LIGHT AND ELECTRON MICROSCOPIC STUDIES OF *ESCHERICHIA COLI*-COLIPHAGE INTERACTIONS*

II. THE ELECTRON MICROSCOPIC CYTOLOGY OF THE *E. COLI* B-T2 SYSTEM

by

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The preparative technique used in this work was selected and developed because, of all the techniques available, it appears to lead to minimal disturbance of the physical relationships in the specimen and to minimal loss of organic components. Unfortunately, the resulting specimens present the electron microscopist with a number of difficulties which cannot be avoided without sacrificing too many of the more valuable attributes of the preparative technique. It is necessary, therefore, to approach the problem by appropriate adaptation of the electron microscope.

Each of the specimens, with the exception of the uninfected cells and latest stages of lysis, can be considered as two specimens in one, each requiring different electron optical procedures for optimal results.

1. There are the cells in various stages of infection and lysis consisting of organic components with only slight variations in density but larger variations in packing. Since these cells have dried thicknesses in the range approximately 0.05 to 0.5 microns they are considered "thick" and require electron optics in which resolving power is intentionally limited in order to gain contrast. Since most of the structures of interest are intracellular, shadow-casting has only very limited value as a means of enhancing contrast. However, for these areas of the specimen shadowing was most valuable for distinguishing adhering material from intracellular structures.

2. The remainder of the specimen consists of the areas between the cells and is largely the supporting collodion membrane on which are dispersed the residual infecting bacteriophage and various other organic products of the cells and of the handling. In these areas high resolution can be achieved but the electron microscopist has little control over contrast. Shadow-casting can be of considerable value provided it is done in conjunction with unshadowed controls.

These considerations led to the use of a modified form of the wide-field high-contrast

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objective described elsewhere[1]. In its original form this objective had a focal length of approximately 12 mm which led to a maximal magnification of only 5000 ×. This meant that resolutions of 30 A could not be attained because of the limitations imposed by photographic grain and by the increased sensitivity (concomitant with the use of a low power objective) of the instrument to varying external magnetic fields. In order to correct this, the focal length of the lens was shortened to 7 mm which gave a maximal magnification of 9,000 × and sufficiently reduced the effects of stray magnetic fields to make 30 A resolution possible. Under these conditions it was found that the lens asymmetries which had not been visible previously now required compensation.

Concurrently with this work the advantages of the use of small condenser diaphragms were recognized. When used in conjunction with this lens the small diaphragms permitted the use of still smaller objective apertures and greatly increased their life by reducing by a factor of 4 the total electron current passing through them. The majority of the micrographs included in this report were obtained with a centered condenser aperture of 250 μ diameter (angular aperture of illumination $8 \cdot 10^{-4}$ radian) and an objective aperture of 30 μ diameter (objective angular aperture $2 \cdot 10^{-3}$ rad.). The electronic magnification was either 7500 × or 9000 ×. Eastman Medium Lantern Slide Plates were used throughout. Under these conditions a well-centered objective diaphragm was found to remain sufficiently clean to provide asymmetry-free images for at least one hundred exposures.

Shadow-casting was in all cases with chromium at a calculated thickness between 3 A and 6 A and with a shadow length-to-height ratio of approximately 3:1. Great care was taken to obtain pressures in the evaporating chamber of *below* 10^{-5} mm Hg before the evaporation was carried out.

It is notoriously difficult to make good photographic prints from high contrast electron micrographs and even more difficult to produce satisfactory half-tone reproductions. In the present work* these problems were greatly intensified by the peculiar character of the specimens where the important information was contained in both the thinnest and thickest areas with little in the intermediate range. In the case of the shadowed specimens, where photographic reversals were required, the situation became hopeless. To overcome these difficulties, we found it necessary to make duplicate micrographs with different exposure times of many electron microscope fields. For purposes of study the original negatives or, in some cases, series of enlargements with graded exposure times were used. Since these methods were obviously impractical for purposes of reproduction, a photographic masking process was devised which made it possible to provide on one print all of the pertinent details in a given field.

The method finally adopted involved making a transparency at a somewhat lower magnification than was to be used for the final enlargement. This transparency was then used as a mask during part of the exposure time of the final print. A stand was arranged above the easel of the enlarger so that when the transparency was placed on it it was in register with the projected image. By choosing the appropriate magnification of the transparency and the correct aperture of the enlarger lens it was possible to avoid the problems of obtaining extremely precise registering. Furthermore, in this arrangement, the masking did not interfere with the fine details of the picture. The transparency always had the same sense (positive or negative) as the final print and was made with

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* We should like to acknowledge the technical assistance of Miss ARTEMIS KURKJIAN in the photographic processing.

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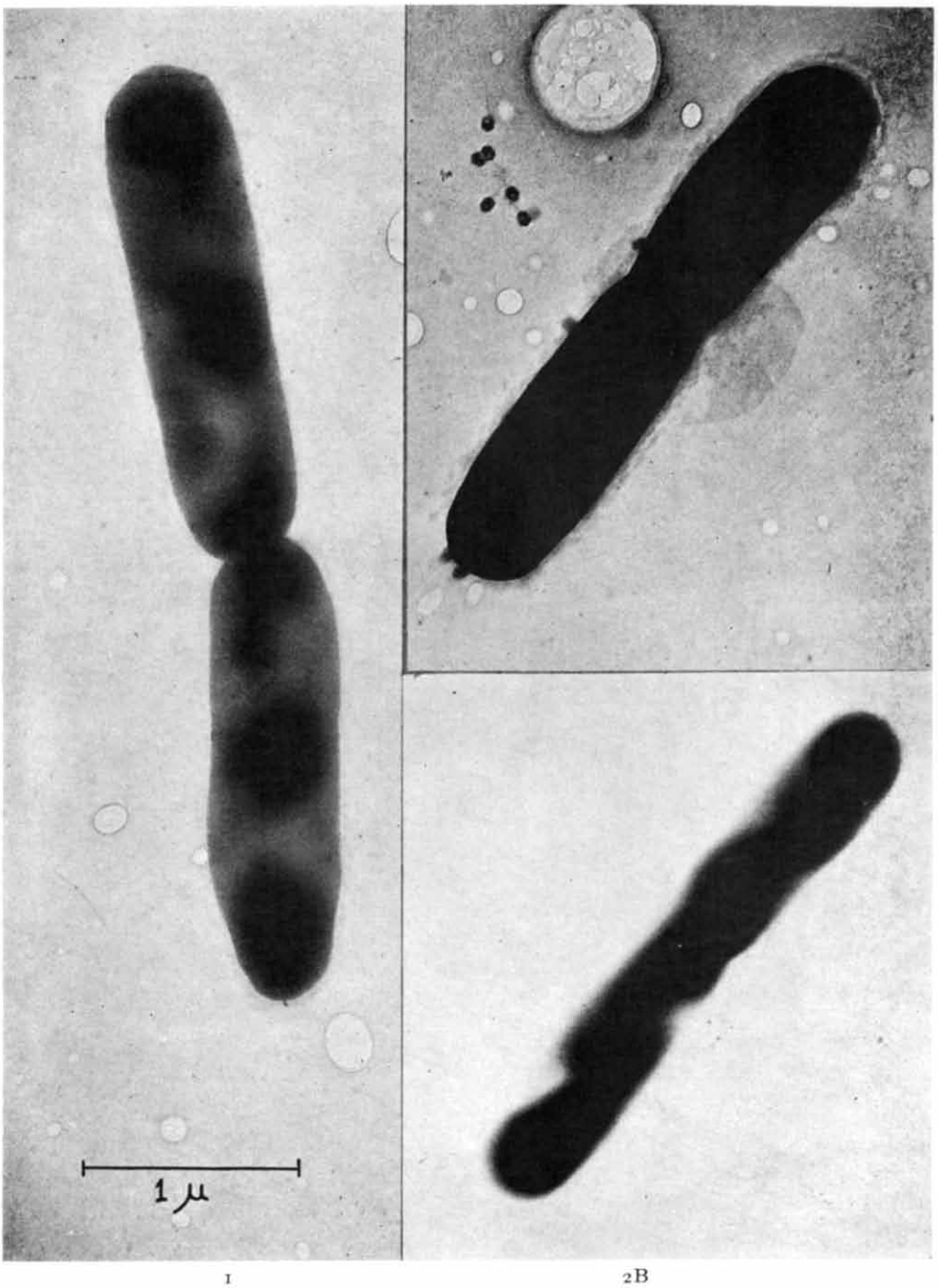

Fig. 1. # 5868a. Uninfected *E. coli* B. × 30,000 (see p. 163).
Fig. 2A. # 5874e. *E. coli* fixed 1 minute after infection with T2r. × 30,000 (see p. 164).
Fig. 2B. # 5874e. Same field more lightly printed. × 30,000 (see p. 164).
The serial numbers in the legends are RCA Laboratories plate numbers, included for identification.

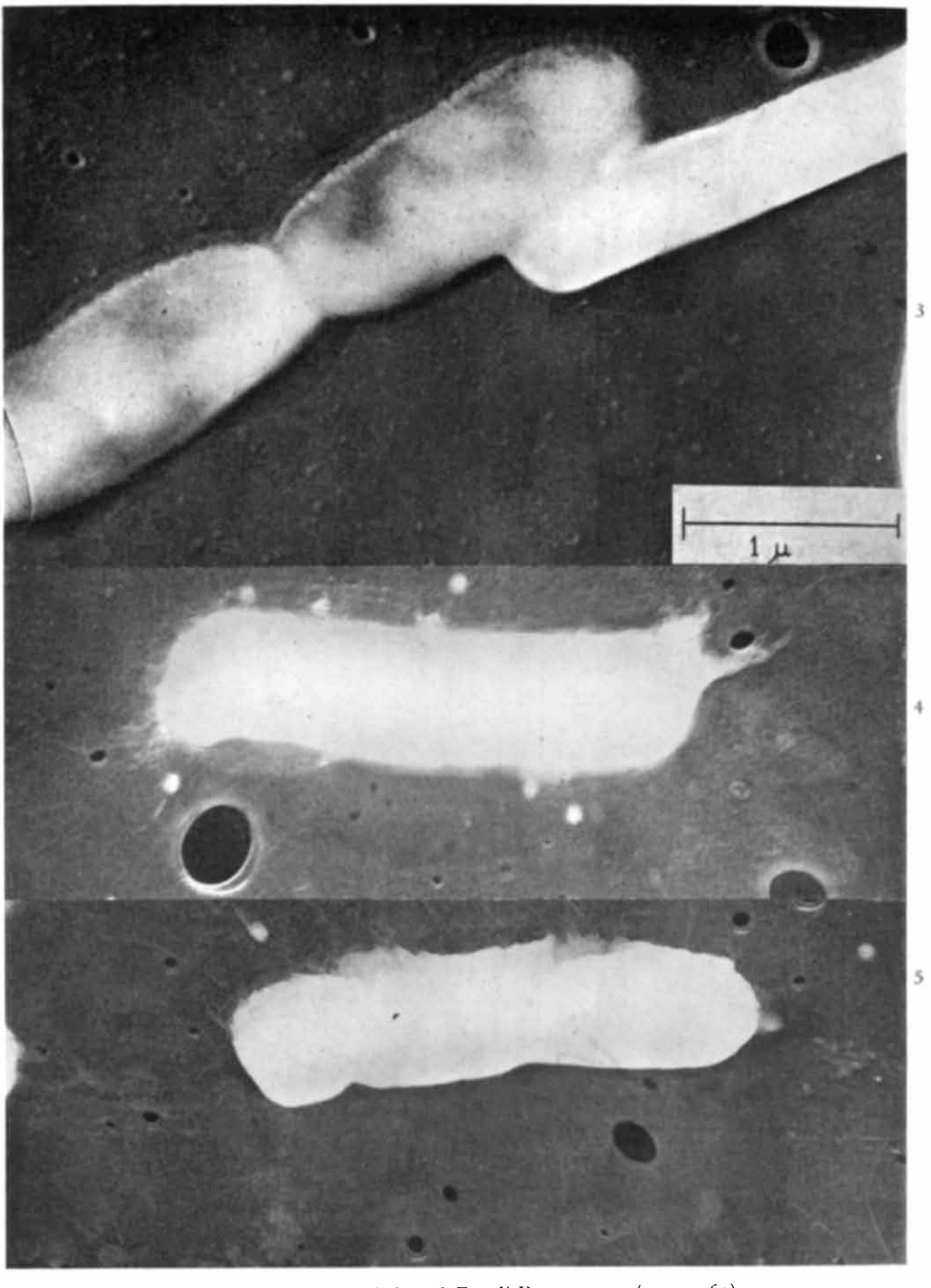

Fig. 3. # 5983 c. Uninfected *E. coli* B. × 30,000 (see p. 163).
Fig. 4. # 5982 c. *E. coli*, fixed 1 minute after infection with T2r. × 30,000 (see p. 164).
Fig. 5. # 5981 d. *E. coli*, fixed 5 minutes after infection with T2r. × 30,000 (see p. 164).

