

NUCLEIC ACID ECONOMY IN BACTERIA INFECTED WITH BACTERIOPHAGE T2

I. PURINE AND PYRIMIDINE COMPOSITION

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The discovery that the desoxyribonucleic acid (DNA) of bacteriophage T2 lacks cytosine (Marshak, 1951), and contains instead 5-hydroxymethylcytosine (Wyatt and Cohen, 1952), permits one for the first time to study viral growth in terms of the synthesis of a distinctive chemical constituent. Other recent findings suggest, moreover, that DNA plays a dominant role in initiating the infection (Hershey and Chase, 1952). This paper describes the changes in cytosine and hydroxymethylcytosine content of the intrabacterial DNA after infection of *Escherichia coli* with bacteriophage T2.

Materials and Methods

Cultural Conditions.—The variety of T2 known as T2H was propagated on a strain of *E. coli*, designated R2, chosen for its relative resistance to premature lysis following multiple infection. All cultures were grown in "peptone-broth" containing per liter 10 gm. bacto-peptone, 3 gm. NaCl, 1 gm. glucose, 1 mM MgSO₄, 0.1 mM CaCl₂, and 5 mg. P added in the form of phosphate buffer of pH 7.0. Beef extract was omitted because it interferes with the diphenylamine reaction for DNA.

Bacteria were grown with aeration at 37°C. to a concentration of 2×10^8 per ml. in 720 ml. peptone-broth, sedimented, and infected with 5 phage per bacterium in a non-nutrient adsorption medium (Hershey and Chase, 1952). The infected cells were then resedimented and transferred (at "time zero") to warm aerated broth at 2×10^8 cells per ml. Each culture was analyzed to obtain the following data: colony counts of bacteria before infection; plaque counts of input phage; colony counts of bacteria escaping infection, and plaque counts of infected bacteria. In nearly all experiments 95 to 99 per cent of the bacteria were infected, and the total cell counts made before and after infection agreed within 15 per cent, showing that most of the bacteria were capable of yielding phage.

Samples of the culture were taken for estimation of total DNA (diphenylamine reaction) at time zero and at one or more additional times; and for titration of infective intracellular phage at 30 minutes and at one or more additional times. The phage yields were measured from 10⁶-fold dilutions in chilled broth containing 0.01 M NaCN, which gives the yields at the time of dilution (Doermann, 1952). The diluted samples were titrated after warming 30 minutes at 37°C. and again after standing overnight in the refrigerator. The two assays usually agreed within 30 per cent.

Extraction of DNA.—The bulk of each culture was used for the extraction and analysis of DNA at a single time. For this purpose an aliquot of culture measuring about 650 ml. was mixed with one-tenth its volume of 3 M trichloroacetic acid (TCA) and 2 ml. of 1 per cent serum albumin (to improve the packing quality of the precipitate), and chilled. Control experiments with small samples showed that the recovery of diphenylamine-reacting substances was the same whether the culture was cooled before or 30 minutes after acidifying. When thoroughly chilled the culture was spun in angle centrifuges, and the precipitate was rinsed into a single tube with small quantities of 0.1 N NaOH and water. After reprecipitation with TCA, the acid-insoluble material was fractionated by the methods of Schmidt and Thannhauser (1945) and Schneider

TABLE I
Recovery of DNA from Bacteria, Phage, and Mixtures

C units as $\mu\text{M P}$	$\mu\text{M P}$	$\mu\text{M base}$	Guanine	Hydroxy- methyl cytosine	Cytosine	Adenine	Thymine
Bacteria (per 1.55×10^{-14} C units, equivalent to 100 μM thymus DNA-P)							
77	107	63	16.3	0	15.7	15.7	15.7
73	107	65	16.7	0	16.1	15.6	16.7
Phage (per 1.55×10^{14} C units or 100 μM total P)							
97	98	83	13.9	12.8	0	26.4	29.7
96	99	82	14.2	12.5	0	27.0	28.5
Mixture (per 1.55×10^{14} C units each)							
179	218	135	28.1	10.5	12.2	41.3	42.6
185	228	153	30.8	12.3	14.8	49.9	45.5

The results are expressed per 100 μM DNA-P as estimated by the diphenylamine reaction carried out on the starting materials. The data show recoveries of diphenylamine reacting substances (C units), phosphorus, and bases in the DNA extracts, all expressed in micromole equivalents. Bases were separated in two dimensions.

