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Electron Microscopy of Virulent Phages for *Streptococcus lactis*

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Electron microscopic studies were made on eight virulent *Streptococcus lactis* bacteriophages. These phages were taken as representative of eight host range groups established in a study of 75 phage isolates and 253 hosts (213 *S. lactis*, 22 *S. cremoris*, 18 *S. diacetilactis*). The phages studied were shown to have an isometric hexagonal head and noncontractile tails, usually several times longer than the head diameter. The virus heads were octahedral. The phages investigated represented three morphological types on the basis of head diameter, tail thickness, and tail length. These dimensions were approximately: for type I phages, 63, 172, and 11 nm, respectively; type II, 73, 200, and 20 nm, respectively; and type III, represented here by a single phage, 98, 551, and 12 nm, respectively. The tail surface revealed a different arrangement of the structural subunits, which lent a helical appearance to the tails of type I and II phages and a gauffered tube appearance to the tail of type III phage. The number of turns along the tail axis, turn length, axial pitch, and helix angle were: type I, 32, 12 to 13 nm, 7.14 nm, and 11° 40′, respectively; type II, 24, 24 to 28 nm, 40.00 nm, and 32° 30′, respectively; and type III, 120, 12 nm, and no visible slope towards the axis. The morphology types showed complete correlation with serological groups, but not with groups based on host range pattern.

At one time certain researchers believed that lactic streptococci bacteriophages were similar in size and shape and could not be differentiated on the basis of their morphology (5, 9, 10). More recently, other workers reported the existence of phages for streptococci that were obviously different in their overall appearance (2, 4, 6-8, 11, 12, 14; J. A. Nyiendo, Diss. Abstr. Int. B 35:999). Despite extensive literature on bacteriophages, few definitive papers on the morphology of virulent phages for *Streptococcus lactis* exist. Nevertheless, a few reports documented apparent significant differences in their morphology. Bradley (2) reported head dimorphism of a phage of *S. lactis* 3 ML. Habaj et al. (6) studied *S. lactis* phages with spherical and hexagonal outlines of the head. Tikhonenko (12) and Keogh and Shimmin (7) described *S. lactis* phages with contractile and noncontractile tails.

This study was made to define the morphology of some *S. lactis* phages and to determine if phage morphology, serology, and host ranges would provide taxonomic criteria.

MATERIALS AND METHODS

Bacteria and bacteriophages. Experiments were carried out on eight virulent *S. lactis* bacteriophages isolated from white, brined cheese factories in Bulgaria. The phages were representative of eight host range groups established in a study of 75 phage isolates and 253 hosts (213 *S. lactis*, 22 *S. cremoris*, and 18 *S. diacetilactis*). The phage isolates were found in the course of an examination of 113 cheese, 85 milk, 25 whey, and 25 starters taken from different regions of the country. The *S. lactis* strains on which these phages were isolated had been in use in Bulgaria as single-strain cheese starters for many years.

Bacterial strains for testing phage-host relationships were obtained from this Laboratory collection and from the Dairy Pure Culture Laboratory, Poland, Unigate Central Laboratory, England, and Hansen's Laboratory, Denmark.

Preparation of phages for electron microscopy. For the preparation of high-titer phage lysates, sterile skim milk was inoculated with 1% of an 18-h litmus milk culture of the host strain and with 1% of the corresponding phage diluted 10^{-2}. The inoculated milk was incubated at 30 C for 4 h, precipitated with 5 ml of 1 N HCl, and centrifuged for 20 min at 5,000 × *g*, and the supernatant, neutralized with 1 N NaOH, was passed through a Seitz filter. Titers of about 2×10^{9} to 5×10^{9} plaque-forming units/ml were obtained. Further concentration and purification of the phage was carried out by centrifugation at 44,110 × *g* for 2.5 h. The sediment obtained was resuspended in 0.2 ml of neutral 0.1 M ammonium acetate and centrifuged again at 3,500 × *g*.

Specimens were prepared and negatively stained

with 2% (wt/vol) phsophotungstic acid neutralized with potassium hydroxide. Electron micrographs were taken with a Siemens electron microscope, model Elmiscop I, at a magnification of ×28,600. The mounts and micrographs were kindly prepared by D. Bradley, Department of Zoology, University of Edinburgh.

