6 nucleic acids in sodium phosphate buffer of ionic strength 0.1 and at pH 6.8. Patterns were recorded after electrophoresis for 3600, 3500, and 4000 seconds respectively at a potential gradient of 7.1 volts/cm.

absorbtivity $\epsilon(P)$ at 260 mμ and at pH 6.8 varied between 7000 and 7320. Electrophoretic analyses of the three substances showed, in each instance, but one component the mobility of which varied between -18.3 and -17.6×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ when calculated from the ascending pattern (Fig. 1). The analyses were performed on 0.2 per cent solutions in sodium phosphate buffer at an ionic strength 0.1 and at pH 6.8, and a potential gradient of 7.1 volt/cm. In each instance the ascending boundaries were sharp and essentially planar. In the descending channel the refractive index gradients at the center of the channel migrated more rapidly than at the walls, leading to a markedly non-planar boundary which could not be used for the estimation of mobilities.

Chemical analyses of several preparations of the viral nucleic acids are given in the Table I. It will be seen that the substances differed slightly yet significantly in their nitrogen and phosphorus content. The percentage of these elements was highest in the nucleic acid of T_2, intermediate in that of T_4, and lowest in that of the T_6 virus. The molar ratios of N/P varied between 3.72 and 3.86, and were slightly higher than the value, 3.69, calculated from the molar proportions of the nucleic acid bases reported earlier (1). Since all the nucleic acid preparations contained small quantities of protein (0.9 to 1.4 per cent), it is possible that the elevated value of the N/P ratio might be due to this component.

When the cysteine color reaction for sugars (21) was carried out, it was observed that the absorption spectra of each nucleic acid showed maxima at 410 and 470

TABLE I

Chemical Composition of Nucleic Acids of T_2, T_4, and T_6 Viruses

Nucleic acid	T_2	T_4	T_6
No. of preparations analyzed	2	4	4
Nitrogen, *per cent*	14.3 ± 0.20	13.9 ± 0.13	13.1 ± 0.19*
Phosphorus, *per cent*	8.45 ± 0.05	8.03 ± 0.08	7.62 ± 0.10
N/P‡	3.75 ± 0.03	3.83 ± 0.02	3.83 ± 0.02
Protein, *per cent*	0.8 ± 0.3	1.4 ± 0.4	1.0 ± 0.1
Deoxyribose, *per cent*	19.8 ± 0.0	19.6 ± 0.2	18.7 ± 0.2
Glucose, *per cent*	6.55 ± 0.13	8.32 ± 0.31	12.50 ± 0.38
Glucose/P‡	0.133 ± 0.002	0.178 ± 0.008	0.282 ± 0.007

* Mean value and standard deviation.

‡ Molar ratio.

mμ and a slight inflexion at 380 mμ, when measured 2 hours after adding the cysteine (Fig. 2, diagram *A*). On comparing these spectra with those of thymus nucleic acid and with that of glucose (Fig. 2, diagram *B*), it is evident that the maximum at 410 mμ agrees precisely with that of glucose. Similarly, the maximum at 470 mμ and the inflexion at 380 mμ correspond to the maxima caused by deoxyribose of the thymus nucleic acid. These observations indicate that a deoxypentose and a hexose are components of each viral nucleic acid.

The hexose was identified chromatographically. As can be seen in Fig. 3, acid hydrolysates of each of viral nucleic acid showed but one spot on chromatograms developed with aniline hydrogen phthalate. Since these spots had the same R_f as glucose, it is apparent that only this hexose is present in the viral nucleic acids.

The deoxypentose was not further identified. It has been shown by Cohen, however, that purine nucleosides of T_{6r+} nucleic acid on hydrolysis, liberated a deoxypentose having the same R_f as the deoxyribose of thymus nucleic acid (22). It can be assumed therefore that the related T_2 and T_4 phages also contain this deoxypentose. Finally, it will be noted that all the preparations of viral nucleic acids gave a color with orcinol reagent. Since the amount of deoxypentose and of glucose in these materials cannot account fully for the coloration, it is possible that the viral

nucleic acids contain traces of pentose. The color tests for uronic acids and glucosamine were negative.