(1945) as follows: The precipitate was warmed for 15 to 18 hours at 37°C. in 5 ml. N NaOH, and reprecipitated cold with 5 ml. N HCl and 1.0 ml. 3 M TCA. After washing with cold 0.3 M TCA, the precipitate was extracted twice in 0.3 M TCA at 90°C. for 15 minutes. An aliquot of the extract was used for the diphenylamine reaction. The remainder was heated for 1 hour at 100°C. to decompose most of the TCA, and evaporated to dryness by further heating in a current of air and finally in a vacuum desiccator.

The recovery of DNA by this procedure was checked by analyzing phage and mixtures of bacteria and phage (Table I). These tests showed that the extracts contained phosphorus, diphenylamine-reacting substances, purines, and pyrimidines in amounts indistinguishable from those contained in the whole phage.

In several experiments the recovery of DNA was further checked by using P^{32} -labeled phage for the infection. 90 per cent or more of the adsorbed P^{32} regularly appeared in the DNA extracted from the cells 10 or 20 minutes after infection. The larger losses were traceable to failure to obtain complete sedimentation of cells during the first centrifugation of the acidified bulk culture.

Diphenylamine Reaction.—The diphenylamine reaction (Dische, 1930) was carried out by heating 2 ml. samples with 4 ml. reagent for 20 minutes at 100°C., and allowing the tubes to stand at least 30 minutes at room temperature. A Corning filter of 590 $m\mu$ was used for the colorimetric determinations. Samples of whole bacterial culture were precipitated cold with 0.3 M TCA before analysis. Resuspending the precipitate

TABLE II
Recovery of Bases from Phage DNA after Hydrolysis for Varying Times

Time of heating	Optical densities of eluates at spectral peaks			
	Guanine	Adenine	Hydroxymethylcytosine	Thymine
<i>hrs.</i>				
1	0.251	0.550	0.215	0.420
3	0.251	0.540	0.232	0.440
6	0.255	0.490	0.215	0.390
12	0.251	0.440	0.215	0.420
24	0.253	0.345	0.237	0.415
Blanks	0.06	0.07	0.04	0.11

DNA was extracted from T2 and hydrolyzed by heating for various times at 100°C. in 6 N HCl as described in the text. The bases were separated by one dimensional chromatography in isopropanol-HCl. Hydroxymethylcytosine showed the same absorption spectrum (peak 278 $m\mu$) after 1 and 24 hours' heating. A rate of destruction of pure adenine similar to that shown above was observed under the same conditions.

in dilute alkali and reprecipitating, or boiling the cells before precipitation, had no effect on the readings. Thymus DNA was used as a working standard after calibration against phage DNA of known P content.

Hydrolysis of DNA.—The extracted and dried DNA was hydrolyzed by heating for 3 hours at 100°C. in 3 ml. redistilled 6 N HCl under CO_2 in sealed tubes. HCl was removed from the hydrolysate in a vacuum desiccator over KOH and $CaCl_2$, after which the residue was dissolved in 0.1 N HCl and sampled for determination of total P (Fiske and SubbaRow), and for chromatographic analysis of bases. The yield of bases from whole phage was not sensitive to the time of heating (Table II): only adenine was appreciably decomposed (40 per cent in 24 hours). The stability of hydroxymethylcytosine under these conditions is notable because, in our experience, about 50 per cent of this pyrimidine and also some of the thymine are decomposed by heating for 1 hour in 70 per cent perchloric acid. Our recoveries appear to be at least as good as those reported by Wyatt and Cohen (1952), who hydrolyzed with formic acid.

Mixtures of guanine, adenine, cytosine, and thymine behaved similarly to the bases from phage when heated in HCl either alone or admixed with whole phage (data not shown).

The same method of hydrolysis was used without further test for DNA from bacteria, since our results for the composition of bacterial DNA (Table III) agree well with those of Smith and Wyatt (1951).

