ELECTRON MICROSCOPE STUDIES OF BACTERIAL VIRUSES[1]

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Physico-chemical (Schlesinger, 1933; Northrop, 1938; Kalmanson and Bronfenbrenner, 1939) and biological (Ellis and Delbrück, 1939) studies of bacterial viruses (bacteriophages) in the last few years have led to a revival of interest in this group of viruses, particularly as a material on which one can study under very favorable conditions properties which may be common to all viruses. Ease and accuracy of titration and the possibility of working under biochemically controlled conditions make bacterial viruses an ideal object for such investigations.

The electron microscope, recently introduced as a tool for biological research, has been applied to the study of animal and plant viruses, and also of bacterial viruses (Ruska, 1940; Pfankuch and Kausche, 1940; Ruska, 1941; Luria and Anderson, 1942). Ruska (1941) published micrographs of suspensions of bacterial viruses, in which "sperm-shaped" particles can be seen. Ruska suggested that these particles should be interpreted either as the virus itself or as bacterial constituents.

In December 1941 and March 1942 Luria and Anderson (1942), through arrangements made with the National Research Council Committee on Biological Applications of the Electron Microscope, were enabled to study several bacterial viruses with the RCA electron microscope. They found sperm-shaped particles in the virus suspensions, and identified them as virus particles on the basis of considerations which will be more fully developed in the discussion of the present paper.

During the summer of 1942 the present authors availed themselves of the presence of the RCA electron microscope at the Marine Biological Laboratory, Woods Hole, to study the interaction of bacterial viruses with their bacterial hosts. Previous growth experiments (Delbrück and Luria, 1942) had served to analyze the various stages of the interaction (specific adsorption, latent period of virus multiplication, virus liberation and lysis of the bacterium). This analysis formed the basis for the interpretation of the results.

The present paper describes and discusses the results of this whole series of electron micrographic studies of bacterial viruses.

MATERIAL AND METHODS

The RCA electron microscope and its mode of operation have previously been described (Anderson, 1942). A suspension of the material to be studied is deposited on a thin (10–20 mμ) collodion membrane, which in turn is supported by a fine wire mesh screen holder. The suspension is left for a few minutes on the membrane in order to permit the particles to settle out and to adhere to the membrane. The holder is then "washed" by dipping it a few times into distilled water; the "washing" is necessary to remove salts, which otherwise crystallize and spoil the preparation.

Several strains of bacterial virus were studied using 60 kV electrons. Two of them, α and γ, received particular attention.[4] In an earlier paper, the growth of these two viruses, which are active on the same host (*Escherichia coli* strain B), has been described in detail (Delbrück and Luria, 1942). Adsorption of virus α on a growing sensitive cell of strain B at 37°C. produces, after a latent period of 13–17 minutes, lysis of the cell with liberation of about 140 infective units of virus. For virus γ on the same host the latent period is 21–25 minutes; the number of infective units liberated is about 135 per cell.

Crude suspensions of the viruses of a titer between 5×10^9 and 2×10^{10} units/ml. were found to give good results in the electron microscope experiments. Suspensions of virus γ which had been partially purified by differential ultracentrifugation were also investigated.[5]

For the study of the interaction of virus and bacteria, an excess of virus was added to a young broth culture of bacteria under standard conditions. Samples were taken at various intervals with a small wire loop and deposited on specimen holders of the electron microscope. The specimen on the holder was incubated for a time in a moist chamber, so that the growth of the bacteria could continue under conditions similar to those in the broth culture. The holder was then washed in distilled water and allowed to dry rapidly in air; the process of washing and drying takes less than one minute. A specimen prepared in this manner will show the state of affairs in the growing mixture at a definite moment, which can be taken as that of washing. In some experiments, bacteria from a broth culture or from a young slant were mixed with a drop of virus suspension on the holder and, after incubation, were washed and dried as described above.

RESULTS

1. The virus particles

Micrographs of suspensions of virus α and of virus γ show the presence of particles of characteristic shape and size, specific for each strain (figs. 1, 2, 3). Regarding the identification of the particles visible on these pictures, with the

[4] Virus α and virus γ, for which the authors are indebted to Dr. J. Bronfenbrenner, were originally designated P28 and PC. Virus γ = PC has been purified by Kalmanson and Bronfenbrenner (1939). The practical reasons for the change of name were given by Delbrück and Luria (1942).

[5] One of the authors (S. E. L.) is greatly indebted to Dr. D. H. Moore for collaborating in the work of purification.

viruses rather than with inert bacterial constituents, we note: the two viruses were grown at the expense of the same host; the same bacterium then produces particles of type α if acted upon by virus α, and particles of type γ if acted upon by virus γ.

The particles of virus α have a round "head," 45–50 mμ in diameter and uniformly dark in the micrographs, that means, uniformly scattering for 60 kV electrons. To this round "head" is attached a "tail," about 150 mμ long and not more than 10–15 mμ thick. The tail appears either straight or slightly curved.

The particles of virus γ present a very peculiar aspect. To an oval head, 65 $\times$ 80 mμ, a straight tail, 120 mμ long and 20 mμ thick, is attached at one of the narrow poles. The head always shows a structure consisting of light and dark areas. The structure, although striking enough to make the particles immediately recognizable, is quite variable. Four frequent configurations can be described schematically as X-shaped, Z-shaped, inverted Z-shaped, and diplococcus-shaped (●). These various configurations can not all be accounted for by one three-dimensional structure seen under different angles. The interpretation of these structures will be discussed later.

The particles described above are never seen in suspensions without virus activity; their number is in direct proportion with the activity.