Quantitative determinations of the monosaccharide components of the three nucleic acids revealed that, although they contained similar amounts of deoxypentose, they differed considerably in their glucose content (Table I). Thus, the content of deoxypentose, as determined with diphenylamine, varied only between 19.8 and

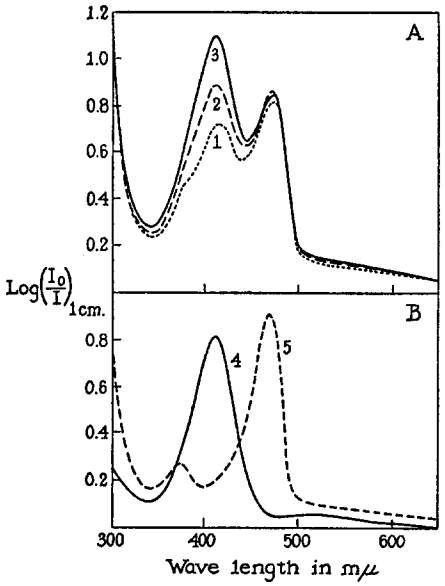

FIG. 2

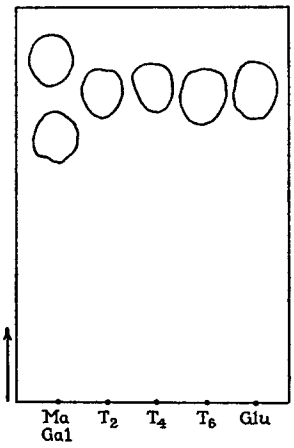

FIG. 3

FIG. 2. Absorption curves of nucleic acids and of glucose 2 hours after the Dische cysteine test. Spectra were measured in a Cary's recording spectrophotometer. Diagram A: curves 1, 2, and 3—1000 μg. of T_2, T_4, and T_6 nucleic acids respectively. Diagram B: curve 4—100 μg. of glucose; curve 5—1000 μg. of thymus nucleic acid.

FIG. 3. Diagram of a paper chromatogram of hydrolysates of T_2, T_4, and T_6 nucleic acids and of mannose (Ma), galactose (Gal), and glucose (Glu). Chromatogram was developed with butanol-pyridine-water (6:4:3) mixture and sprayed with aniline hydrogen phthalate.

18.7 per cent. It was slightly lower, however, in the T_6 nucleic acid than in other two acids. The content of glucose on the other hand was different in each nucleic acid. The T_2 nucleic acid contained 6.6 per cent, the T_4 nucleic acid—8.3 per cent, and the T_6 nucleic acid—12.5 per cent of glucose as determined by the anthrone reaction.

From the data which have been presented, it is apparent that in the three nucleic acids there was present 0.133, 0.178, and 0.282 moles of glucose per mole of phosphorus. Since each nucleic acid (1, 22) contains essentially the same quantity of hydroxymethylcytosine (0.163 to 0.168 moles per 1 mole of phosphorus), it is apparent that the molar ratio of glucose to hydroxymethyl-

cytosine is in each instance different (0.81, 1.08, and 1.71, respectively). It is obvious, therefore, that in the T_2 nucleic acid the number of glucose molecules is less than the number of hydroxymethylcytosine molecules. However, the molar quantities of these two substances are nearly equal in the T_4 nucleic acid, whereas in the T_6 nucleic acid there are more moles of glucose than of pyrimidine.

Enzymatic Degradation of the T_6 Nucleic Acid.—

When the nucleic acids of the even numbered T phages are treated with various phosphatases they are degraded only in part either to mononucleotides or to mononucleosides. Thus it has been shown by Sinsheimer (4) that 68 to 70 per cent of thymidylic, deoxyadenylic, and deoxyguanylic acids and 31 per cent of hydroxymethylcytidylic acid were liberated as mononucleotides following degradation of T_2 nucleic acid with deoxyribonuclease and snake venom phosphodiesterase. Eighteen per cent of the total hydroxymethylcytidylic acid was linked with 1 molecule of glucose and 13 per cent was unsubstituted. Volkin observed (5) that similar yields of the mononucleotides were obtained after enzymic degradation of T_4 nucleic acid. In this instance, only the monoglucoside of hydroxymethylcytidylic acid was liberated and its yield accounted for 17 per cent of the total. On basis of the observation that on acid hydrolysis glucose is readily split from the glucoside of hydroxymethylcytidylic acid, both investigators suggested that this saccharide is linked to the 5-hydroxymethyl group of the pyrimidine base. The composition of the nucleosides, following degradation of T_6 nucleic acid with deoxyribonuclease and alkaline intestinal phosphatase, was studied by Cohen (22). He observed that the digest contained large amounts of thymidine and that a band containing guanine and hypoxanthine was present on paper chromatograms in the position characteristic for their nucleosides. This band contained occasionally very small amounts of hydroxymethylcytosine. The bulk of this pyrimidine was found, however, in the non-motile nucleotide fraction.

