CHEMICAL ANALYSIS OF THE T₂ BACTERIOPHAGE AND ITS HOST, ESCHERICHIA COLI (STRAIN B)*

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A particulate component of characteristic morphology and behaving biologically as the T₂ bacteriophage of *Escherichia coli* (strain B) has been isolated by centrifugation (1) from lysates of the host grown in nutrient broth and in synthetic saline medium. The purified bacteriophage concentrates exhibited a high degree of homogeneity with respect to particle kind in electron micrographs (1, 2), and of uniformity in size, shape, and density of the particles in ultracentrifugal sedimentation diagrams (1, 3). No material extraneous to the particles of characteristic form was evident in significant amounts by either method of examination.

Previous work by other investigators (4–8) on concentrates of various bacteriophages has revealed the presence of protein, carbohydrate, and nucleic acid, the latter in high concentration. Schlesinger (4) demonstrated an undetermined amount of presumably lipide material in concentrates of a phage of *Bacillus coli* and Pollard (7), corroborating the findings of Schlesinger with respect to protein and nucleic acid, found, in addition, much carbohydrate. Preliminary chemical analyses (1) have shown that the T₂ bacteriophage of *Escherichia coli* is a complex of protein, lipide, and a large amount of nucleic acid, findings contrary to those of Kalmanson and Bronfenbrenner (9), who demonstrated only traces (0.07 per cent) of phosphorus. In the work reported here, the chemical analysis of the T₂ bacteriophage has been extended in a more detailed study of the elementary and component constitution of the agent obtained from both broth and synthetic medium lysates. In addition, it has seemed likely that a direct comparison of the chemical nature of the infectious entity, itself, with that of its single cell host organism, might reveal information providing some insight into the host-virus relationship. Consequently, analyses have been made in parallel on washed concentrates of the host organism, *Escherichia coli* (strain B). The present paper¹ is concerned with a description of the results obtained.

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¹ The results reported here were described before the Division of Biological Chem-
Materials and Methods

The derivation of the bacteriophage analyzed in the present work has been described (1); much of the material was from the same preparations as those employed for ultracentrifugal (1, 3) and electron micrographic examinations (1, 2).

Concentrates of *Escherichia coli* were isolated from cultures in broth and in synthetic medium (1) by centrifugal procedures (1, 10) comparable to those used for purifying the bacteriophage. The cultures were prepared in batches of 15 to 18 liters distributed in 1500 ml. volumes in 2 liter Florence flasks. The seed inoculum of bacteria for the large volumes was prepared in 50 ml. of the respective media in 125 ml. Erlenmeyer flasks, which were inoculated with 0.1 ml. of an 18 to 24 hour broth culture of *Escherichia coli* and then incubated for 18 hours at 37°. To each 1500 ml. volume of medium, previously warmed to 37°, there were added 30.0 ml. of the seed inoculum. The flasks were incubated for 8 hours at 37° with vigorous mixing, manually, every 15 minutes. Stained preparations from the individual 1500 ml. cultures were examined microscopically at the end of 8 hours, the cultures were pooled, and isolation of the bacteria was begun immediately. The initial sedimentation of the bacteria was carried out in the standard 160 ml. separator bowl of the Sharples centrifuge, rotating 40,000 r.p.m. (39,000g at the bowl periphery). The culture fluid was passed through at the rate of 14 liters per hour and was followed by 2 liters of chilled, sterile 0.9 per cent NaCl solution to remove the final 160 ml. of culture medium. The bowl was plugged (10) before rotation ceased, removed from the centrifuge, and shaken vigorously for 15 to 20 minutes to resuspend the bacteria in the 160 ml. of saline solution. The concentrated bacterial suspension was washed into a sterile beaker with 20 to 30 ml. portions of sterile 0.9 per cent NaCl solution, with aseptic (with respect to extraneous organisms) precautions. The suspension was diluted to 400 ml., transferred to 100 ml. lusteroid tubes, and cleared of gross aggregates and a small quantity of flocculated, brown pigmented material by spinning in the horizontal centrifuge (International centrifuge No. 2) at 1000g for 5 minutes. The milky white suspension was siphoned into sterile 100 ml. lusteroid tubes and completely sedimented in 30 minutes at 3000g in the angle centrifuge. The clear supernatant fluid was poured off and discarded. The large white putty-like pellets were resuspended in 200 ml.
of sterile saline with the aid of glass beads. The sequence of horizontal and angle centrifugation was repeated three additional times. After the final sedimentation, the pellets were suspended in a volume of 50 to 75 ml. of sterile 0.9 per cent NaCl solution. All transfers were made under ultraviolet light (10), and all centrifugation was carried out with the material in closed sterile tubes.

