THE ELECTRON MICROSCOPY OF DEVELOPING BACTERIOPHAGE

I. PLAQUES ON SOLID MEDIA

by

RALPH W. G. WYCKOFF

Laboratory of Physical Biology, National Institute of Health, Bethesda 14, Maryland (U.S.A.)

Studies of how viruses develop and multiply within their host cells can now be made with the help of the electron microscope; but to do this to best advantage it is necessary to choose a system in which the host cells are small enough to be partly transparent in present-day microscopes. Bacteria and the bacteriophages that prey on them are well suited to this type of work.

Not all bacteriophage systems can be studied with equal profit. Thus the extreme opacity of staphylococci to the 50 kV electrons with which we now work prevents seeing details of the formation of their bacteriophages. Streptococci for which bacteriophages have been isolated commonly produce so much surrounding muccoid and capsular material that, even when the cells themselves are sufficiently transparent, there is no clear vision of their interaction with bacteriophage. As photographs such as those of this paper make evident, the products of bacterial disintegration include an enormous amount of particulate material of the same order of size as the bacteriophage particles themselves. Similar macromolecular material is to be found from other healthy and diseased cells. This fact makes it highly desirable that the bacteriophages selected for investigation shall have distinctive shapes to permit their ready discrimination. The sperm-like bacteriophages fit this requirement well. Though a survey of available bacteriophage systems has shown that it is often difficult to recognize the infectious units of bacteriophage activity, this can readily be done for the tailless as well as for the tailed bacteriophages against E. coli. The electron microscopy to be described in this and the immediately following papers therefore deals with the several well-known strains[1] of this bacteriophage.

Previous examination[2, 3, 4] with the electron microscope has shown the morphology of these bacteriophages. Thus the active elements of the three even-numbered strains T2, T4 and T6 have somewhat elongated heads that are about 65 by 80 millimicra in diameter and thick straight tails about 120 millimicra long. The odd-numbered bacteriophages T1 and T5 have thinner and longer tails that are frequently curved rather than straight. The diameter of the spherical head of T1 has been given as 50 millimicra, that of T5 twice as big. Particles of the remaining two strains, T3 and T7, are still smaller spherical and tailless objects about 45 millimicra in diameter.

Much information about the development of bacteriophage can be gained from growths in liquid media. But to follow many details of its production, host and parasite must be observed in the relationship they have to one another during and after as well as before lysis. This can be done by growing organism and bacteriophage on a solid medium and employing a method of specimen preparation that reproduces the surface

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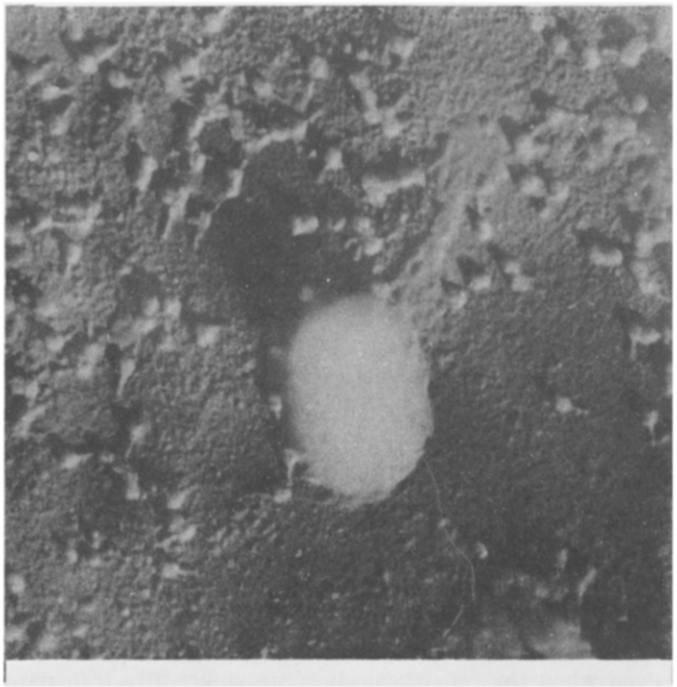