In view of the fact that the T_6 nucleic acid contains more moles of glucose than of hydroxymethylcytosine, it was of interest to determine the manner in which the hexose is linked. The nucleic acid was therefore digested and the products of the enzymic degradation were isolated as follows:—

112 mg. of T_6 nucleic acid dissolved in 20 ml. of 0.2 M sodium acetate and 0.01 M magnesium acetate was digested with 2 mg. of crystalline deoxyribonuclease at pH 7–8 for 24 hours at 37°C. in the presence of chloroform. 3 ml. of a solution of phosphodiesterase, prepared from 110 mg. of *Crotalus adamanteus* venom (23), was then added, and the mixture incubated for an additional 24 hours at 37°C. and at pH 8–9. The digest was now adjusted to pH 4.4 and centrifuged to remove precipitated enzyme protein. The supernate, containing nucleotides, was adjusted to pH 10, diluted to 50 ml., and an aliquot analyzed.

The remaining part of the solution (46 ml.) containing 246 μM of phosphorus was added to a 15 × 0.9 cm. column of dowex 1 × 10 formate. The elution was carried out at the rate of 30 ml. per hour first with 100 ml. of 0.01 M ammonium formate at pH 7.0 and then with 600 ml. of ammonium formate–formic acid buffer. The concentration of the latter was grad-

ually changed by allowing 0.1 M buffer at pH 4.0 to flow into a mixing device (24) containing 500 ml. of 0.01 M buffer at pH 7.0. Elution was continued with 0.2 M buffer (250 ml.) and then with 1.0 M formate buffer (500 ml.) at pH 4.0. Ten ml. samples of effluent were collected and the absorbance of each was measured at 260 mμ. The solutions giving rise to each peak were combined, evaporated, and heated *in vacuo* at 90–100°C. in order to remove ammonium formate. The residues were then dissolved in water, and their phosphorus and glucose content was determined. The absorption spectra of each fraction were measured in 0.1 M HCl, in 0.1 M sodium phosphate buffer at pH 6.8 and in 0.1 M NaOH.

The effluent curve is presented in Fig. 4, where the absorbances of each sample, at 260 mμ, are plotted as a function of the total volume. It can be seen that ten peaks were obtained, numbered I to X. Because the separation of fraction IV was incomplete, it was recycled on a dowex 1 × 10 formate column using 0.04 M ammonium formate buffer at pH 5.4 as the eluent. Two new fractions, IVa and IVb were thus obtained (Fig. 5).

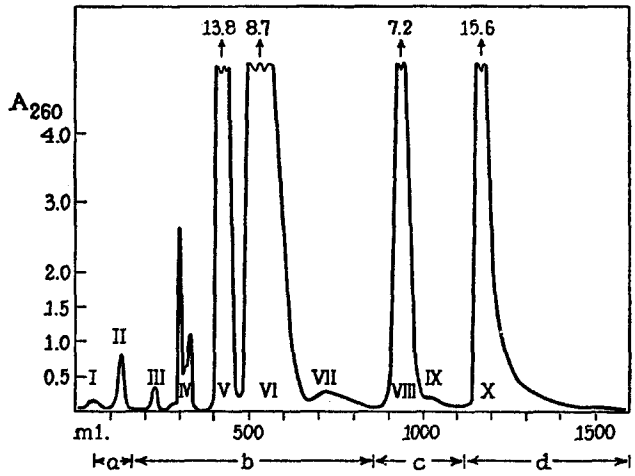

FIG. 4. Nucleotides in the enzymatic hydrolysate of T_6 nucleic acid. The hydrolysate was passed through a 15 × 0.9 cm. column of dowex 1 × 10 formate and the column eluted with ammonium formate–formic acid buffers of the following composition: (*a*) 100 ml. of 0.01 M buffer at pH 7.0; (*b*) 600 ml. of a buffer the molarity of which was gradually changed from 0.01 to 0.1 and the pH from 7.0 to 4.0; (*c*) 250 ml. of 0.2 M buffer at pH 4.0; (*d*) 500 ml. of 1.0 M buffer at pH 4.0.

