

THE PROTEIN COATS OR "GHOSTS" OF COLIPHAGE T2

I. PREPARATION, ASSAY, AND SOME CHEMICAL PROPERTIES

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(Received for publication, November 5, 1956)

With the discovery (1) that the protein coat or "ghost" of coli bacteriophage T2 carried some of the biological functions of the parent virus, it was possible to examine more carefully than heretofore the relationship of biological function to morphological structure and chemical constitution. In the series of papers to follow data will be given to support the statements in the original report (1) and to demonstrate that many properties usually associated with the intact virus are found in the protein component, suggesting a high degree of specialization and organization in this relatively small biological unit.

In this, the first, paper will be described the preparation, fractionation, method of assay, and some physicochemical properties of the ghosts of T2 phage. The biological properties of the ghosts will be described in the second paper while studies of the metabolic changes induced in the host cell by ghosts (2, 3) will appear in the third and fourth papers. The inhibitory effect of divalent cations (4) on the action of ghosts will be presented in the fifth paper of this series.

In the interim since the first report a number of papers (5-14) have dealt to a varying degree with some of the properties of ghosts. These will be discussed in the text of this and succeeding papers.

Materials and Methods

Many of the materials and methods used in the present work are the usual ones (15, 16) described for the T phages of *Escherichia coli* B. The details are found either in the protocols or in the section on Experimental Details near the end of this paper.

Only highly purified phage (titer/ $E_{400} > 2$) (17) was used in the present experiments. In most instances freshly shocked but unfractionated ghosts were used but in certain critical experiments the results were confirmed with ghosts freed of nucleic acid as described in Table I. These instances are noted in the protocols.

* Aided by a grant from the National Foundation for Infantile Paralysis.

† Supported in part by a grant from the Atomic Energy Commission to Dr. Herriott.

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TABLE I

*Preparation and Purification of T2r⁺ Ghosts**T2r⁺ phage: titer 1.8×10^{13} /ml.; titer/ $E_{400} = 2.7 \times 10^{12}$; mg. N/ml. = 1.5; P/N = 0.30.*

Number		Volume	Ghosts/ml.*	Total ghosts	Total <i>per cent</i>
1	2.9 ml. of the above phage + 6 ml. 3 M Na ₂ SO ₄ for 2 min., then + 140 ml. cold water rapidly with agitation Residual infectivity by plaque count was 5×10^8 /ml.	149	3.5×10^{11}	5.2×10^{13}	100
2	Number 1 + 0.15 ml. saturated MgSO ₄ (2.8 M) + 0.15 mg. DNase left at 5°C. overnight	2	3.5×10^{11}	5.2×10^{13}	100
3	Number 2 centrifuged at 3500 for one half hr.; supernatant	149	3.5×10^{11}	5.2×10^{13}	100
4	Number 3 centrifuged at $100,000 \times g$ for 1 hr. in a Spinco refrigerated centrifuge at 10°C.				
5	Supernatant from number 4	134	3.0×10^{10}	4.0×10^{13}	9
6	Residue from number 4 dissolved in cold saline	4	3.0×10^{12}	3.6×10^{13}	70
7	Number 6 centrifuged at 2,000 for 15 min.; supernatant	4	8.3×10^{12}	3.3×10^{13}	64
8	Number 7 centrifuged at $18,000 \times g$ in a Servall SS-2 for 1 hr.				
9	Supernatant from number 8	3.8	3.8×10^{11}	1.4×10^{13}	3
10†	Residue from number 8 dissolved in saline	4	4.8×10^{12}	3.1×10^{13}	60

* See Fig. 3 and the text for discussion of the lytic assay unit.

† Some chemical and physical properties of this and similar preparations are reported in Table IV.

EXPERIMENTAL

Preparation and Purification of T2 Ghosts.—Anderson (18) made the important discovery that rapid dilution of a strong salt solution of the T-even phages leads to a loss of infectivity and of the Tyndall scattering. After using this operation which Anderson termed "osmotic shock" to prepare undegraded

deoxyribosenucleic acid (DNA) it was observed (1) that the shocked phage lysed growing host cells much sooner than did an equivalent quantity of phage. Earlier, Anderson (19) had reported a "lysin" from heavily irradiated phage preparations, but it was not sedimented in an hour at $34,000 \times g$ and its action was detected on *irradiated* host cells. Since in the present instance the lytic factor sediments and lyses normal host cells, it may be different although no direct comparison has been made.

Table I contains the results of an experiment in which the lytic factor in shocked phage was purified by differential centrifugation, after first degrading the nucleic acid with the enzyme, deoxyribonuclease (DNAse). From this and other comparable experiments it was observed that:

1. Osmotic shock destroyed 99 per cent or more of the plaque-forming capacity of a preparation, as had been noted earlier by Anderson (18).
2. The viscous phage DNA appeared in solution as a result of the shock treatment and it was digested with DNAse without any detectable effect on the lytic activity of the preparation. This has been confirmed recently by Bonifas and Kellenberger (11).
3. The lytic property sedimented and was recovered on resuspension of the residue.