Fig. 1. An electron micrograph showing a colon bacillus with surrounding and "adsorbed" particles of T4 bacteriophage. Magnification 19 000 times

of the culture for electron microscopic examination. A technique[5, 6] that achieves this consists in making a collodion replica of the entire surface of growth on a Petri dish, mounting portions containing the desired areas on the usual supporting grids, and metal shadowing for electron microscopy. The replica is made by flooding the surface with a dilute solution of collodion in amyl acetate, draining and allowing several minutes for the resulting film to harden. If conditions are right, the film, carrying embedded in it the top-most layer of growth, will readily float off onto the surface of water. Since it is this growth itself that is then examined in the microscope, such a preparation is not in fact a replica. True replicas of the surface can also be obtained in this general way and these reproduce in collodion the surface structure of many bacterial cells, but they have proved far less instructive than the embedded growths.

Embedded "replicas" are not difficult to make. A proper degree of moisture of the culture surface is essential. If it is too wet the droplets of water covering it will be replicated rather than the organisms; if it is originally too dry or if the film is not floated soon enough, too much growth will adhere to the collodion. The collodion base should be as thin as is consistent with the required strength for subsequent handling. A satisfactory thickness results from using USP collodion diluted with from 5 to 7 parts of normal amyl acetate. The finished "replica" can be shadowed in the usual way with any one of a number of materials. Metallic chromium has been applied to the preparations giving the photographs of this paper.

The plate cultures providing "replicas" for the present work were made by spreading on nutrient or casein digest agar a mixture consisting of a few tenths of a ml of a young broth culture of E. coli and of a dilution of the bacteriophage under investigation. After

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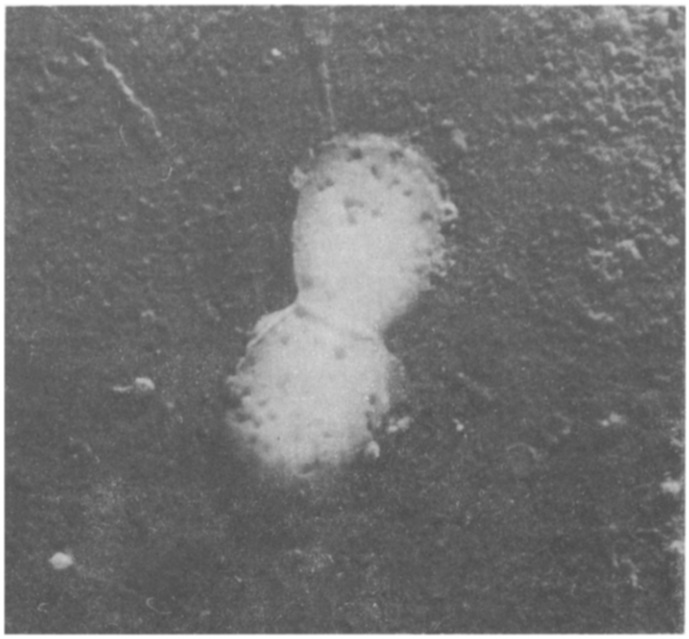

Fig. 2. A colon bacillus (about to undergo fission) from which the cell membrane has begun to peal away exposing the naked protoplasm. At least four particles of the infecting T_4 bacteriophage can be seen at the periphery of the organism. Magnification = 25 000 times

sufficient drying, inoculated plates were incubated for the desired length of time, cooled to room temperature and replicated. The organisms have always been of the classical strain B which is lysed by all seven strains of bacteriophage.

