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Characteristics of Bacteriophage φV-1 Isolated from Live Virus Vaccines (40209)

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The isolation of bacteriophages from live virus vaccines was first described in 1973, and preliminary morphologic characteristics of the most frequent isolate, φV-1, were presented (1). The virus was designated φV-1 to indicate that it was the first phage (φ) isolated from live virus vaccines (V). Since that time φV-1 and two other isolates, φV-2 and φV-3, were more fully characterized with respect to bacterial host range, serologic relationships, and biophysical properties. The results of those studies are presented in this report, and the biologic effects of φV-1 on mammalian cells *in vivo* and *in vitro* are reported separately (2).

Materials and methods. Phage isolations. The standard agar overlay technique with *E. coli* C-3000 as the phage indicator was used as described previously (1).

Bacterial host range. Phage isolates were studied with respect to their relative plating efficiencies in the following *E. coli* strains: 200PS(F^-)K12, C600(F^-)K12, RL16(HRr)-K12, CR63(F^-)K12, B, FF8005(F^-)-K12, FRAG-1(F^-)K12, FRAG-1(F′*lac*)K12, W3350(F^-)K12, and ER58 (Shigella).

Growth kinetics. One step growth studies were performed with isolate φV-1 on *E. coli* CR63 (F^-)K12 using standard techniques (3).

Production of phage stocks. Large quantities of phage were prepared as follows: an overnight culture of *E. coli* CR63 was used to inoculate 1 liter of tryptone broth to produce an absorbance at 650 nm of 0.1. Following growth with aeration at 30° to an absorbance of 0.8, (8×10^8 cells/ml), phage was added to produce a multiplicity of infection (MOI) of 0.1. Aeration of 30° was continued for 90 minutes, by which time the absorbance had fallen to 0.1. Chloroform (50 ml) was added and aeration continued for 15 min. The lysate was then centrifuged for 15 min at 7000 rpm in the Sorvall GSA rotor to remove debris. The supernatant was spun at 21,000 rpm in the Spinco 21 rotor for 135 min to pellet the phage. Pellets were resuspended in 0.01 *M* Tris–Cl, pH 7.5, 0.01 *M* $MgCl_2$ (TM buffer).

Resuspended pellets from a number of 1 liter lysates were combined, centrifuged to remove debris, and re-centrifuged at 21,000 rpm for 135 min. This pellet was again resuspended in TM buffer. Finally, the phage was purified through a CsCl step gradient as follows: 5 ml of phages in TM buffer was layered over a column containing three 4 ml steps of CsCl of density 1.44, 1.54, and 1.64 g/cm^2 in TM buffer. These columns were centrifuged for 130 min at 25,000 rpm in a Spinco SW 27 rotor. The phage bands were collected and dialyzed against TM buffer to remove the CsCl.

Antisera and serologic relationships. Antiserum against CsCl-purified φV-1 was prepared in rabbits by injecting the phage mixed with complete Freund's adjuvant behind the knee. Three weeks later a second injection of φV-1 was given without adjuvant; and 10 days later the rabbit was bled by cardiac puncture. The blood was allowed to clot and the serum was removed after centrifugation. The rates of inactivation of φV-1, φV-2, and φV-3 were determined using suitable dilutions of the antisera in tryptone broth incubated with phage at 30°. The rate constant k was determined from the equation: fraction of surviving phage titer = e^{-kt}, where t is measured in minutes.

Electron microscopy. CsCl purified φV-1 was negatively stained with uranyl formate and examined for morphologic characteristics in the electron microscope. Electron micrographs of φV-1 DNA were prepared from spreads using the formamide method (4). The replicative form of φX-174 DNA served as a standard (1.64 μm long and 3.4×10^6 mol wt).

Thermal denaturation. φV-1 DNA was thermally denatured in 0.03 *M* sodium phos-

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phate buffer, and absorbance was recorded at 260 nm as a function of temperature. T_m values were determined by the method of Marmur and Doty (5).

Buoyant density. The densities of φV-1 and φV-3 were determined by equilibrium centrifugation in CsCl gradients in the preparative ultracentrifuge. Following 40 hr centrifugation at 4° in the SW39 rotor at 35,000 rpm., fractions were collected from the bottom of each gradient tube and assayed for infectivity.

φV-1 DNA was prepared from CsCl-purified phage by gentle extraction in the cold using 0.1 *M* phosphate buffer-saturated phenol. The buoyant density of φV-1 DNA was determined by equilibrium centrifugation in an analytical CsCl gradient using SPO-1 DNA (ρ = 1.742 g/ml) as a reference (6).