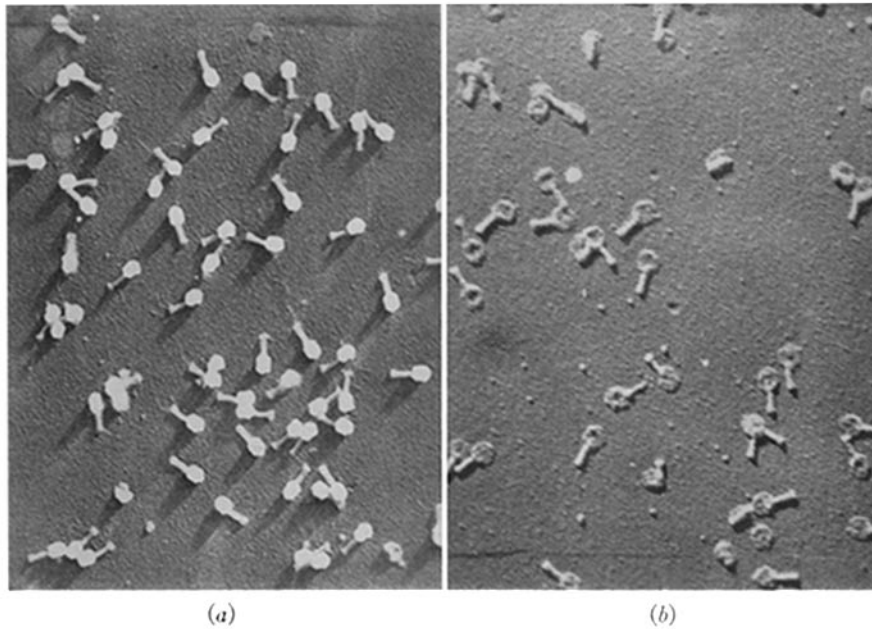


FIG. 1. Electron photomicrographs taken through the cooperation of Dr. James S. Murphy of: (a) T2 coliphage, and (b) the protein coats or ghosts prepared from the T2 phage by osmotic shock.

TABLE II
Phage Titer vs. Lytic Titer of Ghosts from Purified Phage

Sample	Method of converting to ghosts	Titer of parent phage		Lytic ghost titer from standard curve Fig. 3	Killing by an average of 3 ghosts per cell <i>per cent</i>
		1/E 1 cm. 400	1/ml.		
1	Na ₂ SO ₄ -osmotic shock	2.0	4.6 × 10 ¹³	5 × 10 ¹³ /ml.	
2	" " "	2.3	4 × 10 ¹²	4 × 10 ¹²	
3	" " "	2.3	1.7 × 10 ¹³	1.6 × 10 ¹³	
4	" " "	2.7	1.8 × 10 ¹¹	1.7 × 10 ¹¹	
5	" " "	2.6	2.1 × 10 ¹³	1.75 × 10 ¹³	
6*	" " "	2.1	7 × 10 ¹²	5 × 10 ¹²	72
	Glycerin-osmotic shock	2.1	7 × 10 ¹²	5 × 10 ¹²	72
	Polyethylene glycol-osmotic shock			5 × 10 ¹²	73
	Sucrose crenation	2.1	7 × 10 ¹²	5 × 10 ¹²	77
	0.01 M pyrophosphate pH 8.5	2.1	7 × 10 ¹²	5 × 10 ¹²	79

* This and one other sample which had been recently prepared and stored in 0.05 M NaCl, 10 μg. gelatin, and a drop of chloroform showed a decreased lytic ghost titer compared to its infectivity.

4. Through the kindness of Dr. James S. Murphy, a few electron microscope examinations showed that the number of ghosts (shown in Fig. 1 *b*) correlated in a rough manner with the lytic power of the solution. Thus, when sedimentation removed most of the lytic activity of the solution, it had removed most but not all the ghosts. When no lytic activity remained, no ghosts were seen on the electron micrographs.

5. Since the lytic activity could be sedimented in an hour at 18,000 × *g* it must be associated with a relatively large or dense structure.¹

In Table II are the analyses of a number of preparations of ghosts. Many of them were prepared by the usual osmotic shock method, but for a few preparations other methods were used. These results show that the lytic property is relatively constant and is independent of the method of producing the ghosts. Thus far, attempts to dissociate the lytic activity from the ghosts have been unsuccessful. The conclusion is unavoidable, therefore, that the lytic activity of shocked T2 phage is closely associated with the protein coat or ghost.

Lytic Assay.—The curves in Fig. 2 illustrate how the turbidity of *E. coli* B suspensions is affected by the introduction of various quantities or concentrations of shocked phage over the course of 30 minutes. Since a large increase

¹ Ghosts do not sediment from the original "shockate" following DNase treatment as readily as they do after a preliminary high speed sedimentation and resuspension in saline.

