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The Nucleoside Triphosphate Content of Various Bacteriophages*

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In 1959, Kozloff and Lute (1) presented morphological and biochemical evidence showing that one of the major components of the tail of bacteriophage T2 was a contractile protein. T2 was found to contain bound nucleoside triphosphates; 51 molecules[1] of acid-extractable adenosine triphosphate and 17 molecules[1] of deoxy-ATP per T2 particle were determined by an isotope dilution technique (1). T2 has also been found to contain 1 calcium ion for each nucleoside triphosphate (3). Dukes and Kozloff (4) have demonstrated a phosphatase activity in purified preparations of T2, similar in many properties to actomyosin adenosine triphosphatase. Upon interaction of T2 with *Escherichia coli* B cell walls, most of the ATP and dATP is hydrolyzed to ADP and dADP, with the concomitant release of inorganic phosphate and calcium ions. It was concluded that during invasion, this portion of the tail hydrolyzes the bound nucleotides and contracts, and that these reactions aid the entry of the viral deoxyribonucleic acid into the host cell.

The existence of a protein in the T2 tail with contractile properties has been confirmed by Brenner *et al.* (5). They obtained electron micrographs of high resolution showing the T2 tails in both the extended and contracted states. They have also isolated the contractile protein in the shortened condition and have determined its amino acid composition.

The firefly luminescence method (6–8) has now been adapted to measure the micro amounts of ATP present in various bacteriophage preparations. Bacterial viruses which have tail structures different from T2 have been prepared and analyzed. A technique has also been developed to analyze for a second class of high energy phosphate compounds found in bacteriophage preparations. The terminal phosphate groups of these compounds, designated as $X \sim P$, are transferred to ADP by transphosphorylases present in the firefly lantern extracts (9). These compounds produce a slower light response than does ATP. Significant amounts of ATP and $X \sim P$ have been found only in preparations of the T-even bacteriophages. A hypothesis has been advanced to account for the role that these compounds play in both the synthesis of the virus tail and in contraction.

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[1] These values have been recalculated from the originally reported amounts and are based on 1.8×10^{-11} μg of phosphorus per T2 particle (2) instead of the older value of 2.3×10^{-11} μg of phosphorus per T2.

EXPERIMENTAL PROCEDURE

Preparation of Phage Stocks—T2r$^+$, T4r$^+$, T1, T5, and T7 were purified from liquid culture as previously described (1). T6r$^+$ was prepared by confluent lysis (10). A preparation of T3C, a mutant of T3 which has a long tail (11), was kindly provided by Dr. A. Eisenstark of the Department of Bacteriology, Kansas State University.

The temperate phage λ (λ-temperate), was prepared by ultraviolet induction in *E. coli* strain K12 (12) and purified by gradient centrifugation in cesium chloride.[2] A purified suspension of λ-virulent, a strain related to λ-temperate but which causes virulent infection in *E. coli* strain W-1485 and is unable to lysogenize *E. coli* K12, was also prepared (12). A crude preparation of λ-virulent, taken through one cycle of differential centrifugation, was treated successively with RNase, DNase, chloroform, and crystalline lysozyme. The diffusible hydrolysis products were removed by dialysis, and the preparation was subjected to two cycles of differential centrifugation: 20 minutes at $3,000 \times g$ and 5 hours at $12,000 \times g$. The final pellet was covered with 0.067 M Tris buffer, pH 7, and the virus was allowed to leach out overnight. Since bacterial debris in the pellet adheres to the wall of the centrifuge tube more strongly than the virus, it was possible to obtain a relatively pure preparation of the virus.

The T2 protein ghosts were prepared by the osmotic shock method of Herriott and Barlow (13), and the *E. coli* strain B cell walls by the procedure of Salton and Horne (14). Total phosphate was determined by the procedure of Bartlett (15).

Preparation of Firefly Enzyme—The procedure of Strehler and Totter (7) was modified as follows. The dehydrated firefly tails (Schwarz BioResearch, Inc.) were distributed into 300-mg aliquots and placed at −20° immediately upon delivery. Before an ATP analysis, one 300-mg aliquot was homogenized by hand with a glass-Teflon tissue grinder with 30 ml of a 0.1 M sodium arsenate buffer, pH 7.4, for 5 minutes at 0°. After filtration in the cold, 300 mg of $MgSO_4 \cdot 7H_2O$ crystals were added to the filtrate, and the preparation was held for 24 hours at 7–8° to allow the intrinsic ATP level to fall. The pH was adjusted with stirring to 7.5 with 3.1 ml of a 0.175 M glycylglycine buffer, pH 8.5, and the preparation was then placed on ice until used.

Extraction of Nucleotides from Phage Stocks—The ATP and other nucleotides were extracted with 0.6 N perchloric acid in a

[2] R. P. Mackal, F. Meyer, and E. A. Evans, Jr., unpublished information.

