PREPARATION, PURIFICATION, AND PROPERTIES OF E. COLI VIRUS T_2

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PLATE 1

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Many reports (1–9) on the purification of bacterial viruses have appeared since Northrop's paper on staphylococcus phage (24). In general they all agree that these viruses are nucleoproteins and of those analyzed virtually all have contained principally desoxyribose type nucleic acid (DNA). Taylor (11) who made the most complete analysis of purified T_2 virus of *E. coli* B reported the presence of more than 1 per cent pentosenucleic acid (PNA) and lipid in addition to the protein and DNA.

In the present study the method of preparation and the homogeneity of the purified product have been improved. Lysates of T_2 in 6 to 10 liter quantities have been prepared which titered 2 to 5 $\times$ 10^{11} infectious units per ml. Purification was effected by (*a*) decanting the virus solution from the settled cell debris, (*b*) precipitation of the virus in the cold at pH 4.0, (*c*) enzymatic digestion of extraneous DNA with desoxyribonuclease (DNAse), and finally differential centrifugation. These steps resulted in a remarkably stable product having a high specific infectivity yet containing virtually no lipid or PNA. A variety of fractionation experiments designed to furnish evidence on the homogeneity of the purified preparations failed to demonstrate the presence of impurities.

EXPERIMENTAL RESULTS

Preparation of Lysates.—Table I contains data on eight different preparations of synthetic medium T_2 lysates in 6 to 10 liter quantities. Titers of the lysates varied from 2 to 5 $\times$ 10^{11} plaque-forming units per ml. In test tube quantities lysate titers have run as high as 8 $\times$ 10^{11}/ml. Other workers (25, 5, 6) have also reported high titer lysates. Trial runs using the *coli* viruses T_{2r} and T_4 also yielded lysates titering 2 $\times$ 10^{11}/ml. In some preliminary experiments T_2 lysates titering 2 $\times$ 10^{11}/ml. were prepared in nutrient broth of four times the usual concentration.

Fig. 1 is an electron microscope picture of an *unfractionated* high titer T_2

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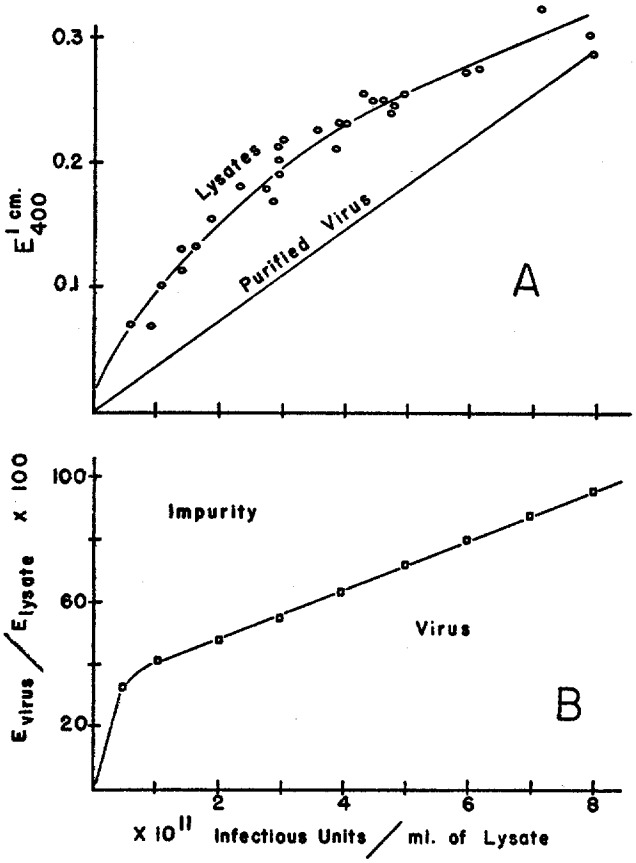
lysate. The presence of a number of smaller but uniformly shaped particles which the writers have circled in Fig. 1 was of considerable interest because of their possible relationship to the virus. Similar pictures of lysates of uninfected bacteria in which rapid lysis from without was brought about by means of T_2 "ghosts" (15) did not show these particles. In an attempt to account for their absence in earlier pictures (2) it seemed probable that the lysates of these workers had been of too low a titer to permit direct photographing and in the process of concentrating the virus the smaller particles were left behind. Quite recently, however, Heden (6) has observed what appears to be the same type particles. Wyckoff (29) reported some particles which he suggests may be immature virus, but it is not clear whether they are the same as those shown in Fig. 1.