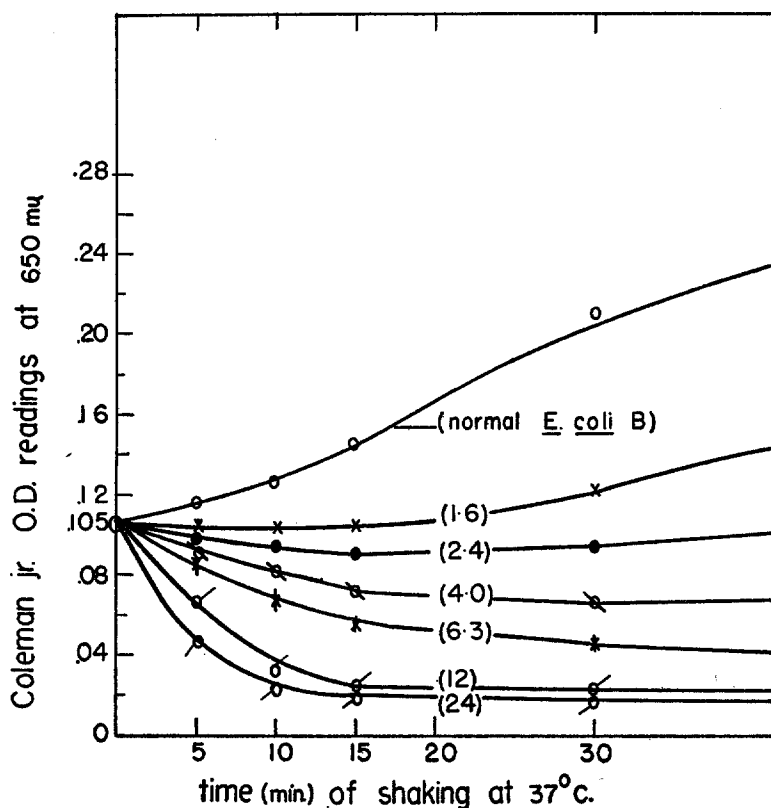


FIG. 2. Changes in turbidity with time of suspensions of nutrient broth-grown *E. coli* B following the addition of various quantities of T2 ghosts. The figures in parentheses represent the average number of ghosts per bacterial cell.

in phage takes only 25 minutes in broth, any evaluation of the ghosts must be made before this time.² The information in these curves was used to develop a convenient assay method for ghosts. The optical density values in Fig. 2 at 15 minutes are plotted in Fig. 3 against the corresponding number of ghosts in the 1 ml. that was added to 10 ml. of standard cell suspension. Since purified phage preparations were used in the preparation of the ghosts, the total number of ghosts was taken from the phage titer prior to shocking.

To analyze an unknown requires only the addition of 1 ml. of a saline dilution of a preparation containing 0.75 to 2.5×10^{10} ghosts/ml. to 10 ml. of 3.8×10^8 *E. coli* B per ml. in nutrient broth (O.D. = 0.115).³ This 11 ml. suspen-

² The action of ghost preparations having too low a phage concentration to complicate the results is shown in Fig. 3 of the next paper (20).

³ The optical density in this system equals $1.5 \times E_{1\text{cm}}^{650\text{m}\mu}$ or a Beckman reading of 0.077.

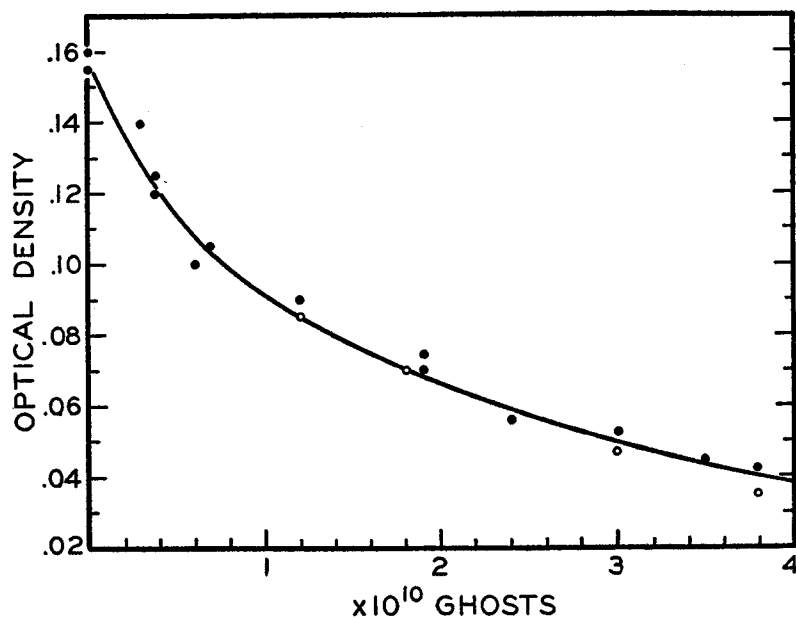


FIG. 3. A standard lysis curve used to evaluate the number of T2 ghosts in an unknown solution. The turbidities as optical densities were read after shaking mixtures of a standard bacterial suspension with the indicated number of ghosts for 15 minutes at 37°C. The initial density reading of the suspensions was 0.105. (See the text for further details.)