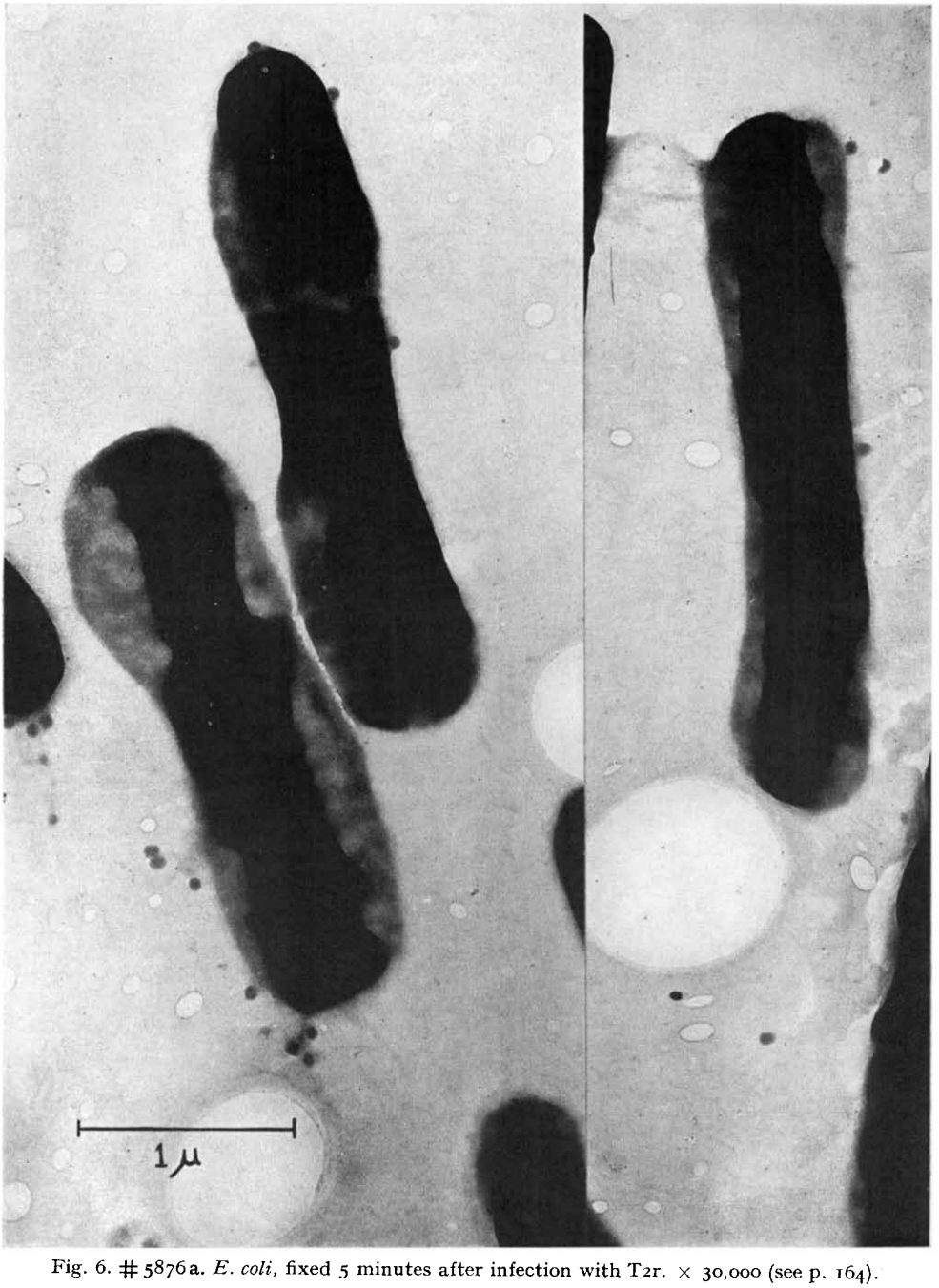

Fig. 6. # 5876a. *E. coli*, fixed 5 minutes after infection with T2r. × 30,000 (see p. 164).

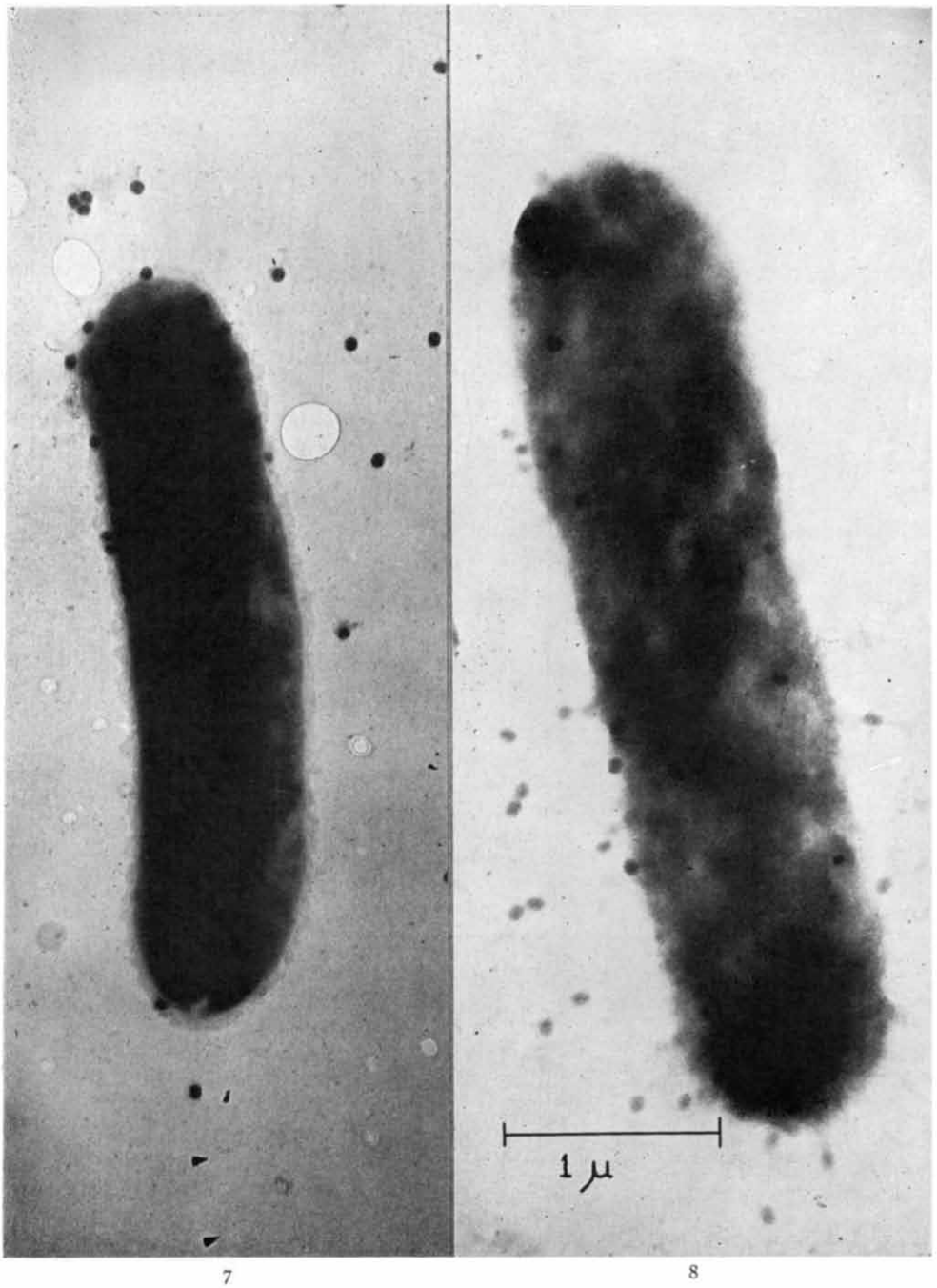

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Fig. 7. # 5878b. *E. coli*, fixed 10 minutes after infection with T2r. × 30,000 (see p. 164).
Fig. 8. # 5858e. *E. coli*, fixed 15 minutes after infection with T2r. × 30,000 (see p. 165).