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modification of a procedure described by Hurlbert *et al.* (16). Cold 1.2 N perchloric acid (0.1 ml) was added to 0.1 ml of a phage preparation containing a minimum of 2×10^{12} particles for the larger phages, T2, T4, T5, and T6, and 1×10^{13} for the smaller, T1, T3C, T7, λ-temperate, and λ-virulent. These phage samples contain 1.0 to 5.0 μmoles of phage P. After ½ hour at 0°, the suspension was neutralized to pH 7 with 0.2 N KOH, and the volume was brought to 1.0 ml with deionized water. After 1 hour at 0°, the precipitated protein and the $KClO_4$ were centrifuged in the cold at $3000 \times g$ for ½ hour, and the supernatant fluid was removed and placed in an ice bath for analysis. This solution is subsequently called the "phage extract."

Test Solution—The following solutions were pipetted into Vycor cuvettes (10 × 10 × 48 mm): 0.5 ml of a 0.175 M glycylglycine buffer, pH 7.5; 0.1 to 0.4 ml of an ATP standard solution with a concentration of 1.6×10^{-11} mole per ml; or 10 to 100 μl of phage extract. Since the firefly luminescent system is inhibited by monovalent anions (6, 7), standard test solutions were adjusted to the same ionic compositions as the phage solutions. Total volume of the test solution was 1.8 ml. The cuvette was placed in a light-proof box, and 0.6 ml of firefly enzyme was then injected through a rubber seal into the cuvette to produce the luminescent reaction. The luminescent light was measured by a 1P21 RCA photomultiplier tube, powered by ten 90-volt batteries, which was connected to a Varian G-11 A-1 recorder. A standard cathode follower, RCA tube No. 956 powered by a 45-volt battery, was included in the circuit to produce a low impedance output.

Standardization of ATP—The disodium salts of ATP, GTP, dATP, dGTP, ITP, CTP, and UTP and the monosodium salt of ADP were obtained from Pabst Laboratories. The ATP and dATP were checked chromatographically and found to contain less than 1% of contaminating nucleoside diphosphate or monophosphate. A typical ATP standard curve and the corresponding traces are presented in Fig. 1. The reproducibility of the method for duplicate samples was approximately 10%. In all analyses, ATP solutions were prepared by dilution of a spectrophotometrically standardized stock solution (17).

The standard curve of Fig. 1, one of the first of the project, has a slope of 1.1 units per 10^{-12} mole of ATP. Later enzyme preparations, made of fresh firefly tails (less than 6 months at −20°), produced deflections of the order of 5 units per 10^{-12} mole of ATP. Thus, if 2 units of deflection are considered the limit of sensitivity, a minimum of 4×10^{-13} mole of ATP is detectable by this method.

