INFECTION AND IMMUNITY, June 1988, p. 1456–1463
0019-9567/88/061456-08$02.00/0

Vol. 56, No. 6

Ultrastructural Study of Endocytosis of *Chlamydia trachomatis* by McCoy Cells

RICHARD L. HODINKA,† CAROLYN H. DAVIS, JOHN CHOONG, AND PRISCILLA B. WYRICK*

Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Received 15 December 1987/Accepted 1 March 1988

The entry of *Chlamydia trachomatis* into McCoy cells (fibroblasts) was studied by transmission electron microscopy. On adsorption of elementary bodies (EBs) to host cells at 37°C, the EBs were bound primarily to preexisting cell-surface microvilli. They were also observed in coated pits located at the bases of the microvilli and along smooth surfaces of the host cells and were internalized within coated vesicles at this temperature. Postembedding immunogold labeling on Lowicryl thin sections with anti-clathrin antibody as the primary reagent revealed the gold marker localized in pits and vesicles containing chlamydiae. Some EBs were present in smooth-surfaced invaginations at or near the bases of microvilli and in vesicles devoid of distinguishable coat material. A similar entry process was observed with centrifugation-assisted inoculation of EBs onto the McCoy cells. Individual EBs were initially internalized into tightly bound endocytic vesicles. However, within 1 to 3 h postinfection, multiple *C. trachomatis* EBs were observed in large, loosely bound vesicles. Evidence suggests that vesicles containing *C. trachomatis* may have fused with one another early in the infectious process. These results indicate that chlamydiae can exploit the specific process of adsorptive endocytosis for entry into host cells and for translocation to a given intracellular destination, which may be different for each species.

Although it has been assumed that *Chlamydia* spp. enter susceptible eucaryotic cells by a classical phagocytic maneuver, these organisms readily enter cells that are not actively phagocytic (4). Although the events responsible for adherence and internalization are poorly understood, it has been shown that the process (i) is parasite mediated (4), (ii) involves a component of the infectious elementary body (EB) that is needed at the time of entry (3, 8, 9, 40), and (iii) is crucial for the intracellular survival of the organism (7–9). This would suggest that chlamydiae have evolved a specialized and effective mechanism for entry and subsequent development within a target host cell.

Few extensive morphological studies have focused on the uptake of chlamydiae by eucaryotic cells (38). Our previous ultrastructural observations revealed that *Chlamydia psittaci* can be internalized into nonprofessional phagocytic L-929 cells by a receptor-mediated endocytosislike pathway (13, 14). This process is used by most eucaryotic cells to efficiently internalize and transport various macromolecules to specific sites within the cell (10, 28, 30). The endocytosis of a number of viral particles (12, 21, 31, 42) and bacterial toxins (22, 25) closely mimics this process. In the present study, the attachment and internalization of *Chlamydia trachomatis* to McCoy cells were also examined by transmission electron microscopy (TEM). Data suggests that both species of *Chlamydia* can utilize a pathway resembling receptor-mediated endocytosis for entry into host cells, but there appears to be a difference in the intracellular destination of these two organisms which may be linked to the molecular features of the internalization process. Such findings may be relevant to our understanding of the pathogenesis of chlamydial infections in vivo.

* Corresponding author.

† Present address: Department of Clinical Microbiology-Immunology, North Carolina Memorial Hospital, University of North Carolina, Chapel Hill, NC 27514.

MATERIALS AND METHODS

Growth, purification and titration of chlamydiae. *C. trachomatis* E/UW-5/Cx, a human urogenital isolate kindly provided by C. C. Kuo and S. P. Wang, University of Washington, Seattle, was grown in McCoy cell monolayers by the methods of Moorman et al. (24) and Newhall et al. (26). The preparation of radiolabeled organisms has also been previously described (24). The infectivity of the purified EB preparations was determined as reported previously (17, 24).

Measurement of attachment and uptake of ^{3}H-labeled *C. trachomatis* with McCoy cells. The procedures for measuring the binding and entry of ^{3}H-labeled chlamydiae to eucaryotic cells have been reported (14, 41). For *C. trachomatis*, McCoy cells were grown to subconfluent monolayers (approximately 10^6 cells per 25-cm^2 tissue-culture flask) in minimal essential medium (MEM) without antibiotics. The cells were washed twice with either cold (4°C) or warmed (37°C) MEM and maintained at the respective temperatures until used. The monolayers were then inoculated with ^{3}H-labeled EBs at a multiplicity of infection of 250 infectious units (IFU) per host cell in 200 μl of MEM per 25-cm^2 flask. Adsorption proceeded for various time periods at 4 or 37°C, and the trichloroacetic acid-precipitable radioactivities of both the unbound and cell-associated chlamydiae were measured.