Sedimentation coefficient. The sedimentation coefficient of φV-1 was measured by conventional boundary centrifugation in 0.01 *M* Tris–Cl, pH 7.5, 0.01 *M* Mg^{2+} (7), and by band centrifugation through 2.7 *M* CsCl (7).

The sedimentation coefficient of φV-1 DNA in 1M NaCl was determined by boundary centrifugation at a DNA concentration of 27 μg/ml (7).

Capsid proteins. Capsid protein patterns were determined by electrophoresis on 10% polyacrylamide gels containing SDS in the phosphate buffer system of Maizel (8). For labeling capsid proteins, ^{35}S-methionine (1 mCi) was added just before infection of bacteria with φV-1. Phage was grown, harvested, and purified as described above, except that the equilibrium centrifugation step in CsCl was preceded by sedimentation of partially purified lysate onto a 10-ml CsCl cushion ρ = 1.53 g/ml (3 hr, 27,000 rpm, SW27 rotor). Gradient purified φV-1 was dialyzed vs 0.01 *M* Na phosphate, pH 7.0, and disrupted by boiling for 3 min in SDS and β-mercaptoethanol as were the markers before gel electrophoresis.

Results and discussion. Vaccine isolations. Our initial phage isolation studies were extended to include a total of 162 lots of vaccines, and as shown in Table I, the frequency of recovery (24%) of phages was in general agreement with our first estimate of 18% (1). On the basis of plaque morphology, one phage, φV-1, accounted for 95% (37/39) of the isolations. The other two isolates, φV-2, and φV-3, were considered possibly different from φV-1 on the basis of plaque morphology.

TABLE I. SUMMARY OF TEST RESULTS FOR COLIPHAGES IN LIVE VIRUS VACCINES.

Vaccine	# positive/total tested	% positive
Measles	20/55	36
Mumps	5/26	19
Rubella	12/45	27
Polio	1/22	5
Combinations[a]	1/14	7
Cumulative total	39/162	24

[a] Measles + mumps; measles + mumps + rubella.

Serology. The *k* values for the three phage isolates using antisera prepared against φV-1 were as follows: φV-1, 835 ± 75; φV-2, 724; φV-3, 745 ± 65. The values for the three virus isolates are identical within experimental error. For comparison, the same antiserum inactivated the unrelated phage, T7, with $k < 0.7$ min^{-1}, which was less than 0.1% of that observed for φV-1, φV-2, and φV-3. While we initially considered that we had three different phages from vaccines based on slight differences in plaque morphology, the serologic studies suggested that φV-2 and φV-3 were, in fact, identical with φV-1.

Biological properties. The host range of the three phage isolates was extremely broad on *E. coli* strains. The phages plated with roughly equal efficiency on *E. coli* B and on Hfr, F$^-$, and F′ K12 strains, as well as on *Shigella dysenteriae.* None were subject to either K or B restriction systems, nor were they restricted by an F′*lac* episome that restricts T7 and its relatives.

One-step growth experiments on *E. coli* CR63 at 30° gave the following results: the first free phage were seen 30 min after infection; and the first round of lysis was complete at 45 min. In low multiplicity infections the second round of lysis was complete in 90 min. The maximum burst size was 30; more frequently it was around 10 phages per infected bacterium. No increase in burst size was observed at lower or higher temperatures in the range 23°–37°. Final titers of lysates were increased two- to threefold by including either gelatin or bovine serum in the growth