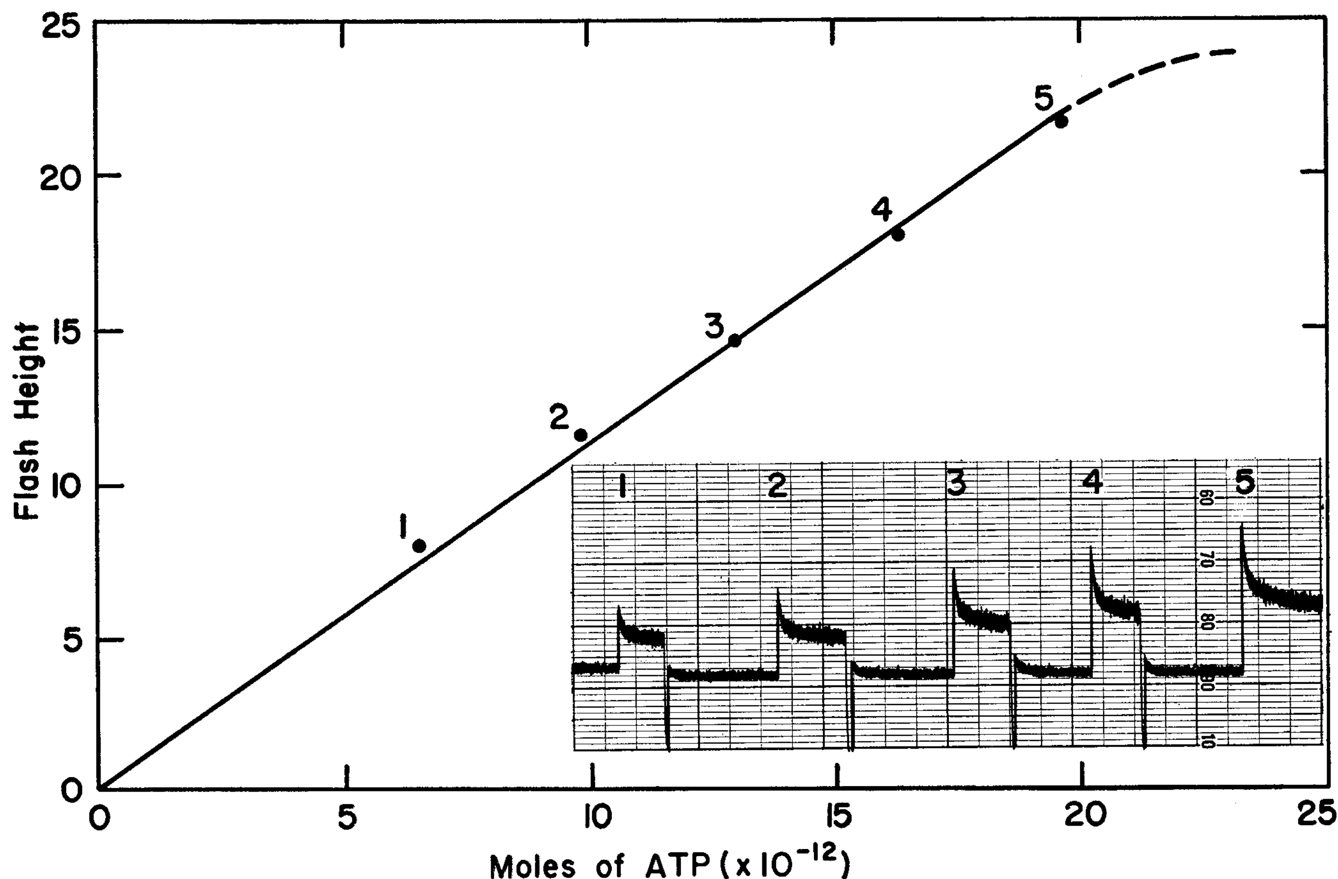

FIG. 1. A typical standard curve for ATP obtained with the firefly luminescence assay method, and the corresponding recorder traces. The flash heights of initial flashes are expressed in recorder deflection units and are corrected for the residual ATP of the firefly enzyme preparation by subtraction of a small blank value. The linearity fails above 25 units, probably because the full scale pen time of 1 second was not rapid enough to accommodate larger flashes, whose half-rise times were only 0.1 second. The size of the aliquots of phage extract and standard solutions were, therefore, always adjusted to give flashes between 15 and 25 units. The *vertical lines* below the recorder base-line are not significant; they are made when the apparatus is turned off to allow the cuvette containing the next test solution to be placed in front of the photomultiplier cathode. The time interval between successive assays on the recorder chart was of the order of 40 to 50 seconds.

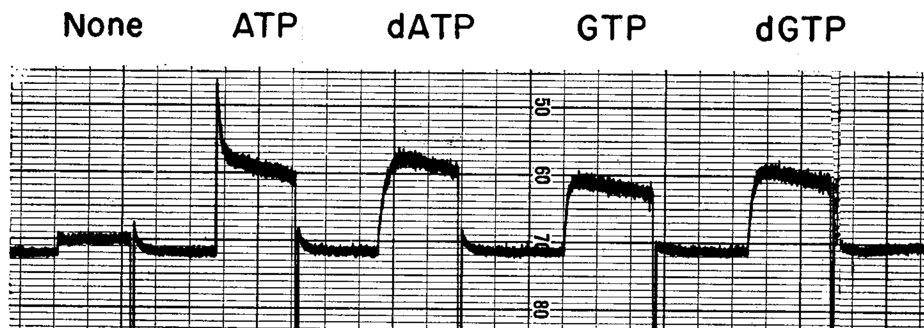

FIG. 2. Recorder traces typical of 10^{-11} mole of ATP, dATP, GTP, or dGTP obtained with the firefly luminescent system. The total time for each flash was of the order of 40 to 50 seconds.

FIG. 3. A typical standard curve for dATP obtained with the firefly luminescent system, and the corresponding traces. The *ordinate* represents recorder deflection units 40 seconds after injection of the firefly enzyme. A correction has also been made for "residual nucleotide" by subtracting the small 40-second deflection at zero dATP concentration.

Standardization of Other Nucleotides—Light was also produced when the firefly enzyme preparation was added to nucleoside triphosphates other than ATP, although the trace patterns were different. ADP did not give any detectable light. Fig. 2 shows traces formed after the addition of ATP, dATP, GTP, and dGTP at a level of 10 $\mu\mu$moles. UTP, CTP, and ITP gave trace patterns similar to that of dATP and GTP. Whereas the ATP produces an immediate light flash upon injection of the enzyme, with a half-rise time of 0.1 second, and falls off to a base level within 10 seconds, all of the other nucleotides require 20 seconds to build up to a maximum, which decays slowly. This pattern suggested that the terminal phosphate was being transferred from the nucleoside triphosphate to the ADP present in the firefly enzyme preparation by transphosphorylases which also occur in the crude firefly enzyme. Balfour and Samson (9) have reported transphosphorylases in the crude firefly extract which react with GTP, UTP, CTP, and ITP.

The concentrations of the dATP, GTP, dGTP, UTP, CTP, ITP, and ADP solutions were also measured spectrophotometrically. A standard curve for dATP is given in Fig. 3, in which

