ELECTRON MICROSCOPE STUDIES OF PLEUROPNEUMONIALIKE ORGANISMS ISOLATED FROM MAN AND CHICKENS[1]

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The characteristic colony morphology is an important distinguishing feature which is used for recognizing members of the group of pleuropneumonia-like organisms. However, characteristic colony morphology may not be apparent when the organisms are freshly isolated from the body or when the growth is crowded on the surface of solid medium. In order to determine similarities or differences among the various strains of these organisms now being isolated from several sources, a study of their cellular morphology is desirable. This information may then aid in a more precise identification of these unusual organisms which is needed for their classification among microorganisms. The average diameter of the smallest phase of the saprophytic strains was shown by Laidlaw and Elford (1936) to be of the order of 125 to 175 mμ. Since this range is beyond the limits of resolution of the ordinary light microscope, the detailed morphology of these organisms needs to be obtained by means other than the light microscope. The electron microscope, especially with the aid of heavy metal shadowing, offers a means of obtaining some of the necessary information. The results of studying with the electron microscope several strains of pleuropneumonia-like organisms from man and chickens are presented.

MATERIALS AND METHODS

The 6 strains of pleuropneumonia-like organisms isolated from man had the following histories:

Strain Campo, isolated by Dr. Louis Dienes, Massachusetts General Hospital, from the urethra of a man with urethritis, was received from him in 1949. This strain has been maintained on artificial medium the longest of any of the strains studied and is the easiest to cultivate.

Strain 07 was isolated by Dr. Milford H. Hatch at the Department of Bacteriology, the Johns Hopkins University School of Hygiene and Public Health, from the human cervix and was received from him in 1948. A subsequent culture of this designation was received from Miss Isabella G. Schaub of the Johns Hopkins Hospital in 1949 and is the one employed in these studies.

The remaining 4 strains were isolated by Dr. Paul F. Smith about 1949 from the following sources: Strain 39 from the prostatic secretion of a patient with prostatitis, strain 48 from the urethra of a male with urethritis, and strains 60 and 110 from the cervices of women with cervicitis.

The 2 strains of pleuropneumonia-like organisms isolated from chickens with chronic respiratory disease were received in 1953 from Dr. Henry Van Roeckel, University of Massachusetts, Amherst, Mass. These strains labeled CRDA 5220 (26 passages) and CRDA 6222 (5 passages) have been designated chicken strains no. 1 and 2, respectively.

The cultures have been maintained by transferring at intervals of approximately 5 to 7 days on bacto-PPLO agar (Morton *et al.*, 1951) enriched usually with bacto-PPLO serum fraction (Smith and Morton, 1951). This is the medium which was employed when it was desired to use cultures grown on solid medium. Medium of the same composition with the omission of the agar was employed when it was desired to use cultures grown in liquid medium. For the basic studies on organisms grown in broth, cultures of approximately 40 hours were employed because it had

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been demonstrated (Lecce and Morton, 1954) that the viability and enzymatic activity of cultures in broth diminish rapidly after the fortieth hour. In the study of organisms grown on solid medium, cultures in their second or third day of incubation were employed.

An RCA electron microscope, model EMU, operating at 50 kilovolts was employed. The conventional screens with a formvar film were employed in preparing specimens for electron microscopy. Some specimens were fixed with osmic acid in a final concentration of one per cent, but this was not entirely satisfactory. The least distortion of the microorganism was obtained by placing the screens inverted on top of the colonies of pleuropneumonia-like organisms. After about 2 minutes the screens were carefully lifted off the colonies and were examined, or shadowed and then examined, without washing with distilled water. Specimens shadowed with chromium by the technique of Williams and Wyckoff (1944) were employed for the major portion of the studies as the specimens ordinarily prepared lacked contrast and details of structure. A few of the earlier electron micrographs were made at an original magnification of 4,670× employing a wide field pole piece, but the majority of the micrographs were made at a magnification of 8,690× with the standard pole piece. The original electron micrographs were enlarged 2.5× for photographic printing, and these have been reduced about 25 per cent in reproduction as half-tones. A contact print on a glass plate was made of all electron shadowed micrographs, and this positive print was used as a "negative" for making photographic prints.

RESULTS

When a broth culture of the Campo strain was mixed with an equal volume of 2 per cent solution of osmic acid, placed on a screen, allowed to partially dry, the remaining portion of the drop removed, the screen washed twice with distilled water, and examined in the electron microscope, the organisms were very few in number. Because of their scarcity and low contrast, examination was very difficult. The broth cultures were concentrated 10-fold by centrifuging for 15 minutes at 13,000 rpm in a Sorvall centrifuge. When this concentrated suspension of cells of pleuropneumonia-like organisms was mixed with an equal volume of 2 per cent solution of osmic acid, the screens prepared in the usual manner, and examined in the electron microscope, the specimens contained numerous cells as pictured in figures 1 and 2. The cells appear individually as nearly circular bodies of less than one μ in diameter. Clusters of 2, 3, and more than 7 cells were observed. The organisms lack contrast except for one or more dense areas of about 75 to 100 mμ in practically every cell. A similar preparation of these organisms was shadowed with chromium, and typical cells are shown in figure 4. The raised areas on the surfaces of the cells are comparable in size and location to the denser areas in unshadowed preparations. A very good example of a dividing cell is shown in figure 8 at higher magnification. A common observation is the small bud on the lower edge of the dividing cell.

If the concentrated suspension of unfixed organisms was used to prepare screens for electron microscopy and the specimens were washed twice with distilled water in the usual manner, the cells were very few in number. Their shape (figure 3) was like that of fixed organisms as shown in figures 1 and 2, and they varied in size from 0.2 to 0.75 μ in diameter. A similar preparation of these unfixed cells was shadowed with chromium before being examined in the electron microscope to give the organisms more contrast. The cells shown in figures 5, 6, and 7 are circular to elliptical in outline and vary from one-third to slightly less than one μ in diameter. Irregularities on the surface of some of the larger cells suggest a collapsed cell membrane.