The present paper describes several of the fields most commonly observed in the plaques that develop on a solid medium. The phenomena of lysis in a liquid medium, and the results of more detailed studies of how bacteriophage particles arise from bacterial protoplasm will be given in subsequent articles. The accompanying electron micrographs provide a considerable insight into steps by which bacteriophage is produced, but the phenomena are of such complexity that it has seemed wisest to present all attainable data before discussing their interpretation.

Physiological age of a bacterium at the moment of infection and the rate at which it is metabolising are important factors in determining the picture it gives of bacteriophage production. Many of the young colon bacilli rapidly growing on the surface of an agar plate are elongated cells whose very fluid protoplasm contains little non-volatile matter. Such cells flatten as they dehydrate and are comparatively transparent to electrons. The protoplasm of older cells is more substantial and in sufficient masses is opaque under the microscope. The sol-like protoplasm of young cells often flows freely and covers large areas when freed by lysis; the protoplasmic gel of an old bacterium may not appreciably alter its shape after lysis has removed its enclosing wall. When seen within sol-like protoplasm particles of bacteriophage are sometimes dispersed, but they are closely packed in their association with denser protoplasm.

The phenomena accompanying lysis depend in striking fashion on the strain of

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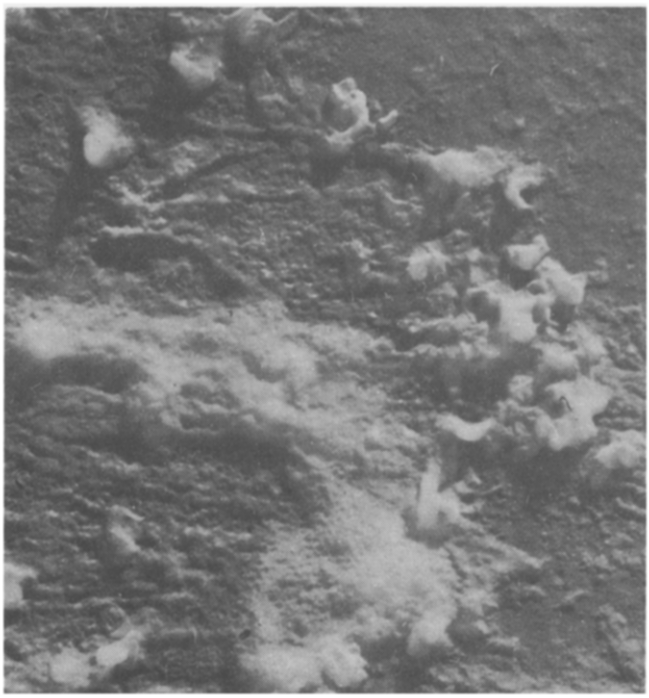

Fig. 3. Filamentous protoplasm resulting from lysis by T3 bacteriophage. The area in the upper right corner is the empty center of a plaque. Separate opaque pieces casting long shadows such as those clustered together at the right of center are bacterial membranes. Structure can be seen in the filaments especially in the region just above the central mass of protoplasm. In the mass at the bottom this structure forms a net. Magnification = 21 000 times

bacteriophage. Infection with the thick-tailed bacteriophages T2, T4 and T6 produces very different fields from those caused by the tailless T3 and T7 or by the thin-tailed T1 and T5 strains. Though macroscopically alike, plaques produced by one strain may look very different under the electron microscope. Thus the floor of one plaque may be completely empty, that of another may be thickly studded with particles of bacteriophage while a third may be covered with a layer of bacterial debris that is sometimes structureless and sometimes possessed of an obvious macromolecular structure. Much study of many preparations is needed for a adequate understanding of the essential phenomena.

It has been difficult to obtain electron micrographs showing initial steps in the infection and disintegration of bacterial cells. Photographs made soon after mixing bacteriophage and growing organisms often reveal particles at cell boundaries (Fig. 1) or embedded in protoplasm from ruptured bacteria, but none has yet demonstrated how a particle can penetrate an intact cellular membrane. On the other hand, bacteria from which the cell membrane is peeling to expose underlying protoplasm are common (Fig. 2). Large areas, of which Fig. 3 is typical, are strewn with these shed and curled-up membranes.