The measurements were determined using polystyrene latex spheres 264 nm in diameter as a calibration standard. Dimensions given are mean values and vary only ±3 nm for individual particles.

Study of the phage structure. Measurements of the structural elements of the phage particles were effected by projection of photomicrographs on a special instrumental microscope, BMI-1 (USSR). The determination of the three-dimensional structure of the head was carried out by plotting a stereographic projection.

Striation of tail surface was estimated from the formula accepted for helical systems: $\text{tg } \alpha = h/\pi d$ (equation 1), where α is helix angle (i.e., the angle made by the helix of the thread at the pitch diameter with a plane perpendicular to the axis of the tail), d is pitch diameter (equal to the tail diameter in our case), and h is axial pitch (pitch of helix; i.e., the distance, measured parallel to the axis, between corresponding points on adjacent thread forms in the same axial plane section and on the same side of the axis). d and $h/2$ were directly measured on photomicrographs with the aid of a special device built into the microscope.

RESULTS AND DISCUSSION

Micrographs reveal virions with more or less clearly outlined polyhedral head shapes and a noncontractile tail several times longer than the head diameter. The overall appearance of the particles show that the *S. lactis* phages studied here can be classified by the system of Bradley (3) into group B, corresponding to Tikhonenko's group IV (12).

Measurements of the particle size and the image of the structural elements indicate that within the limits of these classifications the bacteriophages investigated can be divided into three morphological types. These are illustrated in Fig. 1.

The first phage type (I) (Fig. 2) has a head diameter of 63 nm (from side to side of the polyhedral capsid) and a tail that is 11 nm thick and 172 nm long. A head diameter of 73 nm and tail dimensions of 20 by 195 nm are typical of the second phage type (II) (Fig. 3). The third phage type (III) (Fig. 4) is the largest; it has a head diameter of 98 nm and tail dimensions of 12 by 551 nm.

Phages of the first two types are very similar in their general appearance. Electron micrographs show that their capsids exhibit a regular six-sided outline (Fig. 5). The shape of the three-dimensional structure of the head, determined by plotting a stereographic projection, is nearly octahedral. The slight bending and rounding of the contours are probably due to the existence of some additional small faces on the basic octahedral body. One can see in the center of the empty capsids a three-pointed starlike figure (Fig. 6 and 7). It exists only in the middle part of the hexagon and never goes as far as its periphery. This suggests that the head of the first two phage types represents an incompletely developed combination of three geometrical forms: tetragontrioctahedron, trigontrioctahedron, and octahedron. All these geometrical forms possess a cubic symmetry. It is shown in a diagram (Fig. 8) how the abovementioned solids may congregate to exhibit the same outlines as shown on the electron micrographs.

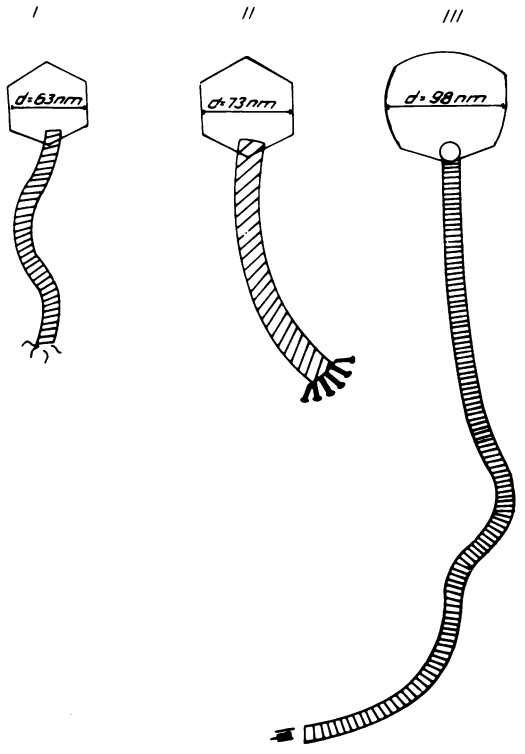

FIG. 1. *Types of bacteriophages: I, morphological type I; II, morphological type II; III, morphological type III.*

The starlike figure could also be explained in another way. If the empty head underwent a slight deformation at the moment of nucleic acid release and the capsid shrank anisotropically, the head symmetry would decrease from a cubic one to a trigonal one, and, as a result of this, an additional outline would appear.