Growth of the Campo strain in liquid medium showed one morphological form of the organisms which was circular to elliptical in outline. To determine if growth on solid medium might affect the morphology, a sterile platinum loop was drawn over the area of solid medium showing numerous colonies, and the organisms thus removed were suspended in a solution composed of equal volumes of 0.85 per cent sodium chloride and 2 per cent osmic acid solutions. Colonies of pleuropneumonia-like organisms are not readily rubbed off the surface of solid medium with a platinum loop as the colonies grow into the medium. However, some organisms were obtained in this manner. Specimens for electron microscopy were prepared in the usual manner. The organisms shown in figures 9, 10, and 11 are comparable in size and shape to those grown in liquid medium but are not of as uniform density.

Denser material is located towards the periphery of the cells. The organisms do not seem to tolerate well being suspended in a solution containing 0.43 per cent sodium chloride and one per cent osmic acid. Similar preparations were shadowed with chromium before being examined in the electron microscope, and representative cells are pictured in figures 12, 13, 14, and 15. The surface of the cells is very flat and uneven.

To obviate the necessity of washing the specimens with distilled water or even to bring the organisms in contact with the solution of osmic acid, screens were placed inverted on top of the colonies of pleuropneumonia-like organisms. After about 2 minutes the screens were removed carefully and examined in the electron microscope without washing with distilled water. The cells of strain Campo in figure 16 and of strain 07 in figure 18 are more opaque than when the cells had been subjected to the fixing solution and washed with distilled water, or when the organisms had been grown in liquid medium. The surface of the cells in shadowed preparations was smoother when the organisms had been subjected to a minimum of manipulations as is evident in figures 17 and 19. The specimen in figure 20 is difficult to interpret. It may be a cell, or it may be material similar to that shown in later pictures and interpreted as nonliving material.

Figure 28 is an unshadowed electron micrograph of an unfixed cell of strain 60. It is similar to the cells of strain Campo in unshadowed preparations.

The shadowed preparations of cells of strains 39 (figures 21 and 22), strain 48 (figures 23 to 27), strain 60 (figures 29 to 34), and strain 110 (figures 35 to 38) are similar to those in comparable preparations of strains Campo and 07.

The two chicken strains were similar to each other and also to the 6 human strains of pleuropneumonia-like organisms. The largest cells in the chicken strains, which were approximately 2 μ in diameter, were larger than the largest cells in the human strains. The cells were predominately circular in outline (figures 42, 43, 47 to 50). Cells with a dense area, as well as budding and dividing cells as in the human strains, are evident in figures 39, 40, 41, 45, and 51. Small structures on the order of 200 mμ in diameter are evident in figure 44 as they are in figure 17 of human strain Campo. Some cells in unshadowed preparations are very dense (figures 16, 18, and 43). The surface of the larger cells in shadowed preparations show an unevenness reflecting differences in structure of the underlying cytoplasm (figures 42, 44, 45, 46, and 50).

In all of the preparations made by placing the screens in contact with the colonies of pleuropneumonia-like organisms and examined in the electron microscope without washing, there were structures markedly different from the cells of pleuropneumonia-like organisms and which appeared to be nonviable in many cases. In some instances these structures were a few microns in their longest diameters and appeared to have developed fissures as from drying (figures 57 to 61, 66 to 69). In some preparations this material was drawn out into thin strands (figures 56, 62, 64, and 65). In other preparations the strands were shorter and thicker (figures 54, 55, and 71). In some areas masses of this supposedly lifeless material can be seen lying adjacent to pleuropneumonia-like organisms as is well illustrated in figures 70, 52, 53, and possibly 63.

No flagella were observed.

No rigid cell wall was observed as exemplified by receding cytoplasm in dried specimens.

DISCUSSION

Many of the earlier studies on pleuropneumonia-like organisms were made on strains isolated from animals. With the invention of the electron microscope, workers utilized this new instrument in their efforts to resolve the nature of the relatively recently discovered microorganisms. As is so frequently the case, the results obtained during the pioneering stage of a science, while dramatic at the time, have to be extended with the development of the science. There have been improvements in electron microscopy and in the cultivation of the pleuropneumonia-like organisms so that a study of these organisms is more feasible. A variety of strains has been studied to date which permits comparisons and some generalizations.

Bovine strains. Two types of organisms have been isolated from cattle; the agent of bovine pleuropneumonia, *Asterococcus bovis*, and the pleuropneumonia-like organisms from the genital tract. In an electron microscopic study of *A. bovis*, Ruska and Poppe (1947) observed three morphological forms. In addition to the nearly round and nearly homogenous organisms of poor contrast and the ring or invaginated forms, as

seen in the saprophytic strains, bizarre filaments of varying thickness were observed. Freundt (1952*a*) published electron micrographs showing branching mycelium with homogeneous filaments and terminal nodes. The "large bodies" observed by Freundt (1952*b*) were "regarded as banal degenerative and involution forms whose significance to the morphology of the peripneumonia organisms can be of a subordinate nature only." The long, branching filaments pictured by Freundt (1952*a*) in his figures 13 and 14 were in cultures grown on the surface of the collodion membrane, used in electron microscopy, floating on the surface of liquid medium. This environment is quite different from that in which the organisms are usually cultivated.

The relationship between the pleuropneumonia-like organisms isolated from the bovine genital tract to the organism of pleuropneumonia has not been established. Edward (1950*a*) reported 2 types of genital strains; the P strains, regarded as possibly pathogenic for cattle, and S strains, which resembled saprophytic strains. All of the cultures investigated by Edward (1950*b*) using light microscopy were stated to have the same general morphology except the P and L3 strains which, in addition, contained peculiar spherical masses. Liebermeister (1953), by means of studies with the electron and light microscopes, concluded that the cultures were made up of round to oval forms of variable sizes. If, in the preparation of the microorganisms for microscopy, the organisms are subjected to lateral displacement or exertion of pressure, either accidently or intentionally, nonsegmented mycelial filaments with terminal consolidations and distended round to oval forms are produced. Because of the ease of altering the size and shape of the cells, it was interpreted that a cell membrane similar to that of bacteria is not present in pleuropneumonia-like organisms. The tendency to assume filamentous forms was found to be greatest in the case of the bovine pleuropneumonia organism and least in the case of the saprophytic strains of pleuropneumonia-like organisms.