sion, which in a 22 x 175 mm. optically uniform test tube measures 0.105 O.D. units in a Coleman Jr. spectrophotometer at 650 $m\mu$, is shaken at 37°C. for 15 minutes and read again along with a control suspension of uninfected cells. The density of the control tube indicates whether the culture has the growth properties of the system used in preparing the curve in Fig. 3. From the optical density readings of the experimental tube the number of ghosts per milliliter added may be found by reading off the curve in Fig. 3. In general, parallel analyses agree within ± 15 per cent though values on different days occasionally differ by as much as ± 25 per cent. Reproducible results are obtained when cells are grown up as needed from concentrations no higher than about 2×10^7 /ml.; i.e., lag phase cells must be diluted below this figure and allowed to grow to 3.8×10^8 /ml. (O.D. = 0.115). Mutants that appear to be more resistant to lysis by ghosts accumulate occasionally after numerous transfers of the stocks so that an occasional isolation of the more susceptible stock is recommended.

The results of analyses of different ghost samples are shown in Table II.

Proof of Lysis of Cells by Ghosts.—In the foregoing paragraphs it has been

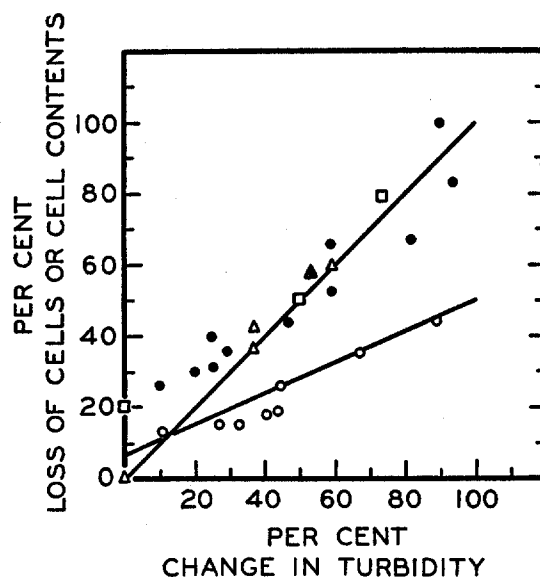


FIG. 4. Changes in turbidity as a measure of cell lysis by ghosts. Lysis has been estimated in several ways. The loss of $260\text{ m}\mu$ absorbing material from cells grown in synthetic medium containing $1 \times 10^{-4}\text{ M}$ magnesium ion is denoted by the solid circles in the above figure. The open circles represent a similar measurement on cells grown in the presence of 2 to $5 \times 10^{-3}\text{ M}$ magnesium ion. The open triangles were obtained by direct microscopic count on cells grown in nutrient broth. The open squares derive from analyses of radioactivity released into the medium by the action of ghosts on broth-grown cells labelled with phosphorus 32.

assumed that changes in turbidity of the cell suspension were a reflection of changes in the number of cells and that a drop in turbidity could be taken as lysis of some cells. It is known (21), however, that changes in turbidity are not always accompanied by changes in number of cells, so that the action of ghosts on coli cells was examined in more detail to decide whether changes in turbidity are correctly a reflection of cell lysis.

Three criteria of lysis were chosen: (1) release of P^{32} from labelled cells; (2) microscopic count of residual cells; and (3) release of material absorbing at $260\text{ m}\mu$. In Fig. 4 are plotted the criteria listed above against the change in turbidity. The microscopic count and P^{32} release experiments were performed with the cells suspended in nutrient broth, the medium used in the assay procedure. For absorbancy measurements the broth could not be used and a synthetic medium (17) was substituted. Since the concentration of magnesium ion is higher in the synthetic medium than in broth, and the divalent cations affect the ghost action (4, 22), another set of experiments was performed in synthetic medium containing 1×10^{-4} molar magnesium ion.

A phase microscope examination showed that many cells were larger and round instead of rod-shaped as a result of the action of ghosts. Since they appeared to be whole cells, they were counted as such without any correction.

Before calculating the percentage change all turbidity readings were corrected for the turbidity remaining at complete lysis obtained by using an excess of ghosts. This correction amounted to 15 per cent of the original cell turbidity.

The results plotted in Fig. 4 show a good correlation between turbidity changes and the number of organisms. Hence, a drop in turbidity is a measure of cell disintegration when the reaction is carried out in nutrient broth. The data also suggest that there is a release of up to 20 per cent of the total phosphorus and absorbing material without a corresponding change in turbidity. This observation is similar to that made by Prater (23) using phage and by Puck and Lee (9) using ghosts. The results obtained in the synthetic medium having the higher level of magnesium ion suggest that about half of the 260 μ absorbing material (probably nucleic acid) sediments in this system with the cell debris even in low gravitational fields.

It is concluded from the above studies that in nutrient broth and probably in a synthetic medium ghosts induce the lysis of cells and that the fall in turbidity is a measure of the cells lysed.

Efficiency of Adsorption of Ghosts.—Although it seemed obvious that if the ghosts induce lysis of the host cells, this effect is preceded by adsorption of the ghosts. Nevertheless, it was important to evaluate the efficiency of this adsorption. The rate of adsorption might have changed in either direction as a result of the change from phage to ghosts.