TABLE I

Titers of Large Volume Lysates

Preparation No.	Volume	Lysate titer	Total phage (infective units)	Titer/E_{400} mμ
	ml.			
11-22-50	8000	3×10^{11}	2.4×10^{15}	1.7×10^{12}
11-28-50	4000	4.7×10^{11}	1.9×10^{15}	2.0×10^{12}
1-7-51	8000	2.8×10^{11}	2.2×10^{15}	1.5×10^{12}
4-13-51	8000	3.0×10^{11}	2.4×10^{15}	1.2×10^{12}
7-1-51	8000	2.7×10^{11}	2.1×10^{15}	1.3×10^{12}
9-19-51	8000	2.9×10^{11}	2.3×10^{15}	1.3×10^{12}
XII A	8000	2.3×10^{11}	1.8×10^{15}	1.1×10^{12}
XIV	10,000	2.3×10^{11}	2.3×10^{15}	

Text-fig. 1A illustrates how the turbidity (E_{400} mμ) of a large number of different filtered lysates varies with the virus titers. Also included is a curve of the turbidity of purified virus calculated from the titer/E_{400} value of 2.8×10^{11} from Table III. The difference between the two curves is a measure of the absorbing or turbid impurities of the lysates. The results are seen somewhat more clearly in Text-fig. 1B in which the virus contribution to the turbidity of these lysates is plotted as per cent of the total turbidity against the titer. It may be seen that the height above the curve in Text-fig. 1B is a measure of the impurity. As the lysate titer rises, the per cent impurity decreases until in a lysate titering 9×10^{11} all the material absorbing or scattering at 400 mμ is apparently virus. This suggests that the virus may be formed at the expense of the non-viral turbid component although there are alternative interpretations. The nature of the non-viral material is not known but at least part of it is a yellow soluble substance, perhaps riboflavin, which has been seen in some supernatants after the virus has been centrifuged out.



TEXT-FIG. 1. A. Turbidity *vs.* titer of many different lysates free of debris. The straight line was calculated from an average value of purified virus (see Table III).

B. Virus turbidity in lysates free of debris as per cent of the total turbidity plotted against the titers.

Experimental Procedures Followed in Experiments Shown in Text-Figs. 1A and 1B

A variety of lysates all prepared in the same media and with the same starting conditions were filtered through hyflow filter cell or centrifuged at 4000 R.P.M. for 20 minutes to remove the cell debris. The extinction at 400 mμ and the infective titers were then determined. Some lysates were prepared in test tubes, some in 6 liter flasks, and others in 40 liter bottles. The results shown in these plots were accumulated over a period of 3 years, but the media and initial cell and virus concentrations were always the same.

Purification.—Methods of purification have been developed which do not involve the long centrifugation in the Sharples ultracentrifuge employed by others (2, 7–9). The essential steps consist of: (*a*) Separation of cell debris by

filtration through hyflow celite (analytical grade) or allowing it to settle overnight at 5°C. (*b*) Cooling to 5°C. and precipitation by acidification to pH 4.0 and allowing the precipitate to settle overnight after which the bulk of the supernatant fluid is siphoned off and discarded. The remaining supernatant fluid is separated by centrifugation at 2400 × *g*. A similar precipitation of T_6 virus at pH 4.2 was noted earlier by Putnam *et al.* (7). (*c*) Resuspension of the residue in saline of approximately $\frac{1}{30}$th the lysate volume is followed by addition of bicarbonate to pH 6.5. This solution is then treated with 1 microgram/ml. of crystalline desoxyribonuclease (DNAse) to digest the free DNA which otherwise makes the solution viscous and the virus less stable (3). (*d*) Differential centrifugation with a selection of the fraction remaining in the supernatant at 5000 × *g* but sedimenting in a half-hour at 11,000 × *g*.