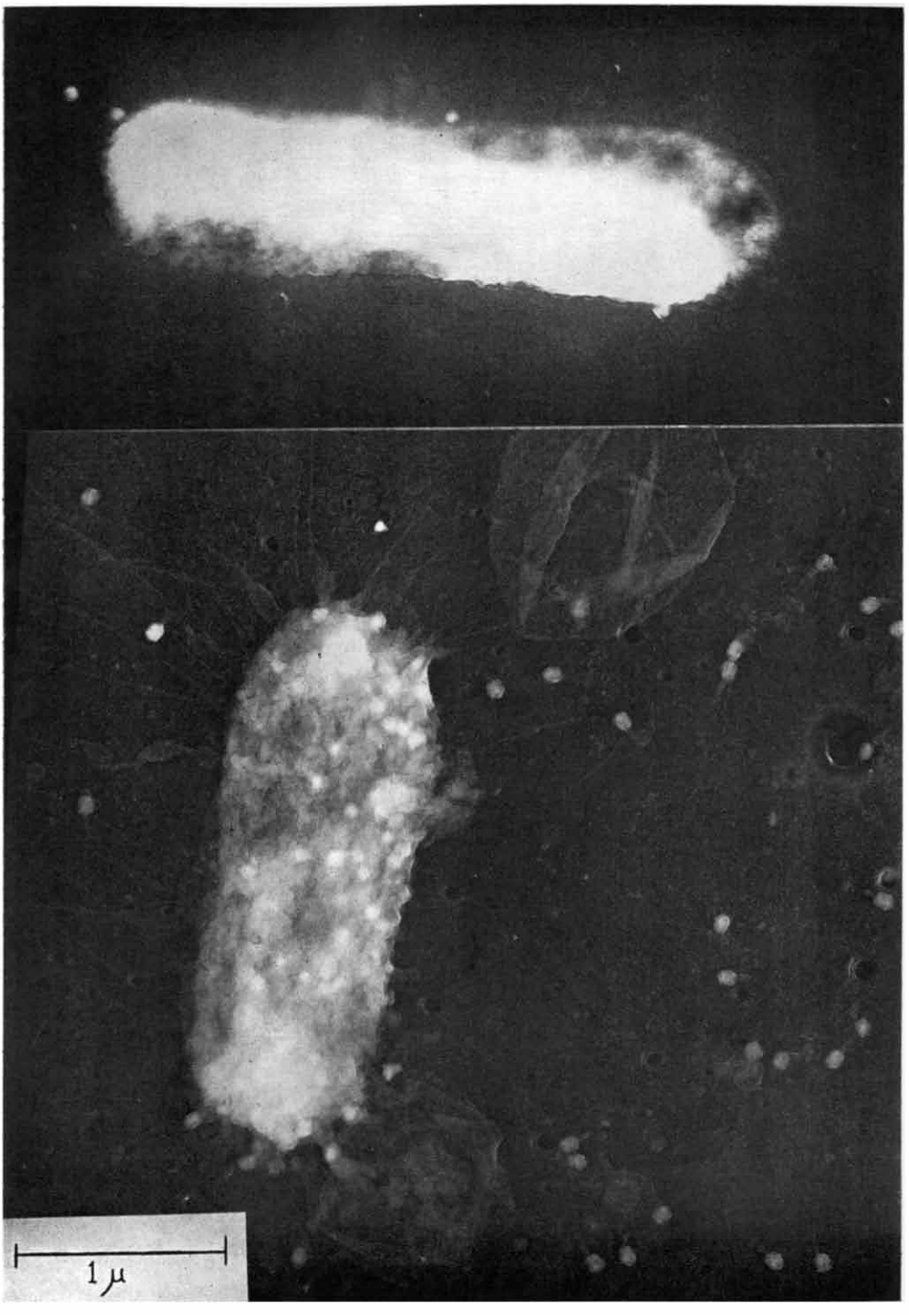

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Fig. 9. # 5980e. *E. coli*, fixed 10 minutes after infection with T2r. × 30,000 (see p. 164).
Fig. 10. # 5953b. *E coli*, fixed 15 minutes after infection with T2r. × 30,000 (see p. 165).

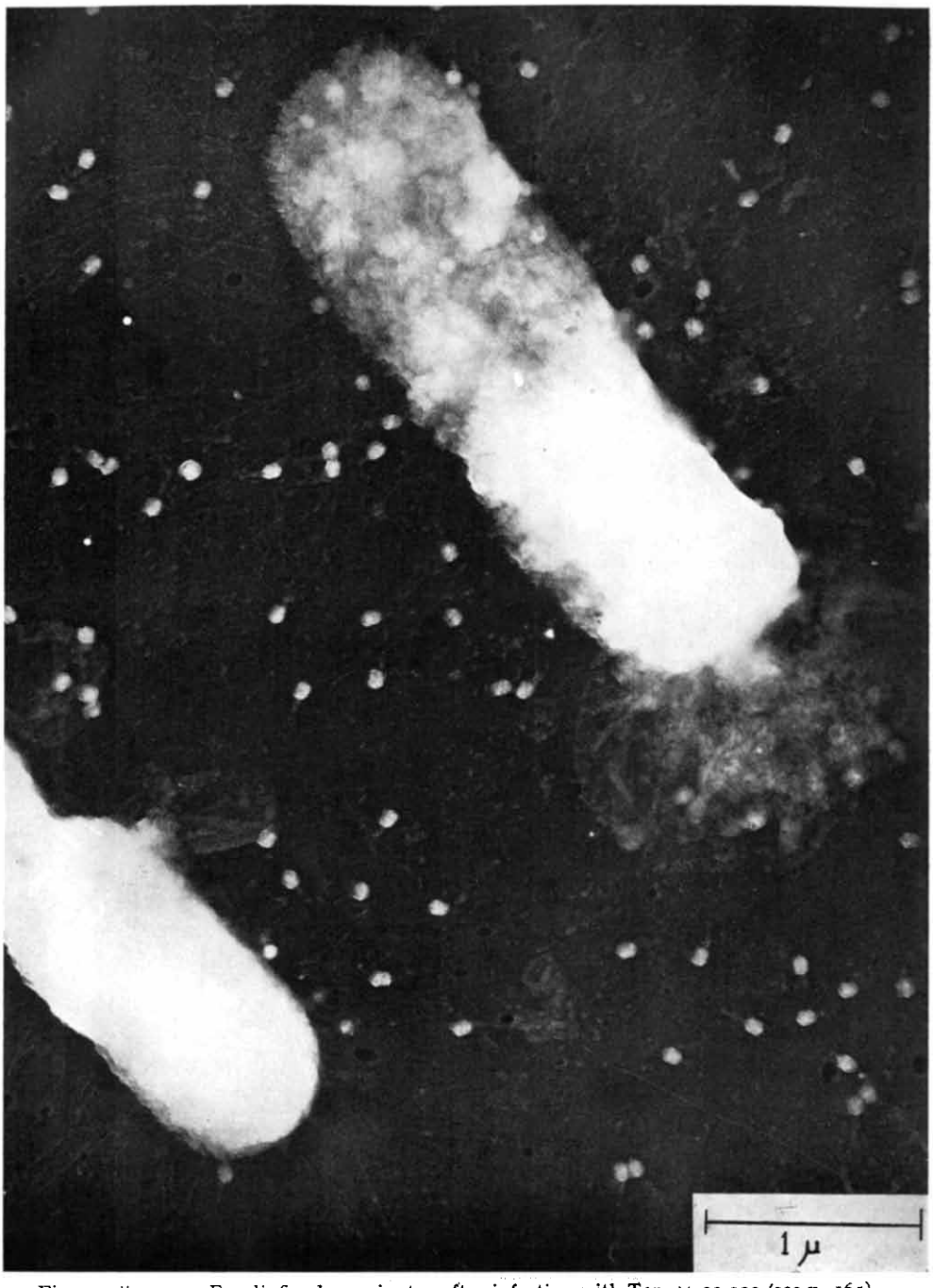

Fig. 11. # 5953e. *E. coli*, fixed 15 minutes after infection with T2r. × 30,000 (see p. 165).

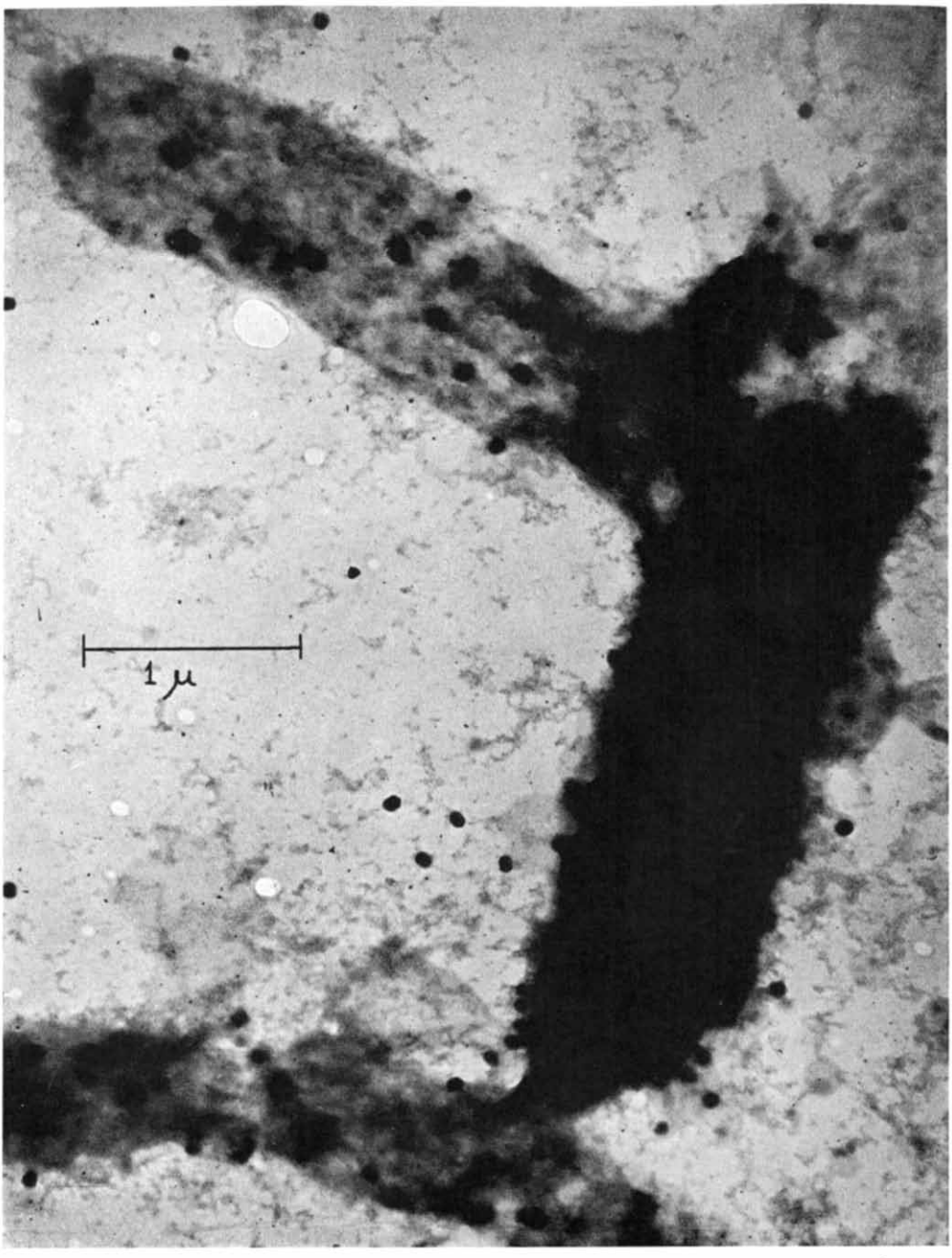

Fig. 12. # 5883c. *E. coli*, fixed 20 minutes after infection with T2r. × 30,000 (see p. 166).