**Qualitative Analysis**—The qualitative chemical findings with concentrates of the bacteriophage from both broth and synthetic medium cultures have already been reported (1). The usual protein tests applied to the bacterial concentrates, biuret, ninhydrin, Millon's, and xanthoproteic, were positive. The Molisch reaction was immediately positive and became progressively stronger with standing of the mixture. The Sakaguchi test was negative, and the Ehrlich benzaldehyde test for tryptophane was positive. A strongly positive pentose reaction was obtained with Bial's reagent (orcinol-HCl-FeCl₃). Both the Schiff and diphenylamine tests were positive. A green color was obtained on heating 10 minutes in the water bath at 100° with tryptophane and perchloric acid (11). Spectrophotometric examination of an isoamyl alcohol extract of the chromogenic material revealed a curve characteristic of ribopentose (sodium thymonucleate and yeast nucleic acids were used as controls). This is in direct contrast to the result of the comparable experiment (1) with bacteriophage in which a red chromogenic material showed a curve characteristic of deoxypentose.

**Quantitative Analysis**—Concentrates obtained from large pools of starting material, as described above, and containing 100 to 200 mg. of bacteriophage from each type of medium and 0.5 to 2 gm. of bacteria from the respective media were dialyzed against running distilled water at 2-8° for 72 to 96 hours, frozen with dry ice, and lyophilized. The bacteriophage and bacterial preparations were treated and analyzed in identical ways. The results of elementary and component analyses of the four materials and fractions are given in Tables I and II.

Analyses for carbon, nitrogen, phosphorus, and carbohydrate were made on the whole bacteriophage complex and on the unfraccionated bacterium. Samples of 4 to 7 mg. of the frozen and dried materials were weighed into small Pyrex boats. After the samples were dried to constant weight at 40-45° over P₂O₅, the weights were taken in closed weighing bottles. Carbon was determined by wet combustion (12), nitrogen by the semimicro Kjeldahl method with the digestion mixture described by Kirk, Page, and Van Slyke (13), phosphorus by the method of Kirk (14), and carbohydrate, as glucose, by the method of Tillmans and Philippi (15).

**Fractionation with Lipide Solvents**—Two samples of 100 to 150 mg. each of the whole bacteriophage or bacterial complex were dried to constant weight, as described above. The samples were transferred to 40 ml.
extraction tubes provided with ground glass connections and extracted with alcohol-ether, 3:1, for 2 hours under a reflux condenser. Details of the manner of the transfer of the alcohol-ether-insoluble material to a

### Table I

**Elementary and Fractional Analyses* of T₂ Bacteriophage and Its Host, Escherichia coli (Strain B)**

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<td>1.81 1.97</td>
<td>1.97 2.16</td>
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<td>12.5</td>
<td>11.2 12.2</td>
<td>11.8 13.0</td>
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</table>

* Each of these values represents the percentage of dry weight of the whole complex or fraction.

† The carbohydrate values are expressed as glucose.

‡ The total lipide values of the bacteriophage = carbon values X 1.3; those of the bacterium = carbon values X 1.5.

§ DNA designates desoxy pentose nucleic acid; RNA designates ribo pentose nucleic acid.
agreement of 1 per cent or better. The percentage of alcohol-ether-soluble material was found by difference. Alcohol-ether-soluble carbon and nitrogen were determined (13) in 2.0 and 5.0 ml. aliquots and carbohydrate was determined (15) in 10.0 ml. aliquots after evaporation to dryness. Aliquots of 5.0 ml. were examined (13) for total cholesterol.