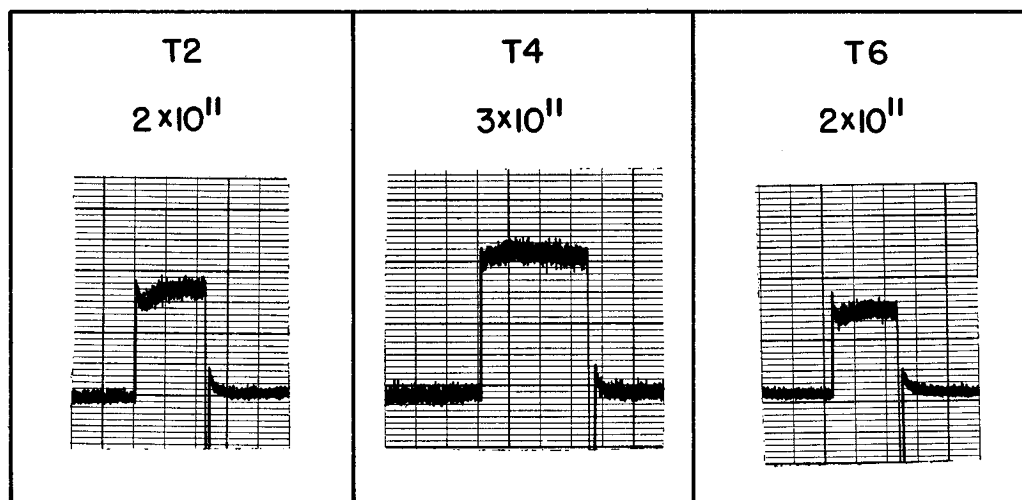

FIG. 4. Representative light flashes produced in the firefly luminescence assay by extracts of T2, T4, and T6 bacteriophage, equivalent to 2×10^{11}, 3×10^{11}, and 2×10^{11} virus particles, respectively.

deflection units 40 seconds after enzyme injection are plotted against moles $\times 10^{-12}$ of dATP. From the data shown in Fig. 2, it was calculated that the dATP maximal deflection reached at approximately 40 seconds was only 50% of an *initial* ATP flash, but that it was approximately equal to the decaying light deflection produced by ATP after 40 seconds. UTP was approximately equal in activity to dATP, whereas GTP, dGTP, ITP, and CTP gave approximately 80% of the light produced by dATP.

Duplication of Trace Patterns of Phage Extracts—Fig. 4 illustrates typical flashes produced by extracts of T2, T4, and T6. These flashes resemble the graphical addition of an ATP trace and the traces of a nucleoside triphosphate with a transferable phosphate (X ~ P). Phage traces could be readily duplicated with mixtures of ATP and other nucleotides such as dATP. Flashes obtained from various mixtures of dATP and ATP are presented in Fig. 5. Each horizontal row represents flashes at a constant ATP concentration and an increasing dATP concentration, yielding dATP to ATP ratios of 0, 0.71, 2.1, and 4.2. By comparing the flashes in a single vertical column (constant dATP to ATP ratio), it is apparent that the shapes of the traces are identical and depend only upon the relative ATP and dATP concentrations. It was found that the initial flash heights at a constant ATP concentration agree within 10% (the reproducibility of the method). It is apparent that the presence of other high energy phosphate compounds with "transferable" phosphate residues does not inhibit the initial ATP flash, making the ATP analysis independent of the presence of these compounds.

Description of Typical Analysis for ATP and X ~ P—The firefly enzyme, phage extract, and standard nucleotide solutions were prepared as described previously. ATP was then determined from duplicates of phage extract and ATP standard. The X ~ P compounds were assayed by preparing several different mixtures of ATP and dATP on the assumption that dATP, a known phage component, was typical of X ~ P compounds in the phage. The concentration of the ATP in the mixtures was kept constant and adjusted to yield a flash height approximating that of the phage extract. The trace patterns were compared, the phage extract trace was matched with the closest mixture trace, and the X ~ P compounds were calculated from the known dATP to ATP ratio and the ATP concentration.

The linearity of flashes from various aliquots of extract was tested in each new phage preparation to detect the presence of an inhibitor of the firefly luminescence system. When no flashes were produced, as in the case of some phage preparations, recovery experiments were performed to detect inhibition. There was no evidence to indicate that any of the phage extracts contained inhibitors.

A blank tube containing no ATP was included in each run, and the data from each run were treated separately because of the slowly decreasing activity of the firefly enzyme.

RESULTS

ATP and Other High Energy Phosphate Compounds (X ~ P) *Extracted from T-even Bacteriophages*

Values for ATP and the other high energy phosphate compounds (X ~ P) extracted by cold perchloric acid from purified preparations of T2, T4, and T6 are given in Table I. The results are expressed as the ratio of ATP (or X ~ P) phosphorus to total phage phosphorus, and as molecules of ATP and X ~ P per phage particle. The first expression makes the ATP and X ~ P values independent of any assumptions concerning the amount of phosphorus present in a single virus. It can be seen that the values for ATP content of T2, T4, and T6 agree well and that there are 42 molecules per virus particle, or 0.037% of the total phage phosphorus. The X ~ P value of approximately 89 molecules per particle, or 0.076% of the total phosphorus given in Table I, is based on the use of dATP as a standard. Of these 89 molecules, only 17 have been definitely identified as dATP. The only information available on the identity of the other compounds is the gradient elution chromatogram of acid-soluble P^{32} compounds from T2 reported earlier (1). Several peaks, in addition to ATP and dATP, were observed but not characterized. Since from the X ~ P values it is apparent that T2 does contain other nucleoside triphosphates, it seems likely that the peaks observed are GTP and UTP (16). It can be estimated from the chromatogram that each phage particle contains 20 to 25 UTP molecules and 15 to 20 GTP