In suspensions of virus γ partially purified by differential centrifugation, the same particles are visible. However, many of them appear to be damaged; the tail is often broken, sometimes altogether missing. Since during the process of purification a large part of the activity had been lost, we believe that the abnormal particles visible in these suspensions have been mechanically damaged and inactivated.[6]

Particles of another coli virus are visible in fig. 16. They are round, 50–60 mμ in diameter, and no tail can be seen. This of course does not preclude the existence of a tail, which might be too slender to be visible in the micrographs.

Particles of a staphylococcus virus[7] are shown in fig. 4. They have a head about 100 mμ in diameter, and a tail about 200 mμ long.

2. The growth of bacterial viruses and lysis of the host

a. Virus γ. Figs. 5–12 are micrographs of samples taken from growing mixtures of virus γ and sensitive bacteria. Under the conditions of the experiment, infected cells yield, after a latent period of 21–25 minutes, an average number of 135 infective units of virus (Delbrück and Luria, 1942). Results from two such experiments will be given. The two experiments differed in the multiplicity of infection, i.e., in the number of virus units available in the mixture for each bacterium, as given by plaque count assays.[8] The first experiment was one of high multiplicity, each bacterium being infected on the average by eight virus units,

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[6] This suggests the opportunity of controlling on crude virus suspensions the results of electron micrographic studies of purified preparations of viruses in general.

[7] Obtained through the courtesy of Dr. H. Zaytzeff-Jern.

[8] The "multiplicity of infection" is defined as the ratio *adsorbed virus/bacteria* (Delbrück and Luria, 1942).

while in the second experiment the multiplicity of infection was between two and three.

Figs. 5–9 refer to the first of these experiments, in which the multiplicity of infection was high. Adsorption experiments (Delbrück and Luria, 1942) had shown that under these conditions practically all the virus is taken up by the bacteria in less than ten minutes, and that more than 99% of the bacteria are infected. Fig. 5 shows one of several micrographs of a sample that was dried after 15 minutes of contact between bacteria and virus (10 minutes in the test tube and five minutes on the specimen holder). At this stage, all bacteria appear normal in structure. Some particles of virus can be seen adsorbed on the edge of the bacterium, on or within the clear peripheral zone of the cell, which arises when the bacterial protoplasm shrinks away from the cell-wall during the process of drying. The existence of this clear zone enables one to see details on about one-third of the total surface of the bacterial cell-wall.

We obtained seven good pictures of bacteria from this sample, and on these seven bacteria, a total of 22 adsorbed virus particles could be seen in all, an average of about three particles per bacterium. Keeping in mind that only about one-third of the surface is in clear view, we can estimate that there are in reality about nine particles adsorbed per bacterium. Within the limit of this rather crude evaluation, we obtain, therefore, fair agreement between the number of particles attached to the surface of the bacterium, as revealed by the electron microscope, and the number of virus units attached to each bacterium, as inferred from plaque count assays. This agreement may be taken as further evidence for the identification of the visible particles with the virus, and as confirming the conclusion previously reached by indirect methods only (Delbrück and Luria, 1942), that infective titers obtained by plaque count correspond closely to the actual number of infective particles present in a suspension. We also note an unexpected and important fact, namely, that the adsorbed particles remain at the surface of the bacterium; at least this must be true for the majority of the adsorbed particles.

No free particles were visible on these micrographs. This is to be expected since, as pointed out above, adsorption is practically complete in ten minutes.

Growth experiments had indicated that lysis and virus liberation occur for virus γ after a minimum latent period of 21 minutes. Electron micrographs taken at 15 minutes confirm this by the absence of lysed bacteria. In contrast to this, micrographs of samples dried at 23 or 26 minutes reveal a completely different picture. By singular chance, we obtained from a series of unusually favorable fields, pictures of a number of bacteria caught in various phases of disintegration.

Figs. 6 and 7 show a long bacterium (not unusual in young broth cultures of this strain), one end of which has burst open and has liberated a flood of material in which several hundred particles of virus γ are visible. Along with the virus particles, a granular material has come out from the bacterium. These granules are of uniform size and are much smaller than the virus particles, being 10–15 mμ in diameter. The increasing transparence of the bacterium from the normal to the bursting end shows how the bacterial content is shed from the bursting

end.   The diffuse mass lying across the bursting end of the bacterium probably contains also the remains of another lysed cell.   It is most noteworthy that the bacterial contents show, besides the virus particles, no other particles of similar size.   The dark spots on the cell-wall of the long bacterium are either single or groups of virus particles.   It is impossible to say whether they are inside or outside of the cell-wall.

It will be seen in figs. 6 and 7 that those parts where the two fields overlap agree in the finest details.   Since these two figures resulted from separate exposures, they show that the objects imaged are not noticeably altered by the very intense electron irradiation necessary for focusing.

The cells shown in figs. 8 and 9 appear to be in a later stage of lysis.   They are "ghosts," empty cell-walls from which all content has been liberated, and are surrounded by virus particles and protoplasmic granules.   Holes of various sizes are visible in these bacterial cell-walls.   Virus particles in large number (80 in one case, 150 in the other) surround the empty cell-walls.   Their location in the immediate vicinity of the cells suggests that lysis has taken place after the specimen had been washed, i.e., during the brief period of drying.   Therefore, the number of visible particles should and does correspond to the average yield of virus per bacterium, as determined by growth experiments, namely 135.

Side by side with the lysed bacteria we find in these specimens bacteria which are not yet lysed.   They show on their edge many more adsorbed virus particles than those from specimens dried at 15 minutes, when lysis had not yet started. These virus particles must have been adsorbed after their liberation from neighboring lysed bacteria.   Since practically all bacteria in the specimen had become infected in the first minutes of the experiment, these bacteria must also be close to lysis.   We conclude that the ability to adsorb virus remains unimpaired until very close to the moment of lysis.   This conclusion had been reached previously on the basis of growth experiments (Delbrück, 1940).   Fig. 10 shows the protoplasmic granular material at a higher magnification.