The efficiency of adsorption to host cells was determined through the use of isotopically labelled ghosts. The experiments are described in Table III, where it is readily seen that *as phage*, 95 per cent of 2, 5, and 8 particles per bacterium were adsorbed in 5 minutes at 37°C. *As ghosts*, however, the value was closer to 80 per cent and this figure did not reach 90 per cent with added time of adsorption. These results are comparable to those reported by Hershey (6, 12) and more recently by French and Siminovitch (14). Using the electron microscope Bonifas and Kellenberger (11) reported that nearly 99 per cent of the ghosts were removed by adsorption. This discrepancy in findings suggests that non-sedimentable ghost protein may be formed following the union of ghosts and cells.

No correction for the fraction of unadsorbed material in terms of ghosts has been made in the ghost titers recorded in this or the other papers of the series.

The Unit of Ghost Action.—Since, as will be demonstrated in the succeeding papers, ghosts have many biological functions, some measure or unit was sought which would be applicable to all functions. The unit selected was the *number* of ghost particles. It has been shown (24, 17) that for purified T2 phage the infectivity titer (plaque-forming units) closely approximates the

number of particles in a sample having a diameter of 50 to 100 μ . Ghosts are prepared directly from phage and can be assayed without fractionation so that any particular ghost property can be correlated with the number of ghosts established from a phage titer. This merely carries the assumption that all or a constant fraction of the phage particles are converted to effective lytic units, which thus far appears to be correct though there will be some comments on this point a little later. To prepare a standard curve such as the lytic curves in Fig. 3, unfractionated ghosts prepared from a relatively pure and precisely titered phage preparation are used. This takes advantage of the relative precision (± 15 per cent) of the phage assay.

The data in Table II indicate the correlation between the ghost lytic titers read from the curves in Fig. 3 and the original infectivity titers of the several parent phage preparations.

Basing the unit of action on the number of ghosts has great convenience and reproducibility, but it conveys a misleading impression. Without presenting the detailed evidence, for it is to be discussed in the next paper, there is evidence for heterogeneity in the ghost preparations. Thus, it has been observed in this and other (11, 14) laboratories that not all the ghosts can "kill" the host cells. With such heterogeneity in the ghost preparations, basing the unit of ghost action on the number would appear to be meaningless. However, thus far three different methods of preparing ghosts have yielded products indistinguishable in their lytic action (see Table II) and until some method permits fractionation of these preparations the present unit has sufficient utilitarian value to recommend its use. Besides, no alternative unit of action offers as much.

Antigenicity.—Since the ghost makes up the bulk of the protein part of the

TABLE III
Adsorption Efficiency of S^{35} -Labelled Ghosts

	Ghosts per cell (average)					
	2		5		8	
	C.P.M.	Per cent	C.P.M.	Per cent	C.P.M.	Per cent
Ghosts* alone.....	5,367	100	11,800	100	19,900	100
Ghost + cells‡; supernatant.....	1,100	20	2,380	20	3,920	20
Adsorbed to cells (by difference).....	4,267	80	9,420	80	15,980	80
Phage alone.....	6,300	100	12,630	100	20,130	100
Phage + cells‡; supernatant.....	220	3.5	460	4	1,120	5.5
Adsorbed to cells (by difference).....	6,080	96.5	12,170	96	19,010	94.5

* These ghosts were prepared by osmotic shock from 2 M Na_2SO_4 solution.

‡ 2×10^8 /ml. log phase *E. coli* B in nutrient broth were incubated with the phage or ghosts for 5 minutes at 37°C., after which aliquots were chilled and sedimented at $5,000 \times g$ for 10 minutes. Aliquots of the supernatant were analyzed for radioactive sulfur.

virus, it was expected that it would be antigenic and that antisera to it would cross-react to some degree with the intact virus. However, in view of the marked changes in antigenic nature which accompany relatively small changes in proteins (25) it was important to confirm our expectations. Hershey and Chase (6), Lanni and Lanni (7), and Nagano and Oda (8) have recently obtained evidence indicating that the ghosts carry the antigenicity of the virus. Before these publications appeared we had prepared rabbit antisera to: (a) purified T2 virus (17); (b) osmotically shocked T2 virus; (c) purified T2 ghosts containing less than 0.01 per cent of the total particles as active virus and no measurable quantity of nucleic acid; and (d) a dilute virus control corresponding to the 0.01 per cent active phage in (c). It was found that the antisera to the first three antigens were nearly the same in their quantitative neutralizing action against either the infectivity of the virus or the lytic property of the ghosts. Velocity constants (15) for the reaction between the antisera and phage or ghosts in saline were 1200, 650, 600, and 22 respectively for the antisera prepared from the materials in (a), (b), (c), and (d). Thus, in agreement with the results of others the ghost is responsible for most of the antigenicity of bacteriophage T2 as tested by the power of the antisera to neutralize phage infectivity.