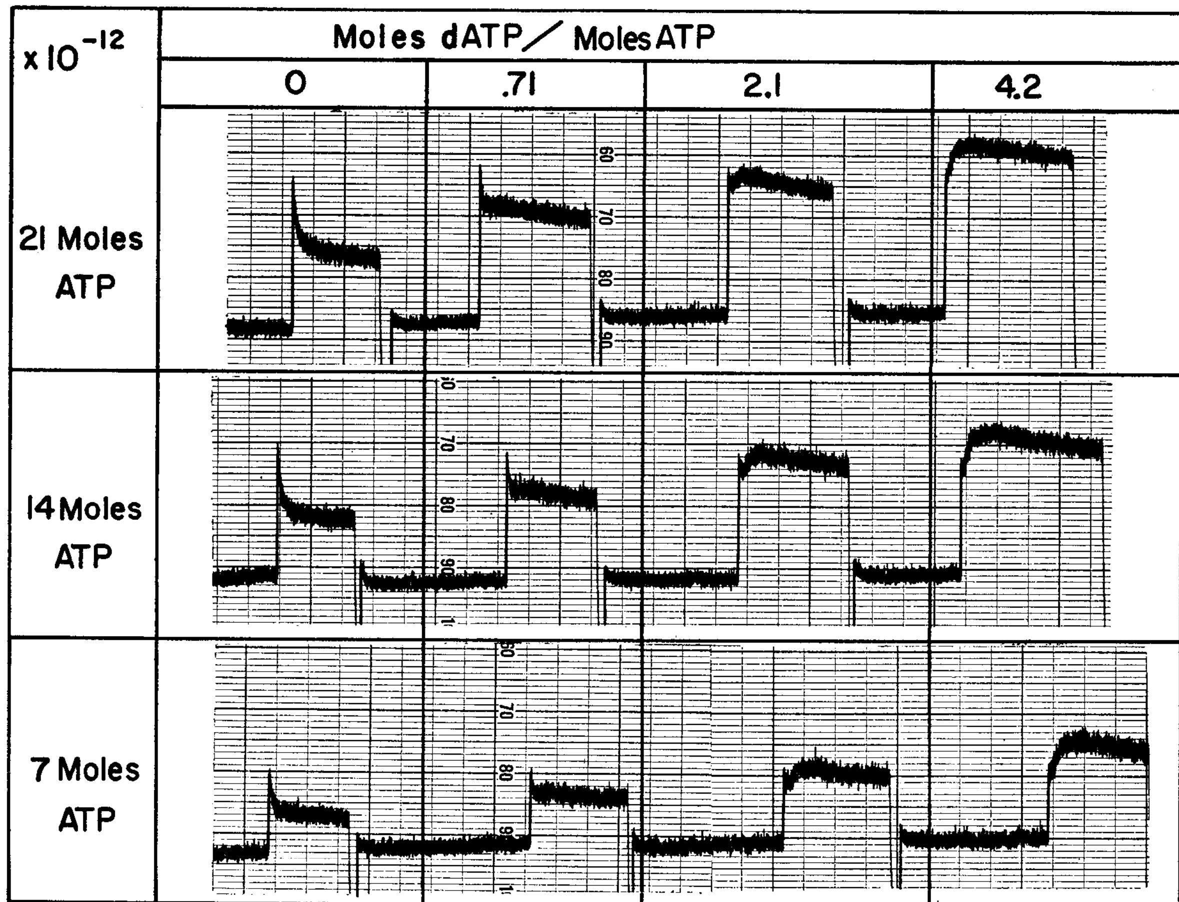

FIG. 5. Light flashes produced by mixtures of ATP and dATP. Each horizontal row represents flashes at a constant ATP concentration (21 $\mu\mu$moles of ATP, 14 $\mu\mu$moles of ATP, 7 $\mu\mu$moles of ATP) and an increasing dATP concentration, yielding dATP to ATP ratios of 0, 0.71, 2.1, and 4.2, respectively. Each vertical column thus represents flashes at a constant dATP to ATP ratio. It can be seen that the shape of the trace depends only upon this ratio.

molecules. In terms of the calculated X $\sim$ P, it should be noted that UTP is as effective as dATP in causing a light flash but that GTP and most other compounds are approximately 20% less efficient. Since the dATP plus UTP amount to only 50% of the X $\sim$ P, the X $\sim$ P values are too low by approximately 10%, and the total X $\sim$ P (dATP + UTP + GTP + others) is close to 100 molecules per phage particle. It can be concluded that the total of high energy phosphate compounds measured by the firefly luminescence method is thus 142 $\pm$ 25 molecules per virus particle.

