THE ELECTRON MICROSCOPY OF DEVELOPING BACTERIOPHAGE

II. GROWTH OF T4 IN LIQUID CULTURE

by

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The first article[1] of this series contained a group of electron micrographs showing typical phenomena seen when colon bacilli, diseased with bacteriophage, are cultured on a solid agar medium. For the most part, these photographs dealt with the action of the spherical and thintailed, or tailless, odd-numbered bacteriophages. Other aspects of the growth of bacteriophage often appear when bacteria are lysed in liquid culture. They are here illustrated by electron micrographs that show various steps in the development of the sperm-like[2] T4 bacteriophage in broth cultures of *E. coli*.

The organisms used, of the B strain sensitive to lysis by all the bacteriophage strains T1–T7, have been grown in either tryptosephosphate or casein-digest broth. Because lysis is so complete and prompt with young bacteria, most work has been done with cultures from two to four hours old. Comparative experiments have, however, been carried through with cultures that were up to 24-hours old at the time of bacteriophage infection.

In an experiment, bacteria were grown for the desired number of hours and then either inoculated directly with a BERKEFELD filtered inoculum of bacteriophage or concentrated by centrifugation before addition of the lytic agent. These phage-diseased cultures were incubated for various lengths of time and centrifuged in an angle centrifuge for 10 minutes at 3000 rpm. The sedimented bacterial mass was suspended in saline, recentrifuged and the washing repeated using formalinised saline and water. The final sediment from this treatment was suspended in fluid having about one percent the volume of the original bacterial culture. In many experiments the infected culture was chilled in ice water after incubation in order to arrest lysis. For rapidly growing and metabolising bacteria, such as those in repeatedly transferred two- or three-hour cultures, lysis has proceeded so rapidly after adding a sufficiently high-titred bacteriophage suspension that few bacteria have remained intact when the interval between addition of bacteriophage and centrifugation was greater than about 20 minutes. In most experimental series this interval has accordingly been taken as 5, 10, 15 and 20 minutes.

In making a preparation for electron microscopy, the final washed suspensions have been diluted with suitable amounts of distilled water, applied as micro drops to the usual collodion-covered screens and allowed to dry. Before observation, they have been shadowed by the oblique evaporation onto them of metallic chromium or palladium, the angle of shadowing being such as to give shadows up to four times as long as the height of the detail causing them. As the photographs amply indicate, particles of bacterio-

References p. 253.

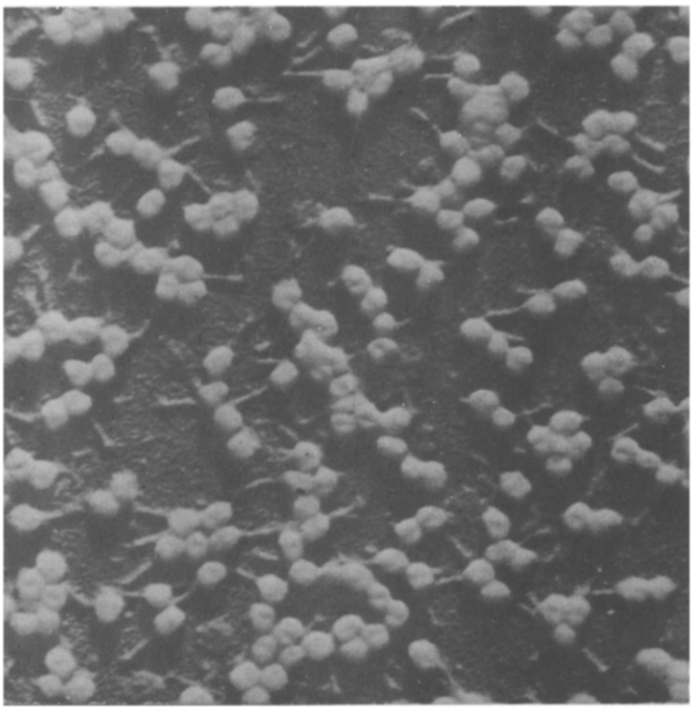

Fig. 1. An electron micrograph of an ultracentrifugally purified suspension of particles of T_4 bacteriophage against *E. coli*. The heads of these particles are ellipsoids with major and minor diameters of about 80 and about 60 millimicra. The tails, all of about the same length, appear to be aggregates of thin fibers that flare out to produce the knob seen on the ends of many tails. Palladium shadowing. Magnification = ca 45000 ×