Table III shows that the mol ratio of bases to phosphorus in hydrolysates of extracted bacterial DNA is only 60 per cent. Probably not all this phosphorus

TABLE III
Mol Per Cent Composition of DNA

Solvent	Guanine	Hydroxymethyl-cytosine	Cytosine	Adenine	Thymine	Mols base per 100 mol P
Bacteria						
Propanol-HCl	23	0	26	22	28	64
“ “	25	0	25	24	27	64
Propanol-NH ₃	25	0	25	25	25	55
“ “	23	0	25	25	27	61
Both	26	0	25	25	25	59
“	26	0	25	24	26	61
Phage						
Propanol-HCl	16	18	0	31	35	100
“ “	17	17	0	31	37	104
Propanol-NH ₃	17	15	0	31	36	88
“ “	17	17	0	31	37	101
Both	17	16	0	32	36	85
“	17	15	0	33	35	83

Data are shown for 6 different hydrolysates of phage DNA, and four of bacterial DNA. The results given for 2 dimensional chromatograms are more reliable. They are taken from Table I.

is DNA. In experiments with P³²-labeled bacteria we have found that treatment of boiled cells with desoxyribonuclease reduces the phosphorus content of this fraction by only 60 per cent. This test may overestimate the non-DNA phosphorus, however. The content of diphenylamine-reacting substances in the extracts (Table I) suggests that our recoveries of bases from phage and bacterial DNA are about the same, namely, 80 to 85 per cent as measured from 2 dimensional chromatograms.

Chromatograms.—Two dimensional chromatograms were prepared using Whatman No. 1 paper, isopropanol-HCl (Smith and Wyatt, 1951), and isopropanol-NH₃. The paper was washed by soaking in water and drying before use. The ammoniacal solvent consisted of 85 ml. isopropanol, 15 ml. water, and 1.3 ml. NH₄OH (28 per cent). The acid solvent was used first in an ascend-

ing run of about 22 cm., followed by a descending run of the same length in isopropanol-NH₃. The second solvent separates guanine, hydroxymethylcytosine, cytosine, adenine, and thymine, in order of increasing *R_f*. Uracil moves with adenine in this solvent. Spots were marked by inspection in ultraviolet light (Holiday and Johnson, 1949), and eluted in N/10 HCl, together with blanks taken from areas adjacent to the spots. The optical densities observed at the spectral peaks were corrected by subtracting the appropriate blank readings, which were small and uniform, and were converted to micromoles of base from the extinction coefficients given by Smith and Wyatt (1951) and Wyatt and Cohen (1952).

One dimensional chromatograms were also made in many experiments. These are of limited application because propanol-HCl fails to separate cytosine and its hydroxymethyl derivative, and because guanine streaks badly in propanol-NH₃, and tends to contaminate the hydroxymethylcytosine spot. In addition, one dimensional chromatograms are contaminated with dirt, partly from the paper and partly from the sample, that can be detected in the eluates (except guanine) by the excessive readings at 250 m μ . Since the dirt runs fast in the acid solvent and slowly in propanol-NH₃, it is largely eliminated from the spots by using both solvents. Eluates from the two dimensional chromatograms give reasonably good absorption spectra even for cytosine and hydroxymethylcytosine coming from infected bacteria at times when the proportion of one or the other of these two bases is small. All the analytical data given in this paper were obtained from 2 dimensional chromatograms unless otherwise stated.

The chromatograms consistently showed a trace spot that moved with cytosine in propanol-NH₃ and just behind adenine in propanol-HCl. Since it was present both in hydrolysates of phage and bacterial DNA, it may be a decomposition product. In any case its light absorption was negligible. A trace spot in the position of uracil was rarely found in preparations from either bacteria or phage.

The composition of phage and bacterial DNA given by these methods is shown in Table III. These data agree with those published by Smith and Wyatt (1951) and Wyatt and Cohen (1952) except that we find a significantly higher proportion of hydroxymethylcytosine and a somewhat greater ratio of bases recovered to total P (using their solvent) from phage DNA. This is presumably due to the different methods of hydrolysis.

The DNA Content of T2.—Our current preparations of T2 usually contain 1.8 to 2.2 $\times 10^{-11}$ μ g. P per plaque-forming particle. This is 20 per cent less than earlier estimates (Hershey *et al.*, 1951), and may be the effect of using a different bacterial host for the preparation of stocks. Table IV shows DNA equivalents per phage estimated by various methods for several preparations, and also shows that the diphenylamine color equivalents of thymus and phage DNA per unit of phosphorus are about the same.