Several lines of evidence argue against the possibility that the ATP found in the T-even bacteriophages is an artifact resulting from contaminating bacterial debris. All preparations were purified to a constant level of ATP, and the values did not change upon another cycle of centrifugation. The T6 preparation, which was made by confluent lysis on agar plates, would be expected to reflect a different contamination level from that of the T2 and the T4 preparations grown in liquid culture. Yet all three types were found to contain the same amounts and kinds of nucleoside triphosphates. Since the most likely contaminant was cell wall fragments, *E. coli* cell walls prepared by the procedure of Salton and Horne (14) were analyzed and found to contain 1500 $\pm$ 110 molecules of ATP and 1700 $\pm$ 300 molecules of X $\sim$ P per cell wall. To account for the 42 molecules of ATP per T-even particle, 6 $\times$ 10^{11} cell walls per ml would have had to be present as contaminants in the final

TABLE I

ATP and X $\sim$ P extracted from T-even bacteriophages*

Three separate preparations of T2 were analyzed; four separate analyses were made of each preparation. Only one preparation each of T4 and T6 was analyzed, but each was measured twice. Since T4 and T6 are morphologically identical with T2, it was assumed that they all have similar P contents.

Phage	Nucleotide phosphorus to total phage phosphorus ratios			Molecules per particle*		
	ATP P to total P	X $\sim$ P P to total P	ATP + X $\sim$ P P to total P	ATP	X $\sim$ P	ATP + X $\sim$ P
	$\times 10^{-4}$	$\times 10^{-4}$	$\times 10^{-4}$			
T2	3.7	8.0	11.7	42 $\pm$ 3	94 $\pm$ 25	136 $\pm$ 25
T4	3.6	7.0	10.6	42 $\pm$ 4	82 $\pm$ 21	124 $\pm$ 24
T6	3.7	7.7	11.4	42 $\pm$ 2	90 $\pm$ 24	132 $\pm$ 24

* See the text for a discussion of the nature of X $\sim$ P. These results are calculated by using the known phage component, dATP, as a standard. If, as seems likely, the phages also contain UTP, GTP, and possibly other compounds, the X $\sim$ P values are approximately 10% low. Thus, the total X $\sim$ P is probably closer to 100 molecules per phage particle, and the total ATP + X $\sim$ P is probably close to *142* molecules per phage particle. The error given with the X $\sim$ P values was obtained by calculating the maximal and minimal possible values for X $\sim$ P from the two mixture traces bordering the "best matched" trace actually used for calculating X $\sim$ P.

purified phage preparations. At most, cell walls appear at a level of 5×10^8 per ml in these preparations and would contribute 2 $\mu\mu$moles of ATP per ml, which would amount to only 0.05 molecule per T2 particle in a typical preparation.

Kozloff and Lute (1) found that T2 ghosts contain only 6 molecules of ATP and 2 molecules of dATP per particle and suggested that osmotic shock not only disrupts the protein head membrane of T2 but also causes the release of these nucleotides from the virus tail. T2 ghosts are produced by a two-step procedure (13), in which the virus is incubated first with 3 M Na_2SO_4 for 5 minutes and then shocked by rapid dilution with 50 volumes of water. To simulate the osmotic shock procedure, yet preserve the structural integrity of the phage particle, the changes in osmotic strength were carried out by dialysis. A T2 preparation was dialyzed for 17 hours against 3 M Na_2SO_4 at room temperature. The osmolarity of the dialysate was then decreased gradually by stepwise dilutions of the 3 M Na_2SO_4 (less than 1.0 osmole apart) until the phage was in its regular suspension solution of 0.15 M NaCl plus 0.001 M $MgSO_4$. An aliquot of the phage solution was then removed and analyzed for ATP, X $\sim$ P, and phosphate in the usual manner. It was found that the ATP and the X $\sim$ P levels had each fallen by 21%. The remaining "high salt-extracted" phage was then dialyzed against 7.8×10^{-3} M NaCl to approximate the second phase of the osmotic shocking procedure. An additional 15% of the ATP and 27.4% of the X $\sim$ P were released during this phase. The total loss of both types of high energy phosphate compounds was 45% over the entire procedure. Since the phage titer does not fall appreciably during these procedures, it can be concluded that the nucleotide binding to the virus tail structure is sensitive to these extremes of ionic environment.

ATP and X $\sim$ P Analysis of T-odd Series of Bacteriophages, λ-Temperate, and λ-Virulent

The results from analyses of preparations of T1, T3C, T5, T7, and λ are presented in Table II, and they can be summarized by the statement that no significant amounts of ATP or X $\sim$ P are present in purified preparations of these types.[3] T1 and T7 bacteriophage, purified by the usual methods of four cycles of differential centrifugation, contain less than 1 molecule of ATP or X $\sim$ P per particle. The ratios of the T1 nucleotide phosphorus to the total phage phosphorus are only 1% of the T-even series, and the T7 ATP to total P ratio of 0.46×10^{-4} is only 12% of the T-even value of 3.7×10^{-4}. The nucleotide content of the T3C preparation continually fell as the virus was purified, and the molecule of ATP and 2 molecules of X $\sim$ P per particle reported in Table II probably represent contaminant nucleotides.

T5 was found to contain 2 molecules of ATP and 3 molecules of X $\sim$ P per particle and a nucleotide P to total P ratio of 0.82×10^{-4}, or 7% of the T-even value of 11.7×10^{-4}. In view of the results with T3C and λ-virulent and the considerable difficulties in purifying T5, it appears more likely that these low amounts are contaminating bacterial compounds rather than genuine phage constituents.

