OBSERVATIONS ON THE BACTERIOPHAGY OF ERWINIA CAROTOVORA

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The lysis of Erwinia carotovora by bacteriophage has been reported by Coons and Kotila (1925), but the morphological characteristics of the virus could not be studied at that time, prior to the methods of electron microscopy, and some of the important physiological characteristics have not been investigated. In undertaking to obtain further data on these aspects of the problem, we have obtained, by usual procedures, a preparation of bacteriophage active against a strain of this organism that causes a soft rot of onions. Before studying the characteristics of the lytic agent, phage stocks were prepared by successive isolation of single plaques, as a routine procedure of purification. With the final preparation, however, electron micrographs of the material from each of a number of apparently single, well-isolated plaques revealed an extraordinary diversity of phage and phagelike particles, whereas a single type had been expected. Although the interpretation of this phenomenon is not yet clear, the essential facts, which are presented and briefly discussed in this report, are of interest and they invite further investigation. Observations on the morphology of the host cells, as revealed by the electron microscope, as well as data on the one-step growth of the virus (Delbrück and Luria, 1942) are also included.

METHODS, MATERIALS, AND RESULTS

The bacteriophage preparation was obtained, after numerous unsuccessful attempts with other appropriate sources, from a decaying onion bulb with adherent soil from a local garden. Although it might be similar to the one studied by Coons and Kotila (1925), the available data are not sufficient to judge how closely similar. Our preparation caused lysis of the bacterial cultures growing in nutrient broth, at the optimum temperature of 28 C, within 12 hours after adding the phage in approximately a 1:1 ratio with the cells of the culture. After clearing, subsequent growth of a resistant strain usually took place. Plaques in semisolid agar layers always showed considerable variation in size (figure 1). Phage stocks prepared from the large plaques gave rise to apparently the same variability of plaque size as stocks prepared from small plaques. In experiments on one-step growth at 28 C, adsorption on cells in the logarithmic growth phase

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in nutrient broth was found to be slow, requiring about 30 minutes for 20 per cent adsorption, using a ratio between the phage and bacterial cells of approximately 1:5. The latent period was long, about 1.5 hours, and the burst size small, the average yield amounting to only about 5 or 6 infective centers per bacterium (figure 2).

Electron micrographs of specimens carefully prepared from widely separated surface plaques on uncrowded plates of 1.5 per cent agar revealed the familiar picture of phage lysis, with various stages of disintegration of the hosts, from apparently intact cells to phage-littered residues. Figure 3, no. 1, illustrates the appearance of unlysed cells along with some membranous material evidently corresponding to that noted by Hillier, Mudd, and Smith (1949), in all probability representing parts of the bacterial cell wall. The dried prune appearance of the cells is in general similar to that shown by Houwink and van Iterson (1950) for Erwinia sp., but the micrograph in figure 3 indicates also the presence of dense, circular areas in each cell. The significance of these dense spots is unknown, but in some of the micrographs (figure 3, no. 2) they may be seen to occur within lighter areas, which can be identified with considerable assurance as the sites of nuclear structures (Hillier, Mudd, and Smith, 1949; Mudd and Smith, 1950). In one series of micrographs, some membranous, presumably cell

Figure 1. Plaques in soft agar layer of plate culture of Erwinia carotovora.
wall material occurred amid a tangled mass of braids (figure 3, no. 3). A braid is apparent in figure 3, no. 1, also (upper left). The braids are apparently connected to and possibly derived from the cell, but this relationship might be more apparent than real. The braids were encountered on only one occasion, which suggests that they may be artifacts. They resemble the structure of calcium salts of the soap component of certain greases (cf. Ellis, 1947; Mottlau, 1949).

Figure 2. One-step and subsequent multiplication of *E. carotovora* bacteriophage at 28°C in nutrient broth.

A typical specimen showing advanced lysis, with numerous phage and phage-like particles along with disks of membranous material, is shown in figure 3, no. 4. Close examination of the particles reveals that, in addition to some that surely must represent bacteriophage particles, there is a diverse array of other forms that are related in size and appearance to portions of the more characteristic morphological units. Individual units, enlarged photographically from the electron micrographic plate, are shown in figure 4, nos. 1 to 23.