The final product dissolved in 0.9 per cent saline was apparently free of most impurities and was stable for months at 5°C. as noted by others (25). An electron microscope picture of a purified preparation is shown in Fig. 2.

Evidence of Purity.—The purified preparations, having the properties shown in Table III, were next examined for their homogeneity. This examination consisted of a series of fractionation experiments using radically different techniques to separate possible impurities. Since the infectivity of the virus is its most specific property, the infectivity per milligram of nitrogen, per unit turbidity (E_{400} mμ) and per unit absorbancy at 260 mμ (E_{260} mμ), was studied in many fractions. In no instance was any specific property higher than that in Table III. Preparations with lower specific properties responded to fractionation by one or more methods by yielding a product having properties approaching those shown in Table III. This is strong evidence for the homogeneity of our best preparations. The fractionation procedures used included: (*a*) Use of 16 per cent ethanol at −5°C. and pH 5.4 as recommended by Putnam, Kozloff, and Neil (7);[1] (*b*) adsorption onto and elution from alumina or copper hydroxide at pH 6–7; (*c*) differential centrifugation; (*d*) precipitation by the addition of metaphosphoric acid; (*e*) precipitation by the addition of acid; (*f*) fractional adsorption to host cells, similar to experiments of Northrop (24); (*g*) treatment with host (*E. coli.* B) antiserum (8, 10).

Recovery or Yield.—It may be seen in Table II that losses during filtration and acid precipitations were small. Recovery of the virus in the fraction of highest purity varied considerably. Table II shows two extremes, 27 per cent and 85 per cent. The yield appeared to be inversely related to the quantity of dark gray colloidal material present in some preparations which was difficult to separate without a considerable sacrifice in the yield. Lesley *et al.* (25)

[1] Two instances of increased titer/E_{400} ratio were obtained while fractionating at low temperature with 25 per cent ethanol. The values obtained were 3.5 and 3.7 × 10^{12}. However, in spite of numerous attempts we have not obtained these results again.

have satisfactorily solved this problem of the gray impurity. As might be expected from the results shown in Text-fig. 1, it has also been observed that the higher the concentration of virus in the lysate, the greater the yield of purified virus.

Some Properties of the Better Preparations.—Some of the values shown in Table III differ from those previously reported by others (11, 12). The titer

TABLE II

Purification of T_2 Virus

Treatment or solution	No.	Volume	Titer/ml.	Total titer	Titer/E_{400} mμ	Recovery
		ml.				*per cent*
Lysate	1	6500	2×10^{11}	1.3×10^{15}	1.05×10^{12}	100
Acid precipitate redissolved	2	175	6.5×10^{12}	1.2×10^{15}	1.3×10^{12}	92
No. 2 after DNAse treatment	3	175	6.5×10^{12}			
No. 3 centrifuged at 1700 × *g* for ½ hr. at 5°C. then 3900 for ½ hr., residue dissolved	4					10
Supernatant centrifuged ½ hr. at 8700 × *g*, residue dissolved in saline	5	23	1.5×10^{13}	3.5×10^{14}	2.8×10^{12}	27
Lysate 126L	10	6000	3.6×10^{11}	2.2×10^{15}	1.5×10^{12}	100
Treat same as in 2, 3, and 4; then centrifuge at 11,000 × *g* for 1 hr., resuspend residue in saline	11	150	1.25×10^{13}	1.9×10^{15}	2.7×10^{12}	85

per milligram of nitrogen is somewhat higher on the average and the per cent nitrogen is considerably higher. The value given by Taylor (11) for the per cent nitrogen is 13.3, whereas our value is about 16 per cent. Kozloff and Putnam (8) comment on the wide discrepancies in analytical values of T_2 preparations and suggest that the differences result from a lack of homogeneity in the preparations analyzed.