The protoplasm of lysed bacteria ordinarily has an obvious fine structure. This is true no matter whether it retains the form of a single organism or has merged into

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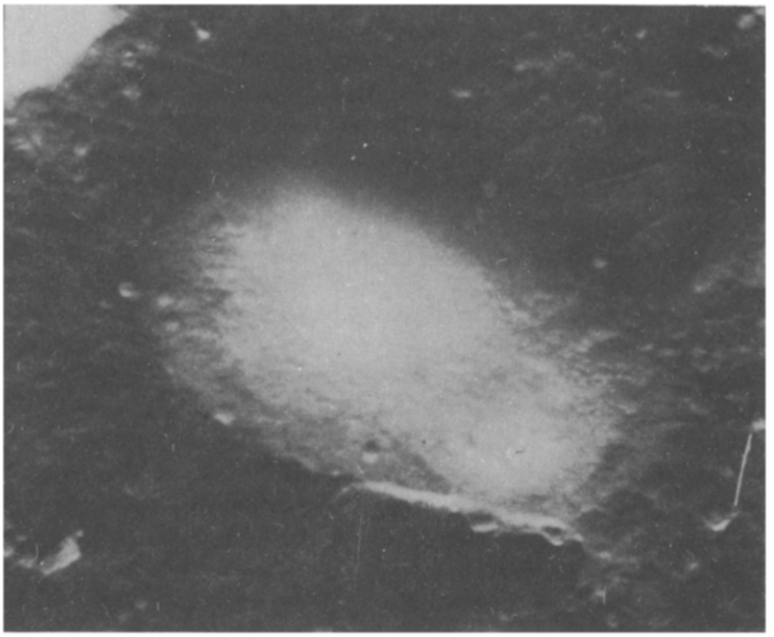

Fig. 4. A bacterium diseased with T3 showing what appears to be an early stage in the formation of the honeycomb structure. Concavities are especially evident at the right hand end of the cell. Several particles of bacteriophage appear on the surface of the organism and on the surrounding background. Note the many flagella-like fibers in the protoplasmic mass of this background. Magnification = 45 000 times

large confluent masses. Various stages can be seen in the development of this structure, which varies from bacteriophage to bacteriophage and under certain conditions has an extraordinary degree of regularity. It consists of elements the size of phage particles. They usually appear as concavities and frequently are lined up in rows or in regular networks throughout a protoplasmic mass. A few of these pits arranged linearly are at the right of the bacterium of Fig. 4; they extend, though seen with difficulty, throughout the cell. The entire protoplasm of many diseased bacteria (Fig. 5) consists of parallel rows of these linear elements which at places seem to be breaking up into separate particles.

Much of the protoplasm freed by lysis is filamentous. It is present in extensive masses on the periphery of plaques and reaches in long fingers into the central clearer areas. Some of it is structureless, other filaments have a fine structure that relates them to the rows of particles just discussed. Structure of this sort is, for example, readily discernible throughout the top half of Fig. 4. Many elongated bacteria, after infection, are largely a bundle of filaments. This is the case with the cell in Fig. 6 where the many longitudinal filaments are also crossed and perhaps held together by similar transverse fibers.

In the lower left-hand part of this figure the two systems of fibers build a regular oblique net. Such nets are frequent after lysis with all but the thick-tailed bacteriophages T2, T4 and T6. Their geometrical regularity is greatest with the tailless bacteriophages T3 and T7, but symmetrical patterns exist in protoplasm diseased with T1 and T5.