All determinations of uptake of ^{3}H-labeled *C. trachomatis* were done after the EBs (multiplicity of infection of 150 IFU per host cell) were centrifuged at 1,000 × *g* for 1 h at 4°C onto the surface of subconfluent monolayers of McCoy cells. This was followed by rapid warming of the McCoy cells at 37°C to allow internalization. At various times (0, 5, 15, 30, and 60 min), surface-associated chlamydiae were removed by treatment with either proteinase K (1 mg/ml), heparin (100 U/ml), or mixed glycosidases (1 mg/ml) for 1 h with the appropriate conditions for maximum activity of each compound. The agent-sensitive and -resistant trichloroacetic acid-precipitable radioactivities were then measured, and

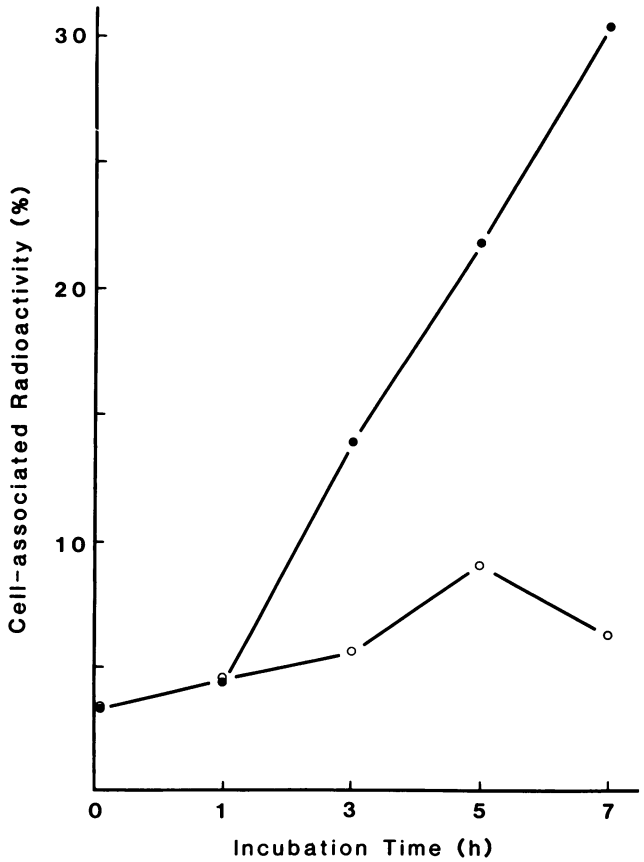

FIG. 1. Kinetics of binding of ^{3}H-labeled *C. trachomatis* to McCoy cells at 4°C (○) and 37°C (●). The values shown represent the means for the percent cell-associated radioactivities of three replicate experiments.

the percent attachment and internalization of radiolabeled chlamydiae to McCoy cells was determined (14). Each sample for the individual assays was performed in duplicate, and the experiments were repeated on at least three separate occasions.

TEM. The standard procedure for TEM with Epon-Araldite-embedded and poststained specimens has been documented in detail (14). Briefly, subconfluent monolayers of McCoy cells were washed and maintained in warm MEM before EBs (multiplicity of infection of 2,000 particles per host cell) were adsorbed to the cells for 1, 3, 5, and 7 h at 37°C. In some experiments, *C. trachomatis* EBs (multiplicity of 250 particles per host cell) were also centrifuged (1,000 × *g* for 1 h) onto McCoy cells at 4°C to synchronize the infectious process. The cultures were removed from the cold and either immediately processed for TEM (14) to observe EB attachment or were rapidly warmed (0, 5, 15, 30, and 60 min) to 37°C to follow the internalization process.