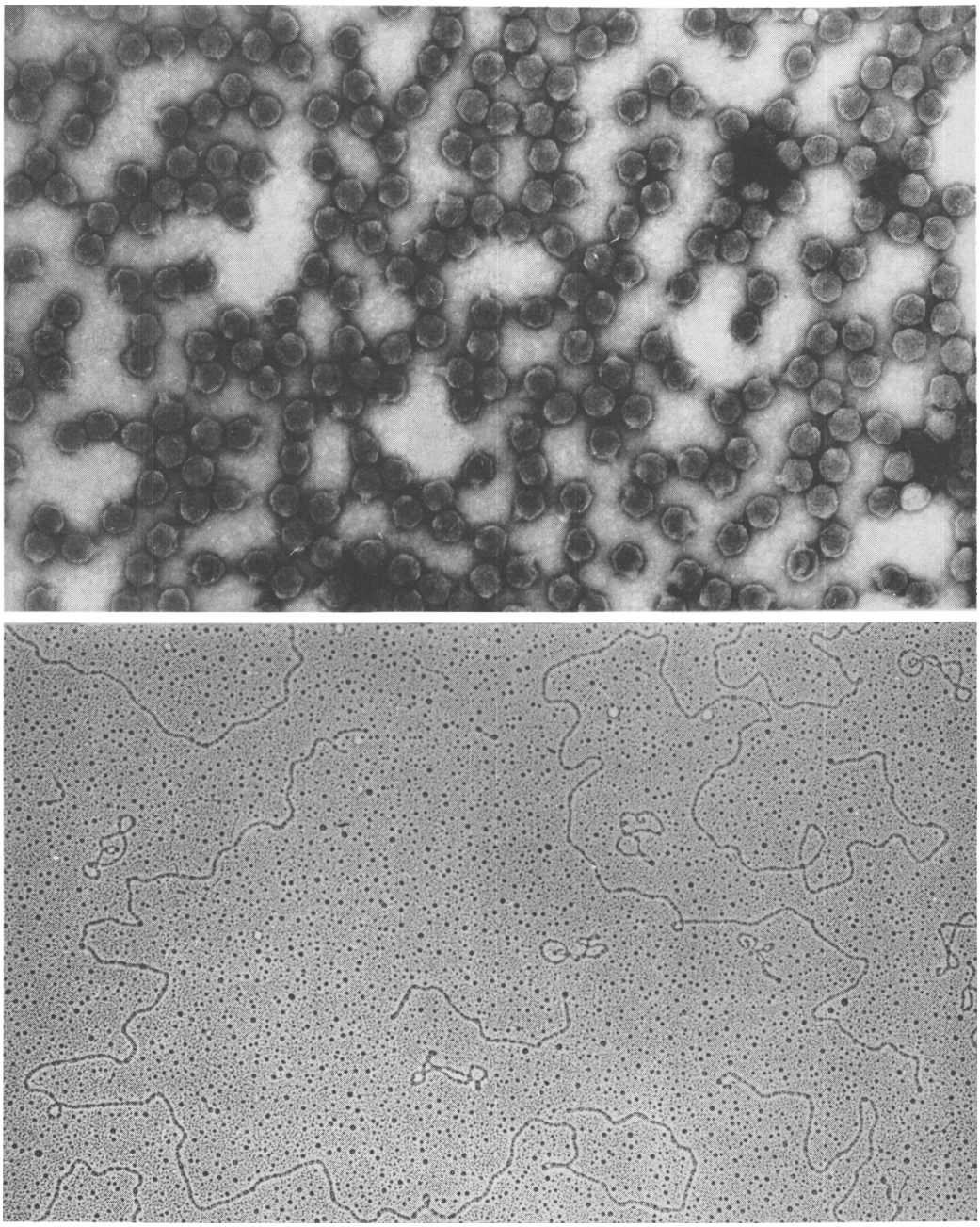

FIG. 1. Electron photomicrograph of (a) bacteriophage ϕV-1 (110,000 ×) and (b) ϕV-1 DNA (25,000 ×). The circles are the replicative form of ϕX-174 which serve as a DNA standard.

medium at concentrations of up to 10 mg/ml. The usual yield of ϕV-1 after lysis of 1 liter growths was 2–4 × 10^{10} PFU/ml. The phage was completely stable in 70% saturated CsCl for at least 20 hr at 4°, but was unstable in polyethylene glycol. ϕV-1 was completely stable in FBS at 56° for ½ hr, and lost only 20% activity after 18 hr.

Physical properties. ϕV-1 DNA melted sharply with T_m = 74.4°. Both buoyant density and T_m values correspond to a nucleotide composition of 49% G + C.

The particles are hexagonal in projection with edge-to-edge distance of 54 nm. Most have a short stubby tail although about 1% appear to have a longer, more complex appendage (Fig. 1). The phage DNA is shown in Figure 1 to be double-stranded. When compared with the replicative form of ϕX-174 as a standard (1.64 μm long and mol wt of 3.4×10^6), the ϕV-1 DNA is 13.5 μm long and has a mol wt of 28×10^6.

The average density at the peak in three ϕV-1 CsCl density gradients was 1.535 ± 0.001 g/ml. Three such gradients run with ϕV-3 gave an average density of 1.536 ± 0.001. For ϕV-1 DNA, ρ = 1.710 ± 0.003 g/ml, the uncertainty deriving from alternative methods of estimating the steepness of the CsCl gradient.