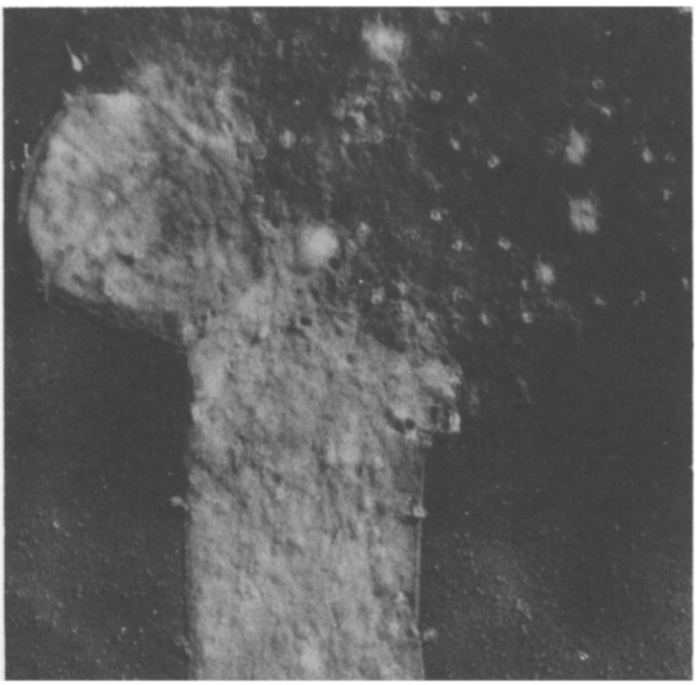

Fig. 2. Part of a colon bacillus infected with T_4 bacteriophage which is just undergoing lysis. Several developing bacteriophage particles are embedded in the protoplasm flowing from the broken cell. Chromium shadowing.
Magnification = ca 18000 ×

Fig. 3. A pair of colon bacilli soon after infection with T4 bacteriophage. Protoplasm which contains many macromolecular globules has been extruded from the bottom lysed cell. These macromolecules are also apparent strewn over the substrate surrounding the two bacilli. Mature particles of phage, probably from the infecting suspension, can be seen around the periphery of the upper organism.
Magnification = ca 18000 ×

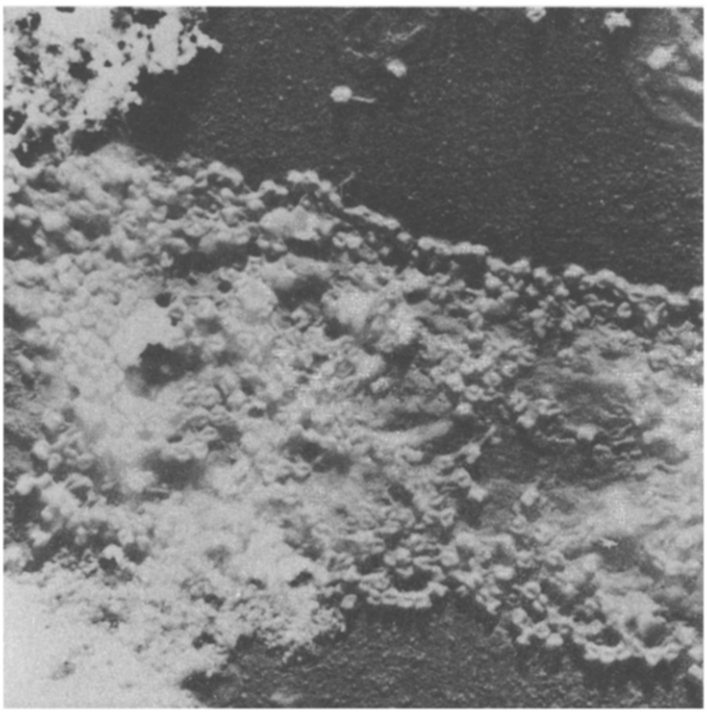

Fig. 4. Part of a colon bacillus whose cellular contents have been almost completely converted into a mass of T4 bacteriophage particles. The lytic process had proceeded for a longer time than for the two preceding figures. Fragments of cellular membranes are seen at the extreme top of this photograph.
Magnification = ca 30000 ×