General Chemical Properties

The physical and chemical properties of a number of preparations of purified ghosts corresponding to sample 10 of Table I have been evaluated and the results are shown in Table IV.

The ghost titer per milligram of nitrogen is a little over two times greater than the corresponding figure for the parent phage preparation. This is in agreement with the value (17) of the protein content of this phage (*ca.* 40 per cent).

TABLE IV
A Comparison of Physicochemical Properties of Ghosts and Phage

	Ghosts	Phage
Titer/ E_{280}	1.8×10^{13}	1.7×10^{11}
Titer/mg. N	$2.8 \pm 0.2 \times 10^{13}$	1.2×10^{13}
R.S.*/ 10^{15} particles	1.6	11
R.S.*/ E_{280}	3.5×10^{-19}	1.9×10^{-19}
Titer/ E_{400}	1.7×10^{13}	2.7×10^{12}
E_{280}/E_{230}	0.92	1.4
P/N	0.01	0.32

* R.S. stands for "Rayleigh slope," the slope of a plot of optical density against the reciprocal of the fourth power of the wave length in centimeters. The measurements were obtained at wave lengths between 1,000 and 700 $m\mu$ in a Beckman spectrophotometer.

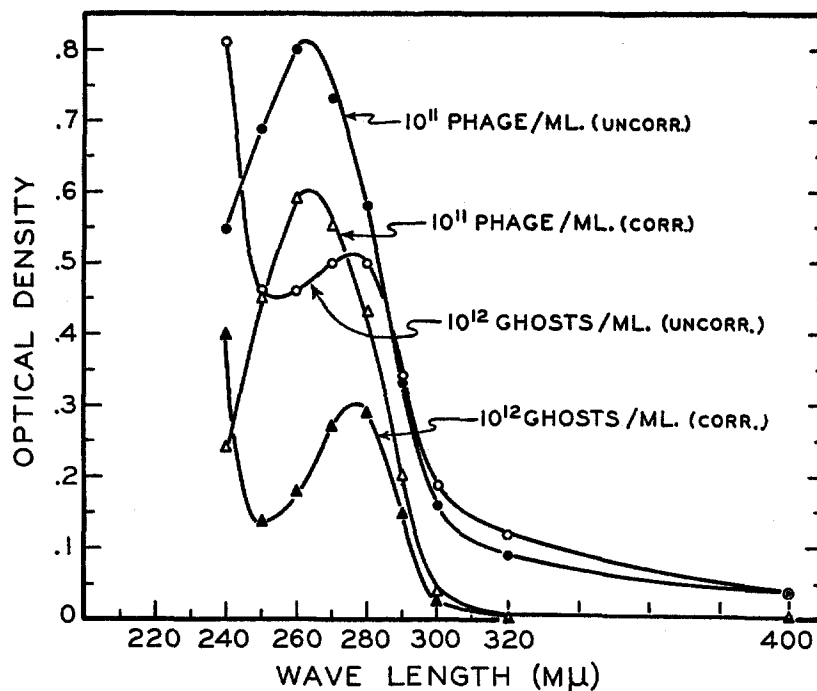


FIG. 5. Absorbancy curves of T2 phage and ghosts. See the text for a discussion of the correction for light scattering.

Absorption Spectrum.—Fig. 5 contains the absorption spectra of T2 phage and its purified ghosts both uncorrected and corrected for scattering. The scattering correction was made by assuming that the optical density value at $320\text{ m}\mu$ represented the scattering at that wave length. The contribution of scattering to the values at the lower wave lengths was then calculated on the basis of Rayleigh's rule (35). According to this rule the scattering increases inversely as the fourth power of the wave length. Rayleigh's expression is applicable only when the particles are smaller than one-tenth of the wave length of the light used (35) and the particles in the present study are borderline with respect to this limitation; hence, it is only a first approximation. The absorption maximum for the ghost preparations was 275 to $280\text{ m}\mu$ which is typical of proteins. Had there been appreciable nucleic acid present the minimum in the region of $250\text{ m}\mu$ would not have been so low for the extinction coefficient of nucleic acids is many times greater than that of proteins. Since 1×10^{12} ghosts per ml. represents $33\text{ }\mu\text{g.}$ of nitrogen and approximately $230\text{ }\mu\text{g.}$ of protein, the corrected $E_{275\text{ m}\mu}^{1\text{mg./ml.}} = 1.3$, and the comparable value for phage, $E_{262\text{ m}\mu}^{1\text{mg./ml.}} = 13$. The corresponding uncorrected values are 2.2 and 17.5 respectively.

Effect of pH on Lysis.—Soon after the lytic action of ghosts was first observed, Quersin and Dirx (30) reported two lytic factors from lysates of phage infected *E. coli* B. Their evidence for two agents was based on the finding of two pH maxima for lysis of coli, one at pH 6.8–7.2 and the other at pH 8.

Studies of the effect of pH on the lysis of coli by ghosts in nutrient broth showed no evidence of two peaks. Lysis at pH 6.9, 7.3, and 7.7 were indistinguishable. It is possible that the lactate medium used by Quersin and Dirx is an important factor in their observations, but we have not investigated this point.