The third morphological phage type also has

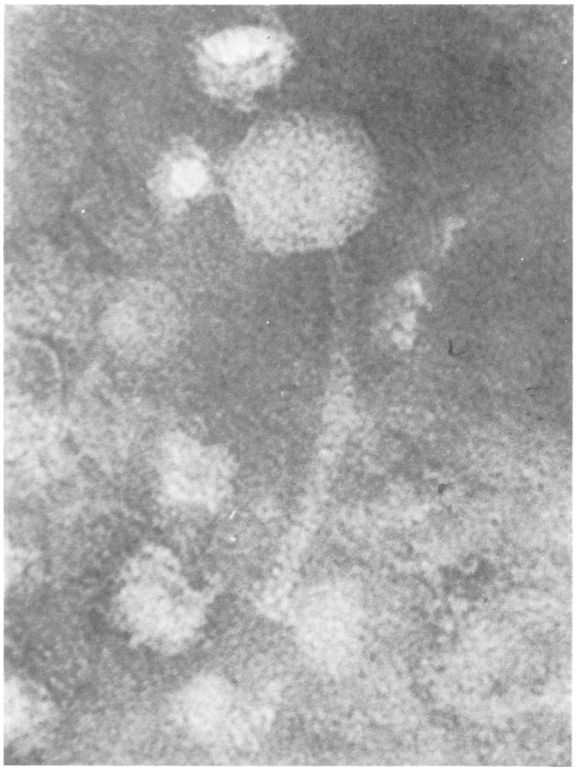

FIG. 2. *Particle of S. lactis phage B, morphological type I. ×300,000.*

a polyhedral head, which on some micrographs is seen to be nearly octahedral, but its outline is more blunted and one cannot make inferences about the actual geometrical form.

The differences among the three morophological types are particularly clear in the tail structure. The most obvious distinction is the arrangement of the structural subunits, which lends a helical appearance to the tail. At this stage of our investigations it is difficult to establish whether the thread turns observed on the tail surface are the real sections of a helix system, as has been shown in the case of other phages (3, 12). They may also emerge optically as a result of a different orientation of the capsomeres in the screw rows, if some capsomeres show up whiter than the others, giving a helical appearance (3). Yet the actual slope of the screw thread may be different.

Nevertheless, we shall consider them as sections of a helix system and, hence, draw conclusions from a mechanical formula, thus illustrating the differences in the features of each morphological type.

The tail striation of morphological phage type I has the following parameters: number of turns, 32; length of a turn, 12 to 13 nm; helix angle, 11° 40′; axial pitch, 7.14 nm. The helix angle and the pitch are graphically represented in Fig. 9a. The tail of phage type I has a cluster of short fibers at the tip.

The tail striation of morphological phage type II has parameters as follows: number of turns, 24; length of a turn, 24 to 28 nm; helix angle, 32° 30′; axial pitch, 40.00 nm (Fig. 9b). Accordingly, the thread would be a multiple-start thread. The terminal appendage of the tail is usually seen to be a cluster of fibers, but on some microphages it looks like a six-pronged disk (Fig. 6). Probably this structure is rather fragile, and the prongs could be easily destroyed into fibers in the course of specimen treatment. The cluster is wider than that of morphological phage type I.

The tail of morphological phage type III is very long and flexible, and reminds one of a gauffered tube. Morphological units are arranged in a series of annuli, equally spaced along the axis. They seem to be superimposed, without a visible slope towards the axis. The number of annuli is 120, i.e., four to six times more than the number of turns of the other two phage types. The length of one annulus equals the tail diameter, 12 nm, the width is 3