To obtain the total lipide values, a 25 ml. aliquot was withdrawn from each of the duplicate alcohol-ether fractions. The two aliquots were combined in a beaker and evaporated to dryness at 58°. The residue was extracted with five 5 ml. portions of redistilled petroleum ether which had been washed with concentrated H₂SO₄. The petroleum ether extracts were filtered into a 25 ml. volumetric flask. Carbon, nitrogen, and phosphorus were determined (13) in 2.0 and 5.0 ml. aliquots. Total lipide values (Table I) for the bacteriophage preparations were calculated from the carbon values of the petroleum ether-soluble fraction, with the factor 1.3 (the factor for neutral fat). The analogous total lipide values for the bacterium were obtained by multiplying the carbon value by 1.5 (the factor for phospholipide).

Alcohol-Ether-Insoluble Fraction—Samples of the non-lipide fraction were dried to constant weight for the determination of carbon, nitrogen,
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and carbohydrate, as described for the samples of whole complex. For the
determination of the total alcohol-ether-insoluble phosphorus and frac-
tionation into DNA phosphorus (desoxypentose nucleic acid phosphorus),
RNA phosphorus (ribopentose nucleic acid phosphorus), and inorganic
or protein phosphorus (designated as "other" phosphorus in Table I), a
modified micro technique based upon the method of Schmidt and Thann-
hauser (17) was employed. The alcohol-ether-insoluble fractions of the
bacteriophage and the bacterium were analogous to Schmidt and Thann-
hauser's extracted tissue powder. Samples of the bacteriophage fractions
weighing 15 to 18 mg. (constant dry weight) and of bacteria weighing 25
to 30 mg. were placed in 20.0 ml. volumetric flasks with 15 ml. of 5 per cent
KOH solution. The flask, closed with a glass stopper, was incubated at
37° for 15 to 18 hours. The material was completely dissolved at the end
of this period. The flask and contents were cooled to room temperature,
and the volume was made up to 20 ml. A 2 ml. aliquot of this solution was
taken for the total phosphorus determination by the method of Kirk (14).
A 15 ml. aliquot was transferred to a tube graduated at 20 ml. and the
desoxypentose nucleic acid precipitated by the addition of 3.0 ml. of 6 N
HCl and 2.0 ml. of 20 per cent trichloroacetic acid. The mixture, chilled
in an ice bath and stirred frequently during a 15 minute period of standing,
yielded a well flocculated precipitate. The latter was removed by filtra-
tion (Whatman paper No. 50, with very gentle suction) and the total acid-
soluble organic and inorganic phosphorus determined in a 5.0 ml. aliquot
of the filtrate. The inorganic or other phosphorus was precipitated accord-
ning to Delory (18) from a 10.0 ml. aliquot of the filtrate. The respective
phosphorus values (Table I) and the nucleic acid values shown in Table II
were calculated as described by Schmidt and Thannhauser (17).

In view of the low content of ribopentose nucleic acid of the synthetic
medium bacteriophage, as shown in Table II, the phosphorus fractionation
of this material was repeated with another 20 mg. sample. The results
were 5.10 per cent total alcohol-ether-insoluble phosphorus, 4.40 per cent
DNA phosphorus, 0.13 per cent RNA phosphorus, and 0.54 per cent other
phosphorus, values corroborating those of Table I. As a further control
experiment, a mixture containing known amounts of DNA, RNA, and
casein was analyzed in the manner described for the bacteriophage prepa-
ations. The mixture contained 1.02, 0.26, and 0.09 mg. of DNA, RNA,
and casein phosphorus, respectively; the amounts found on analysis were
0.98 mg. of DNA, 0.25 mg. of RNA, and 0.1 mg. of casein phosphorus.

Determinations of the nucleic acid content of the bacterium and the
bacteriophage from broth were made also by means of the perchloric acid-
tryptophane method of Cohen (11). The value, 19.1 per cent, obtained
for the bacterium was identical with the amount of ribopentose nucleic acid
determined by the method of Schmidt and Thannhauser. In the instance
of the bacteriophage, the value was 42.0 per cent, which was slightly higher
than the amount of desoxypentose nucleic acid found by the other method.