Tyrosine and Tryptophane Content.—In the preliminary note (1) it was reported that the tryptophane content was 3.5 per cent and that the tyrosine content was low. This was based on a faulty assumption that a failure of the absorption spectra to shift toward the longer wave length and to increase on making the solution alkaline was indicative of a low level of tyrosine. Table V shows the tyrosine and tryptophane content of purified ghosts by several methods of analysis. There is not very close agreement among them nor with the analyses of others (16, 12) for this phage for which no explanation can be offered.

Phosphorus Content.—Three or four ghost preparations comparable to fraction 10 of Table I were analyzed for phosphorus by the King (36) and/or the Fiske and SubbaRow (37) methods. In the latter method the samples after digestion were diluted with 1 ml. of water and boiled for 10 minutes to convert any pyrophosphate to orthophosphate (38). In all the preparations there was residual phosphorus content which relative to the nitrogen content was P/N

TABLE V
Tyrosine and Tryptophane in T2 Ghosts

Method	Per cent of protein	
	Tyrosine	Tryptophane
Microbiological*	4.3	0.9
Radioautograph†	3–9	
Microbiological§	2.0	1.44
Colorimetric		2.0
“		1.5
Spectrophotometric¶	5.8	2.8
Colorimetric**	2.77	1.45

* From Luria's analyses (16).

† Hershey (12).

§ Following the method of Henderson and Snell (41).

|| Following the method of Bates (42).

¶ Following the method of Beavan and Holiday (43).

** Reported by Cohen and Anderson (44).

= 0.01. Since the P/N ratio of T2 phage is about 0.3 (17) the ghosts have about 3 per cent of the original phosphorus.

DISCUSSION

There are many reports (26-34) of bacteriolytic agents produced by phage-infected cells, but in no instance has the evidence been such as to permit a clear understanding of the nature of the agent. In most instances the evidence excludes the whole phage and in some instances phage coats or ghosts from responsibility for the observed lytic activity. In the present instance there can be little doubt about the lytic agent. Highly purified phage was used as the starting point for the preparation of ghosts and all attempts to separate the lytic activity from the ghosts were unsuccessful. It is for this reason that the lytic activity is used as a measure of the number of ghosts. While this holds for coliphage T2 from *E. coli* B, the literature suggests that this would not be true for all systems.

It is perhaps worth making special note that although osmotic shock has been used in the preparation of most of the ghost preparations used in this work, other methods such as crenation or dilute pyrophosphate, two quite different methods, also produce ghosts by releasing the phage DNA. These ghosts are indistinguishable from those produced by osmotic shock.

It will have to remain for future studies to decide whether the route of nucleic acid release is the same in the above methods and whether the same as that utilized by the phage upon infecting its host. The released DNA appears to have no measurable effect on the ghost lytic property for there is no change during the action of DNase. It is of some interest to recall that upon injection into the cell at the time of infection the injected material—largely DNA(12)—must set up some process inhibitory to lysis by the residual ghosts for multiply infected cells do not lyse rapidly unless the multiplicity is well over 50 or the energy metabolism is upset.

Other aspects of this discussion will be deferred to a succeeding paper.

Experimental Details

Preparation of Phage.—The methods of preparing, purifying (17), and titrating (39) phage have already been discussed.

S³⁵-Labelled Phage.—The medium for the growth of labelled phage was the usual synthetic glucose-salts medium (17) except that the sulfate concentration was reduced to one-fifth the usual figure. When the cells had grown to 6×10^8 /ml. and were infected with 1×10^6 T2r⁺/ml., 5 millicuries of carrier-free S³⁵ as sulfate was added per liter of medium. Growth and harvesting were similar to the procedure (17) previously described, except that precautions were taken to prevent excessive exposure of personnel to the radiation.

After purification the virus was found to contain 74×10^8 counts per minute per 10^{12} plaque-forming units.

P³²-Labelled Phage.—T2 phage labelled with 1000 c.p.m./10¹⁰ phage was prepared using the method described for unlabelled phage (17) with the slight modification of adding 20 microcuries of P³² phosphate ion per ml. of culture medium immediately after the cells were inoculated with phage.

Preparation of Ghosts.—

(1) *By Osmotic Shock.*—Most of the osmotic shock experiments were carried out by the method described in Table I. Sodium sulfate could be replaced by sodium acetate or ammonium sulfate but not by magnesium sulfate. In a few experiments non-ionic solutes such as glycerin and polyethylene glycol (a Carbide and Carbon Chemicals Co. preparation) were used in producing osmotic shock. Dilution with cold water gave better results as was noted by Anderson *et al.* (40). In our experience over 99 per cent of the particles were inactivated and sometimes it was 99.9 per cent. In one or two instances phage preparations were found to be more resistant than usual to osmotic shock. The 400 m μ turbidity value of a phage preparation drops to 15 to 20 per cent of the original, following osmotic shock.

(2) *By Crenation.*—Anderson, Rappaport, and Muscatine (40) noted that the Tyndall scattering of a phage preparation disappeared when mixed with a strong sucrose solution. Contrary to the osmotic shock experiments, when strong sucrose has been added, dialysis or slow dilution does not leave phage in an infective form.

