ELECTRON MICROSCOPE STUDIES OF BACTERIOPHAGE ACTIVE AGAINST STREPTOCOCCUS LACTIS[1]

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Owing to their very small size, bacteriophage particles were not observed or photographed until the advent of the electron microscope. Electron micrographs of club-shaped objects resulting from the lysis of *Escherichia coli* by phage were first reported by Ruska (1940) and Pfankuch and Kausche (1940). Ruska (1941) found the various phages with which he worked to measure 250 to 400 mμ in total length and 60 to 100 mμ in breadth of head. Luria, Delbrück, and Anderson (1943) reported the head of the α virus of *E. coli* was 45 to 50 mμ in diameter; that of the γ virus was 65 by 80 mμ, with a tail 120 mμ long. The phage affecting the motile *E. coli* is 50 to 60 mμ in diameter and apparently has no tail. The electron micrograph of *Staphylococcus* phage presented by Luria, Delbrück, and Anderson (1943) indicates that it has a spherical head 100 mμ in diameter and a straight or curved tail 200 mμ long.

Hook, Beard, Taylor, Sharp, and Beard (1946) found that the T_2 bacteriophage of *E. coli* (strain B) in broth had a total length of 211 mμ and width of head piece of 80 mμ, and in synthetic medium a total length of 248 mμ and width of head piece of 86 mμ. Among others who have studied the phage of *E. coli* with the electron microscope are Luria and Anderson (1942), Wyckoff (1948), Mudd, Hillier, and Smith (1948), and Hillier, Mudd, and Smith (1948). The bacteriophage of *Salmonella pullorum*, as observed by Baylor, Severens, and Clark (1944), is 40 to 45 mμ in diameter and has no tail. Apparently no results of electron microscope studies of the bacteriophages active against *Streptococcus lactis*, except for the preliminary reports on the present work (Parmelee, Carr, and Nelson, 1948*a*,*b*), have been published.

METHODS AND MATERIALS

Electron micrographs of bacteriophage particles (strain F. 43) active against *Streptococcus lactis* (strain 122-1) have been made with an R.C.A. type E.M.U. electron microscope. All mounts were made on collodion membranes prepared on the surface of water and stretched over 200-mesh wire screens.

The first micrographs of the phage particles were taken of a bacteria-free whey filtrate that had a titer of 1×10^{10} phage particles per milliliter. The micrographs of phage particles in the presence of bacterial cells were made from mounts prepared from a mixture of a cell-free whey filtrate containing bacteriophage and a 24-hour culture of the susceptible bacteria in 5 per cent whey solution, the mixture having been incubated at 30 C for varying lengths of time. The number of phage particles added always was in excess of the number of

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bacterial cells. The mounts were dried on the collodion membrane at room temperature for 12 hours, immersed in distilled water for 30 minutes, and again dried. The long drying period seemed to give better fixation of the material to the membrane in the presence of the high sugar content of the whey. Washing by immersion was necessary to remove soluble whey constituents, which otherwise prevented clear observation of the specimen. The mounts for all of the electron micrographs presented were shadowed by a modification of the method of Williams and Wyckoff (1944, 1945, 1946). The shadowcasting was done with gold in a vacuum, at an angle of about 75°. Exposures were made at a magnification of 8,500 diameters, and the figures are at a magnification of 15,000 diameters except for figure 11, which is at 30,000, and figures 15a and 15b, which are at 18,000 diameters.

RESULTS

The normal cells of *S. lactis* are shown in figure 1. The mount was prepared from a 24-hour culture in 5 per cent whey, incubated at 30 C. The organisms generally occur in pairs or very short chains in this medium. The spade shape of the organisms at this magnification is of interest, because it explains their appearance as stretched cocci when observed with the optical microscope. The clear area around the cells is probably due to shrinkage of the cells during drying, as was suggested by Umbreit and Anderson (1942). Some normal cells of *S. lactis* with phage particles around them but not attached in any particular order are shown in figure 2. There is an indication of capsular material around the cells in this figure, as well as around those in some of the other figures. This picture was taken from a mount of an *S. lactis*-phage mixture that had been incubated at 30 C for 15 minutes. The length of the shadow cast by each phage particle indicates the relative height of the head and the tail. Apparently some of the bacterial cells have flattened somewhat during preparation, as the shadows vary in length and are not all consistent with the observed size of the cell.