A summary of the analyses performed on the fractions thus obtained is presented in the Table II. It can be seen that fractions I, II, and III were free of phosphorus. The character of the absorption spectra indicated that these fractions contained bases and nucleosides which accounted for 1.2 per cent of the total absorbance at 260 mμ. The absorption spectra of fractions IVa and IVb were characteristic for hydroxymethylcytidylic acid. The absorption maxima of these two fractions in alkaline and in neutral solution were at 275 mμ; which shifted to 283 mμ when measured in 0.1 M acid. The ratio of the absorbances at 260 and 280 mμ was similar to that reported for hydroxymethylcytidylic acid (4). Both fractions had nearly identical

absorbtivity per mole of phosphorus, a fact which indicates that equal quantities of hydroxymethylcytosine were present in these mononucleotides. Furthermore, both fractions gave a positive Stumpf reaction for deoxyribose (25). They differed, however, in that the fraction IVa contained nearly 2 moles (1.91) of hexose per mole of phosphorus, whereas only traces were found in fraction IVb. The hexose itself was identified as glucose by the cysteine reaction (21). On the basis of the evidence presented it can be concluded that fraction IVa contained hydroxymethylcytidylic

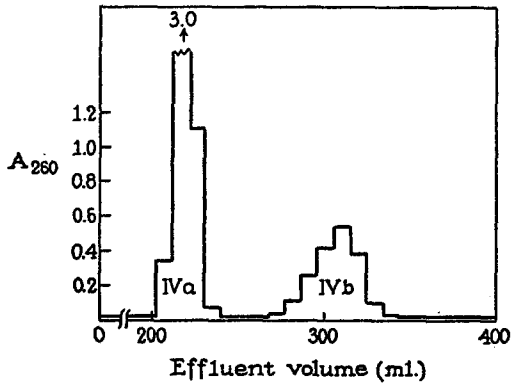

FIG. 5. Fractionation of hydroxymethylcytidylic acids (fraction IV) on a 10 × 0.9 cm. column of dowex 1 × 10 formate with 0.04 M ammonium formate buffer at pH 5.4.

TABLE II

Nucleotides Obtained from Enzymatically Digested T_6 Nucleic Acid

Fraction		$\frac{A260}{A280}$ at pH 6.8	$\epsilon(P) \times 10^{-3}$ at 260 mμ and pH 6.8	$\frac{\text{Moles glucose}}{\text{Mole P}}$	Per cent of P of digest	Per cent of ultraviolet (260 mμ.) absorption of digest
I	Bases and nucleosides	2.71	—	—	0	0.2
II		1.37	—	—	0	0.8
III		1.53	—	—	0	0.2
IVa	Digluco-hydroxymethylcytidylic acid	0.79	6.07	1.91	2.8	1.8
IVb	Hydroxymethylcytidylic acid	0.80	6.12	0.02	1.1	0.7
V	Thymidylic acid	1.34	8.6	0.02	19.3	17.7
VI	Deoxyadenylic acid	6.58	14.6	0.01	19.1	30.0
VII	Oligonucleotides	1.39	9.4	0.97	1.7	1.7
VIII	Deoxyguanylic acid	1.48	11.9	0.09	11.2	14.2
IX	Oligonucleotides	1.33	8.1	0.71	1.4	1.2
X	Polynucleotides	1.63	8.6	0.40	22.2	20.2
				Total...	78.8	88.7

acid linked with 2 molecules of glucose and that fraction IVb contained unsubstituted hydroxymethylcytidylic acid.