The λ-temperate preparation purified by cesium chloride gradient centrifugation yielded an ATP value of less than 1 molecule per particle. The λ-virulent preparation, which was purified by the series of enzymatic digestions and centrifugations described earlier, was analyzed at various stages in the purification process. The amount of ATP and X $\sim$ P decreased at every stage, but purification could not be continued further because of the inactivation of λ which occurs upon each centrifugation. Although 4 molecules of ATP and 9 molecules of X $\sim$ P were found per particle, these most likely represent contaminant compounds.

Table II

ATP and X $\sim$ P extracted from T-odd bacteriophages and from λ phage*

Phage	Nucleotide phosphorus to total virus phosphorus ratios		Molecules per virus particle		
	ATP P to total P	X $\sim$ P P to total P	ATP	X $\sim$ P	ATP + X $\sim$ P
	$\times 10^{-4}$	$\times 10^{-4}$			
T1	0.081	0.081	0.2	0.2	<1
T5	0.32	0.50	2.1	3.2	5.3
T3C	0.46	0.76	1.1	1.7	2.8
T7	0.46		1.0		1†
λ-Virulent	2.4	4.5	4.0	9.0	13‡
λ-Temperate	0.13		0.3		<1†

* See Table I and the text.

† The ATP values for these preparations were calculated from analyses in which the flash heights were between 0 and 2 units. When there was no deflection, a maximal possible value was calculated, assuming a flash height of 1 unit. Analysis of the X $\sim$ P was not possible in these two cases. However, in all the other experiments reported, the method was sensitive enough to yield significant deflections (10 units) in spite of very low ATP and X $\sim$ P levels.

‡ This value appears to be largely due to contamination.

Phosphatase Activity of Various Phage Preparations

Although T1 and λ phages have long tails and essentially no nucleoside triphosphate, preparations of these phages were tested for their ability to hydrolyze ATP (Table III). Preparations purified only by differential centrifugation were enzymatically active on ATP, but when these virus particles were purified by density gradient centrifugation,[2] 95% of the activity was removed. The very weak remaining activity appears to be due to still unremoved host cell material and to have no physiological significance. These long tailed particles have no attributes of a contractile system.

Additional experiments were carried out to extend the initial observation of Dukes and Kozloff (4) that T2 preparations could hydrolyze ATP (Table III). Efforts to purify T2 by density

[3] In order to calculate phage concentrations, it was necessary to assume a value for the phosphorus content per particle for each virus type. These values were obtained by calculating the head volumes from dimensions available from electron micrographs (18) and multiplying the value of 1.8×10^{-11} μg of phosphorus per T2 particle (2) by the ratio of the volume of the given type to that of T2. The estimated phosphorus contents of the various other bacteriophages, expressed as micrograms of P per virus particle, are: 0.45×10^{-11} μg of P for T1; 0.36×10^{-11} μg of P for T3 and T3C; 1.0×10^{-11} μg of P for T5; 0.36×10^{-11} μg of P for T7; and 0.40×10^{-11} μg of P for λ-temperate and λ-virulent. Assays of all of the phage stocks agreed fairly well with the calculated P content. When stocks of T2, the only well characterized particle, are assayed, the virus concentration obtained is routinely 40 to 60% of that calculated from the P content. Assays of the other stocks gave viral concentrations 30 to 50% of those expected from the P values. Since all of the preparations shown in Table II have very little ATP, any error in the estimated P content will not significantly affect the conclusions presented in this paper.

TABLE III

ATP breakdown by various phage preparations

The purification of the phage particles is discussed in the text. The general procedure was similar to that described by Dukes and Kozloff (4). The incubation mixture contained 0.001 M Na_2H_2ATP, 0.08 M NH_4Cl, 0.04 M Tris, pH 7.0, and usually 2 to 10×10^{12} phage particle per ml.

Phage	Molecules of P_i formed per minute per phage particle
λ-Temperate	2.9
T1	3.1
T2	51
T2 ghosts	40

gradient centrifugation in cesium chloride were largely unsuccessful, since most of the phage was inactivated. ATPase assays of the fraction from T2 preparations recovered at the density expected for phage were, however, carried out. These fractions had significant enzymatic activity. Further attempts were made to purify T2 by blending the phage with 1,1,2-trichloro-1,2,2-trifluoroethane to remove any contaminant protein that might be present, by using the procedure of Frick and Albertsson (19). Although proteins such as albumin added to phage preparations were readily precipitated by this procedure, the enzymatic activity of the phage was essentially unchanged after blending. The possibility that a host cell enzyme might be fortuitously enclosed within the phage head was examined. Phage ghosts were found to be almost as active as whole T2. This finding supports the conclusion that the enzymatic activity in these preparations is a property of the phage contractile protein (Table III).