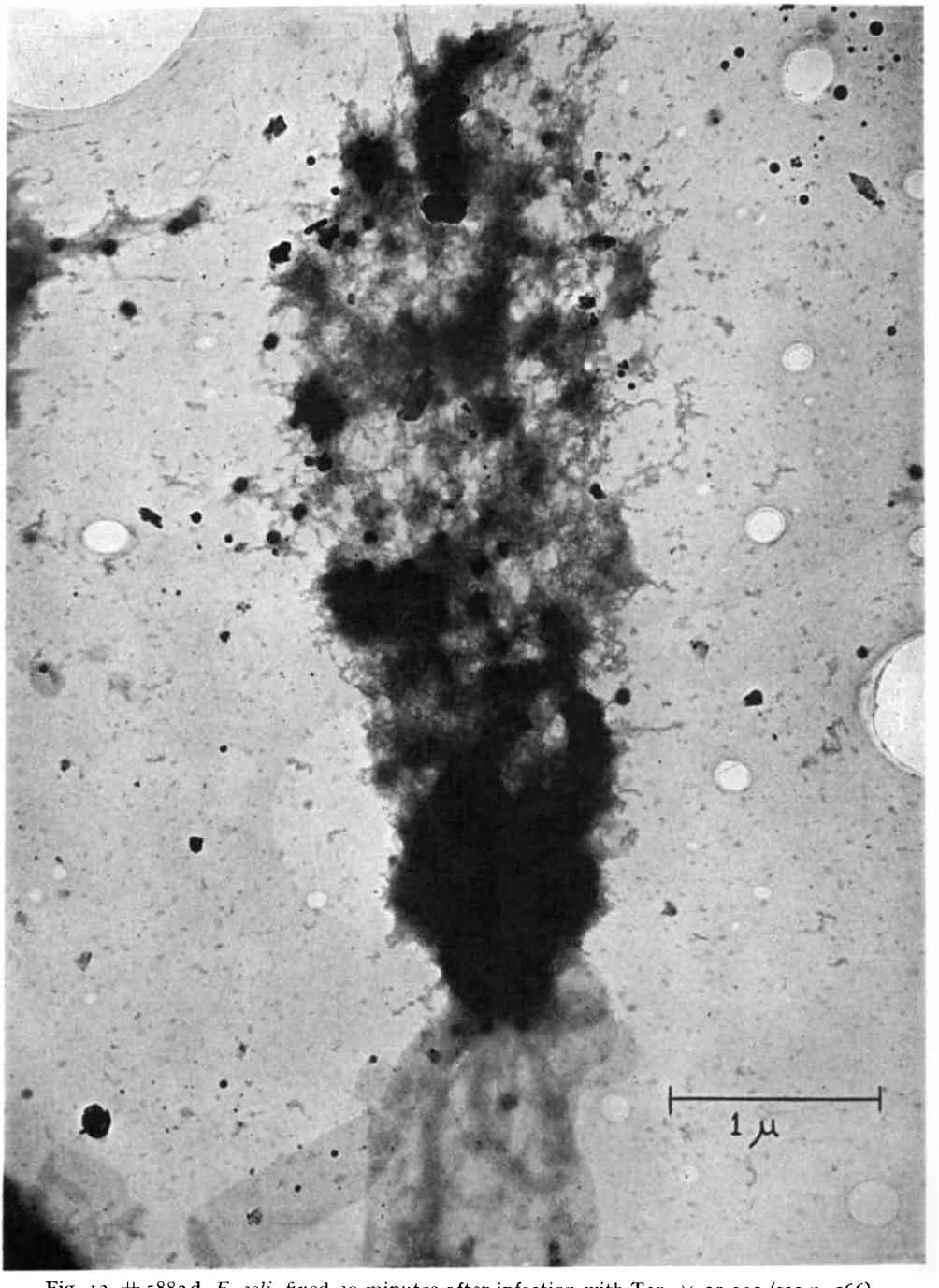

Fig. 13. # 5883d. *E. coli*, fixed 20 minutes after infection with T2r. × 30,000 (see p. 166).

high contrast and low density. Thus, areas on the transparency corresponding to the dark areas of the final print were nearly opaque whereas those corresponding to the light areas were nearly transparent. By using this mask for the appropriate fraction of the exposure time of the final print it was possible to bring both the dark and light areas of the picture within the range that can be reproduced without affecting the detailed contrast in either.

THE CYTOLOGICAL PICTURES AT SUCCESSIVE TIME INTERVALS

Uninfected cells. Fig. 1 shows two cells of *Escherichia coli* B before infection. Each cell has two relatively light areas and three dark areas. The dark areas are approximately circular to oval, are from 0.3 to 0.5 μ in diameter, and are situated at the two poles and in the centers of the respective cells.

Fig. 3 shows two cells of *E. coli* lightly shadowed with chromium. In the two cells in process of division the characteristic patterning of dark and light areas is just discernible through the metal film. The shadows are very close to the cell boundaries, indicating the low profiles of flattened cells. On the sides opposite the shadows the protoplasts may be seen to have shrunken away from the cell walls. The third cell shows no patterning and has a relatively wide shadow. The width of this shadow is greater at the pole and toward the center of the cell, narrower in the intermediate region.

The dark and light patterning of coliform cells has repeatedly been demonstrated in electron micrographs[2–7]. Similar patterning has been demonstrated also in living coliform rods with the phase microscope[8–11], indicating that the corresponding structural differentiation is present in the living cell. The several investigators concerned are in agreement that the light areas are the sites of the bacterial nuclei.

The nature of the dark cytoplasmic areas at the poles and centers of the cells was not clear until it was demonstrated that in the cytoplasm of these dark areas there were contained electron-scattering spheroidal or ellipsoidal bodies which reduce the tetrazols[12–14], oxidize the Nadi reagent and give the range of colour changes with Janus Green B characteristic of mitochondria[13–14]. The granules give these characteristic mitochondrial indicator reactions whether within the cells or freed from the cells by phage or other lysis[13–15]. There is obviously also electron-scattering cytoplasmic material around the mitochondrial granules.

The two cells in Fig. 3 which show the dark and light patternings* have low profiles, as indicated by narrow shadows. We interpret these facts as indicating that these cells have relatively fluid protoplasm which is flattened down by surface tension in drying. WYCKOFF[16] and HEDÉN AND WYCKOFF[17] have remarked upon the relative fluidity of young cells of *E. coli*. The third cell in Fig. 3 has a relatively high profile, as indicated by the broad shadow, has a smaller diameter and shows no patterning. All of these appearances are interpretable as indicating that this cell was approximately cylindrical in shape and was not so much flattened in drying. Under the conditions of our uninfected preparations the cylindrical cells occurred in the proportion of about one cylindrical to three flattened cells. Even the cylindrical cells, which must have been less fluid than the flattened cells, have been depressed somewhat in drying, as indicated by the undu-

* All pictures of shadowed preparations are as usual reproduced as negative prints, and values of dark and light contrast are correspondingly reversed from those of the unshadowed pictures.

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lated marginal shadow. The shadows of this and other cells have hills corresponding to the mitochondrial sites and dales corresponding to the nuclear sites. As previously pointed out[5] the nuclear sites in the living cells must be areas of less density than the enveloping cytoplasm.

Cells one minute after infection. In Fig. 2 a cell which was about to divide is shown one minute after infection with T2r. The great electron scattering by the cell obscures all detail in 2A. The lighter print of the same cell in 2B reveals dark mitochondrial granules at the poles. The light nuclear sites appear closer to the margins of the protoplast than in uninfected cells. The whole protoplast is more electron scattering than before infection.

In Fig. 4 the shadowed cell is obviously of the cylindrical type, with shadow broader at the poles and in the center, depressed in the intermediate regions. The protoplast has shrunken away from the cell wall particularly at the (reader's) left end of the cell.

In fields of cells one minute after infection examined at low power, it was strikingly apparent that practically every cell was of the cylindrical type. The fluid uninfected cells under the conditions of our experiment had therefore undergone change, probably gelation*, within a minute after infection with T2r. This we believe to be a new observation.

Cells five minutes after infection. Fig. 6 shows typical cells five minutes after infection with T2r. The protoplasts in the majority of cells appear as dark, electron-scattering masses with less dense vesicles along the margins of the cells. Amorphous-looking material is present in these marginal vesicles. The cell in Fig. 5, with its wide undulated shadow, is still of the cylindrical type.

Cells ten minutes after infection. Figs. 7, unshadowed, and 9, shadowed, of cells ten minutes after infection with T2r, show that much of the dark protoplasm has shrunken away from the cell walls. The marginal vesicles contain amorphous-appearing granular material.

LURIA AND PALMER[18], LURIA AND HUMAN[19], MURRAY, GILLEN AND HEAGY[20] and the present authors[21] have all demonstrated that the nuclear chromatin in a few minutes after infection is found in marginal areas along the boundaries of the infected cells. The nuclear staining techniques used do not reveal the relation of this marginal chromatin to protoplast and cell wall. The general correspondence in position between the "marginated" chromatin in light pictures and the marginal vesicles in the above electron pictures is described in the preceding paper[21].

The first half of the latent period of phage infection has come to be known as the "eclipse period", or "dark period", in part because of the discovery of DOERMANN[22] and DOERMANN AND DISSOSWAY[23] that cells broken open during this period do not yield either the original infecting phage or phage progeny in infective form. In extension of this, LEVINTHAL AND FISHER[24,25], have examined the contents of broken-open cells electron microscopically and conclude that "during the first half of the latent period no structures are found except those which are also in the uninfected organisms". This evidence is congruent with biophysical data indicating that resistance of T2 phage to inactivation with ultraviolet radiation increases markedly to a maximum at the middle of the latent period and thereafter decreases[26].

Direct evidence has most recently been obtained by HERSHEY AND CHASE[27] that

* The term "gelation" is used without implication as to whether or not the process is reversible, as in the usual sol-gel transformation in normal cells.

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the sulphur-containing protein of the casings of the infecting phage particles remains attached to the outside of the wall of the host cell, and only the desoxyribonucleic acid (or desoxyribonucleoprotein) contents of the head are introduced into the host cell proper. Evidence had previously been obtained indicating that the viral DNA is in part at least broken down[28] and its phosphorus incorporated into the DNA of the phage progeny, *i.e.* about 30% of the ^{32}P of the isotopically-labelled parent phage is transferred to the progeny phage[27, 29, 29a]. During the first half of the latent period genetic determiners from the infecting phage are replicated in linkage groups and undergo genetic recombination; during the second half of the latent period they are incorporated into the maturing phage particles[30–32]. In the first half of the latent period, also, first protein and later phage DNA are being synthesized from components of the external medium[33]. The nuclear DNA of the host cell is being broken down and incorporated into the phage progeny, as discussed previously[21].

These stirring events have been reviewed admirably by several of those who are most active in the experimental work[30–38]. Unfortunately we have not succeeded in visualizing in any detail the intracellular organization through which these extraordinary phenomena are mediated. The electron scattering of the cylindrical protoplast during the first half of the latent period regrettably makes this in all literalness a "dark period" also for electron microscopic observation.

Cells 15 minutes after infection. In Fig. 8 some intracellular detail can again be seen. The texture of the cytoplasm is very coarse and the internal organization of the cell is greatly altered from the texture and organization of normal cells[3]. Relatively large dark granules are distinguishable near the poles and in the center of the cell. A considerable number of dark particles resembling phage are seen either within the infected cell or superimposed upon it. Nothing suggestive of nuclei can be seen.

In the shadowed cells of Figs. 10 and 11, the coarse texture of the cytoplasm is again apparent and particles resembling phage are discernible within the cytoplasm, in addition to the phage particles obviously adsorbed to the bacterial cell wall. In Fig. 10 curious extensions of the cell wall may be seen. These may take the form of ellipses, or in one place a chain of three ellipses connected by thin tubes or strips of membranous material resembling the bacterial cell wall in physical properties. The elliptical extensions of the cell wall may terminate in exceedingly delicate thread-like extensions, or the filiform processes may extend outward directly from the cell wall. At the lower ends of the cells in Figs. 10 and 11 outpouchings of cell wall equal to or exceeding the diameters of the cells are seen to contain some of the coarse-textured cytoplasm and in Fig. 11 particles resembling phage. Membranous discs which appear to be empty sacs occur in the extracellular debris.

Fragments of the cell walls of coliform bacteria, in the shape of roughly circular discs, have been observed by SALTON AND HORNE[39] following rupture of the bacteria by heat. As previously suggested by HILLIER *et al.*[3], it is likely that the cell wall may comprise a mosaic of these circular and elliptical segments which become evident only following stress.

By way of interpretation, several points may be commented upon. The coarsened texture of the cytoplasm is very characteristic of cells during the second half of the latent period of infection. The overall impression of mixed-up internal organization certainly represents a real and profound alteration from that of normal cells. The "confusion" is of course subjective and reflects the fact that the human observer does

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not really understand the processes in progress, which must be assumed on other grounds to be highly ordered.

The elliptical extensions of the cell wall have been described before (see HILLIER *et al.*[3], Fig. 15), chiefly in connection with phage-infected cells. WEIDEL[40], also, observing the interaction of T2 phage particles and the separated cell walls of T2-susceptible *E. coli* B, has noted that the phage particles erode the cell walls with the production of circular and elliptical perforations and of pseudo pod-like extensions of the cell wall. The filiform extensions of the cell wall have also appeared in various pictures of uninfected bacteria, (HOUWINK AND VAN ITERSON[41]). They are certainly suggestive of the filiform, myelin forms which often are seen under dark-field observation, or electron microscopically[42,43] as extensions of the erythrocyte surface.