The protein and nucleic acid contents are also appreciably different from those reported earlier. Thus in Table IV the protein nitrogen is only 40 per

cent of the total virus nitrogen, while the nucleic acid is about 53 per cent. The nucleic acid content as determined by the diphenylamine test (13), the cysteine color method (14), or the phosphorus analysis was over 50 per cent of the whole virus. Carrying along of non-constituent DNA is unlikely in the present instance since DNAse has been used in the purification procedure and

TABLE III

Some Properties of Purified Virus T_2

Preparation No.	Titer/E_{400} mμ	Ti er/E_{260} mμ*	Titer/mgN	P/N	P	N	Lipid	PNA
					per cent	*per cent*	*per cent*	*per cent*
7-14	2.6×10^{12}	1.3×10^{11}	1.2×10^{13}	0.33				
367LP9	3×10^{12}		1.15×10^{13}	0.33	5.4	16.4		
					5.3	16.3		
1.8P934	2.6×10^{12}	1.1×10^{11}	1.0×10^{13}	0.32	5.35	16.7	<0.2	0.4
XII A_3d	2.7×10^{12}	1.25×10^{11}	1.24×10^{13}	0.31	5.0	16.0	0.2–0.6	0.3
XIV 9ad	2.7×10^{12}	1.2×10^{11}	1.2×10^{13}	0.33	5.1	15.5	0.2	0.7

The specific viscosity per milligram of N = 0.12 ± 0.05

* The E_{260} is an observed value and no correction for scattering has been made.

TABLE IV

Distribution of Phosphorus and Nitrogen

Preparation	Per cent of total phosphorus				Per cent of total nitrogen		
	TCA-soluble		Protein	Nucleic acid	TCA-soluble	Protein	Nucleic acid
	Inorganic	Organic					
XII A_3d	0	1	3	97	7	40	53
	1	3			6.6		
XIV 9ad		0.8	3	100	8	39	53
					5.5		

the final concentrated preparations are not viscous as would be expected if even small quantities of free DNA were present.

The figure for the micrograms of phosphorus per infective particle which can be calculated from the data in Table III is in very good agreement with the average value of 3.0×10^{11} reported by Lesley, French, and Graham (25).

The distribution of the phosphorus and nitrogen of the virus may be seen in Table IV. The acid-soluble phosphorus is about 2 per cent of the total phosphorus whereas the acid-soluble nitrogen is close to 6 or 7 per cent. Even after 2 weeks' dialysis against saline and 3 days against distilled water, cold trichloracetic acid (TCA)–soluble nitrogen remains with the virus. Its nature

is being studied. The protein residue after the hot TCA extraction of Schneider (23) appears to hold onto about 3 per cent of the phosphorus. This is probably linked as phosphoprotein.

The lipid and the pentosenucleic acid (PNA) content are both less than had been found by Taylor for T_2 preparations (11). Without any further fractionation the PNA content of the preparations was found to lie between 0.3 and 0.7 per cent while the total lipid ranged from less than 0.2 per cent to 0.6 per cent. In the PNA analyses the Schmidt-Thannhauser procedure (16) for separating the PNA from protein and DNA was used before determining it with the Bial-orcinol test (17). With more or special treatment the PNA content might well be further reduced although we have no data bearing on this point. It should be emphasized, however, that the present values for the lipid and PNA contents represent maximum values. In the lipid determination the procedure used by Taylor (11) was followed, except that we have weighed the alcohol-ether–soluble residues after removal of the solvent by evaporation.

Materials and Experimental Procedures

Viruses.—This T_2 virus, the wild type (r^+), has been used for several years. It differs from T_{2r} in its action in synthetic media on 1×10^8 *coli* B/ml. when multiply (5.0) infected. A comparable study in broth showed less difference. A sample of T_2L, the original T_2 of Demerec and Fano (31), kindly sent by Dr. A. D. Hershey behaved essentially like our T_2.

Culture Medium.—This is essentially the M-9, the glucose–ammonium ion medium, described elsewhere (26), but since we have handled it somewhat differently and little details affect this type of work, the procedure is included. Two concentrated stock solutions are made up and diluted as needed:—

Solution A: 150 gm. of anhydrous Na_2HPO_4 + 75 gm. KH_2PO_4 diluted to 1 liter with distilled water.

Solution B: 20 gm. $MgSO_4 \cdot 7H_2O$;[2] 50 gm. NaCl; 100 gm. NH_4Cl; distilled water to 1 liter.