Human strains. Two strains of pleuropneumonia-like organisms from the human cervix were studied by Smith, Hillier, and Mudd (1948). The strains were obtained from Dr. Louis Dienes in whose laboratory they had been maintained on artificial medium for several years. The L50 strain showed round bodies 0.5 to 3.0 μ in diameter, rod forms, and filaments up to 5 μ in length and 0.5 μ in width. The rod forms, which were numerous, averaged 1.3 by 0.5 μ and showed a well defined cell wall. Well-developed cell walls were not detected on the round bodies. In the case of strain L4330, variation in morphology was shown to be associated with crowding of the colonies on the medium. Where the growth was confluent due to crowding of the colonies from heavy inoculation, large round bodies, bacillary forms, or filaments could not be found. The extremely small colonies, when examined with the oil immersion lens, were found to consist of a rather compact mass of tiny, deeply stained granules growing down into the agar. Some of the granules were connected by a fine thread. In colonies which were less crowded, large round bodies 5 to 7 μ in diameter were observed along with the granules growing down into the medium. Colonies more widely separated appeared similar, but some of the "granules" were distinctly rod shaped instead of round. One to five small dense granules were observed along the limiting surface of the round bodies. The electron micrographs of strain L50 shown in figures 1 to 4 are different from any others which have been pictured for pleuropneumonia-like organisms. A recent attempt was made to obtain a subculture of this strain from Dr. Dienes, but unfortunately the strain no longer exists. Strain L4330 appears more like a pleuropneumonia-like organism. Figures 7, 8, and 9 are comparable to figures 12, 13, and 15 in this report.

Electron pictures of an oral strain and of a genital strain of pleuropneumonia-like organisms isolated from man were published by Dienes (1953). The cultures consisted of round elements, considerable less than one μ in diameter.

Rat strains. Electron micrographs were made by Weiss (1944) of a gram negative bacillus isolated from the fluid of swollen arthritic joints of a rat. From cultures incubated four hours, bacillary cells one μ or more in length were observed clumped together and containing a light spot or vacuole. From cultures incubated 24 hours, ring forms were observed. Filaments, giving the impression of streaming protoplasm, were observed attached to some of the ring forms. This rat strain appears to be slightly different from other strains. In one respect it differs from the human strains in that appreciable growth took place

within the first four hours of incubation. Lecce and Morton (1954) and Keller and Morton (1954) reported human strains to have a rather long lag period, of the order of eight hours. The generation time for the human strains was estimated by Keller and Morton (1954) to be 3.25 ± 0.75 hours. The generation time for one saprophytic strain was calculated by Laidlaw and Elford (1936) to be 1.6 hours at 30 C and 1.1 hours at 37 C. Weiss pointed out that the preparation of the organisms for electron microscopy, such as the washing with water, subjected them to be repeated changes in surface tension. In spite of this harsh treatment the organisms are very numerous in the fields.

Mouse strains. Gonnert's virus of bronchopneumonia in mice was studied in the electron microscope by Ruska (1944). The size, shape, and very poor cell wall morphology placed the organisms with the pleuropneumonia-like group. Nelson (1950) stated that the coccobacilliform bodies isolated from mice were so similar to pleuropneumonia-like organisms that he saw no reason for separating the two. Edward (1947) reported that the two mouse strains examined for motility were nonmotile which is in keeping with the failure to find flagella on the human and chicken strains.

Poultry strains. In previous electron micrographs of the chronic respiratory disease agent from poultry (Reagan *et al.*, 1953), round to elliptical bodies as well as filamentous forms were observed. However, the material was collected from infected chick embryos where it was possible for more extraneous material to be present in the specimens.

Saprophytic strains. Ruska and Poppe (1947) published electron micrographs of Seiffert's strains. The organisms were mainly round objects 0.4 to 0.7 μ in diameter, nearly homogenous and of poor contrast. The organisms often showed pairs of cells of equal size suggesting binary fission, but there was also evidence of budding. Evidence of filaments, such as found in cultures of bovine pleuropneumonia cultures, was not found. Some protoplasmic extensions were observed, but the authors stated that it seems doubtful that the pure filamentous formations have any reproductive properties. A membrane appeared to border the organisms, but it differed so much from that observed around bacteria that it was considered a plasma lemma. Liebermeister (1953) also found that round forms predominated and that filamentous forms, as produced in the bovine strains as the result of harsh treatment of the organisms, were not encountered. In spite of the fact that the saprophytic strains show less tendency to form filamentous shapes than strains from cattle, goats, rats, mice, and man, their surface does not exhibit the characteristics of a bacterial cell wall.

Like Liebermeister (1953) we have found the organisms from broth cultures to be quite uniform in shape and size. This is not the case when organisms from colonies growing on solid medium have been studied. The large masses of amorphous material, such as shown in figure 69, dry out unevenly in the electron microscope, and smaller masses produce vacuoles as shown at the top of figure 71. Vacuoles such as this were observed to develop during examination in the electron microscope. In figure 70 the material appears to have assumed the shape of the letter S between cells of pleuropneumonia-like organisms. In other instances the amorphous material appears to have formed short thick filaments as in figures 52, 54, 55, 61, and 71. In figures 56, 62, 64, and 65 the filaments have become very long and thin. It is difficult to visualize large bodies with fissures in them, as though due to the effects of drying, such as pictured in figures 59, 60, and 66, as having been viable structures. The majority of the strains of pleuropneumonia-like organisms isolated from animals require for growth *in vitro* the presence of some body protein such as ascitic fluid or blood serum. Many of the saprophytic strains do not require a body fluid for growth *in vitro*. Some of the globules may be cholesterol liberated from the culture medium by enzymatic action of the organisms as suggested by Partridge and Klieneberger (1941) for the L1 variant of *Streptobacillus moniliformis*.

The presence of pleuropneumonia-like organisms has taken on added significance with the finding by Freundt (1954) that 2 strains from humans and a strain of the bovine pleuropneumonia organism were similar serologically. The 3 strains were somewhat similar in their biochemical reactions.