It is also evident from the spectral data that thymidylic, deoxyadenylic, and deoxyguanylic acids were present in fractions V, VI, and VIII respectively. These substances accounted for 19.3, 19.1, and 11.2 per cent of the total phosphorus. Only traces of glucose were found in fractions V and VI, whereas a significant amount of the hexose (0.09 moles per 1 mole of phosphorus) was present in fraction VIII. As can be seen in Fig. 4, that fraction VIII was not completely separated from fraction IX. An aliquot of fraction VIII was therefore recycled on dowex 1 × 10 formate column (8 × 0.9 cm.) using 0.2 M ammonium formate buffer at pH 3.4 as eluent. Two new fractions were now obtained. The first, eluted at 30 to 90 ml. contained a mixture of glucose-containing oligonucleotides, the second, eluted at 110 to 210 ml. contained deoxyguanylic acid. From this it is apparent that the hexose present in fraction VIII can be attributed to a contamination with the oligonucleotides. Fractions VII, IX and X contained 0.97 to 0.40 moles of glucose per mole of phosphorus and accounted for 25.3 per cent of the total phosphorus. Since the absorption spectra of these fractions indicated that they contained hydroxymethylcytosine and other nucleic acid bases, it was concluded that they also contained oligo- and polynucleotides.

From the data which have been presented it is clear that the nucleic acid of T_6 phage is degraded by enzymes in a manner similar to the nucleic acid of the T_2 and T_4 phage. It can be calculated that, following treatment of T_6 nucleic acid with enzymes, 59, 59, and 61 per cent of the total thymidylic, deoxyadenylic, and deoxyguanylic acids and 23 per cent of the hydroxymethylcytidylic acid were liberated as mononucleotides. Six per cent of the total hydroxymethylcytidylic acid was recovered in the unsubstituted form and 17 per cent in the form of a diglucoside. The structure of the latter is not yet known. It is believed, however, that two molecules of hexose are linked as a disaccharide to the 5-hydroxymethyl group of the pyrimidine. The quantity of the glucose bound to hydroxymethylcytidylic acid accounted for 21 per cent of the total hexose. The main portion of the latter remained bound to polynucleotides, but the site of its attachment could not be identified by the method employed.

Acid Hydrolysis of the Phage Nucleic Acids.—It is clear from the studies described above that in T_2, T_4, and T_6 nucleic acids at least 20 per cent of the total glucose is bound to hydroxymethylcytidylic acid. In order to learn the manner in which the remaining glucose is linked, the three nucleic acids were hydrolyzed with dilute mineral acid and their apurinic acids investigated.

An aqueous solution of the viral nucleic acid containing 5 mg./ml. was adjusted to pH 1.6 with 0.2 N sulfuric acid (25). The mixture was dialyzed at 37° against dilute sulfuric acid at pH 1.6 for 24 hours in order to remove purine bases. The dialysis residue was neutralized, redialyzed at 4°C., then concentrated *in vacuo* and finally freeze-dried. Several preparations

of each nucleic acid were hydrolyzed in this manner. In each instance the yield of apurinic acid was approximately 70 per cent of the starting material.

The apurinic acids obtained were readily soluble. When measured in phosphate buffer at pH 6.8 their absorption spectra showed maxima at 267 to 268 mμ and minima at 237 to 238 mμ. Their molar absorbtivity ϵ(P) at 260 mμ varied between 4050 and 4280 and their specific rotations $[\alpha]_D^{24^\circ}$ between +41.5 and +38.7°. Electrophoresis of 0.7 to 0.8 per cent solutions of the apurinic acids in sodium caco-