DISCUSSION

ATP and X ~ P have been found so far only in the T-even phages which have tail diameters of 25 mμ but have not been found either in the long, thin-tailed phages with tail diameters of only 10 mμ, such as T1, T3C, and λ, or the very short tailed, phage T7. The absence of both nucleoside triphosphates and phosphatase activity from long tailed virus particles such as T1 and λ indicates that the tail proteins of these viruses probably cannot contract. It should also be noted that contracted tails have not been observed in studies of λ, T1, or T3C. Although T5 in general seems to occupy an intermediate position and has many properties similar to the T-even phages, it still parallels the pattern of the T-odd types in the absence of significant levels of ATP and X ~ P. The T5 tail, 10 mμ × 170 mμ, has also failed thus far to show contractile properties, and although an ATPase activity has been demonstrated in T5 preparations, the enzyme resembled bacterial ATPase more closely than does T2 ATPase (4). The role of the tail protein of λ and the T-odd phages in aiding the injection of DNA into the host cell is not clear.

The function of the contractile step in the process of T2 invasion is still a matter of conjecture, but several possibilities have been advanced. These include the exposure of the phage lysozyme (20), the mechanical penetration of the host cell membrane by the viral tail core (21), and the widening of the diameter of the inner hole in the contractile protein from 70 A to 120 A (21). This considerably wider channel would provide the space necessary for the rapid release of the DNA from the head of the virus. Since the diameter of the DNA double helix is 25 A (22), an opening of 120 A would permit the passage of several (at least 10) tightly packed strands of T2 DNA. Thus the 6×10^5 A long (22) T2 DNA molecule could be rapidly released in a folded state. This possibility is more attractive than the alternative one, in which the 25 A diameter DNA molecule would have to pass through the 25 A hole in the center of the core.

Enough chemical and morphological information is now available to explain some features of the contractile mechanism in T2 tail protein. According to Brenner *et al.* (5), the volume of the contractile protein (either relaxed or contracted) is 1.3 to 1.4 × 10^7 A^3. Making the conventional assumptions that 25% of the volume is occupied by bound water and that the protein has a density of 1.3 g per cm^3, one can calculate a molecular weight of 7.8×10^6. Brenner *et al.* (5) calculated a minimal molecular weight of 54,000 for the subunits making up the contractile protein. The contractile tail structure of each virus particle would appear, then, to contain 144 ± 20 protein subunits, 142 ± 25 molecules of nucleoside triphosphate, and 160 ± 30 calcium ions (3). This stoichiometry strongly suggests that 1 nucleotide molecule and 1 calcium ion are associated with each subunit.

The similarity of the relative amounts of ATP, dATP, and UTP in the T2 particle to that in the T2-infected *E. coli* cells (23) suggests that these high energy compounds are incorporated at random from an intracellular pool during the assembly of the virus. During viral assembly, it might be thought that the subunits making up the contractile tail protein aggregate in circular rings about the tail core, forming a continuous helix of low pitch. The ATP or X ~ P molecule and calcium ion bound to each subunit appear to be concerned in this arrangement. The presence of the nucleotides seems to cause the aggregated subunits to assume an extended form corresponding to the relaxed state of the contractile protein. In the final stages of tail assembly, the distal end of the relaxed contractile protein appears to be attached to either the end plate or the core of the tail. The proximal end of the relaxed tail contractile protein is probably also fixed to some protein component at the "neck" of the virus particle.

For contraction to occur, two chemical changes are necessary, although the sequence is not clear. First, the ATP must be removed. This may occur without hydrolysis of the ATP after treatment with various reagents (1); or the ATP may be hydrolyzed to ADP and P_i by the viral phosphatase, as occurs upon the interaction of T2 with *E. coli* cell walls. Second, the distal end of the contractile protein must be freed. The removal of ATP alone is not sufficient to cause contraction. T2 "ghost" particles contain no ATP, yet they are normally extended. However, the tails of T2 "ghosts" will contract when the bonds fixing the tail to its distal end are broken, as, for example, upon incubation with *E. coli* cell walls. It is apparent that contraction will occur only in the absence of ATP and that it is independent of ATP hydrolysis. Contraction of the tail protein must, therefore, involve interaction between the protein subunits and their subsequent spatial rearrangement.

SUMMARY

1. Forty-two molecules of acid-extractable adenosine triphosphate (ATP) per virus particle were found in preparations of T2, T4, and T6 bacteriophages with the firefly luminescence assay.

2. These bacteriophages also contain a second class of high

energy phosphate compounds, X ~ P, which react with transphosphorylases present in the crude firefly enzyme preparation to transfer their terminal ~ P to ADP. A method was devised to measure these compounds, yielding an approximate value of 90 to 100 molecules per particle. The X ~ P class includes deoxy-ATP and very probably uridine and guanosine triphosphates.

3. The number of ATP and X ~ P molecules per viral particle equals the number of subunits in the T2 contractile protein. This stoichiometry suggests that 1 nucleoside triphosphate molecule is bound to each protein subunit of the contractile tail protein.

4. The very short tailed phage, T7, was analyzed and found to contain no nucleoside triphosphate.

5. The long, thin-tailed phages, T1, T3C, T5, and λ, contain little or no ATP or X ~ P, and it thus seems unlikely that their tail proteins are similar to the contractile tail protein in T2. Further, T1 and λ have no enzymatic activity on ATP, in contrast to the strong activity of T2 phages.

6. The properties of the T2 contractile protein have been discussed, and a mechanism has been proposed for the T2 tail contraction which requires that the nucleoside triphosphates act as agents to keep the quaternary structure of the tail protein in the extended state.

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