For low-temperature preparation of samples for immunoelectron microscopy in Lowicryl K4M, we used the procedure of Carlemalm et al. (5). The fixative was 2% paraformaldehyde–0.25% glutaraldehyde prepared in 0.1 M Sorsenson buffer (pH 7.2). Lowicryl thin sections were cut with glass knives, picked up on nickel grids, and immunolabeled by a procedure adapted from that of Timms (35).

Specificity of the anti-clathrin antibody. A modification of the procedure of Moore et al. (23) was used to demonstrate the specificity of the commercially obtained anti-clathrin antibody by detecting the presence of clathrin in McCoy cells via indirect immunofluorescent staining. McCoy cells in Dulbecco MEM with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid at a concentration of 10^6 cells per ml were placed in 1-dram (ca. 3.7-ml) vials containing poly-L-lysine (1 mg/ml; Sigma Chemical Co., St. Louis, Mo.)-coated cover slips. After 24 h, cells were exposed to conditions for enhancement of coated pit assembly (23). Sheets of membrane (unstripped) were prepared as described or, alternatively, stripped of coated pits and fixed for fluorescence (23). Unstripped and stripped membranes were incubated at 37°C for 30 min with polyclonal anti-clathrin (goat) immunoglobulin G followed by fluorescein isothiocy-

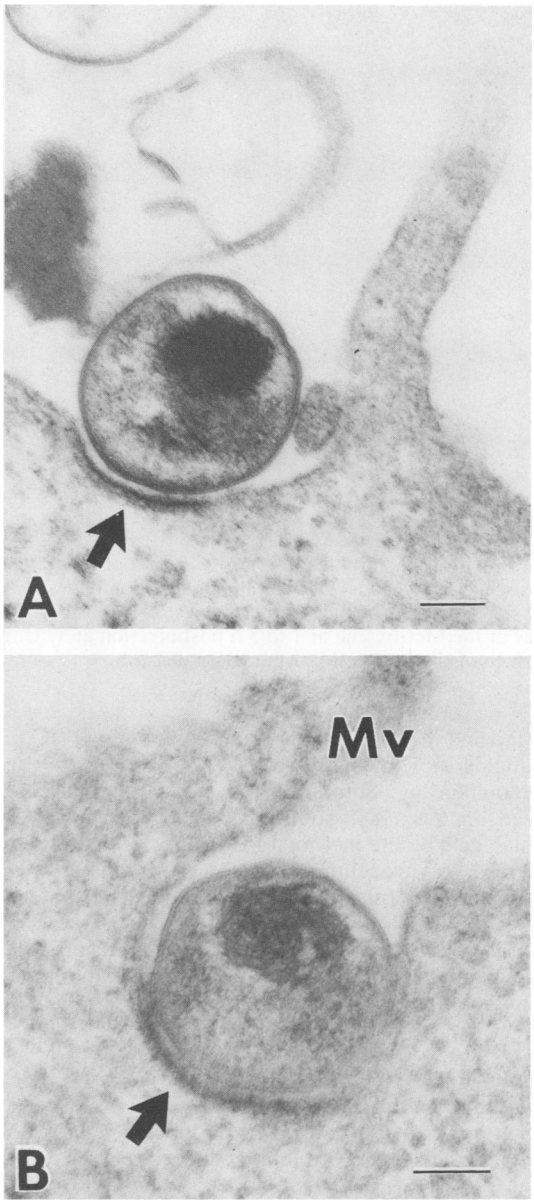

FIG. 2. TEM micrographs depicting the association and internalization of *C. trachomatis* EBs at electron-dense, coated invaginations (arrows) of the McCoy-cell plasma membrane at 37°C. Note the characteristic dark, fuzzy, bristle appearance of these coated pits. Mv, Microvilli. Bars, 0.1 μm.