phage are clearly apparent within the protoplasmic masses of such metal-shadowed colon bacilli, as well as on their surfaces.

RESULTS

Mature particles of the T4 bacteriophage are the sperm-like objects shown in Fig. 1. Their bluntly elliptical heads are terminated by tails which commonly show broadened ends. Fine detail can, under favorable circumstances, be observed both within the heads and in the tails of these bacteriophages and a subsequent article of this series will be devoted to a description of fine structure of this and the other strains of the coli bacteriophages.

Many cells undergoing lysis have been found in cultures centrifuged only 5 to 10 minutes after introduction of bacteriophage. Examples are the bacillus of Fig. 2 and one of the pair of Fig. 3. Both these cells have obviously ruptured with the extrusion of much of their protoplasm. Many photographs have been made which show bacteriophage particles either within these residues of exploded bacteria and within masses retaining the original bacterial outlines. Though they have always had the characteristic sperm-like shape of Fig. 1, these particles have had heads that varied all the way from ellipsoidal bodies of Fig. 1 to nearly empty sacks of equal diameter. An early evidence of bacteriophage within recently infected cells seems to have been the appearance of such sacks, or holes, in the bacterial protoplasm. Some of the holes may have been left when infecting particles have pealed with the membrane from underlying protoplasm. The sacks have sometimes appeared in clusters and sometimes been distributed singly throughout the bacterium. Their connection with bacteriophage is demonstrated by the tails attached to many of them. Several of these holes or nearly empty bacteriophage particles are seen in the two bacteria of Fig. 3. Similar concavities, having the diameter of particles of bacteriophage, have been common in the protoplasm of colon bacilli diseased with the odd-numbered bacteriophages; many photographs have shown them packed with an almost crystalline regularity throughout infected cells[3]. With the tailless bacteriophages it has not been possible to be sure if these holes represented developing particles or places from which matured particles had already escaped. In experiments with T4 bacteriophage the association of relatively thick tails with the vacuities has reduced this uncertainty.

Many of the holes in the protoplasm of recently infected bacteria have been quite empty (see the top cell of Fig. 3). The heads of other particles, especially those in cells diseased for a longer time, have been more substantial. Nearly empty sacks have often contained one or occasionally two granules, or a short rod-like process that sometimes has seemed a continuation of the "tail" (bottom cell of Fig. 3). These granules are less apparent in those bacteriophage particles with fuller heads that look like grains of barley (Fig. 4). There is a marked uniformity in the appearance of most particles within a single cell. If, as seems probable, the amount of matter within a bacteriophage head increases as the particle matures, then this uniformity suggests that the various particles within a cell may all be in about the same stage of development.

It is well known that bacteriophage particles are easily damaged by the desiccation involved in preparing them for electron microscopy. This raises the question of whether the sack-like particles seen in newly infected bacteria may be damaged rather than immature forms. Though no categorical answer can now be given, the difference that

References p. 253.

usually exists between mature "infecting" particles on the periphery of a diseased cell and the "developing" forms within its protoplasm lends weight to the view that the latter are stages in particle development.