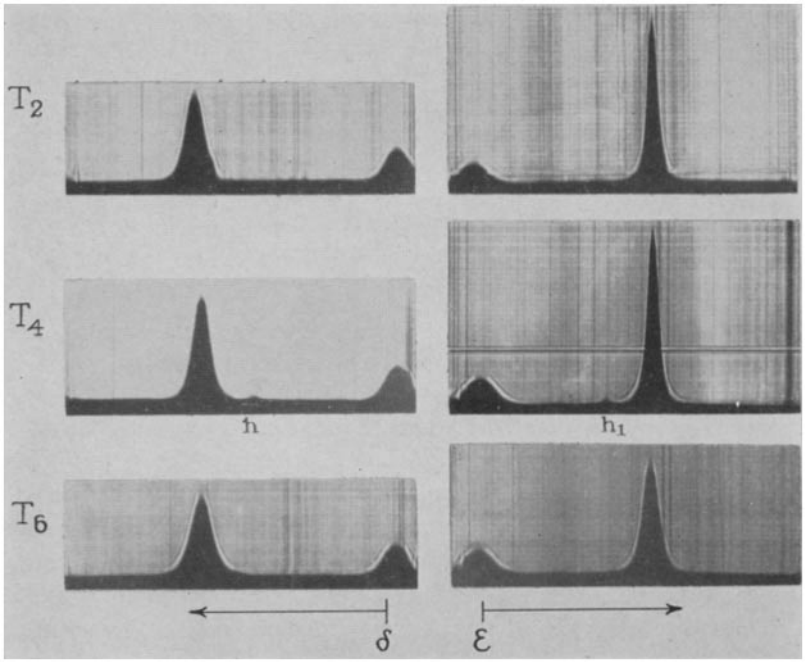

FIG. 6. Electrophoretic patterns of 0.7 to 0.8 per cent solutions of the T_2, T_4, and T_6 apurinic acids in sodium cacodylate buffer of ionic strength 0.1 and at pH 6.8. Patterns were recorded after electrophoresis for 4000 seconds at a potential gradient of 6.8 volts/cm. The perceptible gradients at h and h_1 in the pattern of T_4 apurinic acid are due to an interruption of the current and do not indicate a trace impurity.

dylate buffers of ionic strength 0.1 and pH 6.8 showed but a single symmetrical boundary (Fig. 6). Mobilities of -11.9, -11.7, and -11.3×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ were calculated from the descending patterns for the T_2, T_4, and T_6 apurinic acids respectively. The T_4 apurinic acid was also analyzed electrophoretically in 0.1 M sodium acetate buffer at pH 4.6 and in 0.05 M sodium borate buffer at pH 9.2. In each instance a single and symmetrical boundary was formed. It is evident from this that the apurinic acids are electrophoretically homogeneous in monovalent buffers. When the analyses were performed in sodium phosphate buffer of ionic strength 0.1

and pH 6.8 asymmetric patterns and bimodal gradients were formed (Fig. 7). The reasons for this are not fully understood but may be due to the interaction of the apurinic acids with phosphate ions. It should be pointed out that thymus apurinic acid also forms a symmetrical boundary in cacodylate buffer and an asymmetric boundary in phosphate buffer (26). It would seem that this property is characteristic of all apurinic acids.

Chemical analysis of the three apurinic acids are presented in Table III. It is seen that these substances differed slightly in their nitrogen and phosphorus content; it was highest in the T_2 apurinic acid and lowest in the T_6. The pyrimidine content was similar to that of the nucleic acids themselves (1), but the purine con-

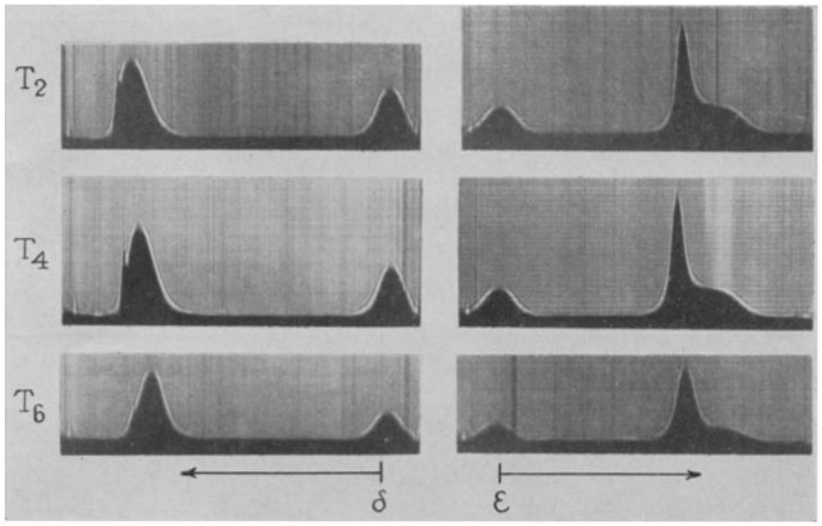

FIG. 7. Electrophoretic patterns of 0.6 to 1.0 per cent solutions of the T_2, T_4, and T_6 apurinic acids in sodium phosphate buffer of ionic strength 0.1 and at pH 6.8. Patterns were recorded after electrophoresis for 4000 seconds at a potential gradient of 6.9 volts/cm.

tent was greatly decreased. The content of deoxyribose, as determined by the diphenylamine reaction, is greater than that of the corresponding nucleic acids as was that of glucose. The molar ratios of glucose to phosphorus are essentially the same as those found in the viral nucleic acids themselves, a fact which indicates that glucose is attached only to the acid resistant part of the nucleic acid molecule.