The determined or calculated component constitutions of the bacterio-
phage isolated from the two media and of the corresponding preparations
of bacteria are given in Table II. The total lipide values for the phage
preparations were calculated from the carbon values of the petroleum ether-
soluble fractions, while the total lipide values for the bacterial prepara-
tions were calculated from the petroleum ether-soluble phosphorus values.
Total non-lipide is expressed as the difference between the whole complex
and the total lipide value. The protein value represents the total non-

| Apparent Partial Specific Volume of T₂ Bacteriophage of Escherichia coli (Strain B) |
|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Broth Bacteriophage 33          | Broth Bacteriophage 34        | Synthetic Bacteriophage 39    |
| Ultra-centrifugal cycle*        | Concentration of phage        | Apparent partial specific      | Ultra-centrifugal cycle*      | Concentration of phage        | Apparent partial specific      |
|                                | mg. per ml.                   | volume                        | mg. per ml.                   | mg. per ml.                   | volume                        |
| 1                               | 7.94                          | 0.6542                        | 1                             | 7.33                          | 0.6588                        |
| 1                               | 7.94                          | 0.6551                        | 1                             | 7.33                          | 0.6589                        |
| 1                               | 7.94                          | 0.6538                        | 1                             | 7.33                          | 0.6584                        |
| 2                               | 7.47                          | 0.6542                        | 2                             | 6.63                          | 0.6584                        |
| 2                               | 7.47                          | 0.6559                        | 2                             | 6.63                          | 0.6544                        |
| 2                               | 7.47                          | 0.6533                        | 2                             | 6.63                          | 0.6553                        |

* The number of times the material was sedimented in the vacuum type ultra-
centrifuge; each preparation had been sedimented one additional time (the initial
preliminary concentration) in the Sharples centrifuge.

lipide value minus the total nucleic acids. The carbohydrate values are
those of the respective whole complexes.

**Apparent Partial Specific Volume of T₂ Bacteriophage**—For the estima-
tion of partial specific volumes, concentrates containing 5.0 to 8.0 mg. of
bacteriophage per ml. of suspension in a solution composed of 0.9 per cent
NaCl and 0.1 per cent KCl (3) were analyzed by the method described in
detail for the influenza viruses (19). The partial specific volume was
calculated by means of the equation given by Kraemer (20). The values
obtained for the apparent partial specific volume of two individual batches
of T₂ bacteriophage from broth lysates are shown in Table III. After the
first ultracentrifugal cycle, replicate determinations were made; the mate-
rials were then diluted to 120 ml., angle centrifuged, and again ultracen-
trifuged. The replicate determinations on the material after the second
ultracentrifugation were virtually identical with those obtained after the

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first ultracentrifugation. Partial specific volume determinations on the synthetic medium bacteriophage were made on material ultracentrifuged only once, owing to the instability of the agent. The replicate values obtained with a representative batch of bacteriophage from synthetic medium are shown in Table III. The reciprocal of the average apparent partial specific volume of the broth bacteriophage indicates an approximate dry density of 1.52, which is in accord with the chemical composition of the material.

**DISCUSSION**

The ultracentrifugal and electron micrographic evidence of the physical homogeneity of the bacteriophage concentrates selected for the present study has already been described (1-3). An index of the chemical homogeneity is seen in the constancy of the values of apparent partial specific volume, Table III, which indicates a chemical and physical entity of uniform composition. These results with the bacteriophage were similar in this respect to those with the influenza viruses (19), which are entities of constant composition having a limiting partial specific volume characteristic for each strain.

The apparent partial specific volume of the bacteriophage was in accord with its chemical composition, and the level of the values for the broth bacteriophage did not change on repeated ultracentrifugation. Though instability (1) attending repeated ultracentrifugation precluded a like study on the agent from synthetic medium, this material nevertheless gave values after a single ultracentrifugation closely similar to those observed for the broth bacteriophage.

The results of the chemical examinations given in Tables I and II show that the T2 bacteriophage consists of protein, a small proportion of lipide, and a very high content of nucleic acid. The nucleic acid, Table II, consisted of both desoxypentose and ribopentose types, the former in a considerably larger amount than the latter. Significant differences between the bacteriophage derived from broth medium lysates and that from synthetic medium lysates were evident in the distribution of the two types of nucleic acid (Table II), as determined in the alcohol-ether-insoluble fraction (Table I) by the phosphorus fractionation method of Schmidt and Thannhauser (17). Attempts to dissociate the nucleic acid and protein directly in the whole complex suspended in NaCl solution by the method of Hammersten (21) were unsuccessful; the bacteriophage complex remained intact. Though the total amount of nucleic acid of the broth phage, determined on the basis of phosphorus, was essentially the same as that of the synthetic medium phage, the content of the ribopentose type of the former was five times as great as that of the latter. Another significant
difference, Tables I and II, between the broth bacteriophage and that from synthetic medium was in the content of lipide.