On the basis of size and appearance, all the particles shown in figure 4, with the possible exception of the one in number 6, might easily represent phage...
Figure S. No. 1. Chromium-shadowed, unlysed cells of *E. carotovora* from a phage-infected culture. No. 2. Chromium-shadowed preparation from a phage-infected culture, showing small dense spots within the light, nuclear areas of unlysed cells. No. 3. Chromium-shadowed cell from a phage-infected culture, showing some membranous, presumably cell wall material and braids of unknown significance. No. 4. Chromium-shadowed specimen from an apparently single plaque, showing diverse morphology of phage and phagelike particles together with cellular material.
Figure 4. Nos. 1 to 23. Chromium-shadowed phage and phagelike particles from an apparently single plaque.
particles or portions thereof. Structures similar to that illustrated in no. 6 could be found in preparations from control cultures. The others seem to include particles representing only the head portion or only the tail portion of complete units—some units with heads longer than others, some with tails longer than others; some with tails at each end or with more than one tail at each end; and some dumbbell-shaped and mallet head types, as well as specimens with membranous portions at one or both ends.

With regard to the significance of the different types of particles illustrated in figure 4, there are at least four reasonable interpretations: (1) they include true phage particles, i.e., complete, potentially functional units, along with purely accidental cellular debris formed during lysis; (2) they comprise true phage particles plus portions of such particles that have been formed either in the process of phage multiplication or in the partial disintegration of the fully formed units; (3) they include several distinct, morphologically different phage types for the same host; and (4) they represent a polymorphic, single strain of bacteriophage. These possibilities are not mutually exclusive, and unequivocal evidence of the true interpretation will most likely require a great deal of further study. At the moment, however, certain points seem to be of particular interest, as follows:

Numerous lines of evidence support the conclusion that, with some bacteriophages, e.g., the T system for *Escherichia coli* (Delbrück, 1946), the fully formed particles in electron micrographs of a lysed or lysing culture are identical with the fundamental infective units that are normally reproduced in the susceptible host, and that, with a given phage type, these units have a characteristic, essentially uniform morphology (Luria, 1950, Putnam, 1950; cf. also, Ruska and Menze, 1948). Among various types that are distinguishable through physiological and other gross properties, the respective morphological units differ either widely in form, including spheres, rods, and tailed structures, or to only a minor degree in dimensions and shapes (Ruska, 1942, 1943a,b; Kottman, 1942). Thus, it is tempting to dismiss the possibility of a single strain of polymorphic phage and ascribe the diversity of forms in the present study simply to a mixture of phage types. As far as we are aware, however, no such diversity has hitherto been encountered in specimens from single plaques, and although the possibility of confluent plaques arising from different types of infective centers cannot be positively excluded, other facts argue against the presence of a mixture of viruses, e.g., the uniformity of results obtained with different plaques, the efforts towards preliminary purification of the lytic agent, and the difficulty of finding a phage for the organism used.

The characteristics of size, as well as appearance, make it likely that the particles shown in figure 4 are related primarily to phage particles rather than fragments of the host cell itself, although in some instances the latter may be present with the former. Conceivably, the different forms include atypical, non-functional particles of a single phage strain, analogous to atypical sperm cells in animals. If they include only complete and partially disintegrated units of a single type, it is difficult to believe that such diversity in size, shape, number,
and disposition of tails, etc., would arise from a process of disintegration and accidental juxtaposition of separated parts.

The possibility that intermediate stages in the formation of complete morphological units are represented in figure 4 is the most interesting, though it has little support. At the same time, it is not excluded. Forms such as those illustrated might be expected to result if a continuous thread of alternating thick and thin portions was produced in the infected cell and then fragmented into separate units. Elongated, threadlike structures associated with spheres of the same diameter, as well as seemingly transitional stages between the two, have been found in studies of animal viruses (Mosley and Wyckoff, 1946; Dawson and Elford, 1949; Chu and Dawson, 1949). On the other hand, there is recent, though not necessarily comparable, evidence that filamentous and other virus-like particles are artifacts (Angulo, Watson, and Olarte, 1950). With bacterial viruses, appearances suggestive of stages in the formation of complete phage particles from smaller units have been described in electron micrographs (Wyckoff, 1948a,b,c; 1949). For this and other aspects of the problem, however, interpretations that are fully convincing must await further evidence.

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**SUMMARY**

A bacteriophage preparation causing lysis of *Erwinia carotovora* was studied with respect to some characteristics of multiplication of the infective units and to morphology. Electron micrographs of specimens from apparently single, well-isolated plaques revealed a diverse array of phage or phage-like particles, including not only the familiar type with head or body and a single tail, but also dumbbell- and rod-shaped particles, bodies without tails and vice versa, bodies with one or more tails at each end, and others. Some morphological features apparent in electron micrographs of the host cells are also described.

**REFERENCES**