In the second experiment mentioned above, in which the multiplicity of infection was only two or three, micrographs of specimens dried at 5, 10, and 15 minutes show bacteria of normal aspect.   No adsorbed virus particles are visible, corresponding to the low multiplicity of infection.   However, the bacteria are infected; in specimens dried at 25 minutes most bacteria are seen to be lysed, and the remaining ones show numerous virus particles adsorbed on their surface. These bacteria appear to be damaged and near to lysis (fig. 11).

b. *Virus α*.   The interaction between the same strain of bacteria and virus α was studied in an experiment in which the multiplicity of infection was about five.   In specimens prepared at five or ten minutes, the bacteria appear normal, occasionally with one or a few adsorbed virus particles visible on their edge. At fifteen minutes (figs. 13 and 14) we witness the lysis of bacteria, as expected on the basis of growth experiments, which give a latent period of 13–17 minutes for the lysis produced by virus α in this host.   Lysis is accompanied by the liberation of particles of the type characteristic for this virus.   Cells in the process of lysis and "ghosts" are both considerably swollen.

The material which is liberated from the lysed cell along with the virus particles

is again granular, the granules being of the same size as in the case of lysis produced by virus γ (fig. 12). This is strong support of the interpretation of these minute particles as constituents of the bacterial protoplasm.

All bacteria which are not lysed within 15 minutes have several virus particles adsorbed (fig. 15); these obviously come from the lysed cells. The bacterium in fig. 15 has almost completely divided, but the cell-walls of the two cells are still connected by an X-shaped bridge.

c. *Other viruses.* Figs. 16 and 17 illustrate the action of still another virus which is active on a different host, a motile strain of *E. coli*. This virus was studied extensively several years ago by one of the authors (Delbrück, 1940). The bacterium in fig. 16 shows flagella and adsorbed virus particles. Fig. 17 shows an empty cell-wall after lysis. It is clearly visible that the flagella have remained intact. The same can be said of the case of a virus active on *Salmonella sp.* (Poona type)[9] (fig. 18). In these two cases at least, the flagella do not appear to be damaged by the action of the virus.

DISCUSSION

There can hardly be any doubt that the sperm-shaped particles in suspensions of bacterial viruses are the particles of virus. They are present in amounts proportional to the activity and they are never present in suspensions without virus activity. The structure of the visible particles is specific for each strain and a bacterium liberates the particles which are characteristic for the virus which has acted upon it. The use of two different and unrelated viruses acting on the same host eliminates the possibility that the particles might be natural protoplasmic components of the bacterium.

The behavior of the visible particles during the reproduction cycle of the virus, showing their specific adsorption and their liberation in the expected amount after the expected latent period, offers further reasons for their identification.

Size and shape of the particles deserve special attention. The size of the "head" is close to that which had previously been inferred for various bacterial viruses by indirect methods. For viruses α and γ the sensitivity to irradiation with x-rays has been tested quantitatively (Luria and Exner, 1941). From these data the sensitive volume, *i.e.*, the volume within which absorption of energy from the x-rays leads to inactivation of the virus has been calculated. This sensitive volume may be smaller than the true volume. The irradiation data therefore give minimum values for the particle sizes. The results were 36 mμ for virus α and 50 mμ for virus γ, *i.e.*, in both cases about 30% smaller than the sizes given by the electron microscope pictures. We conclude that irradiation experiments do give values close to the real ones, and give correct values for the relative sizes of different viruses.

Kalmanson and Bronfenbrenner (1939) have studied the diffusion of virus γ purified by differential filtration. They find that their virus suspensions contain

[9] Obtained through the courtesy of Dr. M. L. Rakieten.

two fractions with different rates of diffusion. The larger fraction diffuses at a rate corresponding to a particle diameter of 16–18 mμ. A very small fraction diffuses faster, corresponding to a particle size of 3–4 mμ. These authors assume that the small particles are usually attached to larger unspecific carriers (the slower diffusing particles), and that they occasionally become free, still retaining their activity. The electron microscope pictures do not support this view, since the particles visible on these pictures are too regular in size and structure to be interpreted as bacterial debris to which the virus might be adsorbed. The electron microscope could not show particles having diameters of 3–4 mμ. But if such small virus particles existed, they should show up in irradiation experiments as a very resistant fraction. Careful search for such an x-ray resistant fraction of virus has given completely negative results (Luria and Exner, 1941).

Size determinations of the strain of staphylococcus virus shown in fig. 4 have not been made by any other method. However, the filtration and centrifugation studies of Elford and others have shown that different strains of staphylococcus virus differ little in size, in contrast to the viruses of the coli-dysentery group. It may therefore be permissible to compare our values with those obtained for other staphylococcus viruses by other methods. Irradiation data have given 50 mμ, somewhat lower than the value here obtained (100 mμ). Ultrafiltration studies gave values between 50 and 78 mμ, in fair agreement with the electron microscope value. Northrop (1938) has made extensive studies with purified preparations of a staphylococcus virus. From the rate of sedimentation in the ultracentrifuge a value of 60–90 mμ (mol. weight 3×10^8) was obtained, again in good agreement with our value. Diffusion experiments on Northrop's virus gave results which depended on the concentration of virus. In concentrated suspensions the diffusion rate corresponded to a molecular weight of about 3×10^8, in agreement with the centrifugation value. However, in highly diluted suspensions the rate of diffusion was found to be faster, corresponding to a particle size of about 10 mμ. In explanation Northrop proposed a reversible equilibrium between small particles and large particles. If this were true, plaque count assays should give titers corresponding to the number of small virus particles, since these assays are done at extreme dilution. The titer of large particles which are presumed to be present at high concentrations should then be almost a thousand times smaller than the plaque count titer. In contrast with this, we find approximate quantitative agreement between the plaque count titer and the number of particles visible on the electron microscope pictures. Therefore, Northrop's idea is not applicable to our cases.