Plaques caused by the thick-tailed bacteriophages T2, T4 and T6 also contain

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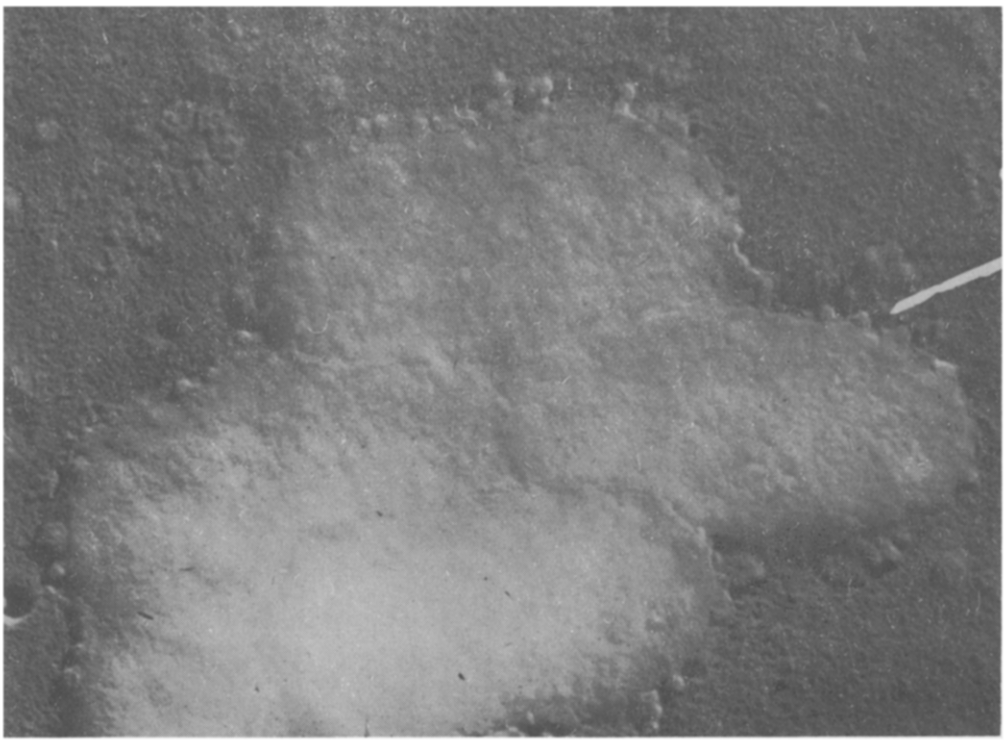

Fig. 5. A small group of bacteria on a plaque produced by T2. Their protoplasm consists of parallel diagonal rows of concavities. Nearly free spherical particles at the periphery of the bacteria have the shape and size of the particles of bacteriophage. Magnification = 35 000 times.

groups of concavities (Fig. 7) which sometimes maintain the outlines of a single bacterial cell and at other times are found in larger masses. Frequently these concavities are in a linear array; they have never been seen to form symmetrical nets. Many of them have tails and among them are all stages between empty-appearing concavities and fully formed sperm-like particles.

An electron micrograph reproduced in an earlier note[7] showed a symmetrical net extending throughout an entire bacterium infected with T3. Another honeycombed bacterium is shown in Fig. 8. In the previously shown cell the net is regular and little distorted over large areas of cell; in the bacterium of Fig. 8, on the other hand, the network has a constantly changing direction.

These nets are also found in protoplasmic masses which do not retain the outlines of the bacteria from which they have been derived. Such extracellular nets occur at the bottom of Fig. 3 and in the second figure of the earlier note. They often cover such large areas that their development must have proceeded after cellular disruption had occurred.

As the electron micrographs indicate, the meshes of these nets are for the most part shallow concavities with a few deep holes and some mounds that have the diameter of isolated bacteriophage particles. Small regions are occasionally encountered in which convexities predominate.