Measurements of a number of particles of this strain have shown that the total lengths of the particles vary from 180 to 280 mμ. The spherical heads range from 60 to 90 mμ in diameter; the tails are 20 to 40 mμ wide and 120 to 190 mμ long. Most particles have a head that is 70 mμ in diameter and a tail that is 30 mμ wide and 150 mμ long.

Observations of two strains of phage from New Zealand, four strains from England, one strain from Canada, and two strains isolated at the Iowa Agricultural Experiment Station indicate that the nine strains are so nearly alike in shape and size that they cannot be differentiated on that basis.

A point of much controversy has been the mode of attack of the bacterial cell by the phage particle. Ruska (1943) reported that the tadpole-shaped "d'Herellen" adsorb tail first on the host cell. The bacillus-shaped phages of Kottmann (1942) oriented themselves perpendicularly to the surface around the periphery of the host cell, suggesting that they are adsorbed in a narrow band or that on drying they orient that way because of surface tension forces. Figure 3 shows the phage particles oriented with the tail toward the bacterial cell, which is in

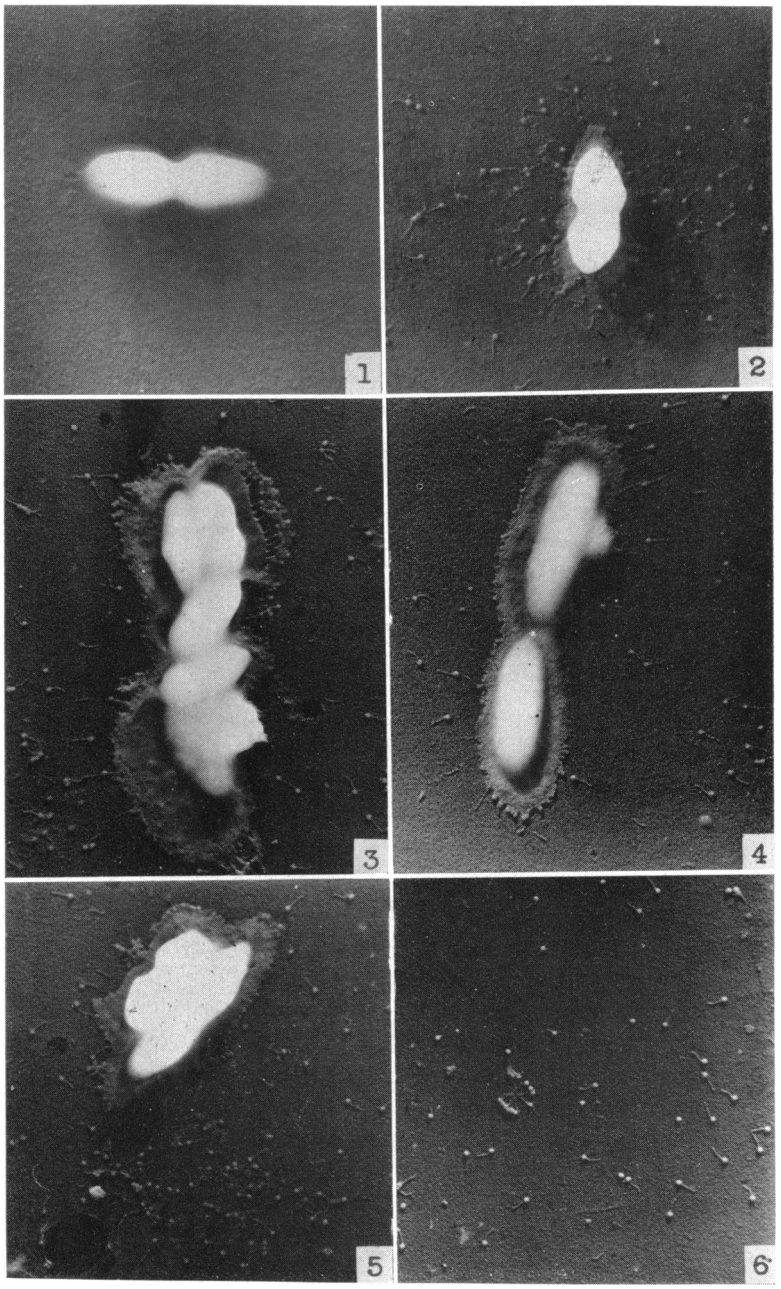

Figure 1. *S. lactis* cells from 24-hr, 30 C culture in 5 per cent whey. × 15,000.
Figure 2. *S. lactis*-phage mixture, 15 min, 30 C. × 15,000.
Figure 3. *S. lactis*-phage mixture, 1 hr, 30 C. × 15,000.
Figure 4. Phage from *S. lactis*-phage mixture, 4 hr, 30 C. × 15,000.
Figure 5. *S. lactis*-phage mixture, 4 hr, 30 C. × 15,000.
Figure 6. *S. lactis*-phage mixture, 4 hr, 30 C. × 15,000.