Shape and structure of the virus particles, as revealed by the electron microscope, deserve special consideration. The pictures here reproduced give the impression of a somewhat more complex organization than the pictures of plant viruses (Stanley and Anderson, 1941) had indicated. These had shown either straight rods or round particles. Of the animal viruses, influenza virus showed very small, round particles (Chambers and Henle, 1941). Papilloma virus (Sharp, Taylor, Beard, and Beard, 1942a) and equine encephalomyelitis virus (Sharp, Taylor, Beard, and Beard, 1942b) have both round particles, but while

the particles of papilloma appear to be homogeneous, those of encephalomyelitis virus show an internal structure. The majority of the much larger bodies of vaccinia virus (Green, Anderson, and Smadel, 1942) show five granules of more scattering material.

The structure in the head of virus γ cannot be ascribed to the presence of material of higher specific scattering power. The most likely material could be phosphorus, which, per atom, should scatter about four times more strongly than carbon, nitrogen or oxygen. In nucleic acid, for instance, there is one phosphorus atom to 20 of the lighter atoms. Pure nucleic acid would therefore scatter only about 20% more than a similar compound without phosphorus. Since we can hardly assume that the dark regions are composed exclusively of compounds of such relatively high phosphorus content, the maximum contrast due to differences in specific scattering power could not be more than a few percent and could not show up in our pictures. We believe therefore that the dark parts represent regions of greater thickness. It is possible that the particles in the native state are oval, but upon drying the more aqueous parts collapse while the solid parts retain more scattering material, which forms the dark areas of the head. The images obtained therefore indicate that the distribution of solid material in the head of the particle of virus γ is not uniform. A detailed analysis of the structure visible in the heads of these particles will be given elsewhere.

A word may be added regarding the tendency to speak of viruses as molecules. This tendency received its greatest momentum from Stanley's discovery in 1935 that paracrystals of tobacco mosaic virus could be obtained by simple methods, and from the great number of subsequent studies of this and of other viruses along the lines of protein chemistry. Also the electron microscope pictures of plant viruses, revealing simple rods and spheres, seemed to encourage such a tendency. While it is true that no strain of bacterial virus has yet been crystallized, chemical studies on purified preparations have indicated that chemically, bacterial and other viruses are closely related (Northrop, 1938). It is only natural that chemical and physical studies of viruses have led scientists to the habit of thinking of viruses in terms of molecules. However, one should keep in mind that the concept "molecule" is flexible when applied to structures such as viruses. When we speak of a long chain compound as a molecule, neither its configuration nor even its composition is to be taken as necessarily definite. The ambiguities will be multiplied if still more complicated structures are considered, the parts of which are not all connected by primary valencies. Such "molecules" will share with living things the impossibility of delimiting unambiguously which atoms belong to them and which do not. While no harm is done by calling viruses "molecules", such a terminology should not prejudice our views regarding the biological status of the viruses, which has yet to be elucidated.

The study of the interaction of virus α and virus γ with their host has confirmed in every detail the picture of the process which had been deduced on the basis of growth experiments. As d'Herelle (1926) had early suggested, and as quantitative studies had shown (Delbrück, 1942), the infection of a bacterium, after a latent period characteristic of each virus, is followed by lysis of the bac-

terium with liberation of a large number of new virus particles. Quantitatively, too, the agreement between electron microscopic and growth experiments is excellent, both regarding the length of the latent period and regarding the amount of virus liberated by the bacteria. Several points, however, receive further light from the electron micrographs.

After lysis of a bacterium a cell-wall remains which, in contrast to those obtained from intense sonic vibrations (Mudd, Polevitzky, Anderson, and Chambers, 1941), is always more or less damaged and variously lacerated. The new virus is liberated from the interior of the bacterium. The pictures, however, give no indication in which part of the bacterium the virus is produced, whether in the deep interior of the cell or close to the inner surface of the cell-wall.

The pictures of lysed bacteria show, besides the particles of virus, also granular material of very regular units, 10–15 mμ in diameter. If these are to be interpreted as molecules, their size corresponds to a molecular weight of the order of 10^6., These particles are liberated from the cell in great abundance, and seem to constitute the bulk of its protoplasm. The absence among the bacterial components of elements of size comparable to that of the virus particles explains why the latter can be studied so favorably in crude suspensions. It also explains the success of work on the purification of bacterial viruses by means of differential centrifugation and by filtration, and should encourage further work along these lines.

In a series of papers Krueger (1938) has proposed the idea that the bacterial cell contains a precursor of the virus particle, which, upon infection of the cell with a virus particle, is promptly converted into virus. This theory was elaborated as an analogue to the well-known relations between proteolytic enzymes and their precursors. According to this theory an uninfected bacterium of the strain here considered should contain on the average 140 precursor particles of virus α and 135 precursor particles of virus γ. The pictures show clearly that this is not the case, since bacteria lysed under the influence of virus γ show no evidence of particles resembling virus α and vice versa.

Finally, a point may be mentioned which seems to us perhaps of the greatest consequence. We have seen that the new virus is liberated from within the cell. On the other hand, the pictures of bacteria infected with virus γ and taken at fifteen minutes showed that the adsorbed virus particles, or at least most of them, do not penetrate into the interior of the cell but remain on the outer surface of the cell-wall. This observation creates a difficulty in interpreting virus growth. How do the infecting particles reproduce if they remain outside while the new virus is generated in the interior of the cell? One might assume either that the infecting particles act through the cell-wall, or that only one particle can enter the cell. The latter idea seems attractive in the light of results of growth experiments on multiple and mixed infection. These have shown that a bacterium always reacts as though only one particle of virus had been effective. The pictures here reproduced, if interpreted on the assumption that one virus particle enters the cell, would indicate that the entry of one virus particle bars the entry of other virus particles by making the bacterial cell-wall impermeable to them. The highly

peculiar phenomenon of mutual exclusion between virus particles attacking a cell would thus be explained by a mechanism alternative to that proposed in a previous discussion (Delbrück and Luria, 1942). An interpretation of this kind, for the correctness of which the experiments offer as yet hardly more than a hint, would suggest an analogy with the fecundation of monospermic eggs, and would lend support to those theories of the systematic position of virus which consider it as related to the host rather than as a parasite (cf. Hadley, 1928).