From the experimental evidence which has been presented it is apparent that the purines are not the sites of attachment of the hexose molecule because all of the glucose has been shown to be bound to the apurinic acid. This conclusion is corroborated by the observation that the deoxyadenylic and deoxyguanylic acids isolated from enzymatically degraded nucleic acids are free of glucose. It is unlikely that thymine serves as the site of attachment for the

hexose because the thymidylic acid obtained from the enzymatic digest was also free of glucose. It is also unlikely that this saccharide is bound to phosphoric acid as a labile tertiary ester because the glucose content of the three nucleic acids was essentially unchanged after prolonged treatment with 0.05 N NaOH at 37°C. Finally, it will be recalled that all of the glucose-containing fractions isolated from the enzymatic hydrolysates contained hydroxymethylcytosine. In view of these facts it would appear that the glucose component of the three phage nucleic acids is linked only to the latter pyrimidine.

TABLE III

*Chemical Composition of Apurinic Acids of T_2, T_4, and T_6 Viruses**

Apurinic acid	T_2	T_4	T_6
Nitrogen, *per cent*	5.85 ± 0.05	5.80 ± 0.1	5.35 ± 0.15
Phosphorus, *per cent*	9.8 ± 0.0	9.6 ± 0.1	9.2 ± 0.15
Deoxyribose, *per cent*	23.5 ± 0.3	23.3 ± 0.3	22.7 ± 0.5
Glucose, *per cent*	7.7 ± 0.26	9.8 ± 0.3	14.3 ± 0.6
Glucose/P‡	0.136 ± 0.005	0.176 ± 0.007	0.269 ± 0.007
Hydroxymethylcytosine/P‡	0.162	0.151	0.157
Thymine/P‡	0.330	0.344	0.339
Adenine/P‡	0.008	0.006	0.003
Guanine/P‡	0.020	0.011	0.007

* The values presented in the table are mean values of two preparations. The content of nucleic acid bases, however, was determined in but one preparation.

‡ Molar ratio.

DISCUSSION

The wild type strains of T_2, T_4, and T_6 bacteriophages have many properties in common. They are morphologically indistinguishable, their gross chemical composition is similar, and their proteins are serologically related. Their nucleic acids are also similar for they contain nearly equal amounts of the same purine and pyrimidine bases. The phages differ, however, in their serological reactions, for the sera of animals immunized with one neutralizes the others, but at slower rates. Furthermore, the viruses differ in their ability to attack certain bacterial strains. Mutants derived from a common host which are resistant to one of the viruses are still susceptible to the others. Since it is the substance present in the phage tail, possibly a protein, which combines with neutralizing antibody (27), or with the bacterial membranes (28), it would seem logical to suppose that the structure of this substance differs in each phage type.

From the data described above it is now clear that the nucleic acids of the three viruses differ chemically and that these differences are due to the presence of varying amounts of glucose. It is our belief that the content of this

saccharide is a characteristic property of the wild type of each of the three viruses. This attribute seems to be independent of the ability of the virus to form plaques on certain variant strains of *Escherichia coli* B. Thus it has been shown that by crossing T_2 with T_4 a hybrid phage $\overline{T}_2$ is produced which contains the nucleic acid of the T_4 virus but does not plate on *E. coli* B/2 (8, 29).

The presence of glucose and of hydroxymethylcytosine in the viral nucleic acids distinguishes them from other types of nucleic acid encountered in nature. The occurrence of these unusual components may well be due to an unknown metabolic path peculiar to the three viruses. The experimental evidence presented here and that presented by others (4, 5) strongly suggest that the hexose is linked only to the hydroxymethylcytidylic acid and probably to the hydroxymethyl group of the pyrimidine base. Since the molar ratios of these two substances in each nucleic acid are different, it is apparent that hydroxymethylcytidylic acid may occur both in an unsubstituted form, as well as in the form of its glucose derivatives. Indeed, the results of chromatographical analyses of the enzymatically degraded nucleic acids indicate that this is the case. Thus, hydroxymethylcytidylic acid and its monoglucoside were found in T_2 nucleic acid. Only the monoglucoside of hydroxymethylcytidylic acid was detected in T_4 nucleic acid whereas the unsubstituted hydroxymethylcytidylic acid and its diglucoside were isolated from the T_6 nucleic acid.