Extraction of the broth bacteriophage with alcohol-ether resulted in solution of 6.3 per cent (Table I) of the weight of the complex. Of this amount, slightly less than half, 2.61 per cent of the whole complex, was soluble in petroleum ether in subsequent extraction. The nature of the major portion of the non-lipide material soluble in alcohol-ether, 3.7 per cent of the whole complex, was not established; a small proportion, 3.7 per cent of the alcohol-ether-soluble fraction, reacted with orcinol-sulfuric acid. In the instance of the synthetic medium phage, the material extracted from the complex by alcohol-ether was essentially all lipide, completely soluble in petroleum ether.

The total lipide content of the bacteriophage, Table I, was considerably lower than the 10.4 per cent of extractable lipide reported for a Bacillus coli phage studied by Pollard (7) and McIntosh and Selbie (8, 22). The methods used for extraction were not described in detail. Schlesinger (4) recorded the finding of ether-extractable lipide in a Bacillus coli phage but gave no values. This author also stated that after acid hydrolysis considerably larger quantities of ether-soluble material, in excess of that directly soluble in ether, were obtained. In the course of the present work, samples of the alcohol-ether-insoluble fractions of the bacteriophage were examined for firmly bound lipide by reextracting with alcohol-ether, 1:1, acidified with hydrochloric acid (0.1 N total acid concentration). The acid alcohol-ether extracts contained no petroleum ether-soluble lipide but did contain 10 to 12 per cent (of the dry weight of the whole complex) of nitrogenous bases, apparently split off from the nucleic acids. No phosphorus was extracted by the acid alcohol-ether.

In the lipide or petroleum ether fraction of the bacteriophage, there was no nitrogen or phosphorus and, therefore, no phospholipide. Cholesterol was, likewise, absent, and the lipide was calculated as neutral fat. As seen in Table II, the amount of lipide in the broth bacteriophage was significantly greater, 35 per cent, than that in the phage from synthetic medium.

The alcohol-ether-insoluble fractions, Table I, contained the protein and nucleic acids. The weight percentages agreed well with the total lipide found in the petroleum ether-soluble fraction of the synthetic medium phage, but not in the case of the broth bacteriophage, as noted above. The principal differences between the broth and the synthetic medium phage are seen in the total alcohol-ether-insoluble phosphorus values and the distribution of this phosphorus into DNA, RNA, and "other" phosphorus. Phosphorus other than that in nucleic acid, presumably inorganic or associated with protein, was present in both broth and synthetic medium phage, considerably more in the latter than in the former. It might be considered
that this represents inorganic phosphorus carried over in the purification process from the synthetic medium. This is questionable, since the bacteria grown in synthetic medium contain the least amount of other phosphorus, 0.02 per cent, as shown in Table I. There may exist, however, differences in the adsorptive properties of the two materials (bacteriophage and bacterium) for phosphate. The carbohydrate content of the alcohol-ether-insoluble fraction of the bacteriophage was similar to that of the whole complex, Table I, confirming the absence of appreciable amounts of carbohydrate in the alcohol-ether-soluble material.

It is of interest to note that the total carbohydrate content of the bacteriophage is approximately one-half that to be expected if all of the carbohydrate present in the whole complex is associated with the nucleic acids. With orcinol and sulfuric acid, the pyrimidine nucleotides and nucleosides are hydrolyzed incompletely (23). It would thus appear that no carbohydrate is present in the bacteriophage other than that associated with the nucleic acids.

The findings obtained with the bacterium, given also in Tables I and II, show that the \textit{Escherichia coli} organism, too, is a complex of protein, nucleic acid, and lipide. The nitrogen content of the whole bacterial complex was about the same as that of the phage, but the carbon content was considerably greater, related probably to the higher lipide content, Tables I and II. The carbohydrate content of the bacterium was approximately the same as that of the phage, but, as noted below, probably differed with respect to constitutional relations within the bacterium.