SUMMARY

1. Four strains of bacterial viruses have been studied with the electron microscope. In all cases the particles of virus could be identified on the micrographs. Three of these strains show "sperm-shaped" particles, consisting of a head and a tail. For the fourth strain, a tail is not visible on the micrographs. The particles of one of these viruses show a distinct structure in the head. The particle sizes agree well with the sizes inferred by some of the indirect methods.

2. The interaction between the virus and its host has been studied in detail in the case of two viruses which act upon the same strain of *Escherichia coli*. The micrographs demonstrate the adsorption of virus on the host and, after the predicted time, the lysis of the host with the liberation of virus particles of the infecting type. There is quantitative agreement between the numbers of particles visible on the micrographs and the numbers predicted on the basis of growth experiments for which plaque count assays were used. Along with the virus particles, the lysing cells shed protoplasmic material of uniform granular structure. The size of these granules is much smaller than that of the viruses and is independent of the virus under whose influence the bacterium is lysed.

3. Upon lysis the virus particles are liberated from the interior of the bacterial cell, for they are not visible on its surface up to the moment of lysis. In cases of multiple infection, the *infecting* particles of virus, or at least the majority of them seem not to enter the cell but to remain attached to the outside of the bacterial cell-wall.

4. The bearing of these results on the problems of the nature of viruses and of their systematic position is discussed.

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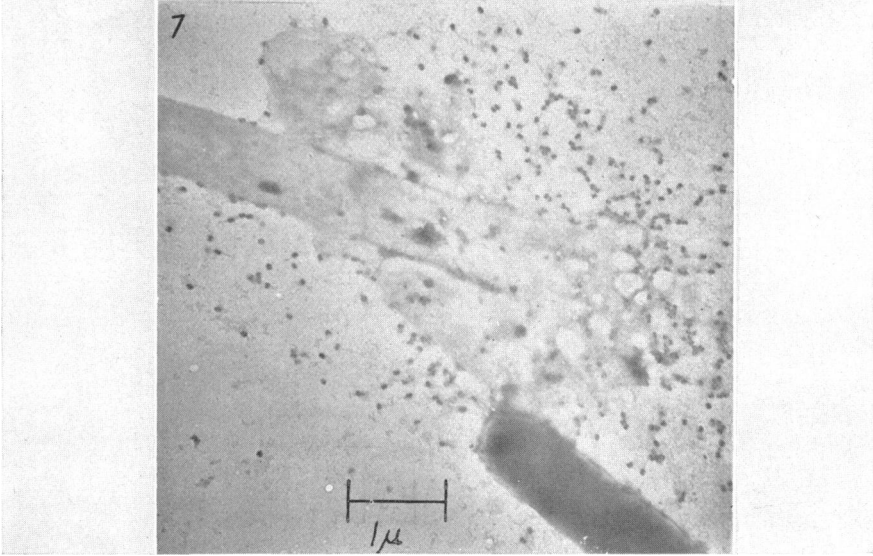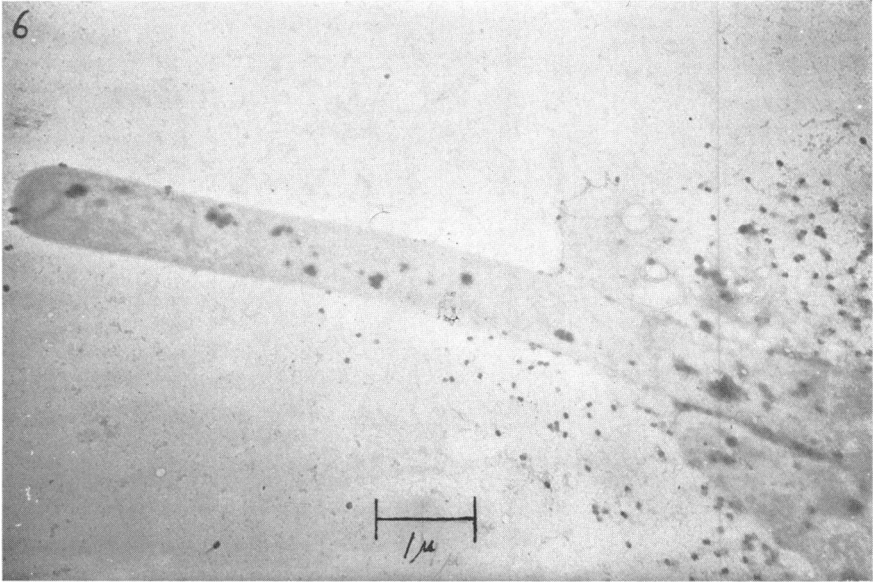
PLATE 1

FIG. 1. Particles of virus γ. $\times$ 36,000.

FIG. 2. Particles of virus γ. $\times$ 40,000.

FIG. 3. Particles of virus α. $\times$ 47,000.

FIG. 4. Particles of staphylococcus virus. $\times$ 20,000.