The average of two determinations of $S_{20,w}$ for ϕV-1 was 508 ± 20 S using the boundary method. By band centrifugation a single run for ϕV-1 gave $S_{20,w}$ = 627 S; and a single run for ϕV-3 gave $S_{20,w}$ = 569 S. The difference in S between ϕV-1 and ϕV-3 and CsCl is within the experimental error of the method; difference in S between ϕV-1 in the two solvents (low salt and high salt) is significant. In 2.7 *M* CsCl the virus particles either shrink or they are less hydated than in low ionic strength medium.

The sedimentation coefficient for ϕV-1 DNA was found to be 30 S. Since the concentration dependence of S has been described (9), the $S^\circ_{20,w}$ for ϕV-1 DNA was calculated to be 33 ± 1.5 S. Accepting this value for S in Freifelder's equation relating S to mol wt for linear DNA ($S = 2.8 + 0.00834 M^{0.479}$) gives a mol wt of 27×10^6. Finally, the DNA content of ϕV-1 can be estimated from the viral density of 1.535, a DNA density of 1.710, and assuming the protein shell is 1.300. If as a first approximation the densities are considered to be additive, then the calculated DNA content is 57.3%. Combining this value with a DNA mol wt of 27×10^6 gives 47×10^6 as the whole phage mol wt. A sphere of mol wt 47×10^6, density 1.535, and diameter 54 nm would have $S_{20,w}$ of about 550 S, in reasonable agreement with the measured value for ϕV-1 of 508. The physical properties of ϕV-1 and its DNA are summarized in Table II.

As shown in Fig. 2, the capsid contained a number of proteins distinguished by electrophoresis on polyacrylamide gels containing SDS. The most abundant capsid protein had a mol wt of 43,000 when compared to internal markers: cytochrome C (13,000 mol wt) bovine serum albumin (69,000 mol wt) the three viral capsid polypeptides of poliovirus type 1, VP-1, VP-2, and VP-3 (37,000; 31,000; and 26,000 mol wt respectively). When the gel was monitored by Coomassie blue staining rather than by radioactivity, only 1 phage capsid band was observed. Table III compares the molecular weights of the major capsid polypeptides of ϕV-1 with those of the

TABLE II. PHYSICAL PROPERTIES OF ϕV-1 AND ITS DNA

	ϕV-1	ϕV-1 DNA
Buoyant density in CsCl	1,535 ± 0.001	1.710 ± 0.003
$S_{20,w}$	508 ± 20 S	33 ± 1.5 S
Length		13.5 μm
Mol wt	47×10^6	27×10^6
GC content		49%

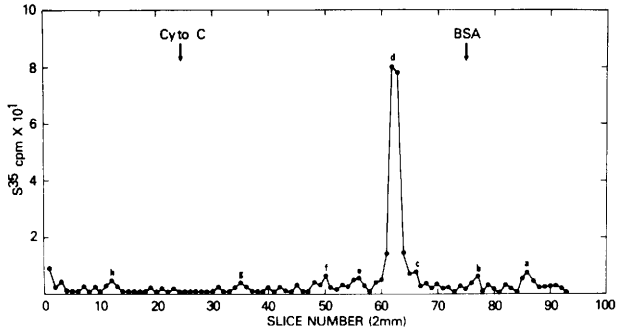

FIG. 2. Polyacrylamide gel electrophoresis of ^{35}S-labeled ϕV-1 capsid polypeptides. Each point is an average of three runs on the same preparation. The major polypeptide has a molecular weight of 43,000.

TABLE III. A COMPARISON OF THE CAPSID POLYPEPTIDES OF ϕV-1 AND T-7.

ϕV-1		T-7 (ref 10)	
poly-peptide	mol wt	polypeptide	mol wt
a	92,000	P_1	150,000
		P_2	93,000 (tail)
b	70,000	P_3	87,000
		P_4	62,000
		a	60,000
		b	
c	50,000		
d	45,000 (major)	P_5	42,000
e	35,000	P_6	35,000 (head)
f	29,000		
g	17,000	P_7	19,000
h	8500	P_8	11,000

coliphage T_7 (10). In its biochemical properties ϕV-1 is quite similar to T_7; but is unrelated to T_7 based on the serology results.

Summary. Three phage isolates from live virus vaccines were characterized and found to be indistinguishable. The phage, ϕV-1, is a small isometric coliphage with a broad host range, an edge-to-edge distance of 54 nm, and contains double-stranded DNA with a molecular weight of about 27×10^6. It is difficult to grow in large volumes; but is very stable in bovine serum.

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