There are several aspects in which the pleuropneumonia and pleuropneumonia-like organisms are similar to each other, and both are different from bacteria. (a) Lack of a rigid cell wall like that found in bacteria has been mentioned by

several workers. (b) The fragility of the organisms to changes in osmotic forces is very pronounced. Most workers who have studied the pleuropneumonia and pleuropneumonia-like organisms in the electron microscope have commented on the destructive action of distilled water on the cells. The organisms are much more susceptible to the action of soaps than are bacteria (Keller, Smith, and Morton, 1952). (c) Growth of the colonies of these organisms into the agar medium differentiates them from bacterial colonies. (d) The colonies are differentiated from bacterial colonies by their characteristic staining with the method of Dienes. (e) The organisms are much more resistant to penicillin than are bacteria. Their resistance is of a different magnitude from that of even the most resistant bacteria. (f) The majority of bacterial species are inhibited by thallium acetate which is without effect upon these organisms. The pleuropneumonia-like organisms are not inhibited by crystal violet which inhibits the majority of gram positive bacteria, nor by potassium tellurite which inhibits most gram negative bacteria. Edward (1954) points out that (g) cholesterol, or certain other sterols, appears to be necessary for the growth of these organisms while it is not known to be required as a nutrient by any other bacteria, and (h) homologous antibody without complement is able to inhibit these organisms.

ACKNOWLEDGMENTS

We are grateful to Dr. Henry Van Roeckel for the 2 chicken strains of pleuropneumonia-like organisms and to Dr. Paul F. Smith for valuable assistance.

SUMMARY

Six strains of pleuropneumonia-like organisms isolated from man and 2 strains isolated from chickens have been studied in the electron microscope.

Cultures grown in liquid medium showed cells which were spheroidal to ellipsoidal in shape.

Cultures grown on solid medium showed, in addition to the coccoid forms seen in cultures grown in liquid medium, a variety of bizarre forms varying from large circular masses to long filamentous forms. These bizarre forms have been observed in electron micrographs of strains of pleuropneumonia-like organisms from other species of animals. Possible explanations of the existence of these forms have been discussed.

No well differentiated rigid cell wall such as seen in bacteria was observed.

No flagella were detected.

The organisms are readily destroyed upon contact with distilled water.

ADDENDUM

While this paper was in press, White, Wallace, and Alberts (Poultry Sci., **33**, 500–507, 1954) published electron micrographs of shadowed preparations of the agents of chronic respiratory disease of chickens and of sinusitis of turkeys. These agents were indistinguishable morphologically and are similar to pleuropneumonialike organisms. The nonfilamentous structures in the two papers are similar.

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PLATE I

Figures 1 to 7 are reproduced at a magnification of 12,000 ×; figures 8 to 20 at 20,000 ×.

Figures 1 and 2. Strain Campo (pictures 1449 c and e), 40 hour broth culture in the early stationary phase was concentrated 10-fold in the Sorvall centrifuge by centrifuging for 15 minutes at 13,000 rpm. Equal volumes of the unfixed concentrated suspension and 2 per cent solution of osmic acid were mixed, and screens were prepared with the concentrated fixed suspension. The preparations were allowed partially to dry, were then washed twice with distilled water, and examined in the electron microscope with the wide field pole piece.

Figure 3. Strain Campo (1450 d). The concentrated unfixed suspension described for figures 1 and 2 was placed on screens, allowed partially to dry, washed twice with distilled water, and examined in the electron microscope with the wide field pole piece.

Figure 4. Strain Campo (1459S e). Conditions were the same as those described for figures 1 and 2 except that the preparation was shadowed with chromium before being examined in the electron microscope with the wide field pole piece.

Figures 5, 6, and 7. Strain Campo (1461S b, c, and d). Conditions were the same as those described for figure 3 except that the preparation was shadowed with chromium before being examined in the electron microscope with the wide field pole piece.

Figure 8. Strain Campo (1481S b). Conditions were the same as those described for figure 4 except that the preparation was examined in the electron microscope at a higher magnification and with the standard pole piece which was used for all subsequent pictures.

Figures 9, 10, and 11. Strain Campo (1482 a, 1483 d and b). The culture had grown on the surface of solid medium for 2 days. A sterile platinum loop was drawn over an area of the medium showing numerous colonies, and the organisms thus removed were suspended in a solution consisting of equal parts of 0.85 per cent sodium chloride and 2 per cent osmic acid solutions. Specimens were prepared from this suspension for electron microscopy in the usual manner.

Figures 12, 13, 14, and 15. Strain Campo (1485S a, b, e, and c). Conditions were the same as those described for figures 9, 10, and 11 except that the preparation was shadowed with chromium before being examined in the electron microscope.

Figure 16. Strain Campo (1484 c). The same culture described for figures 9, 10, and 11. The specimen was prepared by placing the screen inverted in direct contact with the colonies of pleuropneumonialike organisms for about 2 minutes and then carefully removing. Washing with distilled water was not necessary. Specimen examined in the electron microscope in the usual manner.

Figure 17. Strain Campo (1486S c). Conditions were the same as those described in figure 16 except that the specimen was shadowed with chromium.

Figure 18. Strain 07 was grown on solid medium for 3 days. The specimen (1487 b) was prepared and examined as described for figure 16.

Figures 19 and 20. Strain 07 (1495S a and d). Conditions were the same as those described for figure 18 except that the specimens were shadowed with chromium.