FIG. 5. *E. coli* + virus γ. 15 minutes contact. A bacterium with adsorbed particles of virus. $\times$ 20,000.
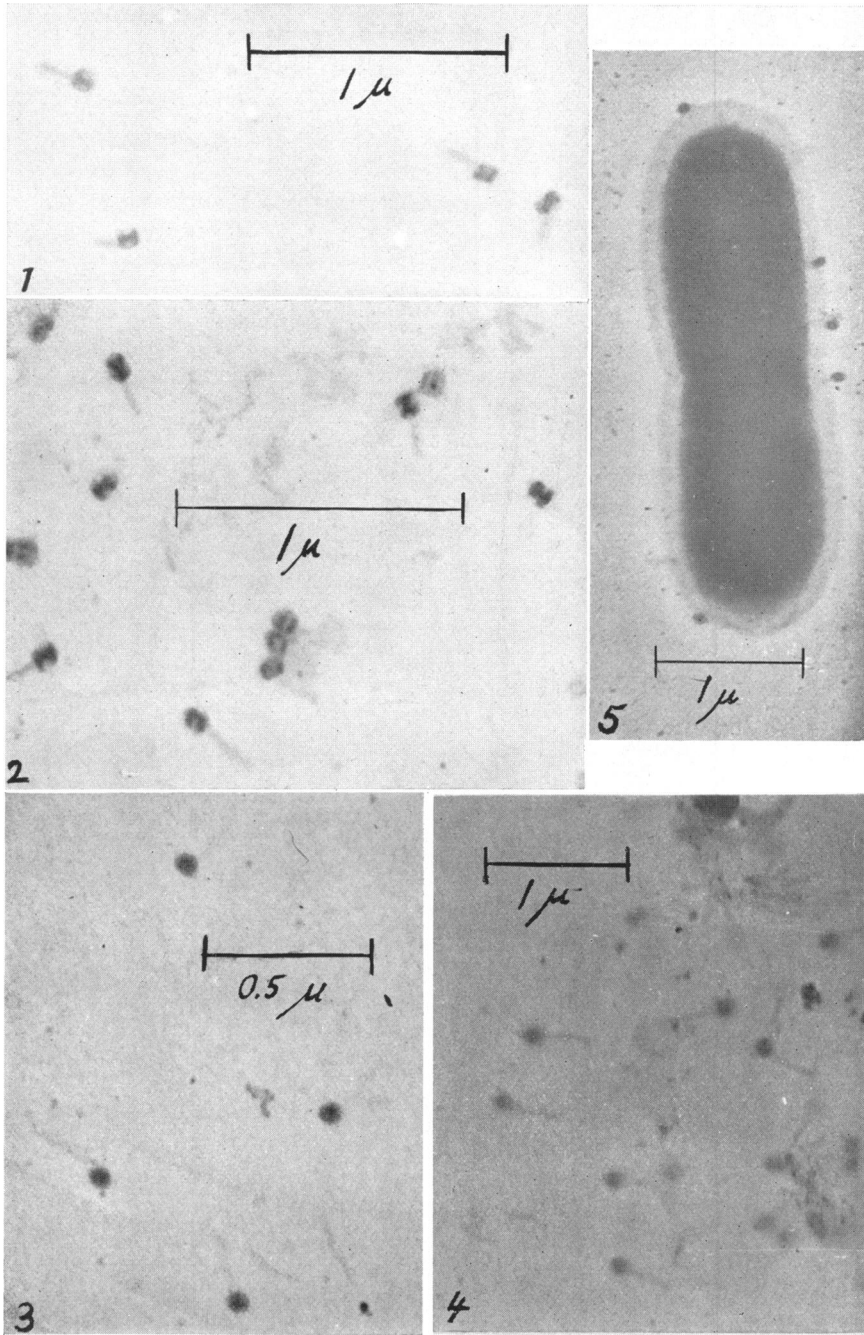
(S. E. Luria, M. Delbrück and T. F. Anderson: Electron Microscope Studies of Viruses)

PLATE 2

FIGS. 6 and 7. *E. coli* + virus γ. 23 minutes contact. A bacterium immediately after bursting, protoplasmic granules and several hundred particles of virus. The fields of the two pictures overlap in part. $\times$ 12,500.