The relatively large dark granules near the poles and the center of the cell in Fig. 8 we believe to be mitochondria. Elsewhere[15] the mitochondria will be shown to persist as active centers of reduction throughout the course of phage infection.

The principal objective of these studies is of course to try to understand phage replication. From this point of view the most important question for interpretation is whether or not some of the granules resembling phage within the infected cells are actually phage particles in process of maturation within the bacterial cytoplasm. Most of the phage particles at the periphery of the bacterial cells are obviously adsorbed to the outside of the cell walls. A few phage particles whose images are superimposed upon the images of the cells we believe also actually to be outside. But close scrutiny of the prints* convinces us that some at least of the phage-like particles are in fact intracellular phage either maturing or already matured. This interpretation is of course in agreement with the findings of DOERMANN already referred to[22,23,37,44], and more recently studied by KAY[58] and JOKLIK[59].

Cells 20 minutes after infection. In Fig. 12 one opaque cell and two transparent cells are shown**. The lowest cell obviously has been ruptured in the middle, and the other transparent cell may be presumed to have lost some of its cytoplasm. In each of the transparent cells there are approximately 20 dark areas slightly larger and slightly less dense than mature phage particles. A series of gradations between rather amorphous-looking dense areas and definitive phage heads can be traced. A very much lighter printing of the dark cell of Fig. 12 shows numerous phage-like particles throughout its cytoplasm.

Fig. 13 shows the remains of a lysed cell. Linear arrays of particles of molecular dimensions*** extend outward from a fabric which evidently includes a mesh-work of such molecular arrays. Throughout this mesh-work are dark particles which again range from those resembling phage heads to larger and less definitive dense areas.

Fig. 14 shows the remains of another lysed cell in the meshes of which a larger number of dark particles appear, many of which are indistinguishable from the heads of mature phage.

Cells 25 minutes after infection. In Fig. 15 are shown the remains of several lysed

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* For instance the negative images of the external phage particles of Figs. 10 and 11 have "high lights" due to the intense shadowing of the external membranes of the phage particles. Such high lights are absent from the particles which we believe to be intracellular phage.

** Lysis of cells before the normal burst time may be attributed to the preparative procedures, which included fixation with OsO_4 vapour and dialysis.

*** Similar particles of molecular dimensions in the debris of lysed cells have been discussed in an earlier paper[3] and their molecular weight estimated as less than 50,000.

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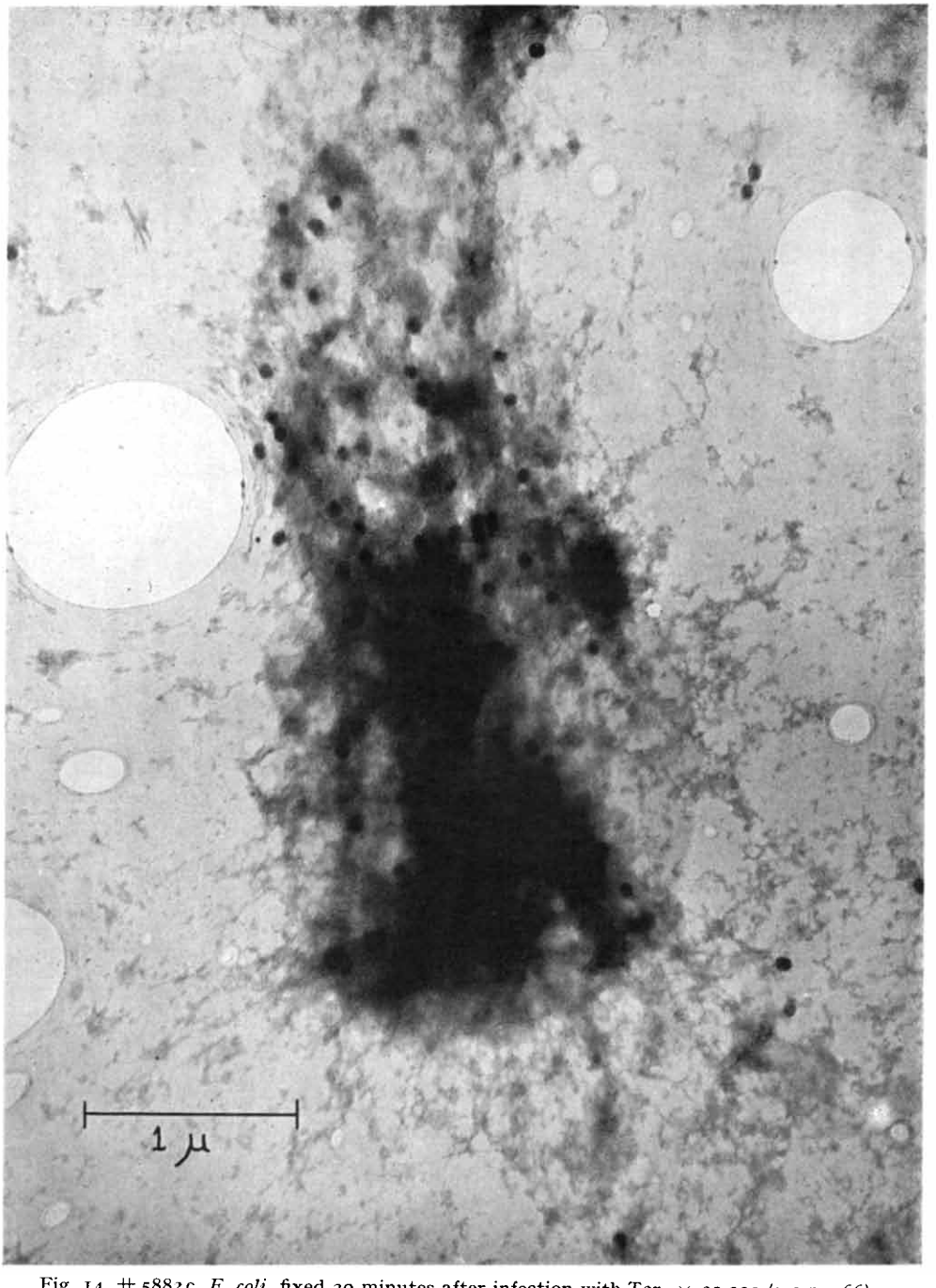

Fig. 14. # 5882c. *E. coli*, fixed 20 minutes after infection with T2r. × 30,000 (see p. 166).

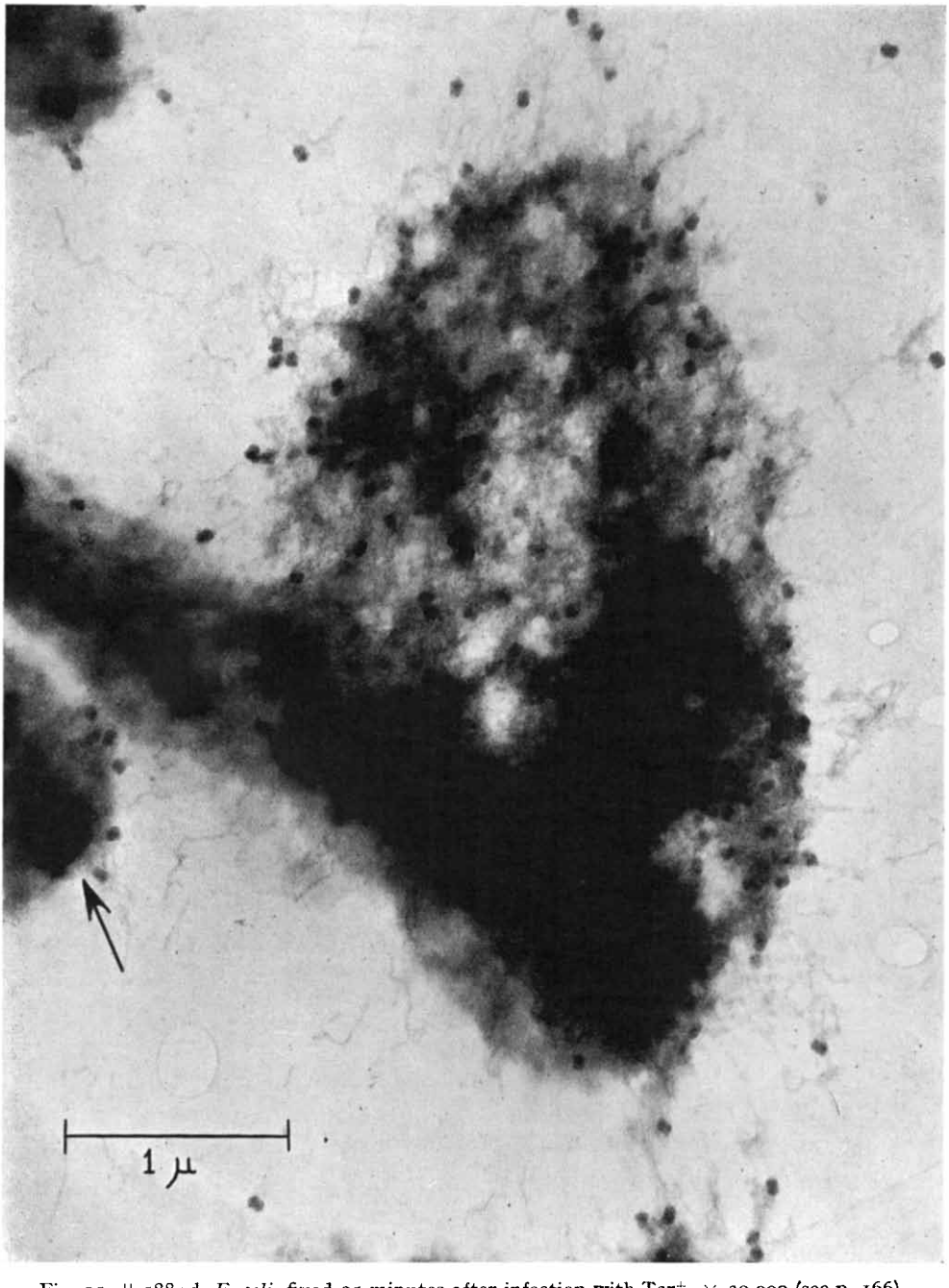

Fig. 15. # 5884d. *E. coli*, fixed 25 minutes after infection with T2r+. × 30,000 (see p. 166).