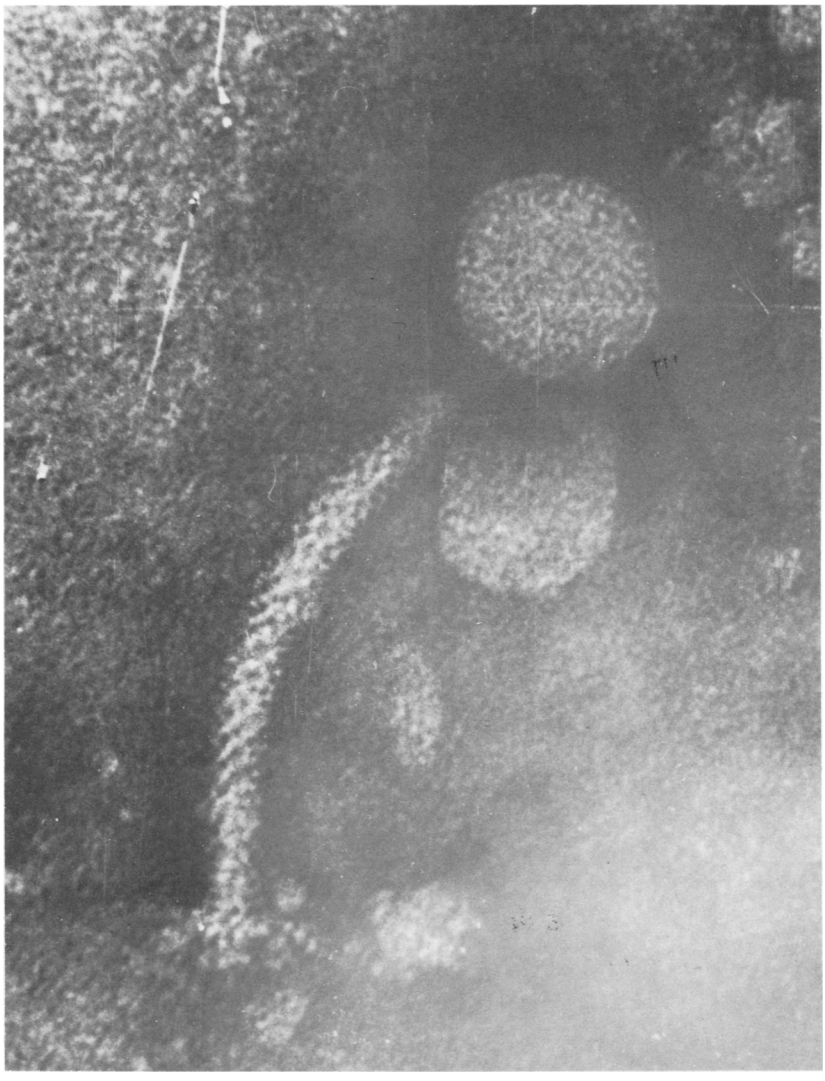

FIG. 3. *Particle of S. lactis phage K, morphological type II. ×430,000.*

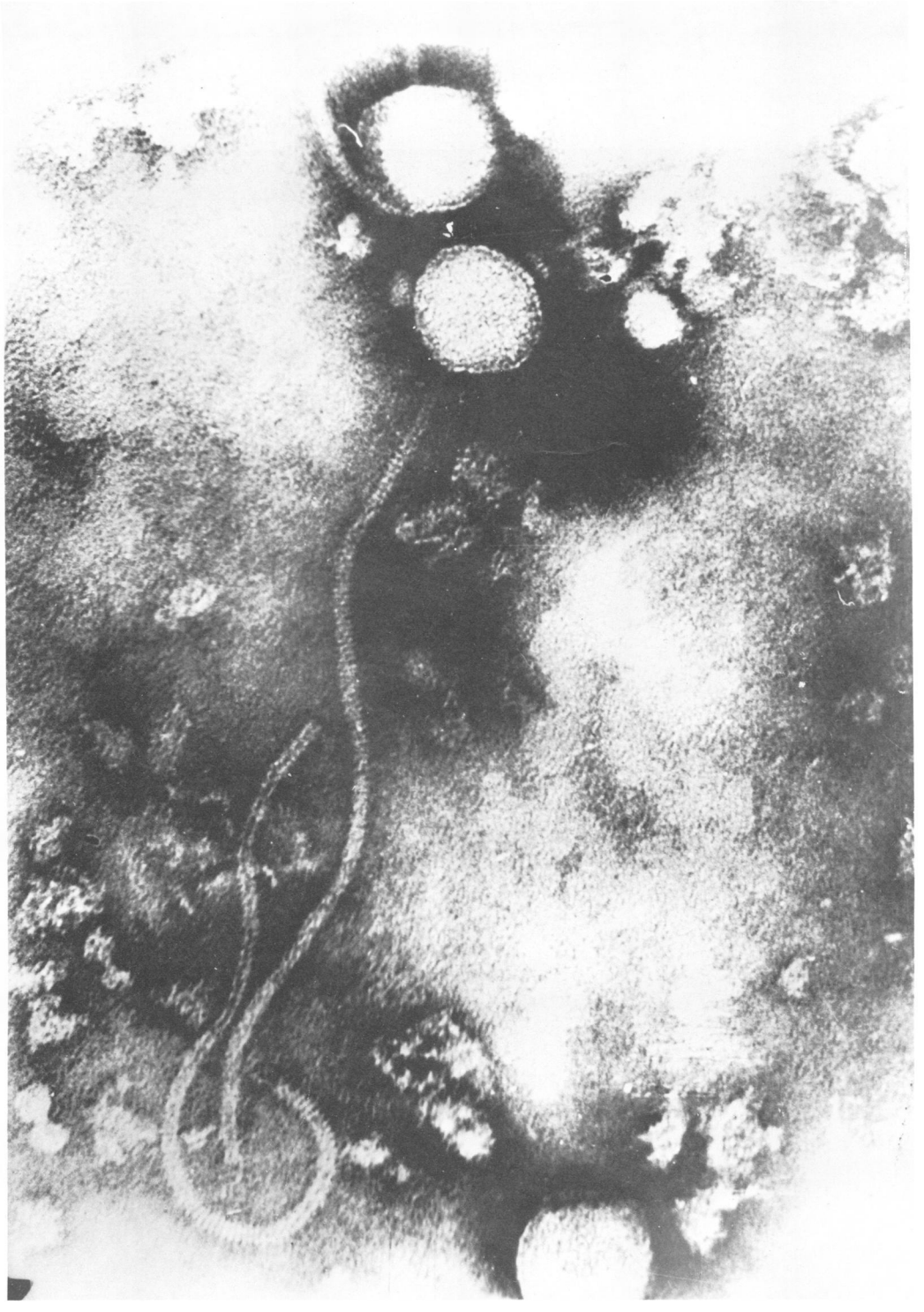

FIG. 4. *Particles of S. lactis phage P, morphological type III. ×290,000.*

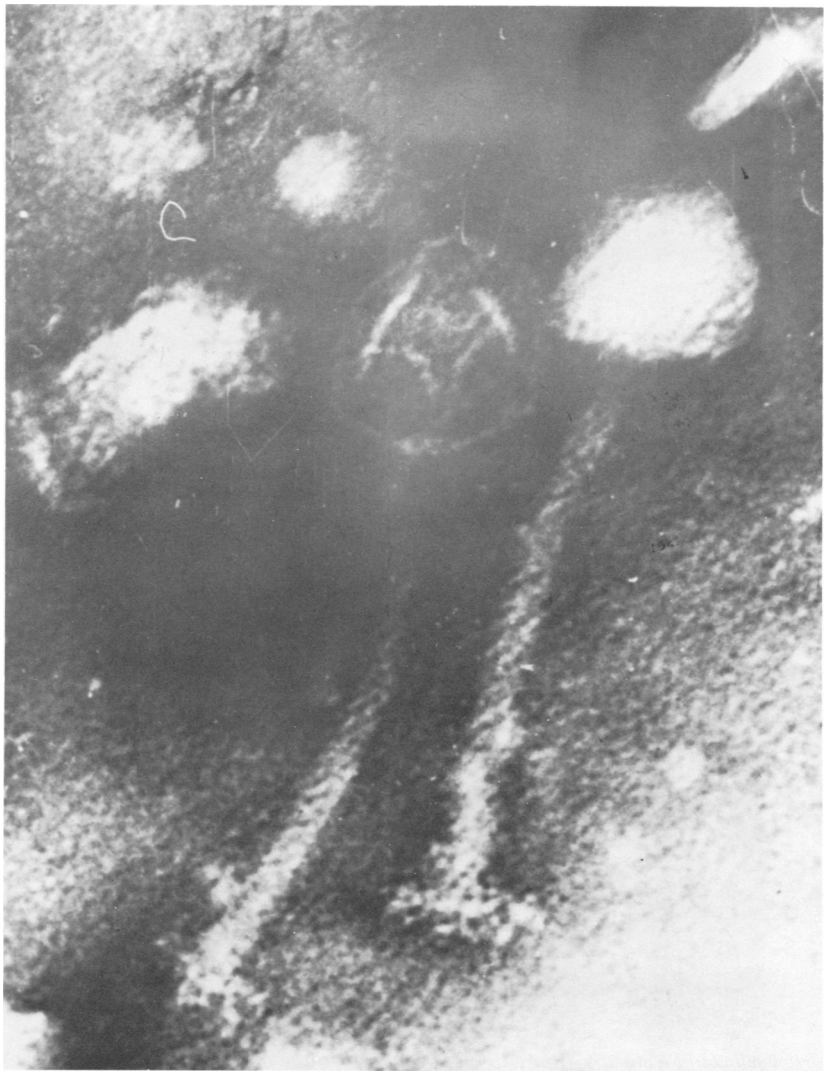

FIG. 5. *Full and empty phage particles of morphological type II; six-sided outlines of the heads visible.* ×*409,000.*

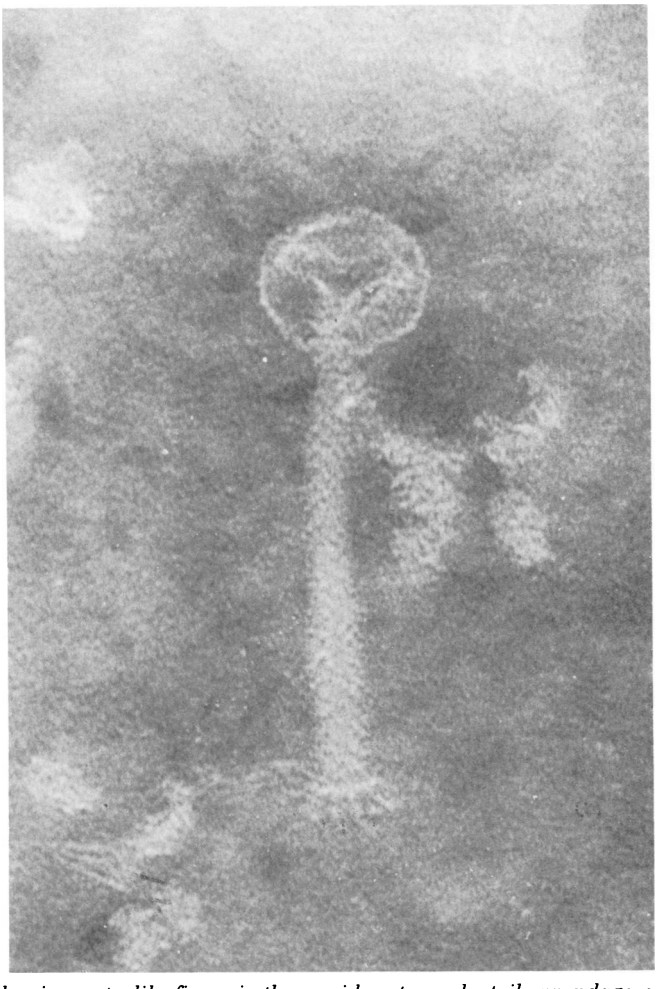

FIG. 6. *Particle showing a starlike figure in the capsid center and a tail appendage, as a six-pronged disk. ×300,000.*

FIG. 7. *Particle whose prongs at the end tail structure are probably half destroyed.* ×*435,000.*

nm, and the space between adjacent annuli is approximately 1.6 nm. There is no obvious terminal appendage, yet similar structures, resembling clusters, are visible on very few micrographs.

It is difficult to determine the tail symmetry of the phages studied, because the important details of the structure remain unknown, especially in the case of morphological types I and III. The structural elements of the phages, fitted in morphological type II, are more distinct. From the six prongs on the terminal appendix one may presume that the tail symmetry of those prongs is a sixfold radial symmetry. That assumption is in agreement with the manner in which morphological subunits are located on the tail surface: they form 24 turns, the visible part of which consists of six subunits.