Since our major objective is to clarify the relationship between intracellular

phage and intracellular DNA, the DNA content of a single phage particle forms an appropriate unit of measurement. For purposes of discourse, we define a unit of DNA as the amount contained in an infective particle of phage T2. In practice this unit has the following relative values as determined by analysis of purified preparations.

TABLE IV
DNA Equivalents per Plaque-Forming Phage Particle Determined by Several Methods

Preparation	Total phosphorus $\times 10^{-11}$ $\mu\text{g.}$	Extinction coefficient at 260 $m\mu$ $\times 10^{-12}$ cm.^2	Total P per diphenylamine color equivalent $\times 10^{-11}$ $\mu\text{g.}$
Thymus DNA, preparation 1			2.0 (standard)
“ “ “ 2			2.2
“ “ “ 3			2.0
T2, preparation 1	2.1	6.4	2.0
“ “ 2	1.8	6.1	1.7
“ “ 3	2.0	6.4	2.0
“ “ 4	2.2	7.1	2.0
“ “ 5	1.9	5.9	1.9

The thymus nucleic acids are commercial preparations. The extinction coefficients are ratios of optical density per centimeter to plaque titer per milliliter, not corrected for scattering. The data shown are selected; unselected data of this type will appear in the second paper of this series.

1 P unit = 2.0×10^{-11} $\mu\text{g.}$ DNA-P (Table IV).

1 C unit = diphenylamine color equivalent of 1 P unit of T2 or thymus DNA (Table IV).

1 D unit = ratio of optical density per centimeter at 260 $m\mu$ (not corrected for scattering) to P units per milliliter = 6.5×10^{-12} cm.^2 (Table IV).

1 B unit = 8.3×10^{-14} μM hydroxymethylcytosine (for T2 DNA) or 1.3×10^{-13} μM cytosine (for bacterial DNA). These follow from the composition of the DNA's (Table I), an assumed recovery of 0.83 mol of base per mol of phosphorus, and the definition of the P unit.

According to data given by Luria, Williams, and Backus (1951), these values must be very close to the values for single particles.

Experimental Results

Table V summarizes analytical data for infected cultures analyzed at different times during the first 30 minutes of viral growth. The results are best appreciated by considering one at a time the several kinds of information they contain.

TABLE V
 DNA Content of Infected Bacteria

Phage yield		Total C units		C units at <i>t</i> min.		DNA-P		Bases $\mu\text{M} \times 10^{11}$	Cytosine $\mu\text{M} \times 10^{11}$	Hydroxy- methyl cytosine $\mu\text{M} \times 10^{11}$
<i>t</i> min	30 min.	0 min.	30 min.	Total	Extract	Units	$\mu\text{M} \times 10^{11}$			
<i>t</i> = 0 min.										
—	86	58	195	58	46	62	4.0	2.5	0.55	0.02
—	245	142	590	142	112	81	5.2	2.5	0.60	0.00
—	65	65	237	65	68	76	4.9	3.1	0.70	0.03
Uninfected		98	—	98	73	104	6.7	4.0	1.00	0.00
<i>t</i> = 5 min.										
—	140	68	—	90	61	87	5.6	3.3	0.65	0.09
—	176	86	—	121	92	121	7.8	4.4	0.78	0.17
<i>t</i> = 10 min.										
1.5	100	83	—	150	105	140	9.0	5.4	0.66	0.36
1.5	100	135	606	257	173	210	14	9.0	1.11	0.65
1.4	100	79	248	151	85	109	7.0	4.8	0.44	0.42
1.9	165	72	365	160	124	150	9.7	6.0	0.53	0.55
<i>t</i> = 15 min.										
18	106	98	—	206	143	175	11	7.4	0.38	0.72
21	109	53	250	155	103	135	8.7	5.4	0.26	0.69
57	230	94	—	246	200	250	16	12	0.48	1.44
<i>t</i> = 20 min.										
20	60	80	388	186	148	181	12	7.4	0.34	0.91
48	107	66	—	170	128	145	9.4	6.3	0.17	0.73
20	77	73	447	240	197	200	13	9.2	0.30	1.20
<i>t</i> = 30 min.										
86	—	58	—	195	147	167	11	7.8	0.05	0.84
245	—	142	—	590	530	540	35	22	0.26	3.2
65	—	65	—	237	208	216	14	9.6	0.25	1.2
123	—	104	—	292	248	291	19	14	0.23	1.8
83	—	74	—	262	217	208	13	11	0.21	1.5

All data are expressed per bacterium. The "unit" is 2×10^{-11} μg . P or equivalent DNA.