PLATE I

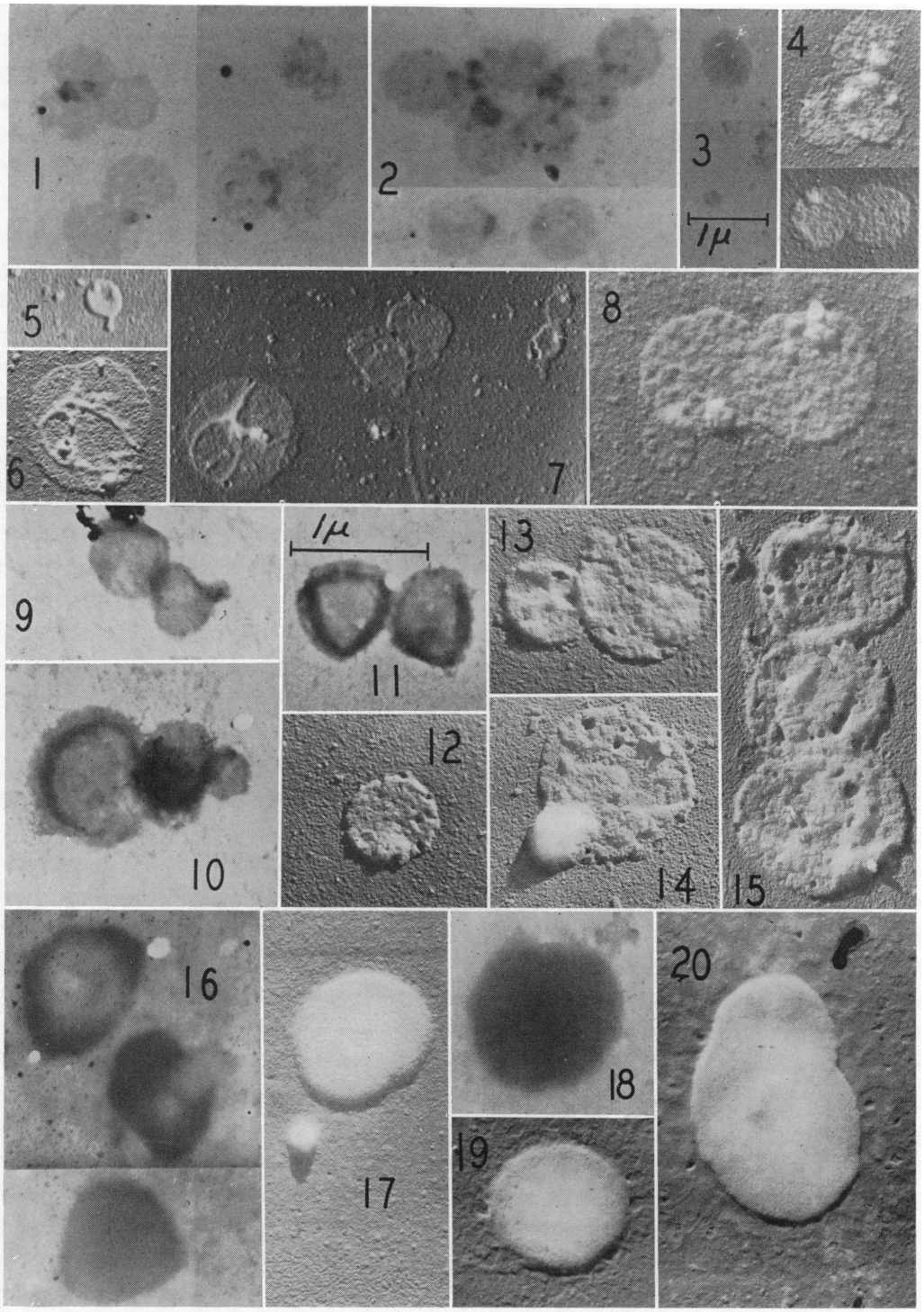

PLATE II

Figures 21 to 38 are reproduced at a magnification of 20,000 ×. All cultures were grown on solid medium for 3 days. All specimens were prepared by placing the screens inverted in direct contact with the colonies of pleuropneumonialike organisms for about 2 minutes and then carefully removing. No washing with distilled water was necessary. All specimens were shadowed with chromium except figure 28.

Figures 21 and 22. Strain 39 (1497S a and d).

Figures 23 to 27. Strain 48 (1496S a, b, and c; 1491S d and e).

Figures 28 to 34. Strain 60 (1489 a; 1493S b, d, and e; 1498S a, b, and d).

Figures 35 to 38. Strain 110 (1499S a, c, e, and b).