The protoplasm released from lysed cells has a definite fine structure. This is well shown in Fig. 3 where the cellular contents evidently consist of innumerable globular particles of macromolecular dimensions embedded in a fibrous matrix. The globules are better seen on the edge than in the center of the protoplasmic mass, and they are most evident strewn thinly over the substrate surrounding the two bacteria of this figure. Delicate fibrous strands appear throughout the liberated protoplasm of the lower bacterium. They are still clearer in the extruded protoplasm around and to the right of the cellular residue of Fig. 6. Many observations suggest that they may be closely related to the bacteriophage tails. Colon bacilli diseased with other strains of bacteriophage exhibit the same differentiation of lysed protoplasm into globular and fibrous components. Fibres are well shown in Figs 6, 8, and 9 of the first article. At present it is not known if there is a similar differentiation in the protoplasm of healthy living bacteria; much research to determine the effects of different chemical and mechanical lytic agents will be needed to settle this question.

The number of "developing" particles in an infected bacterium is highly variable. Sometimes no more than three or four particles can be found in a bursting cell, while at other times the conversion of bacterial protoplasm to bacteriophage is surprisingly complete and yields many hundreds of particles per cell (Fig. 4). Thus the bacterial residue of Fig. 5 is nothing but a mass of developing bacteriophage particles. Observations with the electron microscope demonstrate that the variation in yield per cell is so great that estimates based on biological titration can have only statistical significance.

The photographs already discussed were made of very thoroughly washed cells. When the washing was less drastic, a deposit often was found, adhering intimately to the organisms. This deposit was sometimes in sheets containing many holes the diameter of bacteriophage particles (as in Fig. 7) and sometimes in fragments (Fig. 8). It undoubtedly contains residual salt but its inhomogeneity suggests that it may be in part a product of the bacterial metabolism. Particularly when fragmented it seems to include bits of disintegrated cell walls.

A knowledge of the fate of the bacterial membrane during lysis is especially needed when deciding if a particle is within or on the surface of an organism. The wall evidently disintegrates early during lysis and can sometimes be seen pealing from a recently infected cell (see Fig. 2 of the preceding article). Its fragments appear in most preparations; they are present, for example, at the top of Fig. 4. Instances have been met where one cannot be sure if membrane still covers a bacterium, but careful study of a shadowed preparation ordinarily leaves no doubt on this point.

The completeness with which bacterial protoplasm can, in favorable instances, be converted into bacteriophage is a most impressive result of these studies. Cellular residues demonstrating such completeness are common in preparations from cultures in which bacteria and bacteriophage have been growing together for about half an hour. As already pointed out, the erstwhile bacteria of Figs 4 and 5 have become masses of developing bacteriophage particles.

The manner in which this conversion is effected is of course the crux of the problem of how bacteriophage multiplies. These photographs bear on the problem but do not answer it. Clear-cut evidence for division by fission or by the swelling and splitting shown by

References p. 253.

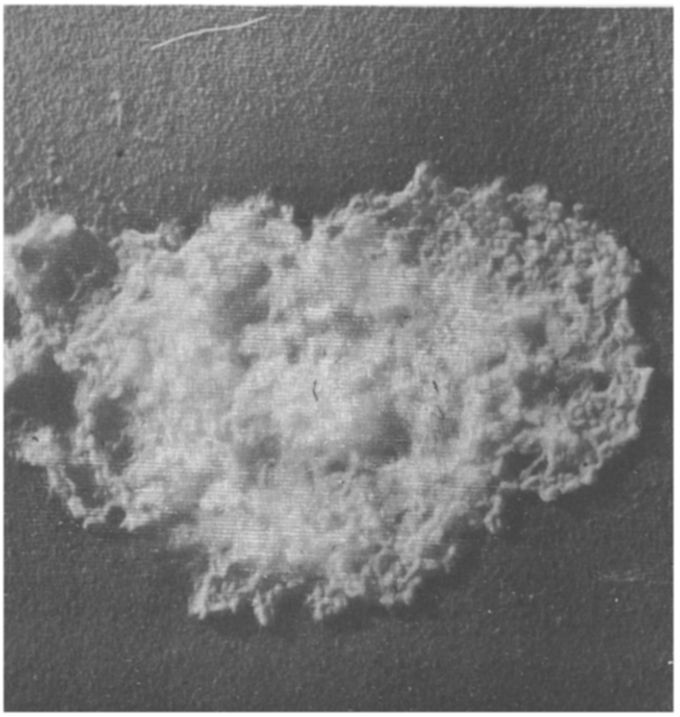

Fig. 5. The remains of another colon bacillus whose protoplasm has been almost completely converted into developing T4 particles. Granulations appear at the center of many of the heads of these developing particles. Magnification = ca 32000 ×