For each liter of final medium desired 40 ml. of A and 10 ml. of B are mixed and 4 gm. of glucose added. This solution is filtered through a sterile number 015 Selas porcelain filter candle and added to an appropriate volume of water previously sterilized by steaming for 2 hours at 100°C. Usually a 10 liter quantity of medium is made up as needed and brought to temperature equilibrium by incubating for 18 hours at 37°C.

Preparation of Lysates.—The organisms are first grown in a 5 liter Blake bottle coated with 250 ml. of Difco nutrient agar. 10 ml. of *E. coli* B strain in nutrient broth in log phase of growth at a concentration of 5×10^7 cells/ml. are spread within the Blake bottle and incubated for 15 hours. The cells are then washed off with the aid of 100 ml. of warm synthetic medium and added to the 6 to 10 liters of

[2] In the paper by Hook *et al.* (2) this is listed as $MgSO_4$. By mistake we used the same weight of $MgSO_4 \cdot 7H_2O$ and have not recently returned to the higher level of $MgSO_4$.

sterile synthetic medium in a 40 liter bottle. This is then agitated vigorously by rotating at 120 R.P.M. on a rotating table designed and constructed by Mr. Paul Huffman of this school. When growth has reached 1×10^8 cells/ml. as determined on a 10 ml. aliquot removed and examined in a Coleman Jr. spectrophotometer set at 650 mμ, 1.5 liter aliquots are siphoned sterilely or poured into each of four 6 liter Erlenmeyer flasks which are then placed on a reciprocating shaker (Arthur H. Thomas Co., number 8922-B). A 10 ml. aliquot is also removed and placed in a sterile 22 × 175 mm. test tube and shaken. The 40 liter bottle containing the 4 liters remaining is rotated at 120 R.P.M. When the culture has reached 5×10^8 cells/ml. as judged by turbidity values ($E_{1\text{ cm.}}^{650\ m\mu} = 0.10$) sterile T_2 virus is added to each flask to make the final concentration 1×10^6 phage units/ml. These flasks are then shaken at 35–37°C. until lysis is complete which is usually within 9 to 11 hours. Some lots have been shaken overnight without noticeable further effects. In many instances titers of both broth and synthetic media lysates have increased twofold or more on standing at 5°C. Usually a maximum was reached in 2 days but sometimes 5 days were required. This observation is qualitatively similar to those of Lesley *et al.* (25) and Luria *et al.* (30).

Purification.—2.5 gm. of hyflow filter-cel[3] (Johns-Manville) is added for each liter of lysate and the whole suspension filtered on a 310 mm. Buchner funnel with a 3 to 5 mm. pad of hyflow filter-cel covering the number 3 Whatman paper. If it is not convenient to filter it immediately, the lysate may be chilled to 5°C. and decanted or filtered later.

Acid Precipitation.—With the filtrate at 5°C., M/1 HCl is added slowly with stirring (approximately 25 ml./liter of lysate) until the acidity reaches pH 3.9–4.0 as measured with a glass electrode. The precipitate that forms is allowed to settle and the supernatant decanted off and discarded. The residue is then centrifuged cold in the International number 2 angle head at approximately 2400 × *g*. This residue is then resuspended in about 200 ml. cold saline and M/1 $NaHCO_3$ added carefully till the residue is completely redissolved and the pH = 6.5.

Digestion with DNAse.—At this stage the virus solution is viscous. Magnesium sulfate, 0.1 ml. of saturated solution (2.8 M), is added for each 100 ml. virus solution to make it approximately 0.003 M followed by the addition of sufficient crystalline DNAse (Worthington of Freehold, New Jersey) to make the final concentration 1 μg./ml. solution. Digestion is allowed to continue at room temperature until the viscosity is nearly that of water, requiring 0.5 to 2 hours. Sometimes the solutions stood overnight at 5°C. but in no case was a drop in infectivity titer observed during or immediately after DNAse action.