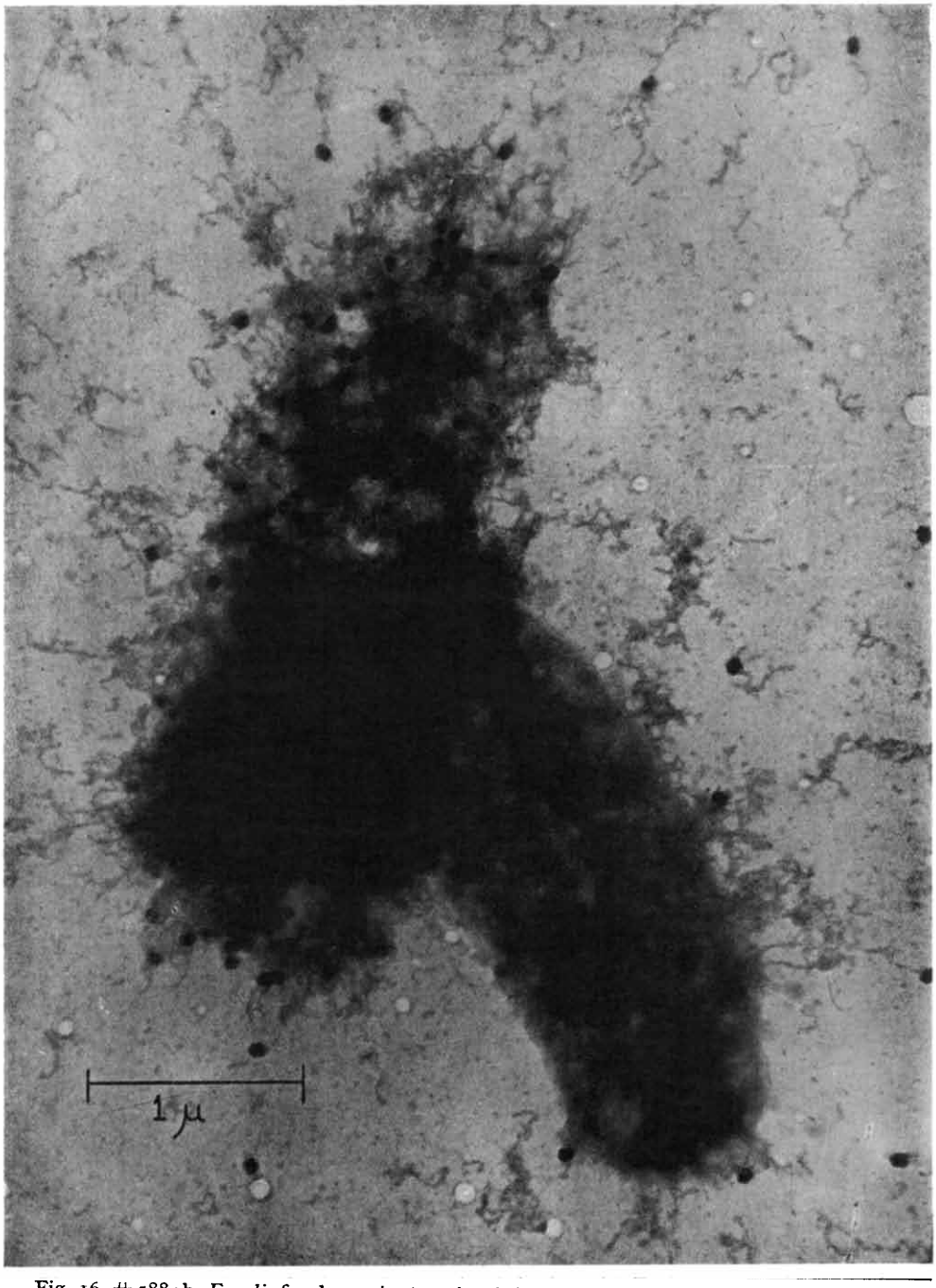

Fig. 16. # 5884 b. *E. coli*, fixed 25 minutes after infection with T2r+. × 30,000 (see p. 176).

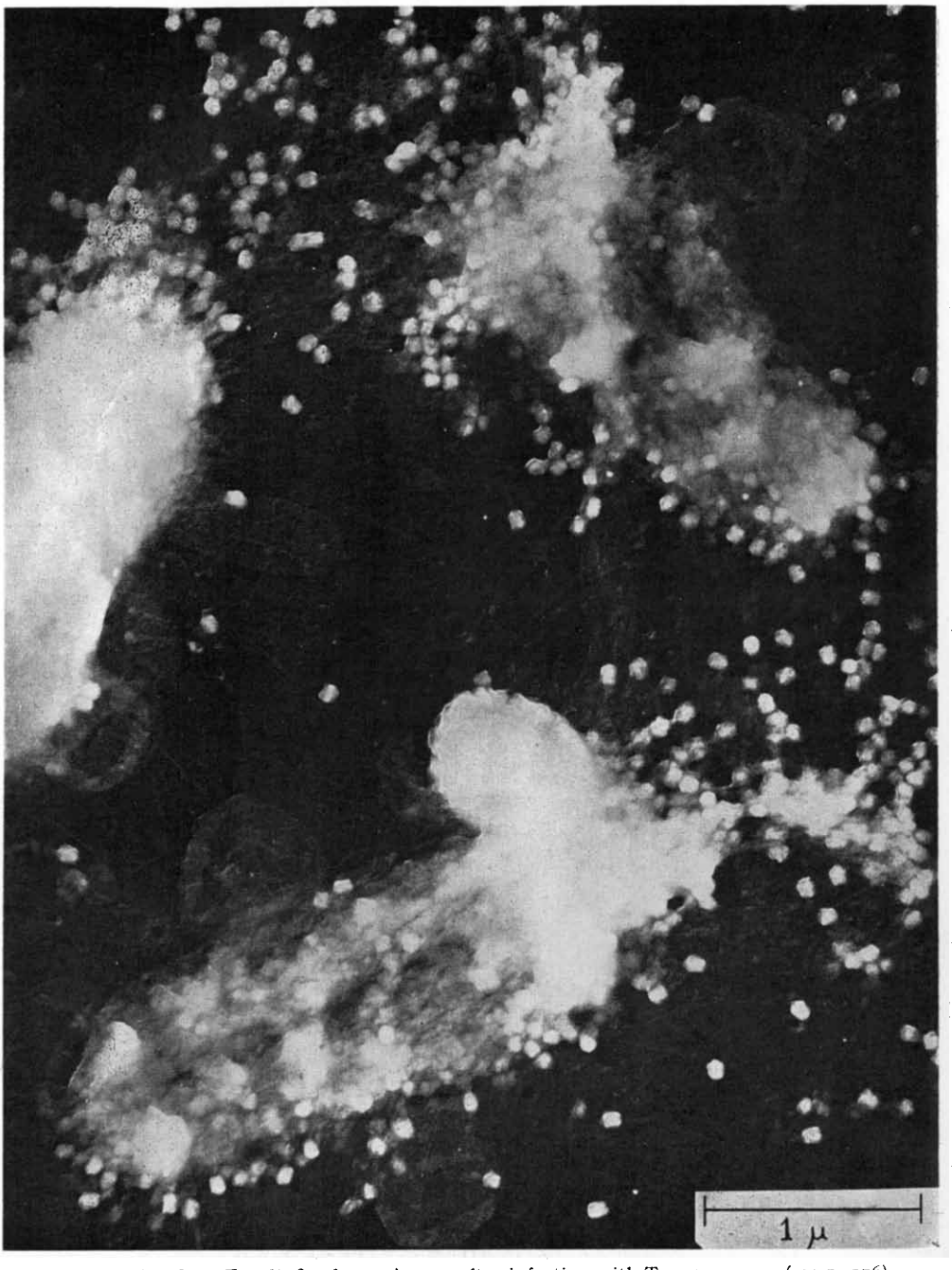

Fig. 17. # 5986c. *E. coli*, fixed 25 minutes after infection with T2r. × 30,000 (see p. 176).

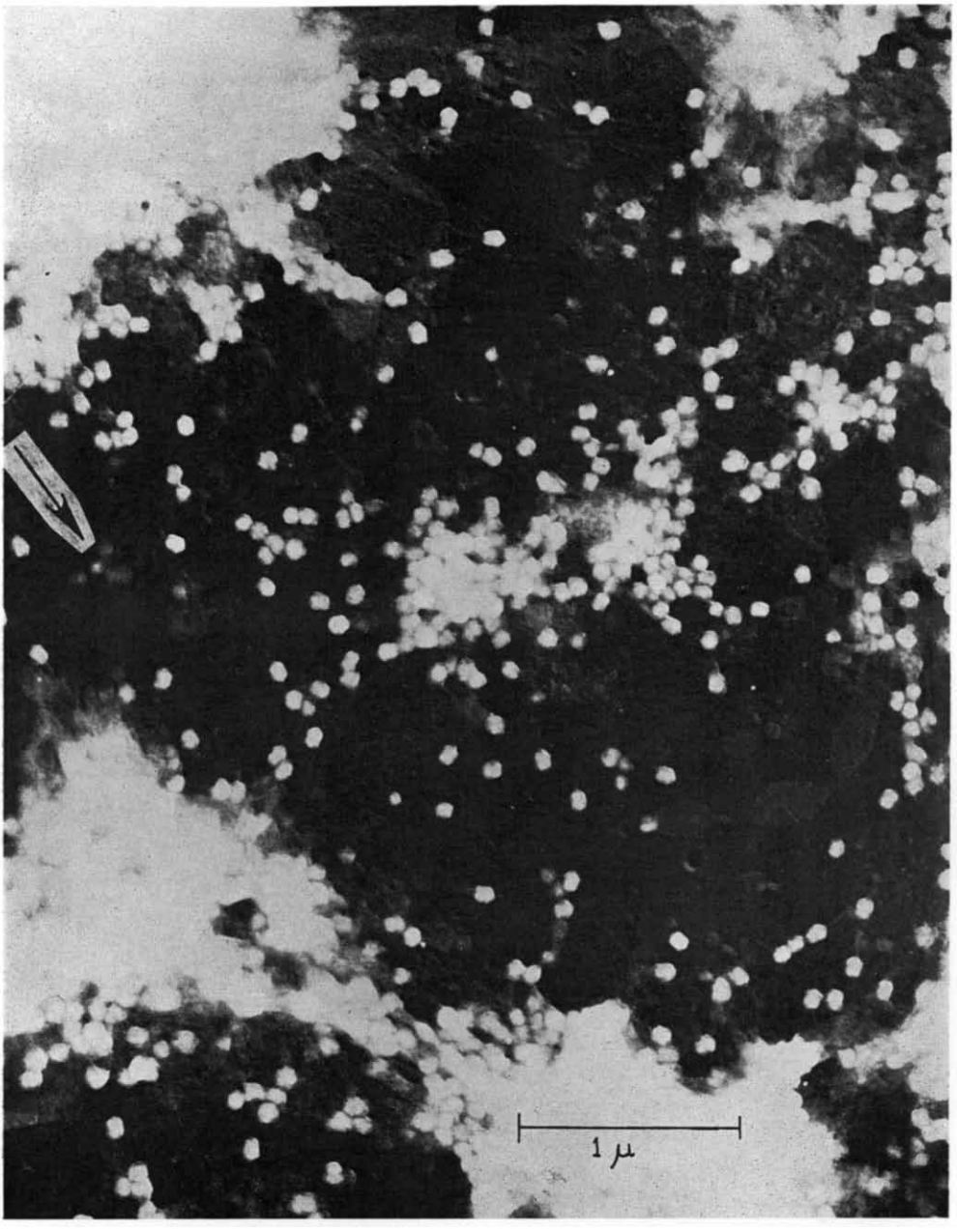

Fig. 18. # 5786b. *E. coli*, fixed 25 minutes after infection with T2r. × 30,000 (see p. 176).