(S. E. Luria, M. Delbrück and T. F. Anderson; Electron Microscope Studies of Viruses)
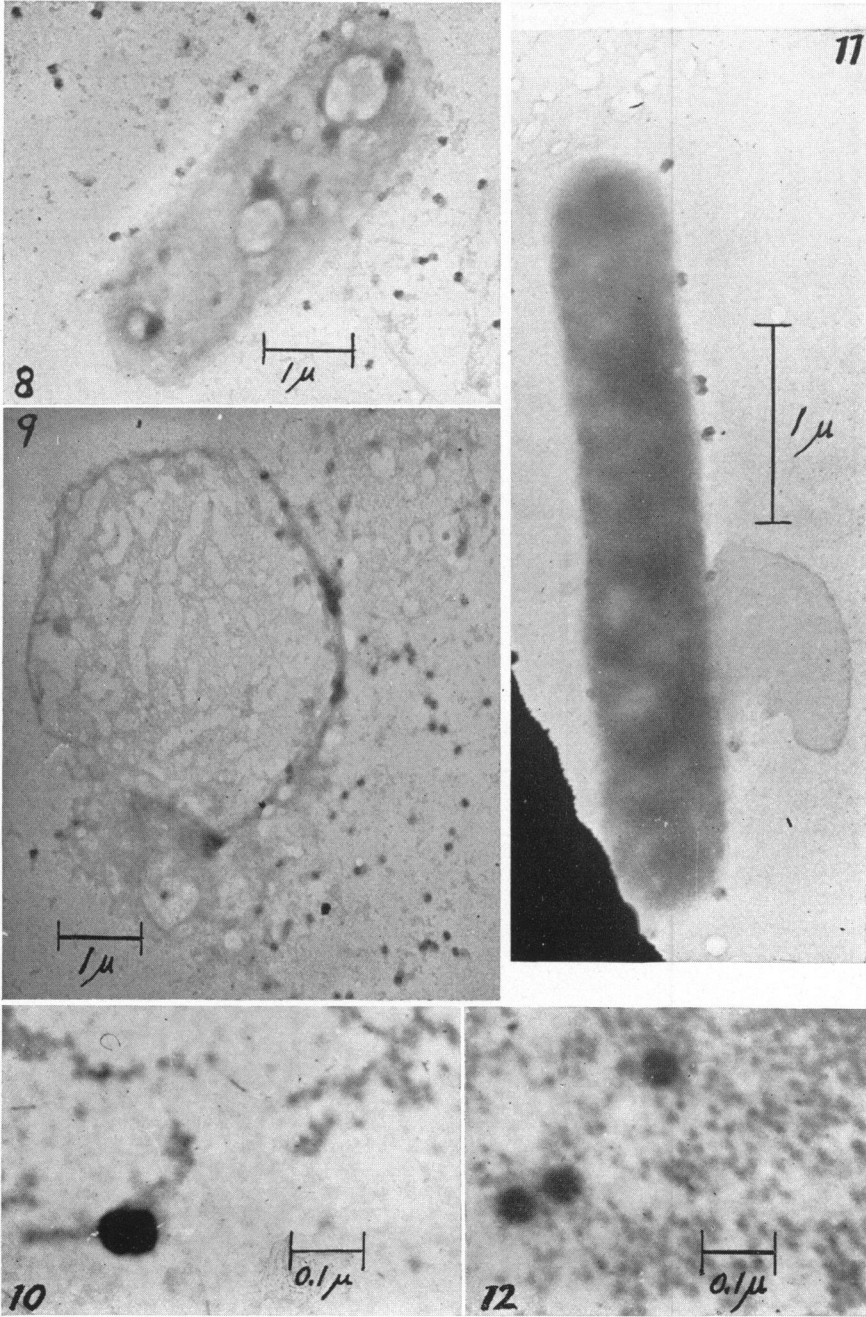
PLATE 3

Fig. 8. *E. coli* + virus γ. 23 minutes contact. "Ghost" of a lysed bacterium. × 12,500.

Fig. 9. *E. coli* + virus γ. 23 minutes contact. Another "ghost". × 11,500.

Fig. 10. *E. coli* + virus γ. 25 minutes contact. Detail of contents shed from a lysed cell, showing protoplasmic granules and one particle of virus γ. × 95,000.

Fig. 11. *E. coli* + virus γ. 25 minutes contact. A bacterium with ten adsorbed particles of virus visible on edges. × 27,000.

Fig. 12. *E. coli* + virus α. 15 minutes contact. Detail of contents shed from a lysed cell, showing protoplasmic granules and three particles of virus α. × 100,000.
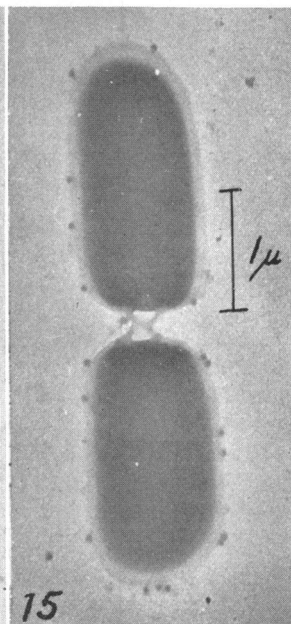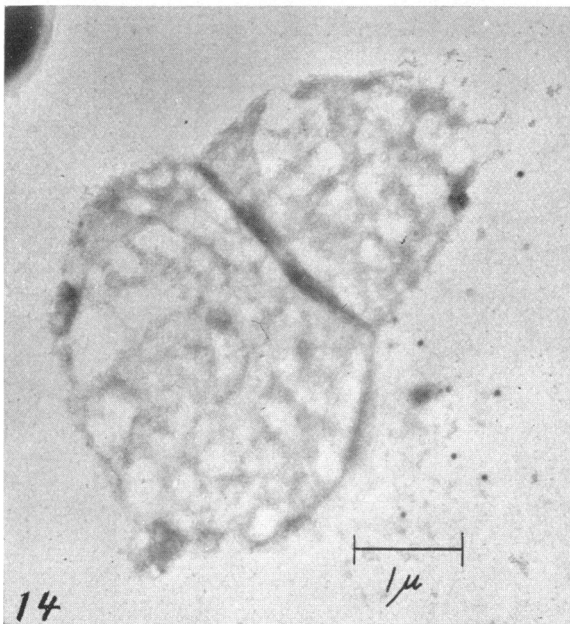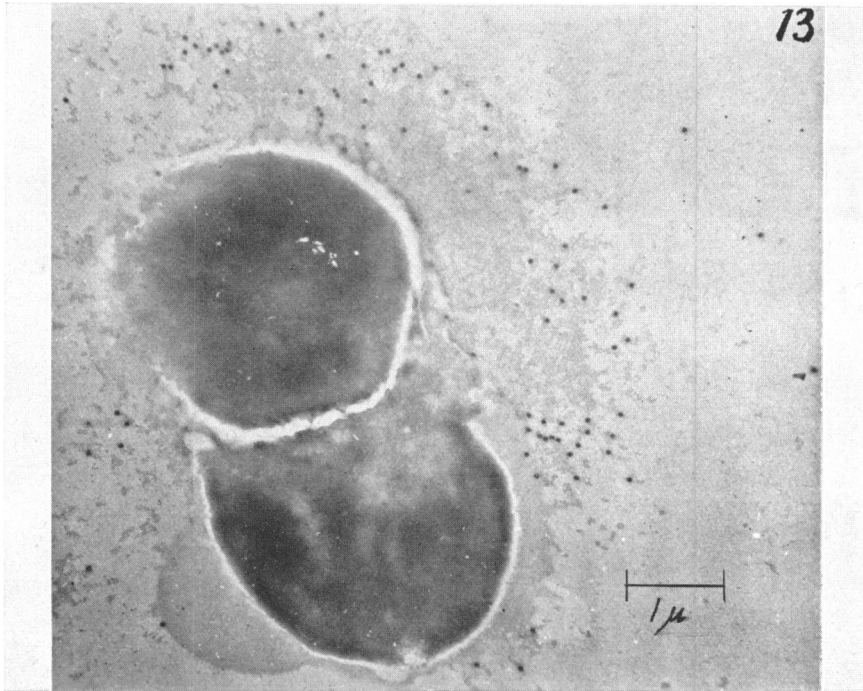
(S. E. Luria, M. Delbrück and T. F. Anderson: Electron Microscope Studies of Viruses)