Differential Centrifugation at 5°C.—With the digestion of the nucleic acid the viscosity decreases, sedimentation becomes easier, and the virus is more stable (3). The virus solution is first spun at 2400 × *g* in an angle head of the International No. 2 centrifuge for 30 minutes followed by a centrifugation of the supernatant at 5000 × *g* (6000 R.P.M.) in a precooled Servall SS-2 angle head for ½ hour. These two pre-

[3] We have had one lot of this product which contributed some color to our virus solution. To prevent this occurrence one may use the refined "Analytical" grade purchasable from Fisher Scientific Co.-Eimer and Amend Co.

liminary treatments remove nearly all visible cells, debris, and filter-cel not removed by earlier filtration. Centrifugation of the supernatant at 11,000 × *g* (9000 R.P.M.) for ½ hour in the Servall produces a clear pellet faintly yellow by transmitted light but bluish and opalescent by reflected light and when dissolved having the properties of the materials shown in Tables III and IV. If this centrifugation is continued longer, *i.e.* 2 hours, a dark gray film often, but not always, collects over the pellet and the ratio of titer to turbidity is proportionally lower indicating that this gray material is an impurity. Lesley *et al.* (25) who also encountered a similar material suggest a simple procedure for its removal.

Experimental Methods

Titration of Virus.—This has been described earlier (18). It has proven a very reliable procedure.

Kjeldahl Nitrogen.—To the sample containing 0.2 to 2.0 mg. of nitrogen in a 75 ml. digestion flask is added 2 ml. of a digested mixture (containing 40 gm. K_2SO_4, 250 ml. H_2O, 20 ml. M/1 $CuSO_4$, 2 ml. $SeOCl_2$ + 250 ml. concentrated H_2SO_4). This is boiled for 1 hour after white fumes appear or it clears.

Total Phosphorus.—This is the method of King (19) except that the color reagent was made up as recommended by Norberg (20). Solutions were read in a Klett colorimeter using a 66 filter. In general the blank read 7 Klett units and 0.02 mg. of phosphorus read 105 before correcting for the blank.

Inorganic Orthophosphate.—The method of Lowry and Lopez (21) was used in this work.

Dry Weight.—The samples were first subjected to still dialysis at 5°C. for 3 days against numerous changes of distilled water. Sample XIV 9ad was dialyzed 2 weeks against saline before the 3 days against water. Analyses showed that not more than 5 to 10 per cent of the phosphorus, nitrogen, or material absorbing at 260 mμ was lost by dialysis. In only one instance was the drop in titer greater than 5 to 10 per cent. Samples were dried to constant weight over sulfuric acid at 37°C. One sample (XIV 9ad in Table III) was dried from the frozen state in the weighing bottle and then further dried to constant weight over H_2SO_4 in a vacuum desiccator at room temperature.

Absorbancy or Extinction $E^{\lambda}_{1\ cm.}$ Values.—These were obtained with the aid of the Beckman quartz DU spectrophotometer standardized as recommended by the manufacturer at 656 mμ with the hydrogen line. In addition, the optical density and wave length scales were checked against a carefully standardized didymium glass (22). The wave length in the region of 260 mμ was standardized against 0.02 per cent benzene dissolved in isooctane. Our instrument gave values within 2 mμ of the standards.

Cell Count.—Bacterial suspensions have been estimated in the Coleman Jr. or Beckman spectrophotometers at 650 mμ wave length. The optical density or absorbancy values varied linearly between 5×10^7 and 5×10^8 cells/ml. and there is very little interference with changes in media. The calibration of optical density in terms of number of cells per milliliter was first established by actually counting the cells in a Petroff-Hausser bacterial chamber. We were unable to distinguish single cells from budding or doublet cells. Consequently, our count may be too low.

The cell count gave values only 20 per cent higher than the colony plate count which also does not distinguish multicellular units from single cells. 4×10^8 cells/ml. had an optical density or extinction $E_{1\text{ cm.}}^{650\ m\mu} = 0.08$.

Details of Experiments in Table IV

Materials.—Both samples of virus were prepared as described in this paper. XII A_3d was then dialyzed for 3 days at 5°C. against saline while XIV 9ad was dialyzed for 14 days.

TCA-Soluble Fraction.—To 1 volume of cold virus solution containing 1.5 to 2.0 mg. of total nitrogen/ml. was added 4 volumes of cold 5 per cent TCA after which the suspension was centrifuged at 2000 R.P.M. for 15 minutes. Total nitrogen and total phosphorus were determined on the supernatant liquid. In a few instances the inorganic phosphorus of this fraction was estimated by the method of Lowry and Lopez (21).