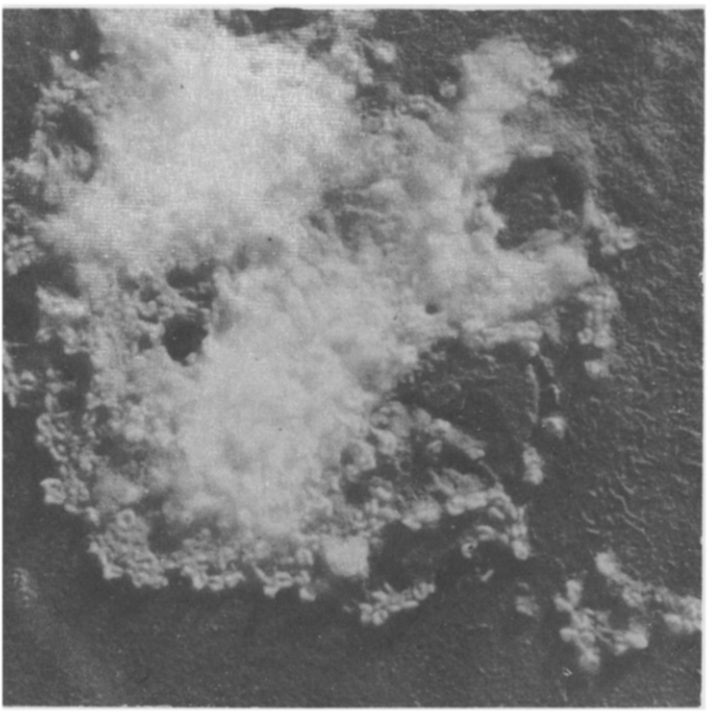

Fig. 6. Another mass of developing bacteriophage particles which marks the site of an infected colon bacillus. Extruded protoplasm, perhaps from other infected cells, covers the substrate at the top and to the right of this mass. The filaments which are a component of this protoplasm can be seen separately at the lower right-hand side of the photograph. Magnification = ca 30000 ×

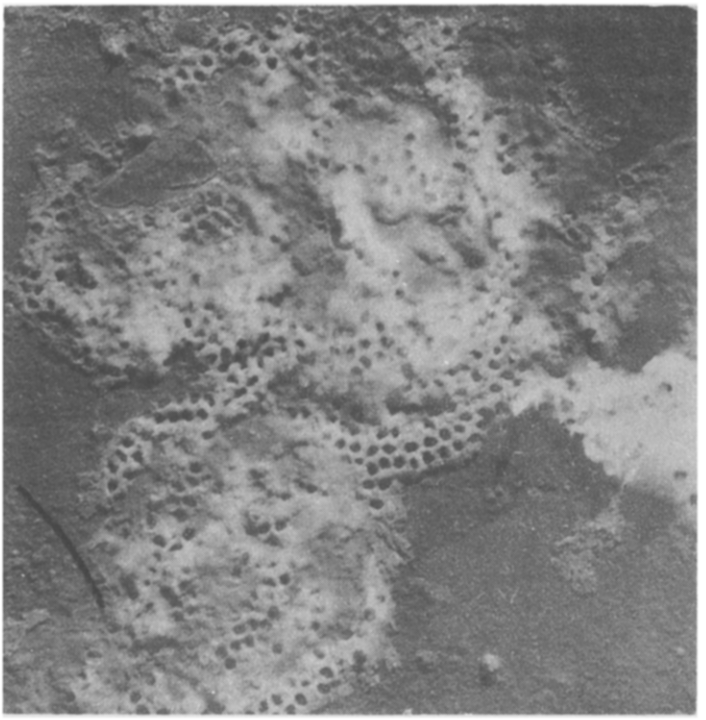

Fig. 7. The type of perforated deposit that can be seen covering incompletely washed lysed colon bacilli. Magnification = ca 18000 ×