PLATE 4

FIG. 13. *E. coli* + virus α.   15 minutes contact.   Lysis of a bacterial cell. Protoplasmic material and 93 particles of virus.   × 13,500.

FIG. 14. *E. coli* + virus α.   15 minutes contact.   "Ghost" of a lysed bacterium, several particles of virus α.   × 15,500.

FIG. 15. *E. coli* + virus α.   15 minutes contact.   Dividing bacterium with 19 adsorbed particles of virus visible on its edge.   × 16,500.
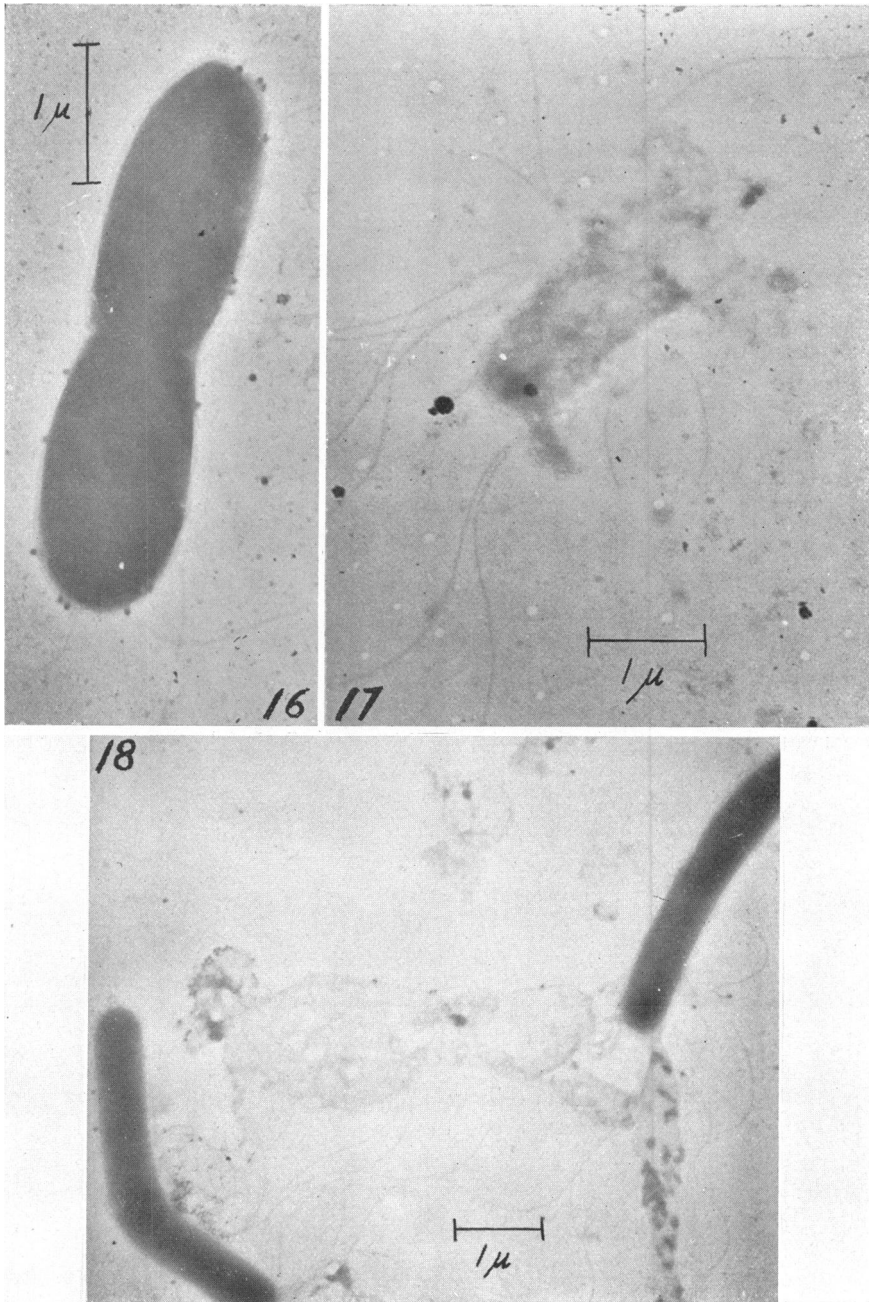
(S. E. Luria, M. Delbrück and T. F. Anderson: Electron Microscope Studies of Viruses)

PLATE 5

FIG. 16. *E. coli* (motile strain) + virus P_2. 22 minutes contact. Bacterium with 12 adsorbed particles of virus. 2 free particles of virus. $\times$ 19,000.

FIG. 17. *E. coli* (motile strain) + virus P_2. 22 minutes contact. Lysed bacterium with flagella. $\times$ 16,500.

FIG. 18. *Salmonella sp.* (Poona type) + virus SP. Centrifugation sediment of an unfiltered lysate. Lysed cells and two apparently normal cells. Flagella. $\times$ 12,000.

(S. E. Luria, M. Delbrück and T. F. Anderson: Electron Microscope Studies of Viruses)