PLATE II

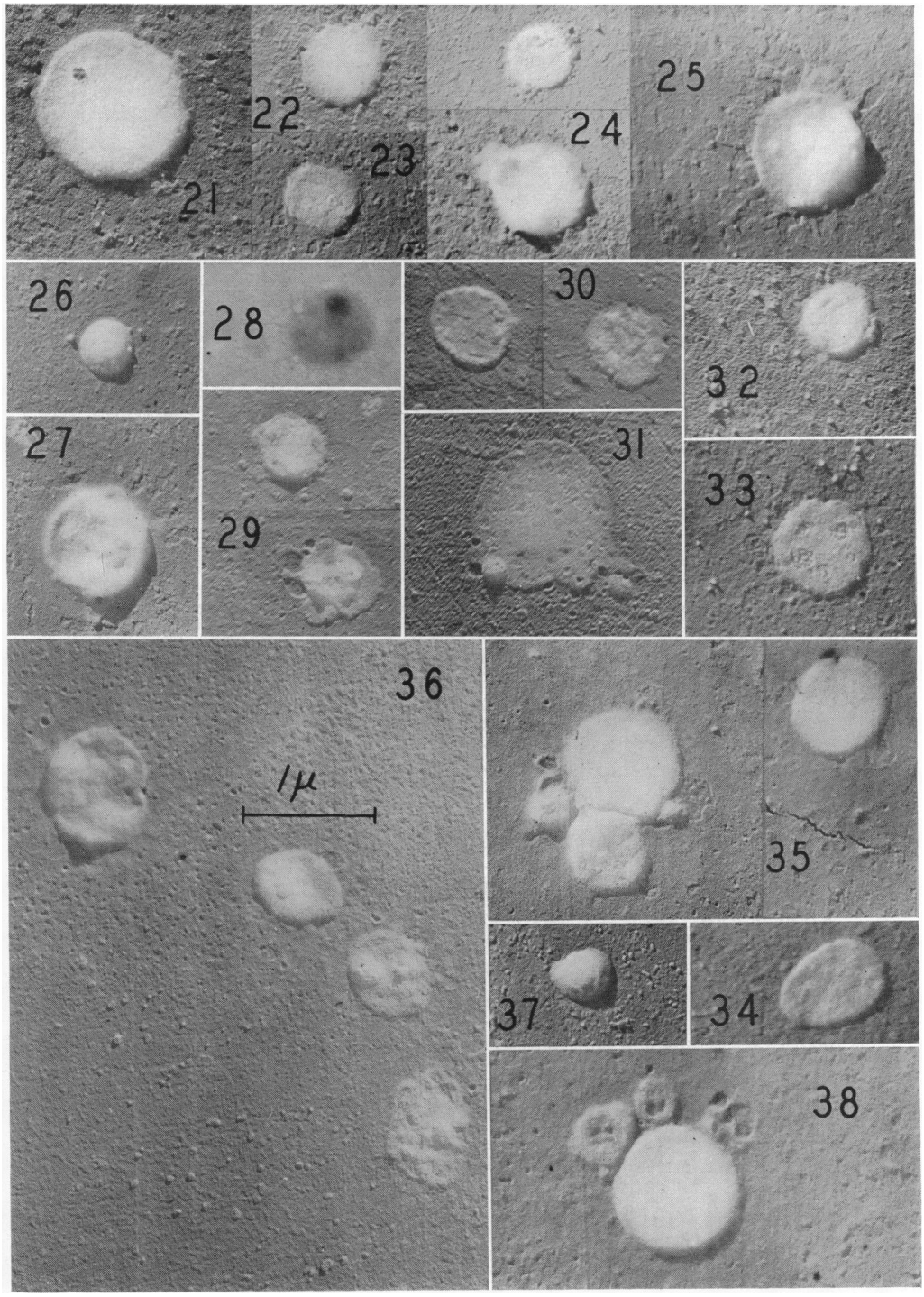

PLATE III

Figures 39 to 50 are reproduced at a magnification of 20,000 ×. All cultures were grown on solid medium for 3 days. All specimens were prepared by placing the screens inverted in direct contact with the colonies of pleuropneumonialike organisms for about 2 minutes and then carefully removing. No washing with distilled water was necessary.

Figures 39, 40, 41, and 43 are unshadowed preparations. Preparations for figures 42 and 44 to 50 were shadowed with chromium.

Figures 39, 40, 41 (1569 a, c, and d), 42, 44, 45 (1577S c, b, and d), 43 (1568 b), 46, 47, and 48 (1578S c, a, and b) are of chicken strain no. 2.

Figures 49 and 50 (1576S a and b) are of chicken strain no. 1.

PLATE III

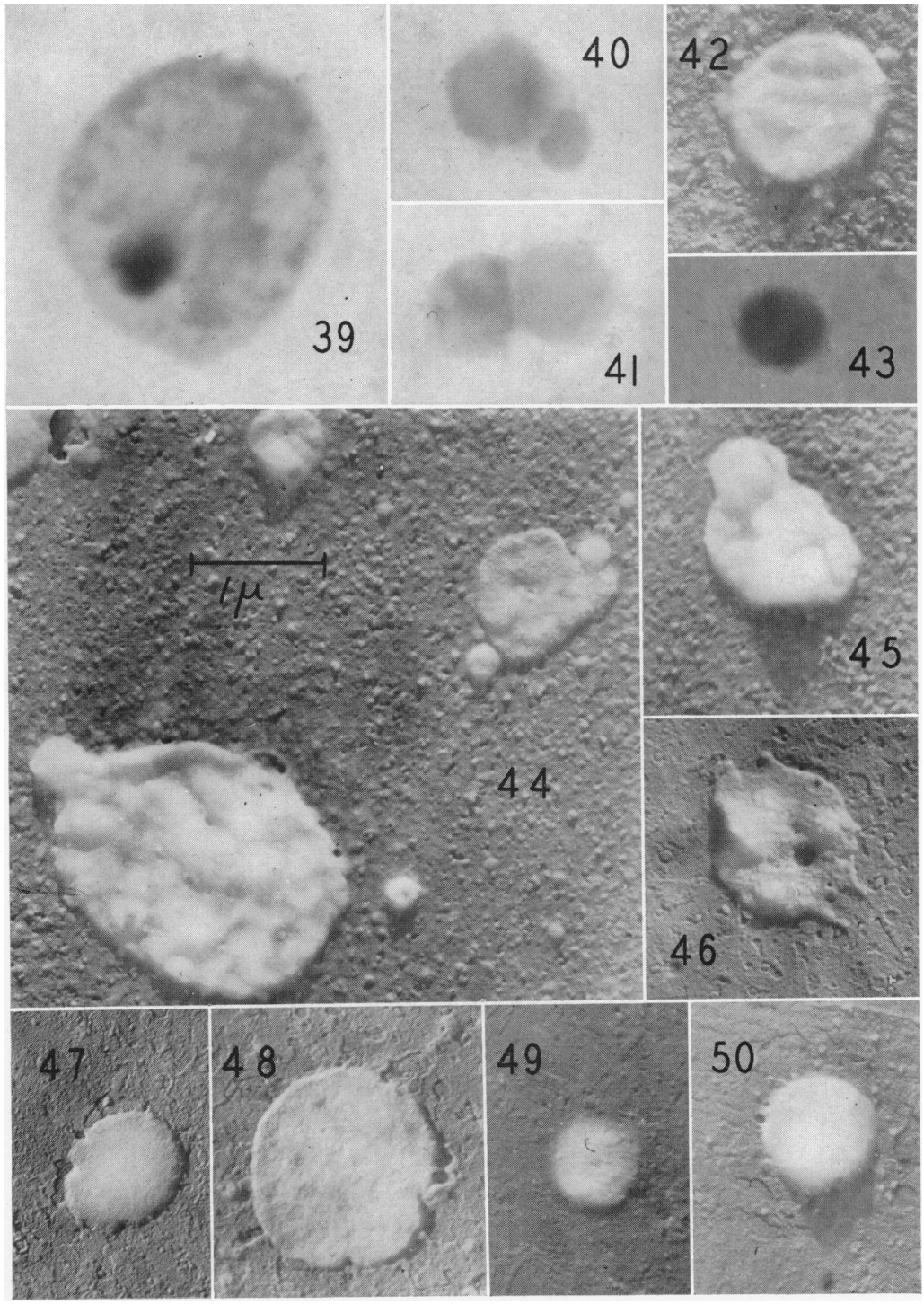

PLATE IV

Figures 51 to 55 are reproduced at a magnification of 20,000 ×. All preparations were prepared by placing the screens in direct contact with the colonies as described for Plate III. All specimens were shadowed with chromium.

Figures 51 to 54 (1577S e; 1578S e and d; and 1580S e) are from a 3 day old culture of the chicken strain no. 2 grown on solid medium.

Figure 55. Strain Campo (1486S e) 2 day old culture grown on solid medium. This is the same culture used for figure 17.