These facts indicate clearly that the three viral nucleic acids differ in the chemical composition of their hydroxymethylcytosine-containing mononucleotides. Since glucose is linked only to hydroxymethylcytidylic acid, it is apparent that the content of this saccharide in the viral nucleic acid is proportional to the content of substituted hydroxymethylcytidylic acids. The content of the latter and of hydroxymethylcytidylic acid itself is, at present, not known for only 20 to 30 per cent of these constituents can be isolated as monomers, following the enzymatic degradation of the viral nucleic acids. A wider understanding of the structure of viral nucleic acids depends, therefore, upon the development of methods which will allow these substances to be degraded quantitatively to their structural units.

SUMMARY

The deoxyribonucleic acids of the wild type strains of the T_2, T_4, and T_6 bacteriophages have been shown to contain glucose as an integral part of the molecule; the amount of hexose present in each nucleic acid differs.

A study of the acid degradation products of the three nucleic acids has revealed that in each instance glucose is linked to the apurinic acid component.

In the case of the T_6 nucleic acid it was found that two molecules of glucose are linked to hydroxymethylcytidylic acid. The other mononucleotides contained no glucose.

From the results which have been presented here, and from data presented

by others, it can be concluded that the three viral nucleic acids differ in that they contain different proportions of free and glucose-substituted hydroxymethylcytidylic acids.

BIBLIOGRAPHY

1. Wyatt, G. R., and Cohen, S. S., *Biochem. J.*, 1953, **55,** 774.
2. Jesaitis, M. A., and Goebel, W. F., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18,** 205.
3. Jesaitis, M. A., Microbial Genetics Bulletin, Cold Spring Harbor, New York, Carnegie Institution of Washington, 1954, **10,** 16.
4. Sinsheimer, R. L., *Science*, 1954, **120,** 551.
5. Volkin, E., *J. Am. Chem. Soc.*, 1954, **76,** 5892.
6. Cohen, S. S., *Science*, 1956, **123,** 653.
7. Sinsheimer, R. L., *Proc. Nat. Acad. Sc.*, 1956, **42,** 502.
8. Jesaitis, M. A., *Nature*, 1956, **178,** 637.
9. Jesaitis, M. A., and Goebel, W. F., *J. Exp. Med.*, 1955, **102,** 733.
10. Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46,** 2066.
11. Allen, R. J. L., *Biochem. J.*, 1940, **34,** 858.
12. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, 1951, **35,** 89.
13. Loewus, F. A., *Anal. Chem.*, 1952, **24,** 219.
14. Knudson, H. W., Meloche, V. W., and Juday, C., *Ind. and Eng. Chem., Anal. Ed.*, 1940, **12,** 715.
15. Dische, Z., *Mikrochemie*, 1930, **8,** 14.
16. Mejbaum, W., *Z. physiol. Chem.*, (Hoppe-Seyler's), 1939, **258,** 117.
17. Dische, Z., *J. Biol. Chem.*, 1947, **167,** 189.
18. Sørensen, M., *Compt.-rend. trav. Lab. Carlsberg*, 1938, **22,** 487.
19. Cohn, W. E., *Science*, 1949, **109,** 377.
20. Longsworth, L. B., *Anal. Chem.*, 1953, **25,** 1074.
21. Dische, Z., Shettles, L. B., and Osnos, M., *Arch. Biochem.*, 1949, **22,** 169.
22. Cohen, S. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18,** 221.
23. Sinsheimer, R. L., and Koerner, J. F., *J. Biol. Chem.*, 1952, **198,** 293.
24. Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1954, **211,** 893.
25. Stumpf, P. K., *J. Biol. Chem.*, 1947, **169,** 367.
26. Tamm, C., Hodes, M. E., and Chargaff, E., *J. Biol. Chem.*, 1952, **195,** 49.
27. Lanni, F., and Lanni, Y. T., *Cold Spring Harbor Symp. Quant. Biol.*, 1953, **18,** 159.
28. Kellenberger, E., and Arber, W., *Z. Naturforsch.*, 1955, **10b,** 698.
29. Streisinger, G., and Weigle, J., *Proc. Nat. Acad. Sc.*, 1956, **42,** 504.