Total C units means DNA determined by the diphenylamine reaction carried out on whole TCA-precipitated culture; extract DNA refers to the isolated fraction. The data on each line come from an independent experiment, except that three cultures were analyzed at 0 and 30 minutes.

Reliability.—The reliability of the analytical data is simply tested by examining correlations between independent measures of DNA content.

If one plots yields of total acid-insoluble diphenylamine-reacting substances (C units of total DNA) against the corresponding assays of the DNA extracts,

the points fall very close to a straight line. The slope of this line shows that *91 per cent of the color-giving substances formed after infection appear in the extracts*. This is to be compared with 75 per cent for uninfected bacteria and 97 per cent for isolated phage (Table I). Evidently bacteria contain appreciable amounts of substances interfering with the colorimetric determination of DNA, but these do not increase very much after infection.

If one plots total phosphorus content against diphenylamine-reacting substances for the extracts, the points again fall very close to a straight line. The slope of this line shows that *the substances synthesized after infection and appearing in the DNA extracts react with diphenylamine to give a color equivalent to phage or thymus DNA containing 92 per cent as much phosphorus*. Extracts of uninfected bacteria contain phosphorus of which only 70 per cent is DNA by the same test (Tables I and V).

Finally, if one plots total bases recovered by chromatography against mol equivalents of extract phosphorus, the points (with one exception) fall very close to a straight line of which *the slope indicates 0.83 mol of base recovered for each mol of extract phosphorus assimilated after infection*. This is precisely the recovery of bases from isolated phage DNA (Table I), which means that most of the phosphorus assimilated after infection and appearing in the extracts is DNA phosphorus. Uninfected bacteria yield only 0.6 mol of base per mol of extract phosphorus (Table I).

Graphs illustrating these correlations are not reproduced here since all the data are contained in Table V.

Variability of Cultures.—The correlations noted above exclude analytical error as an important cause of variability of results. Errors in counting bacteria can also be excluded, since counts made before and after infection usually agree. The considerable variation in results for different cultures prepared on different days (Table V) is therefore due to differences in the behavior of the cultures. Our only clue to the cause of this variation comes from concurrent experiments with otherwise identical cultures of smaller size. Yields of phage and DNA given by a typical culture of this kind are illustrated in Fig. 1.

The following relationships were rather consistently observed:—

1. Large cultures yielded about twice as many bacteria per milliliter at the end of 2 hours as small cultures receiving the same proportionate seeding.
2. The DNA content per bacterium at 2 hours was about twice as great (and less variable) for the smaller cultures.
3. Yields of phage and DNA synthesized after infection under the two conditions were more nearly proportional to initial DNA content than to cell concentration.

All these facts are consistent with the hypothesis that the relatively inefficient aeration of the larger cultures stimulates cell division before infection without having much effect on DNA synthesis either before or after infection. If this

is so the DNA content (nuclear numbers?) of the culture might form a more appropriate basis for calculation than cell numbers. The data of Table V would, in fact, be appreciably improved by computation on this basis. Since the results given throughout this paper are expressed per bacterium, it should be kept in mind that the bacterium referred to is smaller than cells growing under more favorable conditions.

Composition of DNA.—The first question one asks about DNA synthesis is whether the intrabacterial DNA consists solely of DNA like that of uninfected bacteria and DNA like that of resting phage. If so, the composition of the mix-