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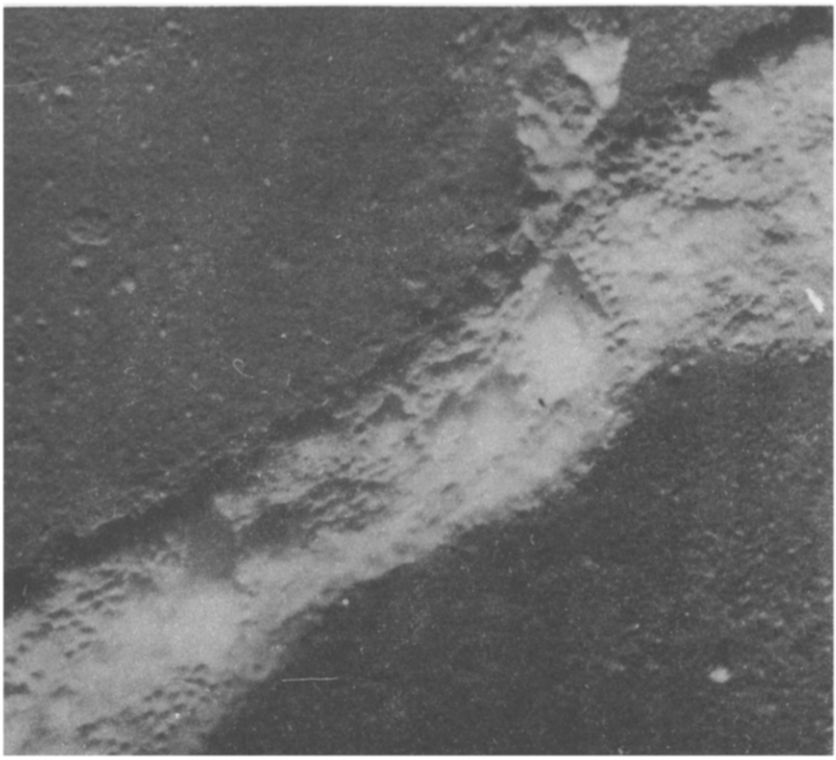

Fig. 6. A portion of an elongated bacillus diseased with T7. The many longitudinal filaments are clear. Several cross filaments, weaving through these, are above and to the right of the oblique net in the lower left corner. Note the free longitudinal strands just below the right hand portion of the cell. Magnification = 36 000 times.

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Fig. 7. Groups of developing particles of T4 resulting from the infection of single bacteria. Many mature particles are scattered over the clear intercellular spaces. Similar fields have been shown in the paper of EDWARDS and WYCKOFF. Magnification = 16 000 times