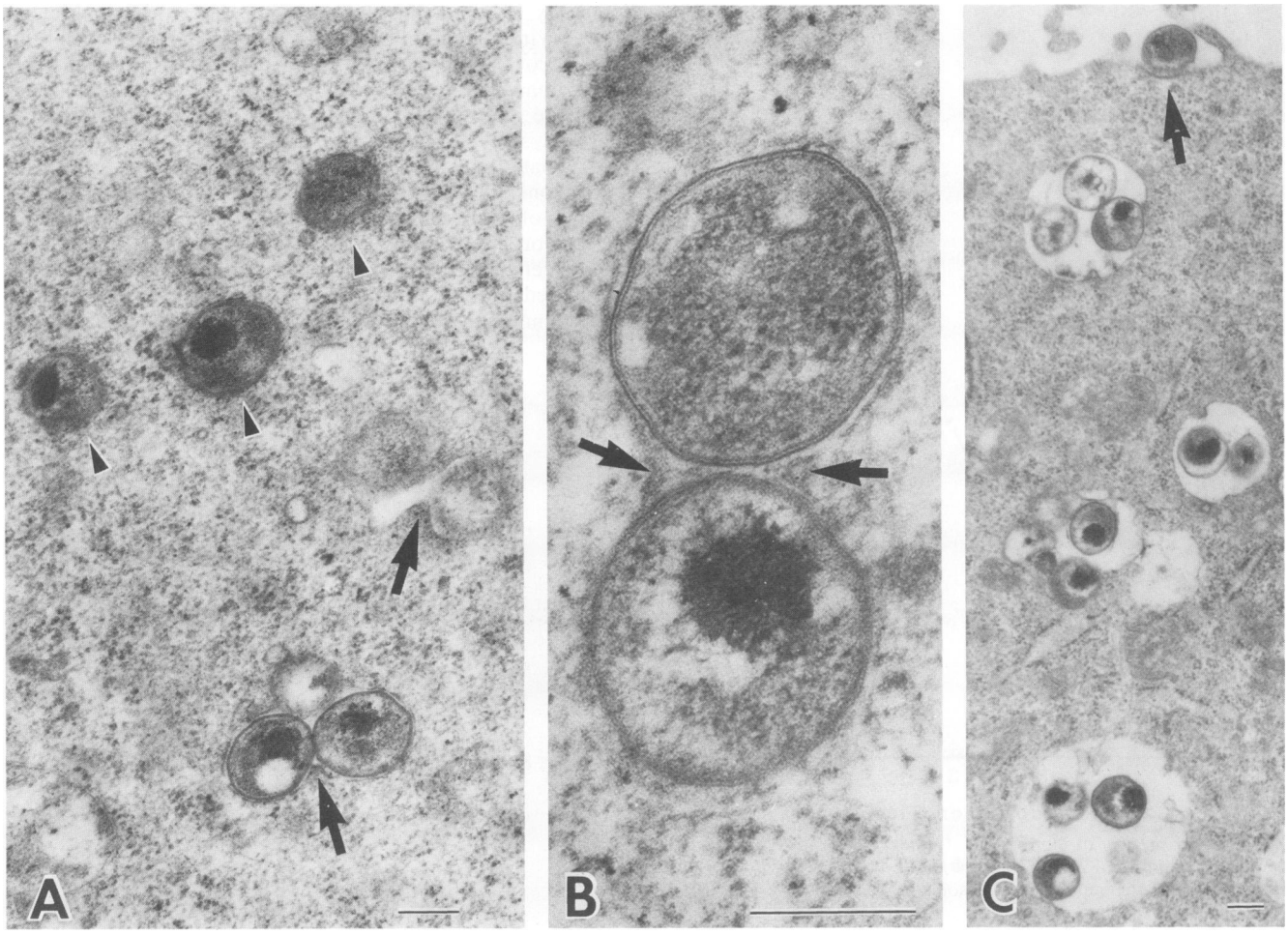

FIG. 3. Ultrastructural features of the fusion of *C. trachomatis* EB-containing vesicles. (A) Individual EBs within endocytic vesicles of the host cell (arrowheads) and the apparent fusion of two or more vesicles to produce single vacuoles containing several EBs (arrows). (B) Higher magnification of the fusion (arrows) of two EB-containing vesicles. (C) Multiple *C. trachomatis* EBs within large, loosely bound vesicles of the McCoy cell at 1 to 3 h postinfection at 37°C. Note the presence of an EB within a coated pit at the base of a microvillus on the surface of the host cell (arrow). Bars, 0.2 μm.

anate-labeled rabbit anti-goat immunoglobulin G (ICN Pharmaceuticals Inc., Irvine, Calif.).

Immunoblotting. Solubilized eucaryotic membranes were resolved on 6 to 18% sodium dodecyl sulfate–polyacrylamide slab gels by the method of Laemmli (19). Proteins were transferred to nitrocellulose in a Hoefer transfer apparatus (Hoefer Scientific Instruments, San Francisco, Calif.). Western blotting was done by the procedure of Blake et al. (2). Antibody bound to clathrin was detected on nitrocellulose by using alkaline phosphatase-conjugated rabbit anti-goat immunoglobulin G (Sigma).