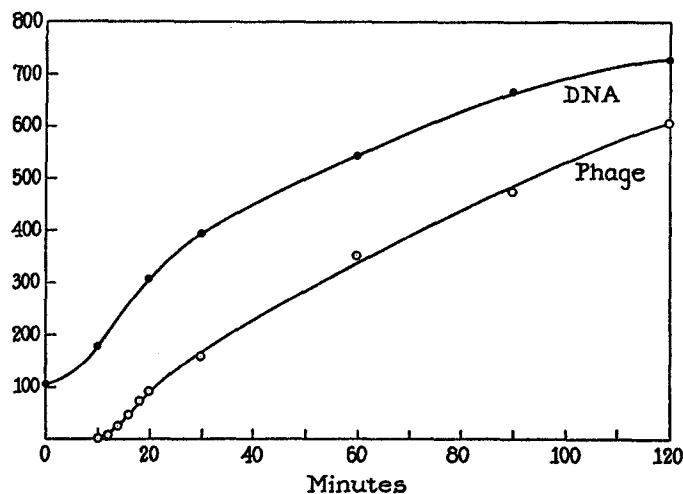


FIG. 1. Content per bacterium of infective phage particles (by cyanide lysis) and DNA (phage particle equivalents determined by the diphenylamine reaction) in a culture measuring 200 ml. total volume. Note that the DNA increment is roughly 1 unit per phage after the 10th minute, and that the total increment is about 80 units in excess of the total phage.

ture can be predicted from its cytosine and hydroxymethylcytosine contents (Table V), and from the composition of the two kinds of DNA (Table III). These predictions are compared with the compositions found in Fig. 2. The tests are most sensitive at the 10 minute points ($x/[x + y] = 0.47$ to 0.63), when the bacteria contain approximately equal amounts of the two kinds of DNA and virtually no mature phage. No significant discrepancy from the hypothesis can be detected for these points. The systematic discrepancy for all times brought out in the upper left quadrant of Fig. 2 can be attributed to slightly overestimated cytosine and hydroxymethylcytosine contents of the two kinds of DNA (*cf.* Table I). We conclude that our data are consistent with the idea that infected bacteria contain a mixture of two kinds of DNA, one

kind having the composition of bacterial DNA and the other having the composition of the DNA of isolated phage.

Temporal Changes.—Fig. 3 shows the changes in amounts of the two kinds of DNA during the course of the infection, as estimated from the cytosine and hydroxymethylcytosine contents. The cytosine-containing DNA decreases to about one-third of its initial value in 20 minutes. The results for the 30 minute cultures cannot be interpreted because some of the bacteria lyse before this time and some of the cultures contain appreciable numbers of uninfected bac-

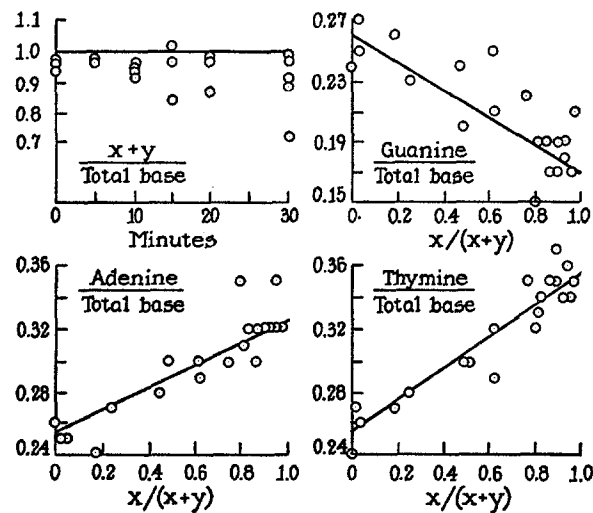


FIG. 2. Composition of the intrabacterial DNA during growth of phage. The straight lines correspond to the hypothesis that the bacteria contain a mixture of DNA's like those found in uninfected bacteria and resting phage. The points show the results of chromatographic analysis. x = micromoles of hydroxymethylcytosine found \div 0.155. y = micromoles of cytosine found \div 0.25. $x/(x + y)$ = fraction of viral DNA in the theoretical mixture.

teria. The one culture containing virtually no cytosine at 30 minutes suggests that destruction continues in unlysed infected bacteria.

The cytosineless DNA increases, after a brief initial delay, to an amount equivalent to 150 or more phage particles at 30 minutes. Evidently this increase could not be accurately determined by measurements of total DNA without taking into account the decrease in bacterial DNA. Total DNA synthesis, as measured by Cohen (1948) and others, nevertheless gives a reasonably good estimate of the accumulation of cytosineless DNA. The prompt initiation of DNA synthesis in our cultures as compared with those studied by Cohen probably depends more on the nutrient media and methods of infection used, than

it does on the masking of DNA synthesis in his experiments by concomitant disappearance of bacterial DNA.