Nucleic Acid Fraction.—The TCA residue from the above separation was extracted with 5 ml. and then 3.0 ml. of 5.0 per cent TCA at 90°C. for 15 minutes (23). Total nitrogen and total phosphorus determinations were made on the combined supernatants.

Protein Fraction.—The residue from the nucleic acid separation procedure was dissolved in dilute alkali and analyses made on it for total nitrogen and phosphorus.

DISCUSSION

The conditions described in the present paper for the formation of lysates lead to high yields of virus per cell. This will be reported in detail in a later paper, but it appears that in several instances the yield was 400 or more infectious units per cell. Even higher yields have been obtained from multiply infected cells in four times the normal concentration of nutrient broth. The results of Lesley *et al.* (25) show that yields of T_2 up to 500 per cell are possible in nutrient broth.

The best purified virus preparations titered 2×10^{15} units per gm. of material or 1.2×10^{16} units per gm. of nitrogen. Using the partial specific volume value of 0.66 (6) and assuming that the particles are spherical, although they are not, the diameter of the infective unit may be calculated from the infectivity per gram. Such a calculated figure when compared to an independently determined value of the diameter may serve as a rough measure of the absolute purity of the preparation. The diameter calculated from the above data was 86 mμ which is lower than the similarly obtained figure given by Putnam (28) of 107 mμ but higher than the 59 mμ this same author arrived at from physical measurements. Considering that any clumping, inactivation, or adsorption of virus particles during measurement will lower the titer, plus the fact that the particles are not spherical and the value being used as reference is an experimental one perhaps the difference is not a true reflection of the inhomogeneity of the preparations.

The data presented show that virus T_2 is composed of about 40 per cent

protein and something over 50 per cent DNA. These values are just the reverse of those usually quoted for T_2 (11, 27) but are similar in many respects to those reported by Kozloff and Putnam for T_6 (8) to which T_2 is immunologically and morphologically related. 6 to 7 per cent of the total nitrogen is present as TCA-soluble fraction. Despite its TCA solubility this nitrogenous fraction does not dialyze free from the intact virus during a 2 week period.

The low values of PNA in several of the better virus preparations will interest several workers who have been concerned with the nucleic acid of the T viruses. Cohen (12, 4) has reported that 99 per cent of the nucleic acid phosphorus is of the DNA type. The quantitative character of this conclusion has been questioned (8) with some reason, but the present studies are not open to the same objection, yet they support Cohen's conclusion.

In the absence of evidence to the contrary the residual phosphorus on the virus after removal of nucleotides with hot TCA is classified as phosphoprotein. This could be checked, for phosphoprotein linkages in general are alkali labile. The phosphorus present as phosphoprotein was 3 per cent of the total phosphorus. This is close to the phosphorus content of the biologically active protein "ghost" of the T_2 virus after removal of nucleic acid (15).

SUMMARY

1. A method for the preparation of 8 to 10 liter quantities of T_2 virus lysates, titering 2 to 5 $\times$ 10^{11} infectious units per ml. has been described.

2. Procedures have been developed for the concentration and purification of virus to a high specific infectivity. No fractionation procedure of the several used succeeded in further raising the specific infectivity of these purified preparations.

3. Some of the general properties of the better preparations have been determined. They exhibited titers of 2 $\times$ 10^{15} infective units per gm. of material or 1.2 $\times$ 10^{16} per gm. of nitrogen.

4. A study of the distribution of nitrogen among the various fractions of the virus showed that about 6 per cent of the total nitrogen is soluble in 4 per cent trichloracetic acid; that the protein nitrogen is about 40 per cent of the total and the nucleic acid nitrogen is 53 per cent. At least 96 per cent of the total phosphorus is in the nucleic acid fraction. Less than 0.5 per cent quantities of lipid and PNA were found.