RESULTS

Quantitation of attachment and entry. Quantitative studies of the attachment and entry of ^{3}H-labeled *C. trachomatis* into McCoy cells met with limited success. Since the genital serovar of *C. trachomatis* employed did not avidly associate with host cells at 4°C upon adsorption (Fig. 1), binding and internalization could not be examined as separate processes. In an attempt to synchronize the infection and to quantitate the entry process, centrifugation-assisted inoculation of the McCoy cells at 4°C was performed. Using this procedure, 90 to 99% of the EBs were readily bound to the cells at 4°C but were not internalized. However, the majority of these surface-associated EBs could not be removed with either proteinase K, heparin, or mixed glycosidases before warming at 37°C (data not shown). Therefore, quantitation of the entry process by this method was not possible. Adsorption of ^{3}H-labeled EBs to McCoy cells at 37°C was slow and inefficient, reaching a level of only 30% in 7 h (Fig. 1). This necessitated incubating large numbers of purified chlamydiae with host cells for extended times at 37°C to adequately observe enough EBs at various stages of attachment and entry by TEM.

Morphological aspects of entry. TEM observations revealed that *C. trachomatis* EBs preferentially bound to preexisting cell-surface microvilli. The chlamydial particles appeared to travel down these structures to their base, where they entered the host cell through specialized invaginated pits of the plasma membrane. Often, the cytoplasmic side of the host-cell membrane beneath the entering EBs was more electron dense than the rest of the cell surface, suggesting a clathrin-coated pit (Fig. 2). Membraneous structures resembling EB envelopes could also be found associ-

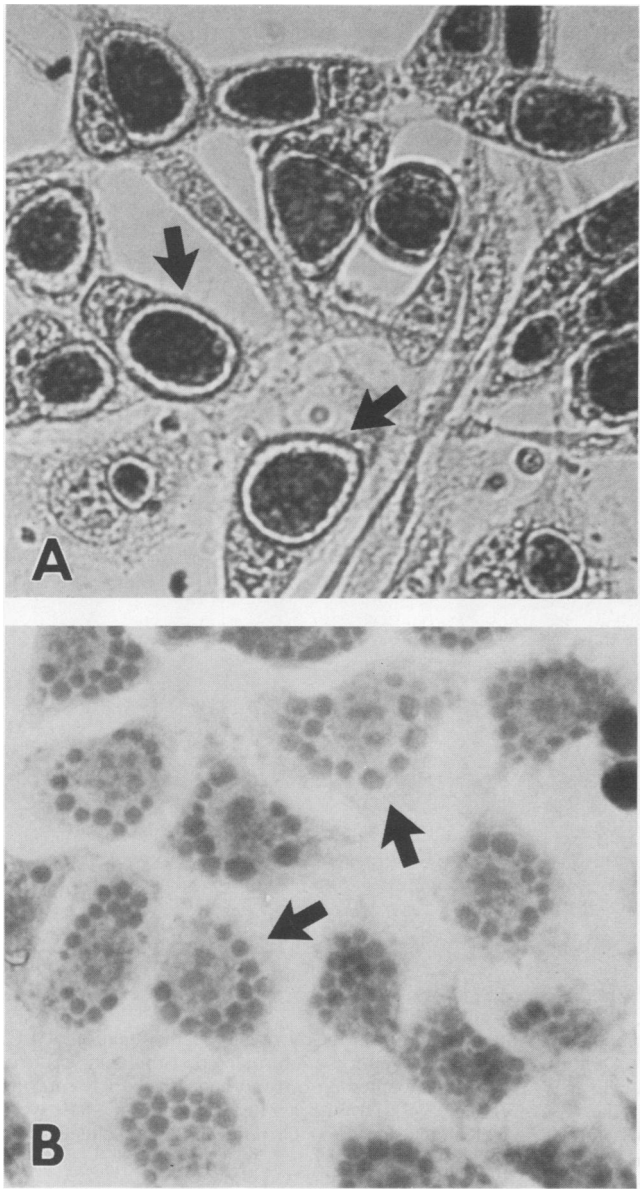

FIG. 4. Light microscopy of *C. trachomatis* and *C. psittaci* inclusions within infected host cells. (A) Single *C. trachomatis* inclusions (arrows) within McCoy cells as revealed by Jones iodine staining. (B) Giemsa stain of multiple *C. psittaci* inclusions (arrows) within L-929 cells.