PLATE IV

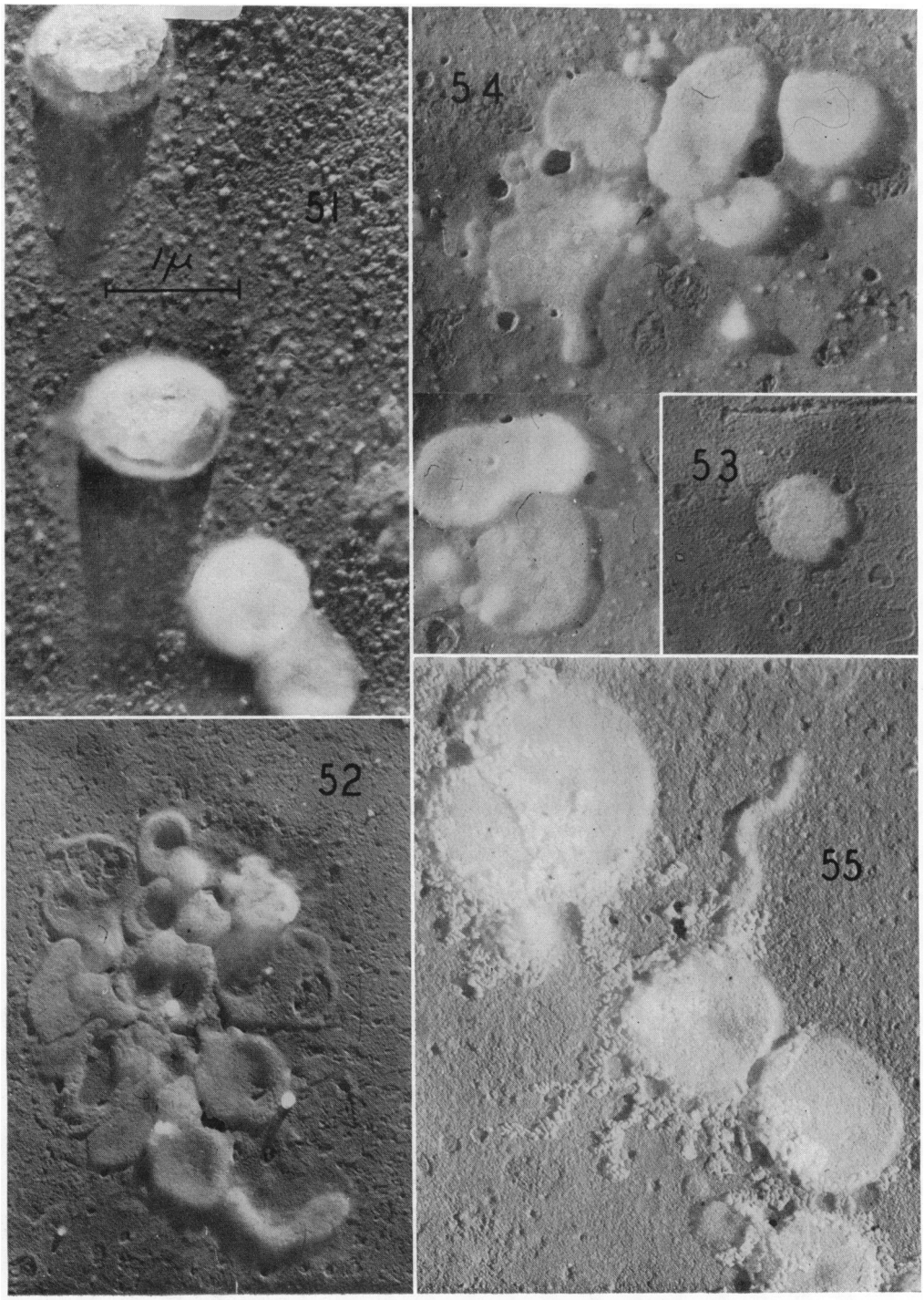

PLATE V

Figures 56 to 61 are reproduced at a magnification of 20,000 ×; figure 62 is at 9,430 ×. All preparations were prepared by placing the screens in direct contact with the colonies as described for Plate III. All preparations, except figure 59, were shadowed with chromium.

Figures 56 and 57. Strain Campo (1486S b and d) grown for 2 days on solid medium. This is the same culture used for figure 17.

Figure 58. Strain 60 (1493S c) grown for 3 days on solid medium.

Figures 59 to 62. Strain 07 (1487 d; 1945S e; 1490S a and c) grown for 3 days on solid medium.