The material of the bacterium soluble in alcohol-ether was soluble likewise in petroleum ether, as shown by the close agreement of the findings, Table I, with these two fractions. In contrast with that of the bacteriophage, the lipide of the bacterium contained nitrogen and phosphorus. The atomic ratio of the elements was 1:1, indicating a lecithin type of phospholipide. Neither neutral fat, found in the phage, nor cholesterol was present. In view of the difference between the bacteriophage and the bacterium, it would be difficult to consider either the neutral fat or the phospholipide as a contaminant carried over in the process of purification. The total lipide content of the bacterium was considerably greater than the lipide content of the phage, and slightly more was seen in the bacterium grown in broth than that in synthetic medium. The total bacterial lipide observed here was much higher than the 1.6 per cent of extractable lipide found by Pollard (7). Leach (24), Dawson (25), and others have shown that the age, conditions of growth, and especially the composition of the culture medium markedly influence the composition of \textit{Escherichia coli}.

The nucleic acid of the bacterium, approximately half the amount found in the bacteriophage, also consisted of both desoxypentose and ribopentose
types, as shown by phosphorus fractionation of the alcohol-ether-insoluble fraction. In the bacterium, however, the ribopentose type was predominant. Schaffer, Folkoff, and Bayne-Jones (26) isolated a nucleic acid preparation from *Bacillus coli* which contained approximately the amount of phosphorus required for a ribopentose nucleic acid. Guanine was found by these authors, but the pentose reaction was reported to be negative. As in the case of the bacteriophage, the ratio of the two types of nucleic acid varied in relation to the medium used, but in contrast with the synthetic medium phage, the deoxypentose nucleic acid was less in amount in the synthetic medium bacterium. The carbohydrate of the alcohol-ether-insoluble material agreed closely with the values for the whole complex. In contrast with the phage, the amount of carbohydrate of the bacterium, 12 per cent, was greater than could have been bound in the nucleic acid present. Since complete recovery of all of the carbohydrate of the nucleic acid was unlikely (23), and judging from the proportion of carbohydrate to nucleic acid found in the bacteriophage, it appears probable that the bacterium contains significant amounts of carbohydrate not associated with nucleic acid.

In so far as comparisons can be made, the constitution of the T₃ bacteriophage of *Escherichia coli* appears similar, qualitatively, to the agents studied by Schlesinger (4), Pollard (7), and McIntosh and Selbie (8). Certain quantitative differences were evident, but it should be borne in mind that the agents studied by these authors may not have been identical with the strain, T₃, examined here. It can scarcely be assumed that all bacteriophages of *Escherichia coli* will have the same constitution and, consequently, a detailed comparison of quantitative differences would not be significant. Cohen,³ however, in a study of the T₃ bacteriophage, has found a smaller amount of phosphorus, 3.8 per cent, than the amounts, 4.84 and 5.22 per cent, found here. The differences may well be related to the type of medium, sodium lactate (Friedlein), used by Anderson (27), who furnished the material analyzed by Cohen,³ and the broth and the sodium lactate-free NH₄Cl media employed in the present work. Cohen³ found only deoxypentose nucleic acid with the perchloric acid-tryptophane method (11), in an amount which was in agreement with the 3.8 per cent of phosphorus. With this method (11), it was not possible in the present work to demonstrate quantitatively small amounts of one type of nucleic acid in the presence of relatively large quantities of the other type. The results found in the present studies are almost entirely at variance with those on the T₃ phage obtained by Kalmanson and Bronfenbrenner (9). These authors found only 0.07 per cent of phosphorus in T₃ bacteriophage.

³ Cohen, S. S., personal communication.
isolated from sodium lactate medium by means of ultrafiltration, and the nitrogen values were too variable for judgment of their significance. The elementary analyses of the T\textsubscript{2} bacteriophage reported here were similar to the results obtained by Northrop (6) with a staphylococcus bacteriophage, with the exception of the carbohydrate value of 1.5 per cent.