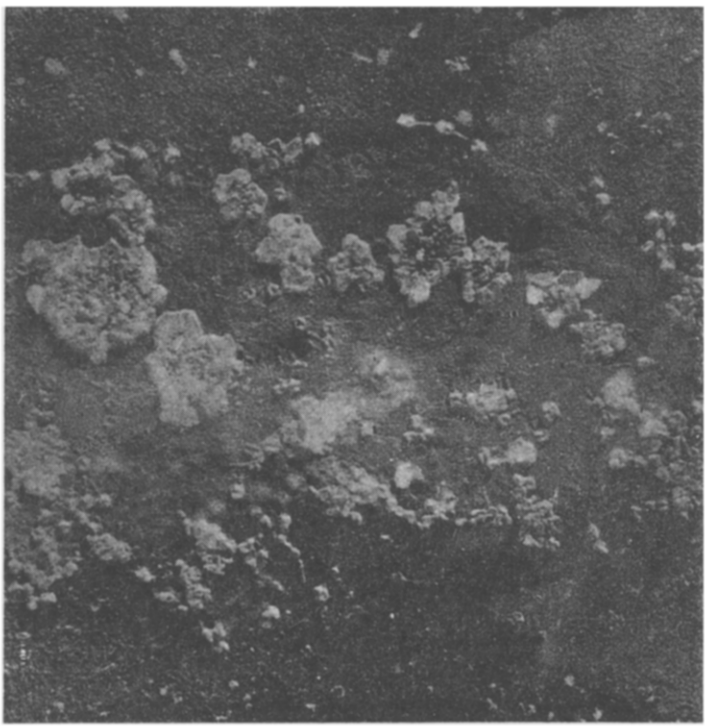

Fig. 8. Part of an infected colon bacillus with many fragments of deposit distributed in clusters over its surface. A few mature particles of T_4 lie on the substrate near the borders of the cell residue; new forms are distributed, with varying degrees of clarity, throughout its protoplasm. Magnification = ca 24000 ×

cocci has been hard to find in the several hundred electron micrographs first made to study this question. In some recently infected bacteria the "immature" phage particles are in clumps, but in others they are well distributed throughout the cells. Linear aggregates of bacteriophage particles are common within bacterial protoplasm and instances have been seen in which the tail of one particle has seemed attached to, and even penetrating, the head of an adjacent particle. But it has not yet been possible to find convincing evidence that this was more than a chance superposition of the particles. There is reason to expect, however, that more definite information about how bacteriophage particles develop can be obtained through modifications in the experiments described above.

SUMMARY

A series of electron micrographs portray steps seen in the development of T4 bacteriophage particles within infected colon bacilli growing in liquid media. These show rupturing cells, the early appearance of bacteriophage as particles with nearly empty heads, the globular and fibrous fine structure of the extruded bacterial protoplasm and instances of practically complete conversion of cellular contents into bacteriophage.

RÉSUMÉ

Une série de micrographies électroniques illustre les différents stades du développement de particules de bactériophage T4 au sein de Colibacilles croissant en milieu liquide. Ces photographies montrent les cellules en cours de rupture, la première apparition du bactériophage sous forme de particules dont les têtes sont presque vides, la structure fine globulaire et fibreuse du protoplasma bactérien s'échappant des cellules, et enfin des exemples de la transformation pratiquement complète du contenu cellulaire en bactériophage.

ZUSAMMENFASSUNG

Eine Reihe von Elektronenmikrographien bildet Stufen ab, die bei der Entwicklung von T 4 Bakteriophagteilchen in infektierten Colonbazillen, die in einem flüssigen Medium wachsen, zu sehen sind. Diese zeigen platzende Zellen, das erste Erscheinen von Bakteriophag als Teilchen mit beinahe leeren Köpfen, die globulare und faserige Feinstruktur des ausgestossenen Bakterienprotoplasmas und Beispiele von beinahe vollständiger Umwandlung von Zellinhalt in Bakteriophag.

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