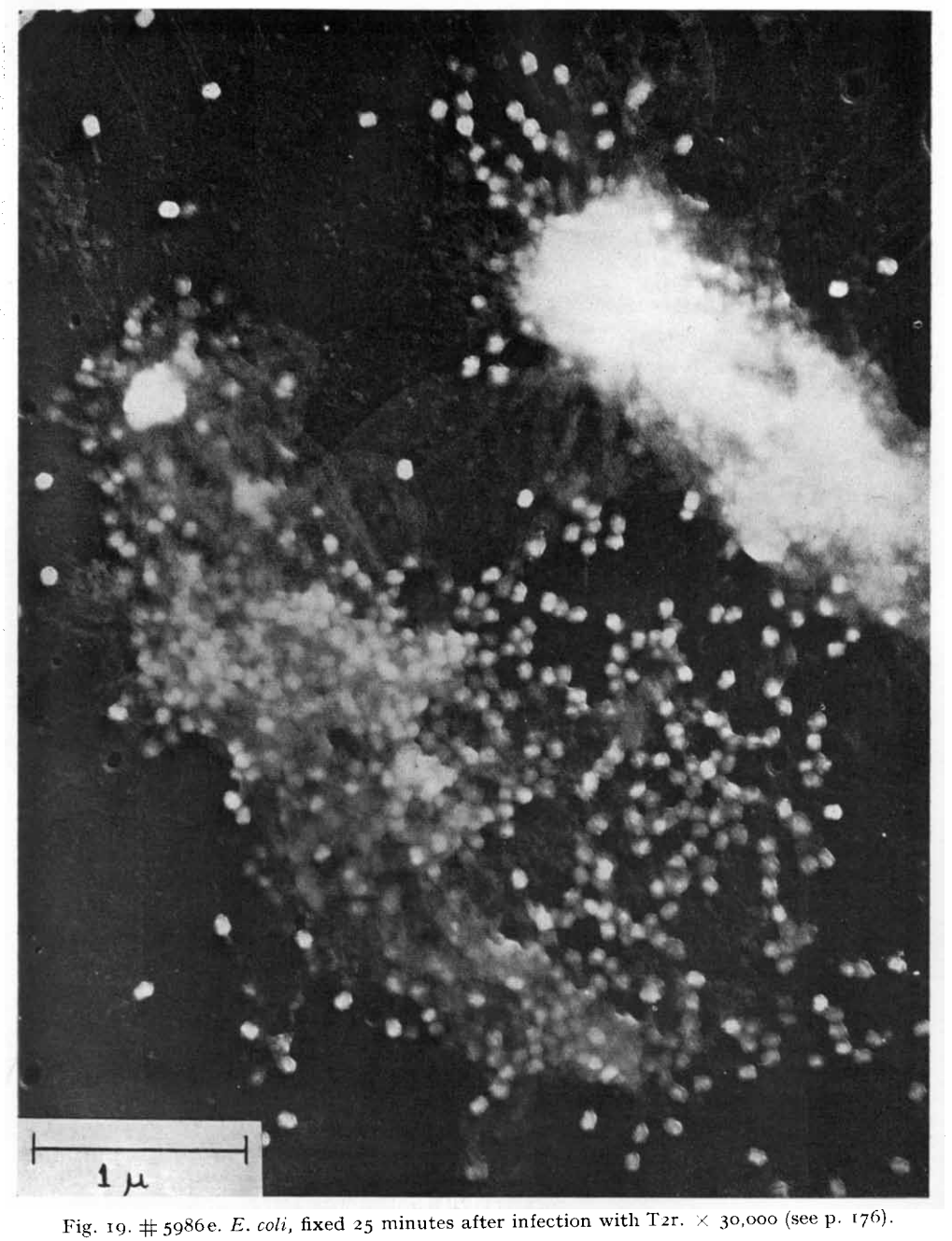

Fig. 19. # 5986e. *E. coli*, fixed 25 minutes after infection with T2r. × 30,000 (see p. 176).

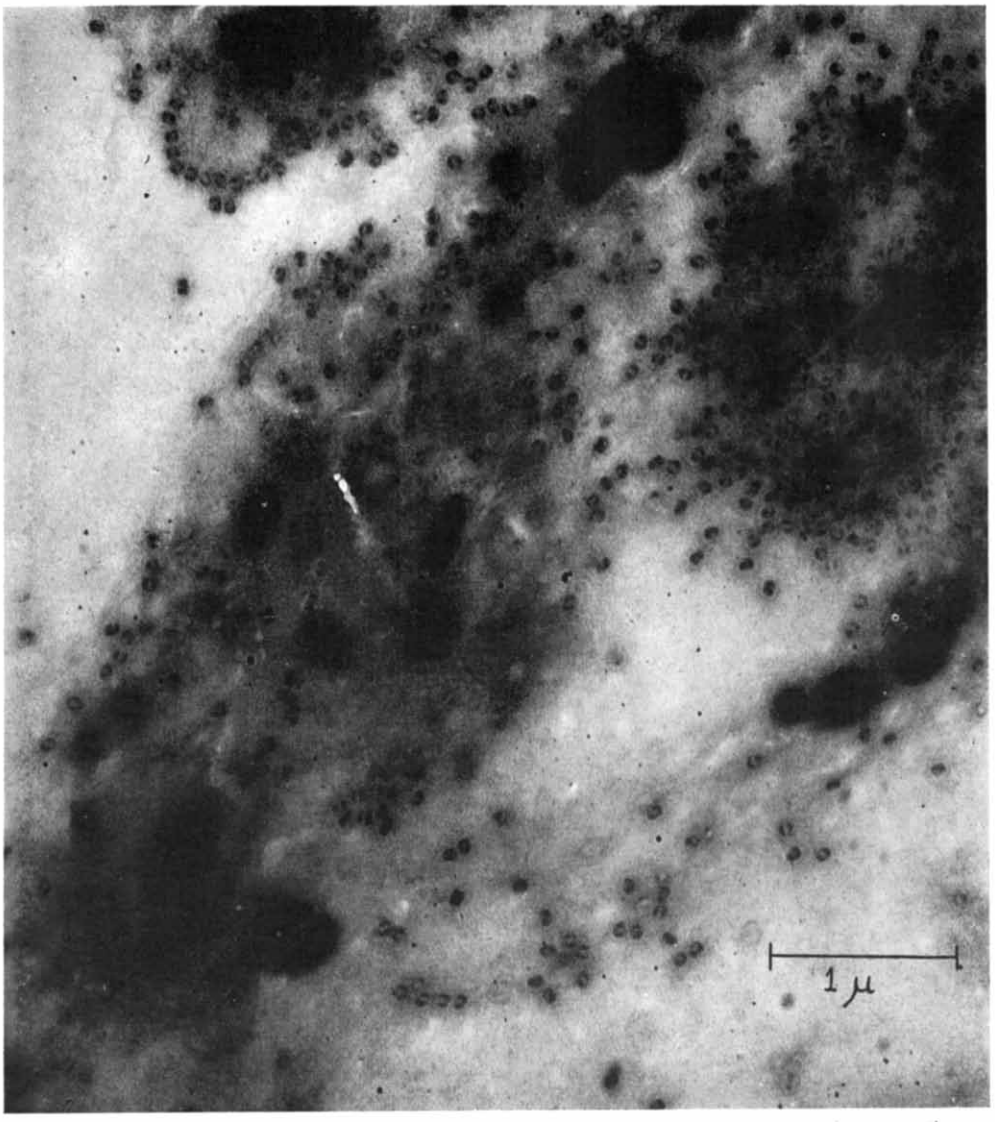

Fig. 20. # 5942 b. *E. coli*, fixed 40 minutes after infection with T2r+. × 25,000 (see p. 176).

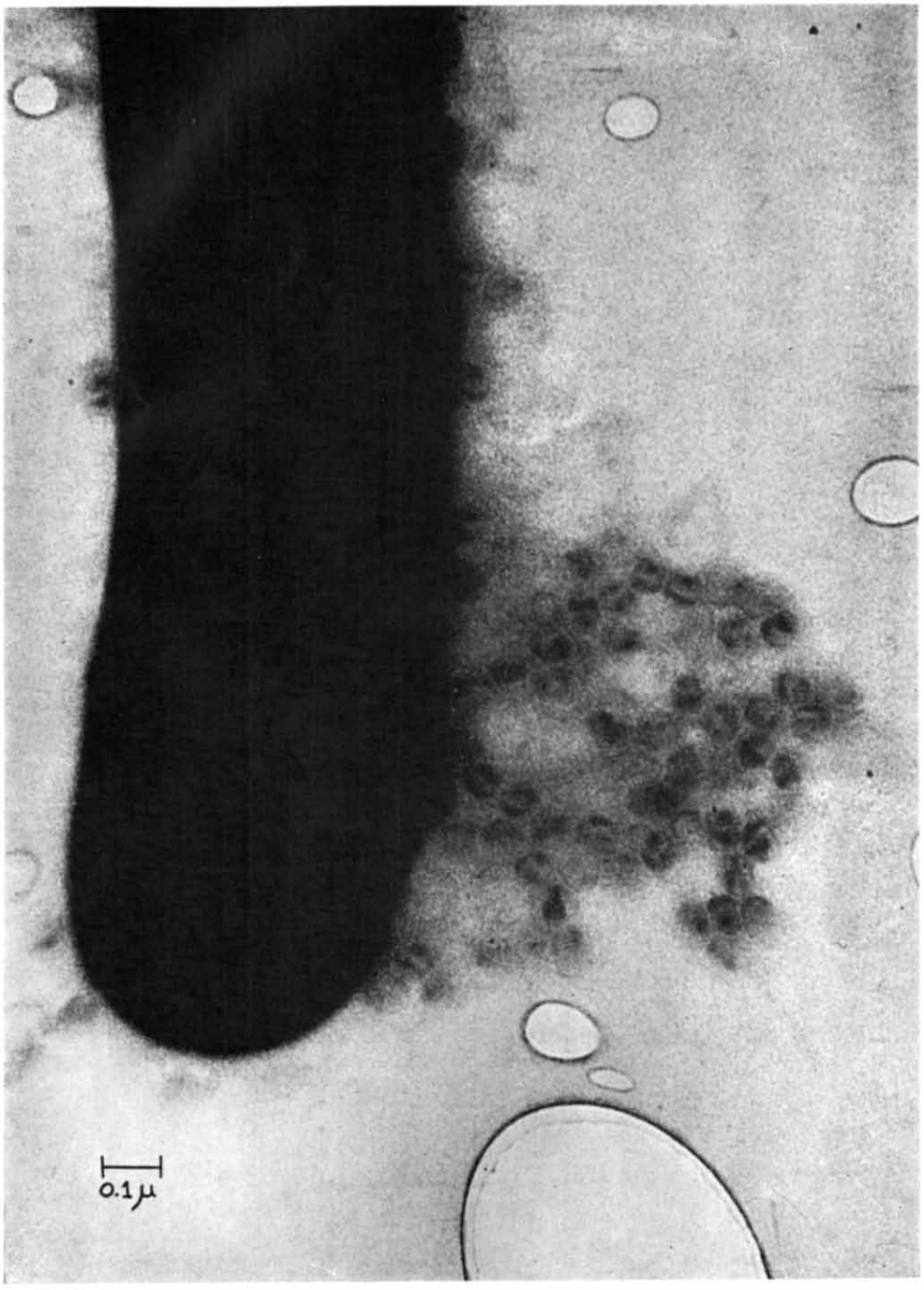

Fig. 21. # 5873a. *E. coli* and T2r phage particles fixed 1 minute after infection. × 83,000 (see p. 177).