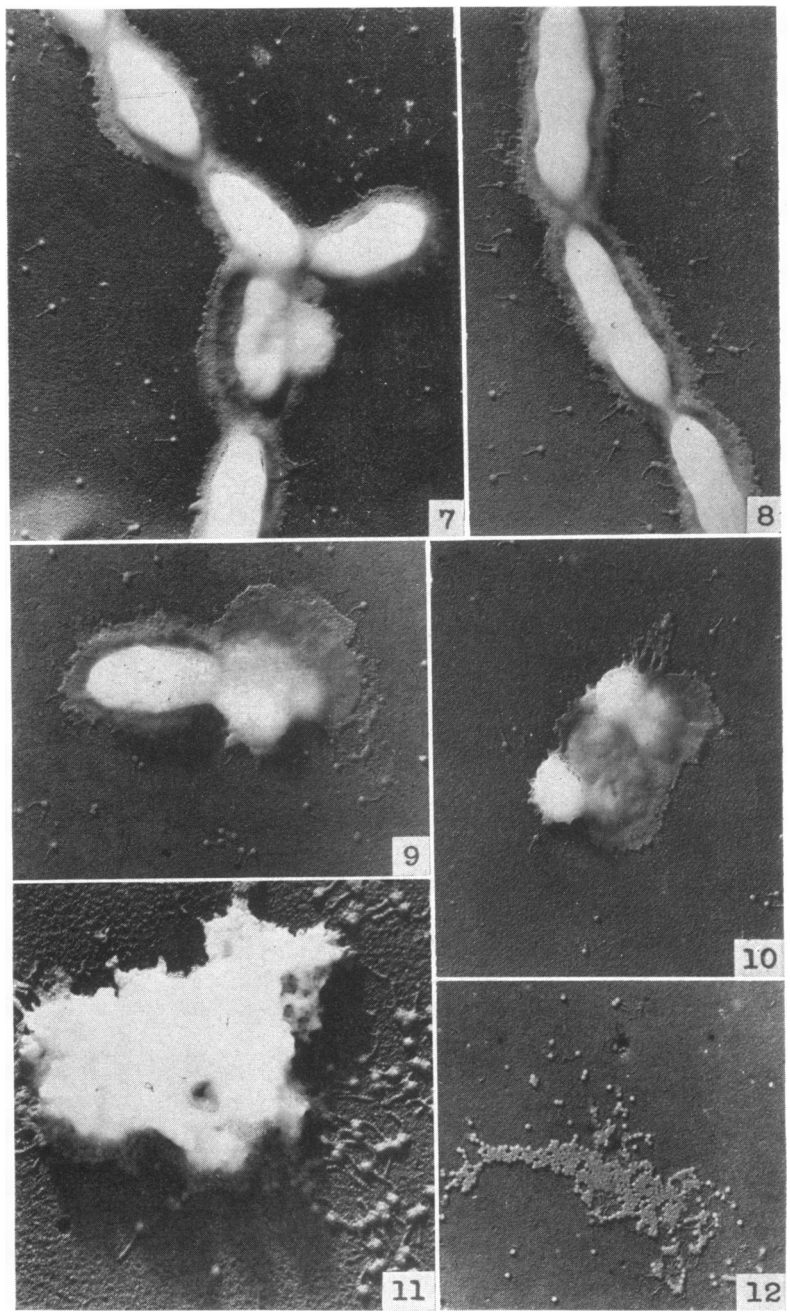

Figure 7. *S. lactis*-phage mixture, 1 hr, 30 C. × 15,000.
Figure 8. *S. lactis*-phage mixture, 45 min, 30 C. × 15,000.
Figure 9. *S. lactis*-phage mixture, 45 min, 30 C. × 15,000.
Figure 10. *S. lactis*-phage mixture, 30 min, 30 C. × 15,000.
Figure 11. Cell debris and phage from *S. lactis*-phage mixture, 5 hr, 30 C. × 30,000.
Figure 12. Phage from *S. lactis*-phage mixture, 1 hr, 30 C. × 15,000.

agreement with the observation of Ruska (1943) and the electron micrographs of coliphage of Anderson (1948). This orientation may be real or it may be an artifact of the drying of the preparation. The shrinkage of the cells in the upper part of figure 3 apparently is due to drying, whereas that in the lower part is