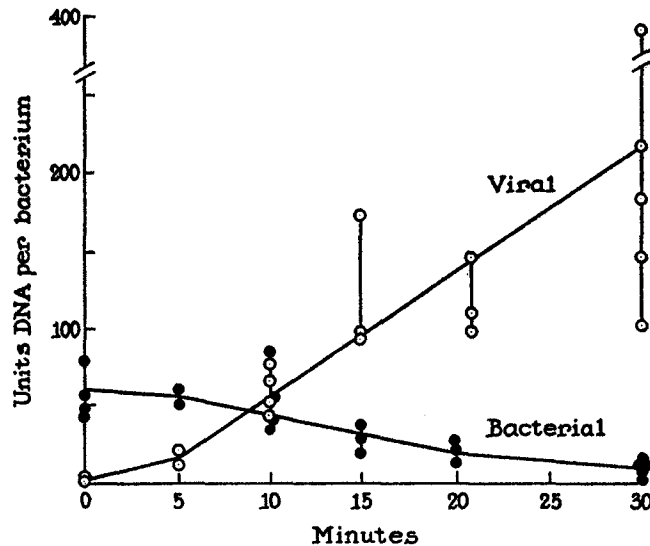


FIG. 3. Amounts of viral and bacterial DNA per infected bacterium during growth of phage. These are computed from the amounts of cytosine and hydroxymethylcytosine recovered chromatographically, and are expressed in phage equivalents (B units) of DNA per bacterium. Each point represents a different culture.

TABLE VI
Cytosineless DNA and Infective Phage per Infected Bacterium

Time after infection	Cytosineless DNA (B units)	Infective phage particles	Difference (B units)
<i>min.</i>			
10	43, 78, 51, 66	2, 2, 1, 2	41, 76, 50, 64
15	87, 83, 174	18, 21, 57	69, 62, 117
20	110, 88, 145	20, 48, 20	90, 40, 125
30	101, 390, 145, 217, 181	86, 245, 65, 123, 83	15, 145, 80, 94, 98

Cytosineless DNA = $\frac{\mu\text{M hydroxymethylcytosine} \times 31}{0.155 \times 0.83 \times 2.0 \times 10^{-11}}$, expressed as B units (equivalent phage particles) per bacterium.

Cytosineless DNA Not Contained in Mature Phage.—The main purpose of this work was to measure the intrabacterial cytosineless DNA in excess of that contained in the mature phage also present in the cell. This can be estimated by subtracting the yield of phage from the total cytosineless DNA expressed in phage equivalents. Table VI gives these estimates. They show that an in-

infected bacterium contains newly synthesized DNA equivalent to 40 to 80 resting phage particles at 10 minutes, when maturation of phage is just beginning, and that this content of non-phage cytosineless DNA remains sensibly constant thereafter at least to the 30th minute, at which time the cells contain in addition 65 to 250 mature phage particles. A similar quantitative situation is indicated by the data of Fig. 1. The metabolic relationship between these two kinds of viral DNA will form the subject of a second paper in this series.

SUMMARY

1. The phosphorus content per infective particle of isolated bacteriophage T2 has been redetermined. It does not exceed 1.8 to 2.2×10^{-11} μg . The equivalent amount of DNA has been defined in terms of several analytical methods and taken as a unit of measurement of intrabacterial DNA.

2. The DNA of *E. coli* contains guanine, adenine, cytosine, and thymine in approximately equal amounts, but no hydroxymethylcytosine. One bacterial cell contains 40 to 150 units of DNA, depending on the conditions of growth and the method of measurement.

3. The DNA of phage T2 (one unit per particle by definition) contains guanine, 5-hydroxymethylcytosine, and relatively large amounts of adenine and thymine, but no cytosine.

4. Infected bacteria contain DNA of a composition that varies systematically during the course of viral growth. At all times it resembles a mixture of bacterial and viral DNA.

5. The characteristic bacterial DNA is decomposed after infection, measuring about one-third its initial amount at 20 minutes. The characteristic viral DNA increases in amount, after a short delay, reaching a level of 100 to 400 units per bacterium 30 minutes after infection. At 10 minutes after infection, the two kinds of DNA are approximately equal in amount.

6. The characteristic viral DNA present in infected cells exists in two forms, one consisting of infective particles and one not. The portion not contained in infective particles builds up to 40 to 80 units per cell during the first 10 minutes after infection and afterwards remains roughly constant in amount. Infective particles begin to appear at 10 minutes and account for all or most of the increase thereafter.

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