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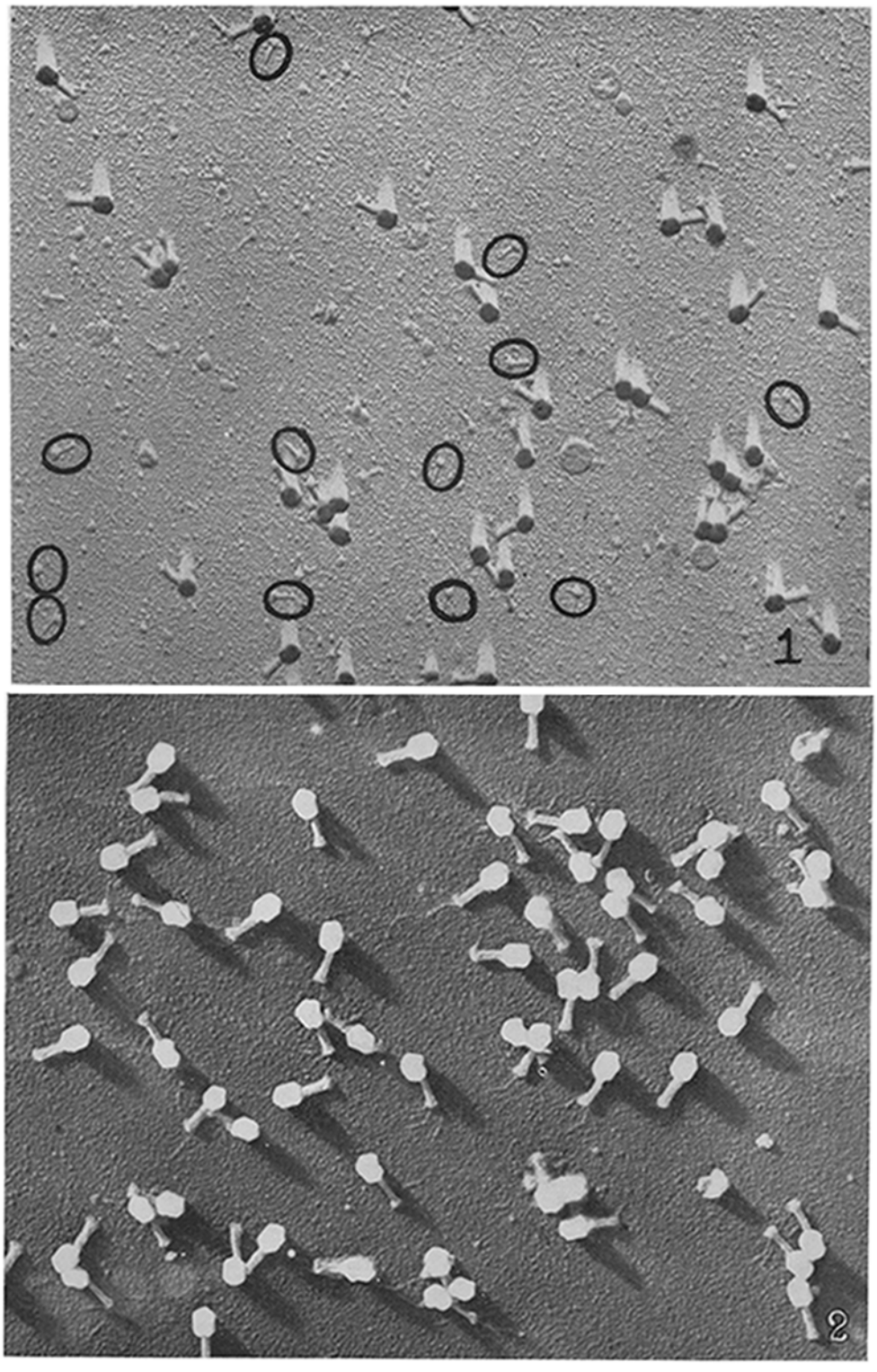
EXPLANATION OF PLATE 1

FIG. 1. An unfractionated T_2 lysate. Circles have been drawn around a number of uniform particles considerably smaller than the virus particles. Electron microscope photograph taken by Dr. James S. Murphy. × 27,000.

FIG. 2. Purified T_2 virus. × 37,000. Photograph by Dr. James S. Murphy after fixing with formalin vapor for 30 minutes followed by chromium shadowing.



(Herriott and Barlow: *E. coli* virus T_2)