The T\textsubscript{2} bacteriophage exhibits a morphological and constitutional complexity resembling that of the animal viruses more closely than that of the corresponding agents of plant diseases, except in the high total content of nucleic acid, which is comparable to the amount found in tobacco ring spot virus (28). The presence of both desoxypentose and ribopentose nucleic acids also constitutes a difference from the animal viruses previously studied; in the vaccinia (29) and rabbit papilloma (30) viruses, only desoxypentose nucleic acid has been demonstrated, and in the agent of equine encephalomyelitis (31)\textsuperscript{4} only the ribopentose type. Knight (32) has reported the presence of the ribopentose type in the influenza virus, though only the desoxypentose type has been found in work (16) in this laboratory. Ribopentose nucleic acid is the type found in plant viruses. The absence of phospholipide and cholesterol constitutes a unique difference between the lipide constitution of the bacteriophage and that of the animal viruses. While the bacteriophage contains only neutral fat, in all other cases in which lipide has been found associated as an integral part of a virus complex, phospholipide, cholesterol, and neutral fat have all been present (16, 29, 31, 32),\textsuperscript{4} usually in definite proportions characteristic of the virus or its strain.

The qualitative constitutional similarities of the bacteriophage and the host organism, \textit{Escherichia coli}, are clearly shown in Tables I and II. An outstanding difference was observed in the kind of lipide associated with the two materials. The atomic ratio, 1:1, of lipide nitrogen and phosphorus in the bacterium is that of a phospholipide of the lecithin type which, incidentally, resembles lecithins similar to those found in vaccinia (29) virus and a normal component (33) of chick embryo tissue. This phospholipide differs from the analogous constituent of the influenza (16) and the equine encephalomyelitis (31)\textsuperscript{4} viruses. Quantitatively, wide differences are evident between the structure of the bacteriophage and that of its host. Especially notable are the total lipide contents, the distribution of the two types of nucleic acid, and the proportional contents of carbohydrate.

A remarkable character of the bacteriophage was the definite variation in size as seen in electron micrographs (1, 2) and chemical constitution as described above in relation to the medium in which the host organism was cultured. These findings and others, previously discussed (1), indicate that the bacteriophage behaves as an autonomous entity, responding like a

\textsuperscript{4} Taylor, A. R., Sharp, D. G., Beard, D., and Beard, J. W., unpublished work.
living organism to a changing environment. The bacteriophage increases only in the presence of the host bacterium and to do so apparently enters the host cell. It appears likely that the influence of medium on the composition of the bacteriophage is indirect and dependent on a primary effect of the medium on the bacterium, an effect disclosed in the variation seen in the composition of the bacterial host in relation to the medium. A lack of parallelism between the variation of the bacteriophage and that of the bacterium was seen in the opposite changes in the proportions of the two types of nucleic acid and in the amounts of lipide. It is evident that the processes involved in the formation of the phage and responsible for its specific constitution are to an extent independent, not only of the qualitative aspects of bacterial metabolism and constitution in a given medium, but also of quantitative changes within the bacterium in relation to medium.

SUMMARY

Analyses were made of the chemical constituents of the T₂ bacteriophage of Escherichia coli (strain B) obtained from lysates of the host in broth and synthetic medium and of the host bacterium cultured in like media. The bacteriophage consists of protein, lipide, and nucleic acid. The lipide fraction exhibited the behavior of neutral fat, and neither cholesterol nor phospholipide was present. There were present both desoxypentose and ribopentose nucleic acids, of which the former was greatly predominant. The amount of carbohydrate found was approximately half the theoretical amount bound in the nucleic acids and was considered to be wholly associated with the nucleic acids.

The bacterium likewise consisted of protein, lipide, and nucleic acid. In contrast with the bacteriophage, the lipide contained nitrogen and phosphorus. The atomic ratio of these elements indicated a phospholipide of lecithin type. Neutral fat and cholesterol were absent. The nucleic acid consisted, as in the bacteriophage, of the desoxypentose and ribopentose types, but, unlike the ratio of these constituents in the virus, the quantity of the ribopentose type greatly exceeded that of the desoxypentose type. The amount of carbohydrate found was greater than the quantity theoretically associated with the nucleic acids.

The apparent partial specific volume of the bacteriophage from broth lysates was 0.655 and that from synthetic medium lysates was 0.669, values in accord with the chemical constitution.

Variation was seen in the composition of the bacteriophage and in that of the host in relation to the type of medium in which the bacterium was cultured. The quantitative changes in certain of the constituents of the bacteriophage, for example the ratio of the two types of nucleic acid, did not parallel analogous changes in the bacterium.
ANALYSIS OF BACTERIOPHAGE

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