(3) *By Pyrophosphate.*—Dr. Helen Van Vunakis of this laboratory observed that dilute (10⁻⁴ to 10⁻² M) pyrophosphate at pH 7 and 49°C. or pH 8.9 at 37°C. brought about a gradual drop in the infectivity and turbidity of aqueous or saline solutions of T2 phage at concentrations of 1 to 3 \times 10¹¹/ml., and this was accompanied by a corresponding increase in viscosity of the solution, presumably from the liberated phage DNA. Such solutions were found to have ghosts that were indistinguishable from those produced by osmotic shock.

Precipitation of Ghosts.—Precipitation and settling of ghosts occur if following step 2 of Table I the solution is dialyzed at 5°C. in Visking membranes against 0.01 M acetate buffer of pH 4.4–0.05 M NaCl. The clear supernatant fluid can be easily separated from the precipitate and the latter sedimented at relatively low speeds. Resuspension of the residue and analysis of the lytic property have indicated a recovery of 85 per cent of the ghosts.

Microscopic Count.—Counts of bacteria were made in a Petroff-Hausser chamber using darkfield illumination and 4 mm. high dry objective lens. Occasionally a phase microscope was used.

Absorbancy Measurements.—These were the usual values obtained with the aid of a Beckman DU spectrophotometer standardized in the ultraviolet with 0.02 per cent A.C.S. reagent grade thiophene-free benzene in isooctane. The maxima were found at 249, 255, and 261 m μ which are within 2 m μ of the values in the International Critical Tables, volume 5, pp. 361–372.

Radioactive Measurements.—Samples of phosphorus and sulfur were dried at temperatures below 50°C. in metal planchets (Buckeye Stamping Co., Columbus, Ohio, Style No. 100) to which a few drops of alcohol were added to promote uniform spreading of the sample. These samples were then counted in a scaling instrument and after deducting the background count they were compared to a standard or ali-

quot of the starting material. The method had been previously shown to be free of the usual difficulties of self-absorption, spattering, etc. The instrument was standardized occasionally with a standard.

Preparation of Antisera.—

Antigens.—Purified phage (17) titering 2.7×10^{13} /ml. with a titer/ $E_{400} = 2.4 \times 10^{12}$ was used in the preparation of ghosts.

“Shocked phage” consisted of shocking osmotically according to No. 1 in Table I followed by dialysis in a collodion membrane against water to remove the sodium sulfate. This was then concentrated through evaporation and addition of sodium chloride to 0.85 per cent. Most of the residual phage was removed by centrifugation at $9,000 \times g$ for 1 hour. The phage titer of this preparation was 5×10^7 /ml. and the ghost lytic titer was 5×10^{11} /ml.

Purified ghosts were prepared in accordance with the directions in Table I. Additional centrifugal fractionation was necessary to reduce the concentration of infective particles in the ghost preparation below 0.01 per cent of the ghost titer. Analyses of this preparation showed a ghost titer (lytic)/milliliter = 1.4×10^{12} ; ghost titer/milligram N = 2.6×10^{13} ; ghost titer/ E_{280} (uncorrected) = 2.1×10^{12} ; and phosphorus/N = 0.01, phage titer = 6×10^8 /ml.

100 ml. quantities of the four antigens—(a) 3×10^{12} /ml. T2 phage; (b) 5×10^{11} /ml. shocked phage; (c) 1.4×10^{12} /ml. purified ghosts; (d) 6×10^7 /ml. T2 phage control—were each mixed with 50 ml. of a suspension containing 10 ml. of 1 per cent $\text{Kal}(\text{SO}_4)_2 \cdot 10\text{H}_2\text{O}$, 0.6 ml./N/1 KOH, 1.5 ml. 1 per cent merthiolate, and 37 ml. of saline. The final pH was 6.0.

Injection Schedule.—On the 1st and 2nd days 0.1 ml. and 0.5 ml. respectively of each antigen were injected intraperitoneally into three 10 pound healthy rabbits. After a 4 day lapse the animals were started with 0.5 ml. aliquots injected intravenously. Gradually increasing quantities were given by the same route 4 days a week for 4 weeks until they were finally receiving 5 ml. per injection. A total of 39 ml. was given to each animal. After a respite of 10 days the animals were exsanguinated from the heart and the serum collected after a clot formed at 5°C . in a large Petri dish.

SUMMARY

A method of preparing the protein coats or ghosts of phage T2 is described along with proof that the lytic action is a property of the ghost.

An assay based on the lytic action toward host cells has been developed which permits a rapid evaluation of the number of ghosts with a reliability of ± 15 per cent.

The antigenic and certain physicochemical properties of the ghost have been determined.

The writers are grateful to their associates, Dr. I. R. Lehman and Dr. Helen Van Vunakis, for their helpful suggestions and for permitting us to report the results of some of their experiments.

To Patsy Miller Perlman and Mary T. Dunn we also owe a debt of gratitude for their patience and valuable technical assistance.

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