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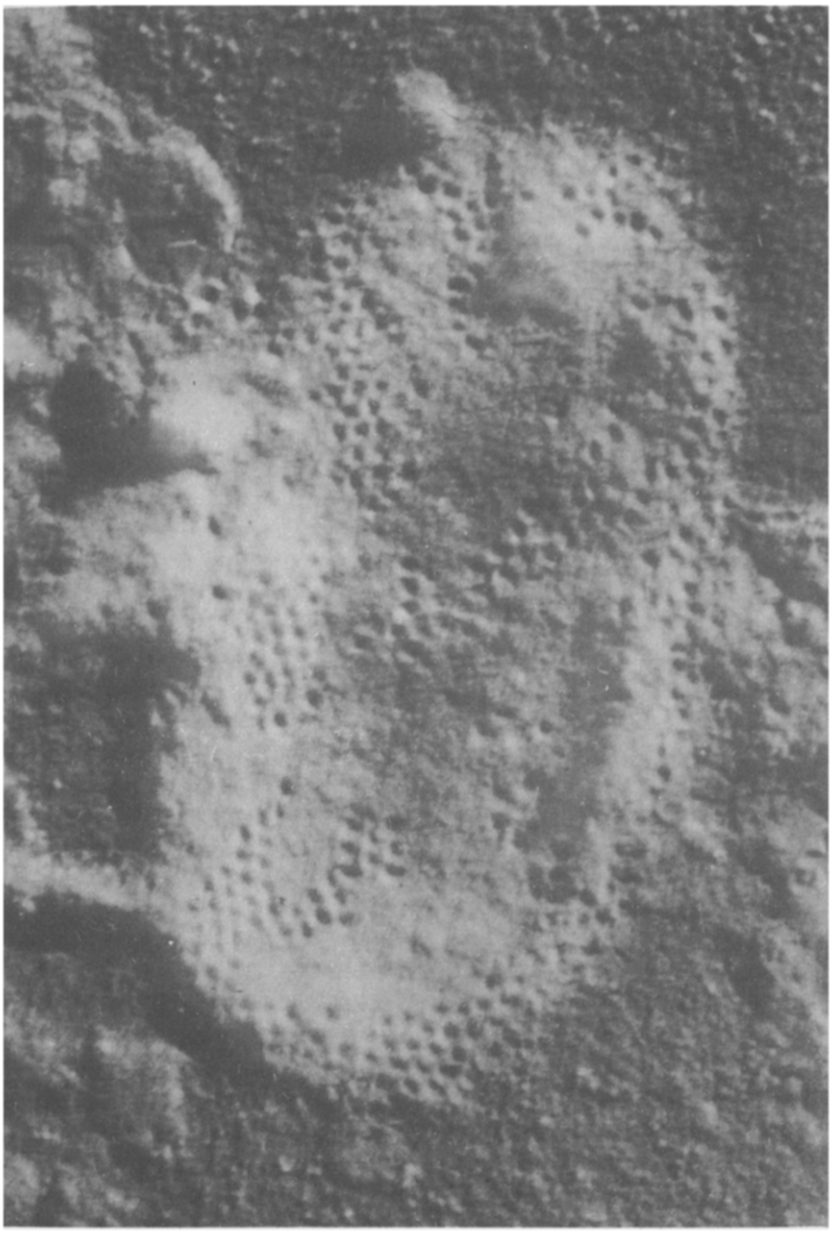

Fig. 8. A single bacillus diseased with T3. Note particularly the many fine fibers that cross one another in the central, nonhoneycombed areas and that apparently remain behind to form walls to the net left by emerging particles of bacteriophage. Magnification = 60 000 times

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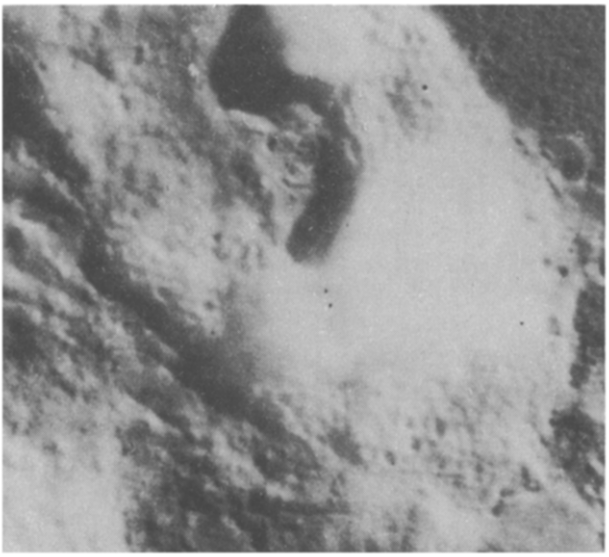

Fig. 9. Part of a bacterium after infection with T3. The fibrous character of the walls of the net and the interlacing of these fibres is evident at several points. Magnification = 40 000 times