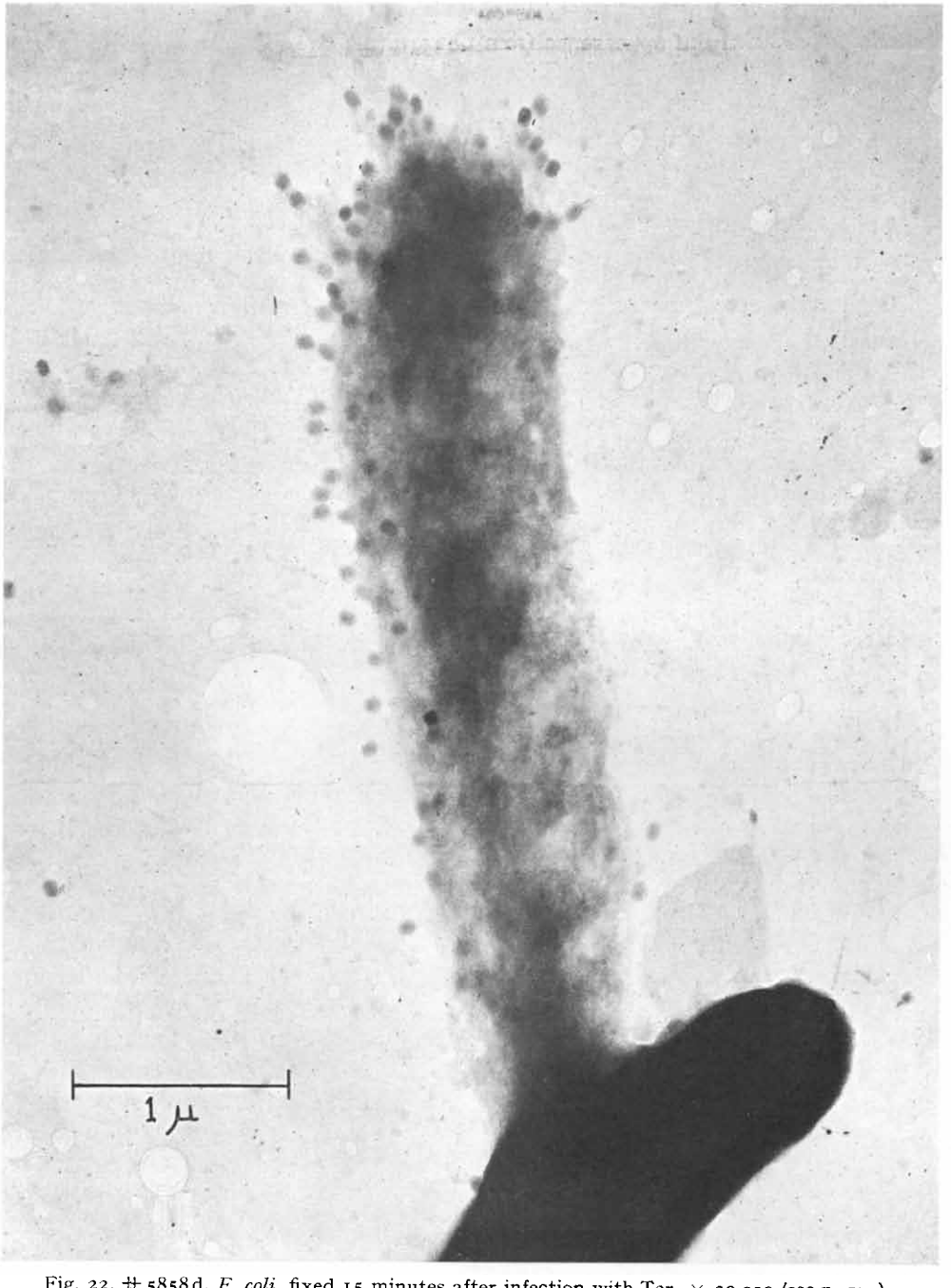

Fig. 22. # 5858d. *E. coli*, fixed 15 minutes after infection with T2r. × 30,000 (see p. 177).

cells infected with T2r+ in the meshes of which many phage heads and dense areas differing only slightly in appearance from phage heads are seen. Areas slightly larger than mature phage heads may be discerned in which a dense circular rim surrounds a lighter circle in the center of which is a dense granule; these are suggestive of the "immature phage particles" described by WYCKOFF[45–47] and by HERCIK[48]; we also interpret them as immature phage heads. The dark particle indicated by an arrow we believe to be a mitochondrion.

Fig. 16 shows the remains of two cells in the meshes of one of which there are a large number of T2r+ phage heads whose appearance is indistinguishable from that of mature external phage heads*. In the second cell are a much smaller number of dense areas; if, as we believe to be probable, these represent phage precursors they must be in quite immature state. This very unequal degree of maturation of phage particles in two cells from the same preparation is in line with the findings of DOERMANN AND DISSOSWAY[23].

In Fig. 17 are three cells infected with T2r in process of disintegration. In each large numbers of intracellular phage particles are discernible and from each numerous phage particles appear to be escaping. From the cell toward the bottom four rounded processes have been extruded, one still filled with cytoplasm, the others empty cell wall. Filiform processes are everywhere. The external phage particles, as earlier shown by ANDERSON[34], may be seen to comprise dense material within an external surface membrane which is continuous with the tail.

Fig. 18 shows for the most part mature phage particles and debris from T2r-lysed cells. This picture is included principally because it shows membranous structures such as have been depicted particularly by WYCKOFF[45] and whose interpretation is very difficult. In the lower right-hand corner are seen two elliptical membranous structures continuous with what is believed to be a segment of cell wall. To the left in the middle are several objects which are very suggestive of phage heads without their usual dense contents. These may well be the "unfilled" heads described by LEVINTHAL[25]. Obviously electron micrography can reveal more than is presently interpretable.

Fig. 19 shows a T2r-infected cell apparently still intact but packed with phage, and, below, innumerable phage particles scattered through the debris of a lysed cell.

Cells 40 minutes after infection with T2r+. Fig. 20 shows phage particles and debris 40 minutes after infection with the lysis-inhibiting strain. The phage particles seem to be uniformly mature. Because of the great amount of lysis at this late time after infection there is much obscuring material. Adsorption of some of this small molecular material to the phage heads is believed to have contributed to the coffee-bean appearance of the phage heads in Fig. 20. Attention is directed to the numerous large dark bodies strewn about among the debris. These are believed to be residual mitochondria (HARTMAN *et al.*[15]).

Study of the pictures of infected cells from the middle of the latent period (15 minutes after infection) to 40 minutes after infection reveals two facts of great importance to understanding of the process of phage replication. In the first place, the ratio of phage-like areas of density to definitive phage particles in the infected cells

* Phage particles without visible tails in the various unshadowed pictures of this study are not to be interpreted as tailless. The shadowed preparations and some unshadowed pictures show the phage particles to be ordinary tailed T2 phage. Photographic processing has purposely been adjusted to the demonstration of intracellular phage heads and the areas which we believe to be the precursors of phage heads.

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and cell debris is much greater than unity in cells near the middle of the latent period. This ratio gradually falls to a small fraction of unity with increasing time post-infection. Indeed in the picture at 40 minutes post-infection almost all of the particles are definitive, mature-looking phage particles. This progression is precisely what one would expect if the phage-like areas represent loci in which the assembly of mature, infective phage particles is proceeding. This we believe to be the most probable interpretation of our findings. This interpretation is compatible with the known facts obtained by other types of experimentation. Furthermore, the progression of phage-like to mature phage particles which we have demonstrated intracellularly seems to be in essential agreement with the succession of particles obtained by LEVINTHAL AND FISHER[24,25] extracellularly by breaking open the infected cells at successive intervals.

The second major fact of observation is that the definitive phage particles appear in increasing numbers as infection progresses, *within the cytoplasm of the infected cells.*

The structure of mature phage particles. The shadowed preparations of Figs. 10, 11, 17, 18 and 19 confirm what was already well known from the work of ANDERSON and others[34, 49, 50], namely that mature phage particles consist of dense material within a casing of membranous material with which the tail is continuous. In the dried preparations the contents of the heads are seen to have shrunken away from the surface membranes. Recent important work of HERSHEY AND CHASE[27] has shown that the sulfur-containing protein of the casing remains attached to the wall of the infected colon bacillus and the desoxyribose nucleic acid or nucleoprotein contents of the phage head, with its genetic determiners, enters the cell.

Fig. 21 shows internal structure within the contents of the phage head at very high magnification and resolution. The dark material, which appears at lower resolution to form a continuous pattern, may be seen in fact to be discontinuous. We have of course no evidence whatever for relating visible fine structures within the head with the linkage groups[30–32] of genetic determiners. Nevertheless it is reassuring to find fine structure of an order of complexity sufficient at least to offer no contradiction to the genetic evidence. T2 phage of exceptional purity has recently been prepared and studied by HERRIOTT AND BARLOW[57].

The adsorption of phage to bacterial cell. Fig. 22 shows a cell 15 minutes after infection with T2r. A large number of phage particles seem to be adsorbed to its surface by their tails. Adsorption of phage to host cell wall by the phage tails has been described previously by RUSKA[51] and by HAARDICK[52] as an occasional phenomenon. Latterly ANDERSON, using the critical point method of preparing specimens, has presented evidence that the normal mode of attachment of the tailed phages to their host cells is by the tips of their tails[49, 53]. The physical-chemistry of phage adsorption is discussed by HAARDICK[52] and by PUCK *et al.*[54–56].

DISCUSSION

The fundamental problem of phage replication has been approached with the T-series of phages and their host cell, *Escherichia coli* B, by diverse methods. Biophysical, genetic, biochemical and morphological procedures have each yielded considerable bodies of well authenticated *observations*. It is perhaps axiomatic that the data in each area of investigation must be authentic in and of themselves. Valid *interpretations* of the mechanisms of phage replication must, moreover, be congruent with all valid data.

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Whereas interpretations based primarily on morphological observations must be in essential agreement they cannot be *authenticated* by the fact of being in essential agreement with currently accepted interpretations of genetic and biochemical data; on the other hand, such morphologically based interpretations can be *invalidated* by their doing violence to well-established data in the genetics or biochemistry of phage replication. These axiomatic considerations have been rather often overlooked in the morphological investigation of phage replication.

The observation that phage particles assume their definitive morphology while within the meshes of the cytoplasm of host bacterial cells we believe to be unequivocal. Certainly this observation is in agreement with data currently available from other areas of investigation. Neither the morphological data nor data obtained by other means are in agreement with the several variants of the hypothesis of extracellular multiplication of phage. Similarly it is clear from many lines of evidence that mature phage particles as such do not multiply by binary fission.

LEVINTHAL AND FISHER[24, 25] and ourselves have described areas or particles present in the early part of the second half of the latent period in high ratio to particles of definitive morphology. These all the observers cited interpret as an immature phase of phage particles, but very likely not the multiplying phase. Phage particles of definitive morphology increase throughout the second half of the latent period. Study of the transition from phage precursors to infective phage is clearly a strategic area for further investigation.

There is much to suggest that insight gained into phage replication may afford insight into virus replication in general. Moreover these involved problems have so much in common with the problems of gene and chromosome replication and of the mechanisms of genetic control as to constitute one of the most challenging and critical areas of investigation in present-day biology.

SUMMARY

High contrast electron microscopic observations were made at known intervals after infection of *E. coli* B cells infected with T2 phage. During the first half of the latent period, marginal vesicular areas replaced the alternating dark and light patterning of normal cells. The latter half of the latent period was characterized by gross changes in the texture of the cytoplasm and by the gradual internal build-up first of phage "Anlagen" and second of mature phage progeny.

RÉSUMÉ

Nous avons étudié, au microscope électronique, des cellules de *E. coli* B infectées avec du phage T2, au bout de périodes d'infection de durées connues et à l'aide d'une technique donnant de forts contrastes. Pendant la première moitié de la période latente, le dessin alternativement foncé et clair des cellules normales se trouvait remplacé par des aires vésiculaires au bord des cellules. La deuxième moitié de la période latente était caractérisée par de gros changements de la texture du cytoplasme et par la formation graduelle interne, d'abord des "Anlagen" de phage puis des phages complets nouveaux.

ZUSAMMENFASSUNG

Mit T2-Phage infizierte *E. coli* B – Zellen wurden in bekannten Zeitabständen nach der Infektion hochkontrast-elektronenmikroskopisch beobachtet. Während der ersten Hälfte der latenten Periode ersetzten randständige blasige Flächen die abwechselnd dunkle und helle Musterung der normalen Zelle. Die zweite Hälfte der latenten Periode wurde durch grobe Änderungen im Cytoplasmagefüge und durch einen allmählichen inneren Aufbau erstens der Phagen-"Anlagen" und zweitens der fertigen Phagennachkommenschaft gekennzeichnet.

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