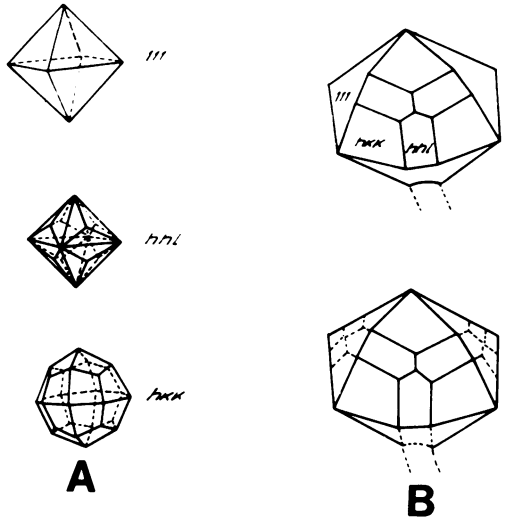

FIG. 8. *Diagram showing a posible congregation of the three geometrical forms: 111, octahedron; hhl, trigontrioctahedron; hkk, tetragontrioactahedron. (A) Outlines exhibited by the phage head as revealed by micrographs; (B) outline that should be seen in a case in which one complete combination of all three geometrical forms is developed (not to scale).*

Apart from the differences mentioned above, a pluglike structure has been observed within the empty heads as a common feature for all three phage types. It is located in the place where the tail is attached. Similar details have been described by other workers (3, 12). Tikhonenko believes that these structures are in some way related to the mechanism of nucleic acid release (12). Also, micrographs reveal a hollow channel along the tail length of some particles, free of their nucleic acid contents (Fig. 10 and 11). According to Bradley, this fact leads to the important conclusion that in the intact phage the nucleic acid strand extends from the head right down to the tip of the tail (3).

The morphological distinction of the phages studied, which has been shown here, is in complete agreement with their serological distinction reported earlier (14), but there is no correlation with groups based on host range patterns (Table 1). Morphological type I phages fall serologically into the SA group. These three phages share a common host strain, *S. lactis* Cup II, but their host ranges are quite different. Morphological type II phages are fitted serologically into the SB group. Three of them share the common host *S. lactis* Lb 7, and one is homologous to *S. lactis* Lb 5. They also belong to different lytic groups. Morphological type III phage is an *S. lactis* phage homologous to the strain 1911. This phage falls serologically into the SC group. By the length and shape of its tail it is similar to an *S. cremoris* phage, reported by Williamson and Bertaud (14), and to the phages of type X, described by Nyiendo (Diss. Abstr. Int. B 35:999).

The morphological, serological, and host range features given here are intended to be used as a stage in the selection of different types of phages for testing cheese starters in a culture rotation system.

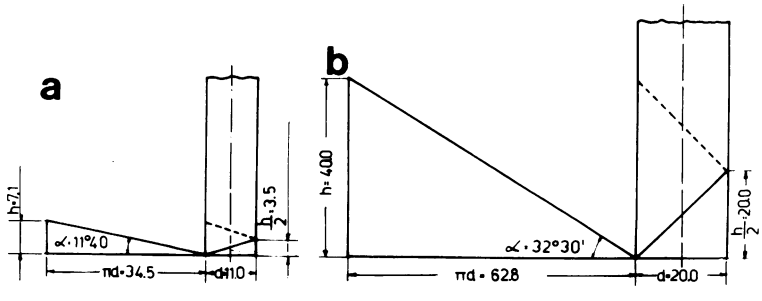

FIG. 9. *(a) Parameters of the helical system at tail of type I phage; (b) parameters of the helical system at tail of type II phage. Measurements are in nanometers. h, Pitch of helix; d, pitch diameter; α, helix angle.*