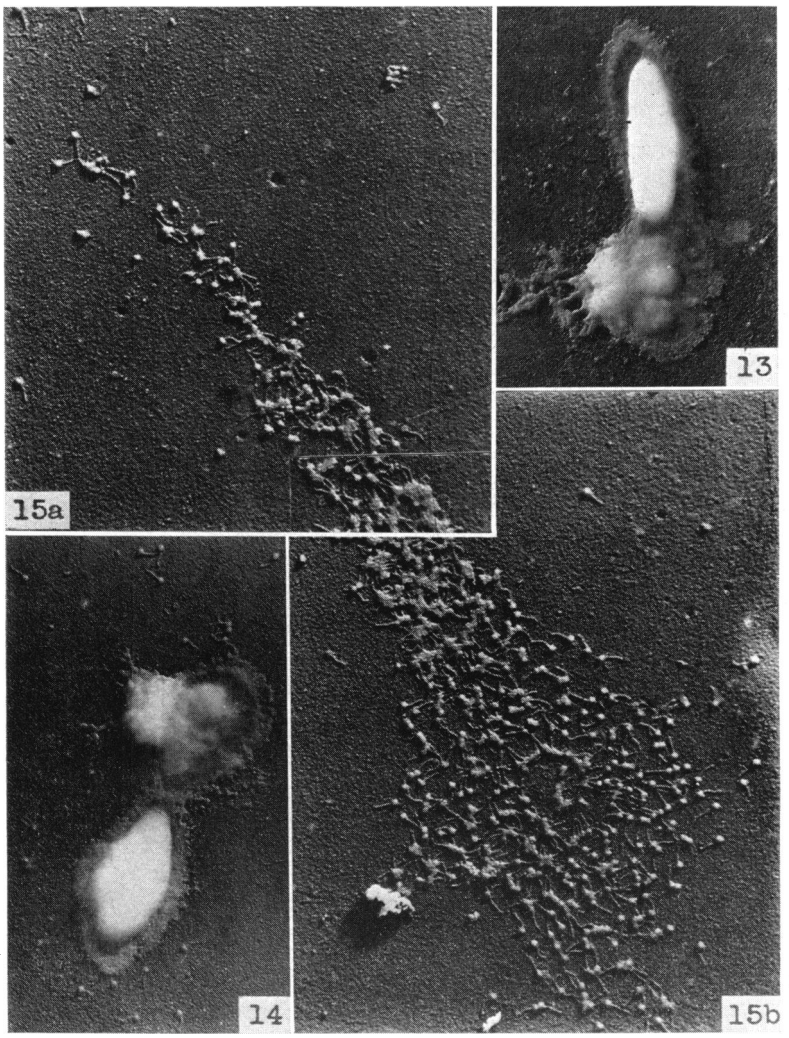

Figure 13. *S. lactis*-phage mixture, 30 min, 30 C. × 15,000.
Figure 14. *S. lactis*-phage mixture, 30 min, 30 C. × 15,000.
Figure 15a. Phage from *S. lactis*-phage mixture, 5 hr, 30 C. × 18,000.
Figure 15b. Phage from *S. lactis*-phage mixture, 5 hr, 30 C. × 18,000.

due to the bursting of the cells as the material remaining is considerably less opaque than normal undisrupted cells appear under similar conditions. The sharp point of protoplasm on the right-hand side also indicates the bursting of cells.

Bacterial cells that have been in contact with homologous phage for a time tend to become elongated, as shown in figures 4, 5, 7, 8, and 13. In figure 5 two of the cells are of normal size and shape and two are elongated. In figure 8 the cells appear to have failed to form cross walls as they should have for normal fission. This may be a stage in the development of the cell just prior to the burst.

The circular arrangement of phage particles in figure 6 has been observed upon several occasions in degrees varying from two phage particles joined by the tips of the tails, through a "Y" formation of three particles, to nearly a complete circle. The varying degrees of curvature of the tails of the phage particles as shown in the figures indicate that the tails are not as rigid structures as are the tails of coli phage, which according to Delbrück (1946) are straight or slightly curved. The curvature of the tails of the *S. lactis* phage appears to resemble that of the *Staphylococcus* phage, as shown by Luria, Delbrück, and Anderson (1943), more closely than that of any of the other phages.

The honeycomb structure which can be observed surrounding the bacterial cells of figures 7, 8, and 9 and around the burst cells of figures 9, 10, 11, 13, and 14 appears to be made of very closely packed heads of phage particles. First observation seemed to indicate that the segments of the honeycomb are smaller than the heads of the free phage particles; closer observation indicates that the dimples in the honeycomb structure are shrunken