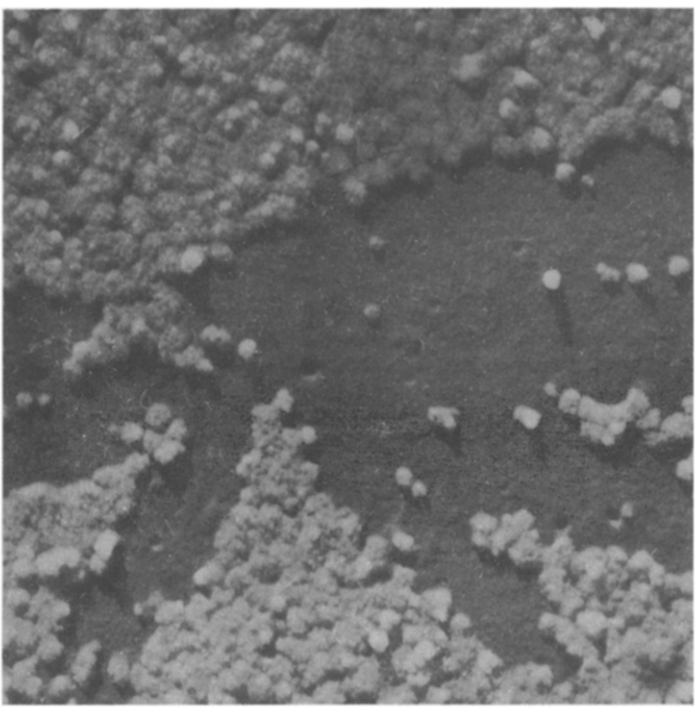

Fig. 10. A field of a plaque obtained by lysis with T5. The granular texture of these fragments of bacterial protoplasm is clear. At the center is a clear area of the plaque. Magnification = 24 000 times

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The filaments that form the walls of these nets are not always apparent, but they are evident in especially clean and well-shadowed preparations. A striking feature is the way these fibers often intertwine, almost as do the warp and woof of cloth. This can be seen in the part of a cell reproduced in Fig. 9 and in the bottom right-hand corner of the cell of Fig. 8.

A close examination of Fig. 8 shows that the parts of its cell which do not partake of the honeycomb also are crossed by fibers similar to those making up the walls of the net. These fibers fall into two groups, one running lengthwise of the cell, the other transversely across it. The members of each group are roughly parallel to one another, but they do not form an equi-spaced grid. Similar systems of threads can be seen on other cells and in the free protoplasmic masses. The omnipresence of these fine filaments raises the question of whether they and the networks they form are brought into being by the process of infection with bacteriophage or are pre-existing structures made apparent as a residue that is not utilized in bacteriophage production. Further evidence bearing on this point will be given in a later paper.

Most of the other fields in plaques consist of lysed protoplasm. Where it is not filamentous, it is in sheets or thick masses. Some of this is devoid of structure, but much of it has a definite macromolecular structure. After infection with T1 or T5 such granular protoplasm often breaks up into particles that pack closely to produce fields like Fig. 10. This fragmented protoplasm exists within seemingly intact bacteria as well as in extracellular aggregates. Bits of regular nets have been observed within some of the larger pieces, but the place of this fragmentation in the general process of bacteriophage production is not yet clear.

SUMMARY

This paper is the first of a series describing electron micrographs made of various stages in the development of bacteriophages from the infected cells of E. coli. It describes the fields most commonly seen within plaques produced when E. coli and bacteriophage grow together on a solid medium.

RÉSUMÉ

Cette étude fait partie d'une série d'articles décrivant des micrographies d'électrons faites à divers stades du développement de bactériophages de cellules de E. coli infectées. L' auteur décrit les champs que l'on voit généralement à l'intérieur des taches produites quand E. coli et le bactériophage croissent simultanément sur un milieu solide.

ZUSAMMENFASSUNG

Die vorliegende Arbeit ist die erste einer Reihe, die Elektronenmikrographien, die von verschiedenen Stadien in der Entwicklung von Bakteriophagen aus den infektierten Zellen von E. coli aufgenommen wurden, beschreibt. Sie beschreibt die Felder, die man am häufigsten in den Flecken sieht, die entstehen, wenn E. coli und Bakteriophag zusammen auf einem festen Medium wachsen.

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