PLATE V

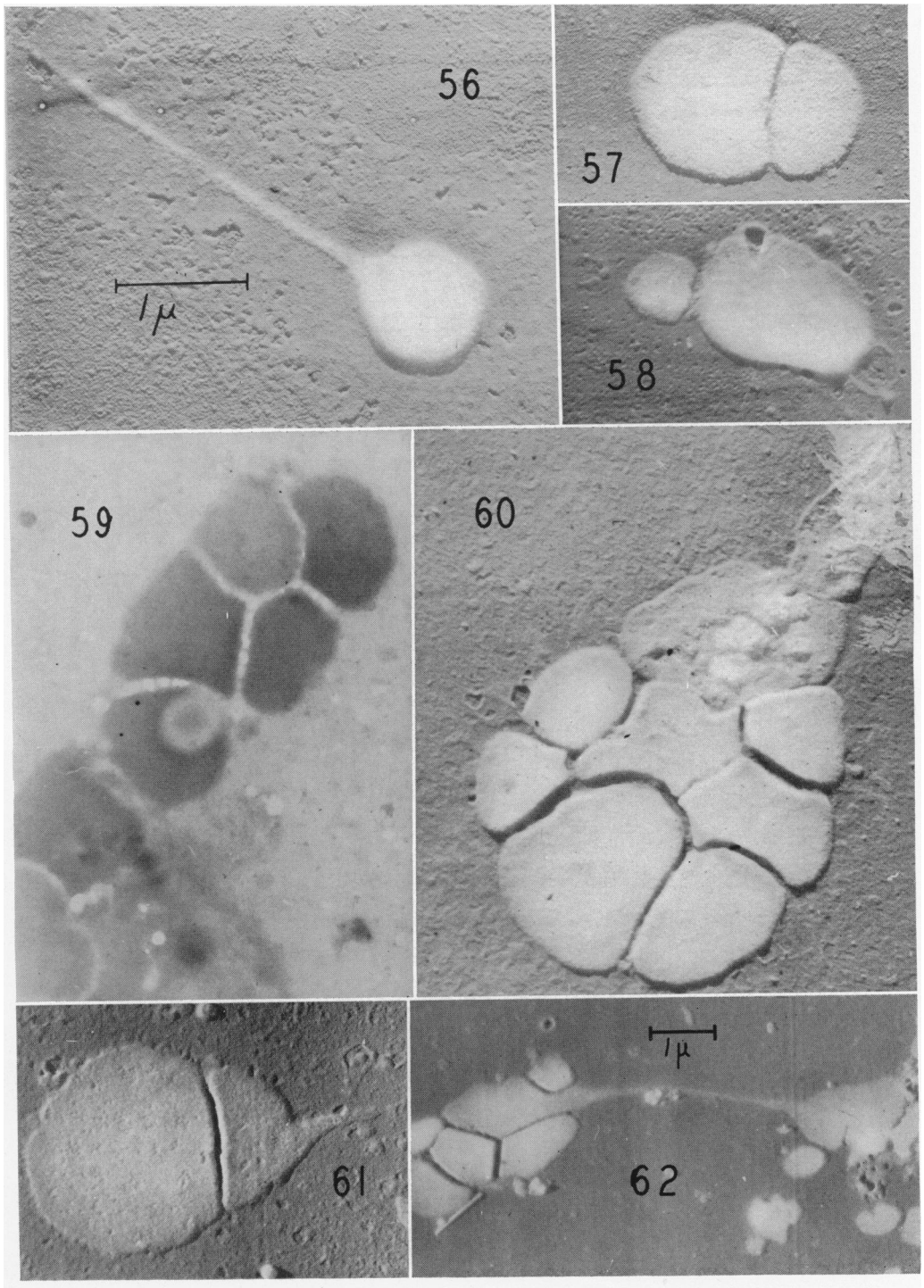

PLATE VI

Figures 63 to 68 are reproduced at a magnification of 20,000 ×. All preparations were prepared by placing the screens in direct contact with the colonies as described for Plate III. Figures 63 to 65 are ordinary electron micrographs. In figures 66 to 68 the specimens have been shadowed with chromium. The cultures were grown for 3 days on solid medium.

Figures 63 to 67. Strain 39 (1488 c, d, and e; and 1492S a and d).

Figure 68. Strain 110 (1499S d). This is the same culture which was used for figures 35 to 38.

PLATE VI

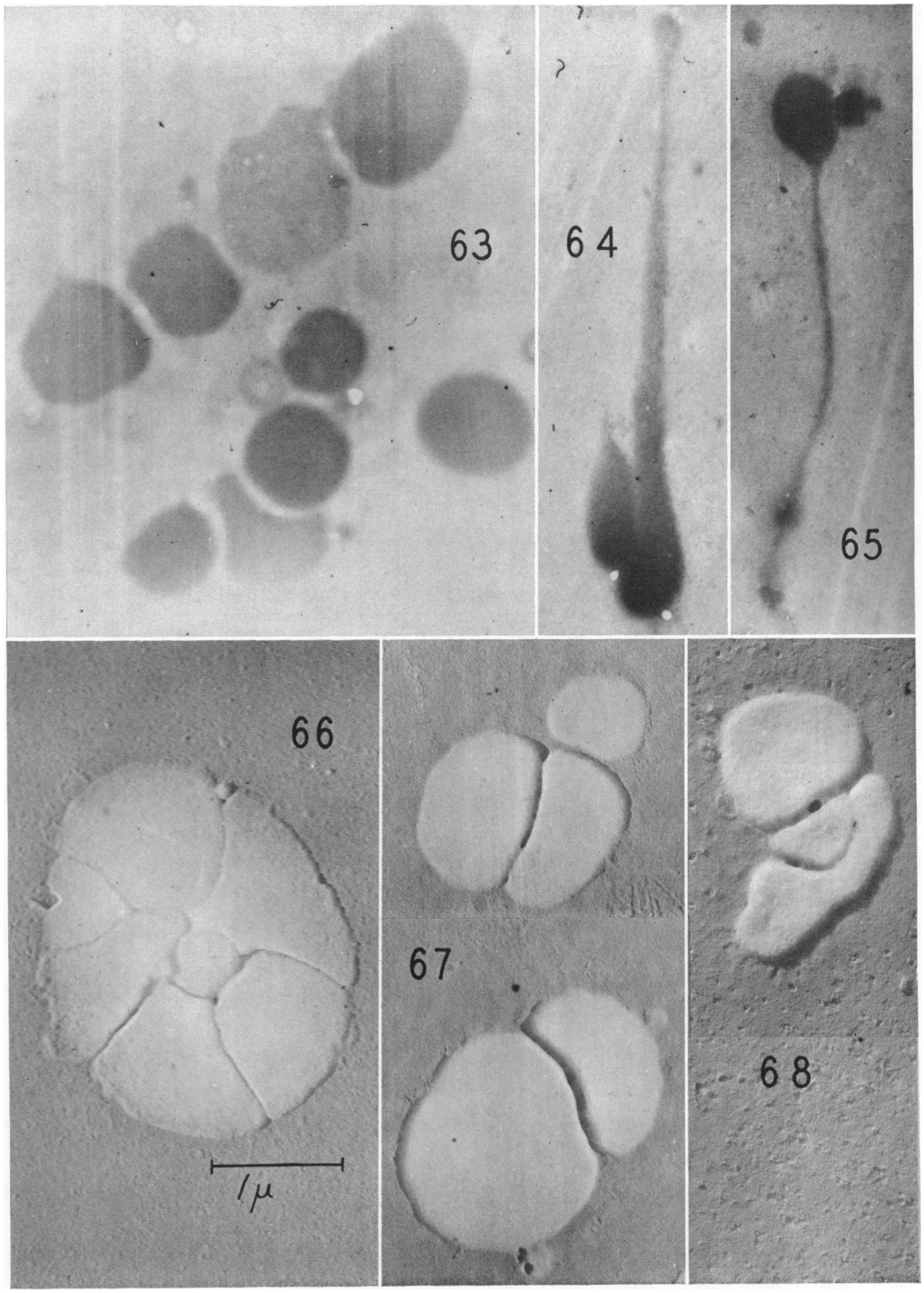

PLATE VII

Figures 69 to 71 are reproduced at a magnification of 20,000 ×. The cultures were grown for 3 days on solid medium. The specimens were shadowed with chromium.

Figure 69. Strain (1496S d). This is the same culture which was used for figures 23 to 25.

Figure 70. Strain 110 (1494S b). This is the same culture which was used for figure 36.

Figure 71. Strain 39 (1497S c). This is the same culture which was used for figures 21 and 22.

PLATE VII