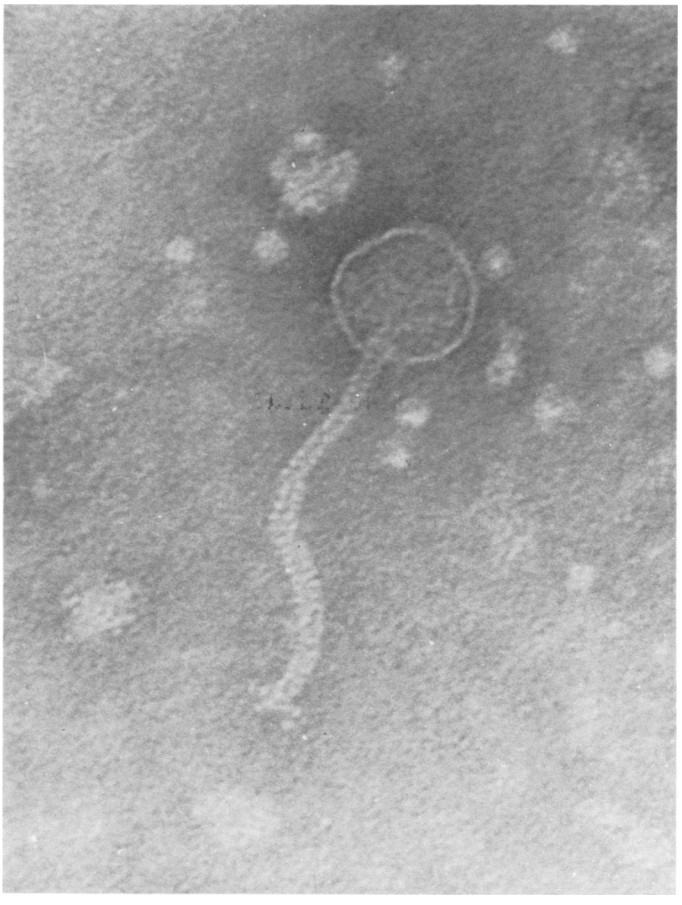

FIG. 10. *Empty phage particle of the morphological type I showing a hollow channel along the tail length.* ×*300,000.*

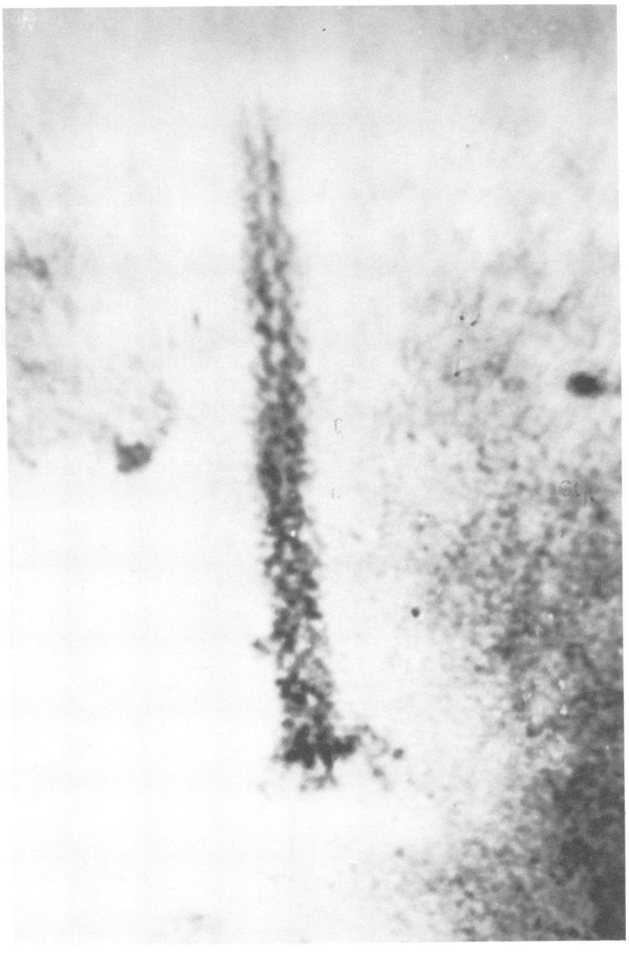

FIG. 11. *Isolated tail of phage K, morphological type II; hollow channel visible.* ×420,000.

TABLE 1. *Morphological, serological, and host range relationships of the phages*

Host range group [a]	*S. lactis* host strain	Serological group	Morphological type
I	Cup II	SA	I
II	Cup II	SA	I
III	Cup II	SA	I
IV	Lb 7	SB	II
V	Lb 7	SB	II
VI	Lb 7	SB	II
VII	Lb 5	SB	II
VIII	1911	SC	III

[a] Group from which a representative phage has been morphologically and serologically studied.

ACKNOWLEDGMENTS

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