spots on the heads of phage particles, rather than interstices between the heads of particles. The shadows of the edges of the honeycomb structures indicate they have about the same height as the edge of the mass of phage heads of figure 12. The closely packed heads of phage particles in the shadow of the cell debris of figure 11 indicate that under some conditions they can be seen when not shadowed with gold. As these micrographs were made of cells in the presence of an excess of phage particles, the honeycomb structure could be the result of adsorption of large numbers of phage particles to the surface of the cell; however, it is doubtful that such large numbers of phage particles would adsorb in such an orderly manner. Similar structures have been shown for the bacteriophage of *E. coli* by Wyckoff (1948).

The bacterial cells that are disintegrating in figures 7, 9, 10, 13, and 14 give evidence that cells actually burst after they have been subjected to the activity of homologous phage.

The mass of phage particles of figures 15a and 15b apparently is another form of the mass of figure 12, both of which probably result from the lysis of one or more bacterial cells. Such masses of phage particles have been observed a number of times on mounts prepared from *S. lactis*-phage mixtures that have been in contact 4 to 5 hours.

Figures 8, 9, 11, 12, 15a, and 15b appear to indicate a possible sequence by which the cells of *S. lactis* are lysed by phage.

SUMMARY

Electron micrographs of a phage active against *Streptococcus lactis* are presented. The phage particles are sperm-shaped, 220 mμ long, have a head diameter of 70 mμ, and a tail that is 30 mμ wide and 150 mμ long. Nine strains of

phage observed with the electron microscope are so nearly alike in shape and size they cannot be differentiated on that basis. Bacterial cells in contact with homologous phage appear to elongate prior to bursting. A possible sequence by which *S. lactis* cells are lysed by phage is indicated by figures 8, 9, 11, 12, 15a, and 15b.

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