ated with these coated regions. Only one EB could be seen in a single coated pit. Some EBs were also present in smooth-surfaced invaginations at or near the bases of the microvilli. Individual EBs were subsequently internalized into tightly bound endocytic vesicles. Although a number of chlamydiae simultaneously invaded a single cell, only one EB was initially present in each vesicle, and it remained membrane bound. Within 1 to 3 h postinfection, *C. trachomatis* vesicles appeared to fuse with one another (Fig. 3A and B), and multiple EBs were observed in large, loosely bound vesicles (Fig. 3C). This could explain why the development of *C. trachomatis* within a cell culminates in a single large inclusion (Fig. 4A). In contrast, cells infected with *C. psittaci* contain multiple inclusions approximately equal in number to the infectious particles internalized by the cells (Fig. 4B).

The ultrastructural features of the attachment and entry of *C. trachomatis* EBs inoculated onto McCoy cells by centrifugation were similar to those observed for adsorption infections (Fig. 5). As observed by TEM, the entry of EBs into McCoy cells at 37°C was rapid with this procedure. The majority of the EBs attached by centrifugation at 4°C were observed to be internalized within the first 5 to 15 min of incubation at 37°C.

Localization of clathrin in coated pits and vesicles by indirect immunoelectron microscopy. The specialized regions of the eucaryotic cytoplasmic membrane termed coated pits consist of clathrin and other associated proteins. To determine whether clathrin was present in the McCoy cell membrane invaginations and entry vesicles containing chlamydial EB, we utilized the technique of postembedding labeling on Lowicryl sections with second affinity gold-labeled antibody amplification.

Purified EBs were adsorbed to McCoy cell monolayers for 3 or 5 h at 35°C and then processed in the cold for electron microscopy, substituting Lowicryl for Epon-Araldite in the infiltration and embedding steps. Thin sections on nickel grids were exposed to polyclonal anti-clathrin antibody (goat) followed by second affinity (rabbit anti-goat) gold-labeled antibody. The gold marker (15 nm) was reproducibly and specifically associated with surface invaginations and internal vesicles of the McCoy cells (Fig. 6A). In infected cells, chlamydiae were frequently observed in the labeled pits and endosomes (Fig. 6B and C). The gold label almost always appeared as a site-specific, aggregated pattern; the pattern could not be made more dispersed to cover the entire coated pit region by increasing the primary antibody concentration. This suggested to us that exposure of antigenically preserved epitopes on the clathrin is very much dependent on the plane of section relative to the orientation of the polygonal lattice.

The specificity of the gold labeling for clathrin-coated pits and vesicles was determined by several control experiments (1). First, verification of the clathrin specificity of the commercially obtained primary antibody was tested by indirect immunofluorescent staining of large sheets of our McCoy cell membranes, manipulated to contain the maximum number of coated pits (23). Numerous characteristic punctate fluorescein dots were seen (data not shown). The clathrin-coated pits were removed by incubation of the plasma membranes in a high-pH buffer under conditions known to strip coat proteins from isolated coated vesicles (23). After this stripping procedure, the coated pits were no longer visible by fluorescence microscopy. Second, eucaryotic membranes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the separated proteins were transferred to nitrocellulose paper for immunoblot analysis. The polyclonal anti-clathrin antibody bound to proteins in the molecular size ranges of 180, 100 to 110, 50 to 60, and 30 to 40 kilodaltons (data not shown). These molecular sizes correspond to the heavy chain (180 kilodaltons) and one of the light chains (36 kilodaltons) of clathrin and to a family of 100- to 50-kilodalton accessory proteins involved in promoting clathrin assembly. Third, in the postembedding labeling procedure, the Lowicryl sections were incubated with both primary anti-clathrin antibody and second affinity gold-labeled second antibody but in the absence of blocking agent (ovalbumin), permitting specific as well as nonspecific binding (Fig. 6D). Fourth, incubation of the thin sections directly with ovalbumin and gold-labeled second antibody,

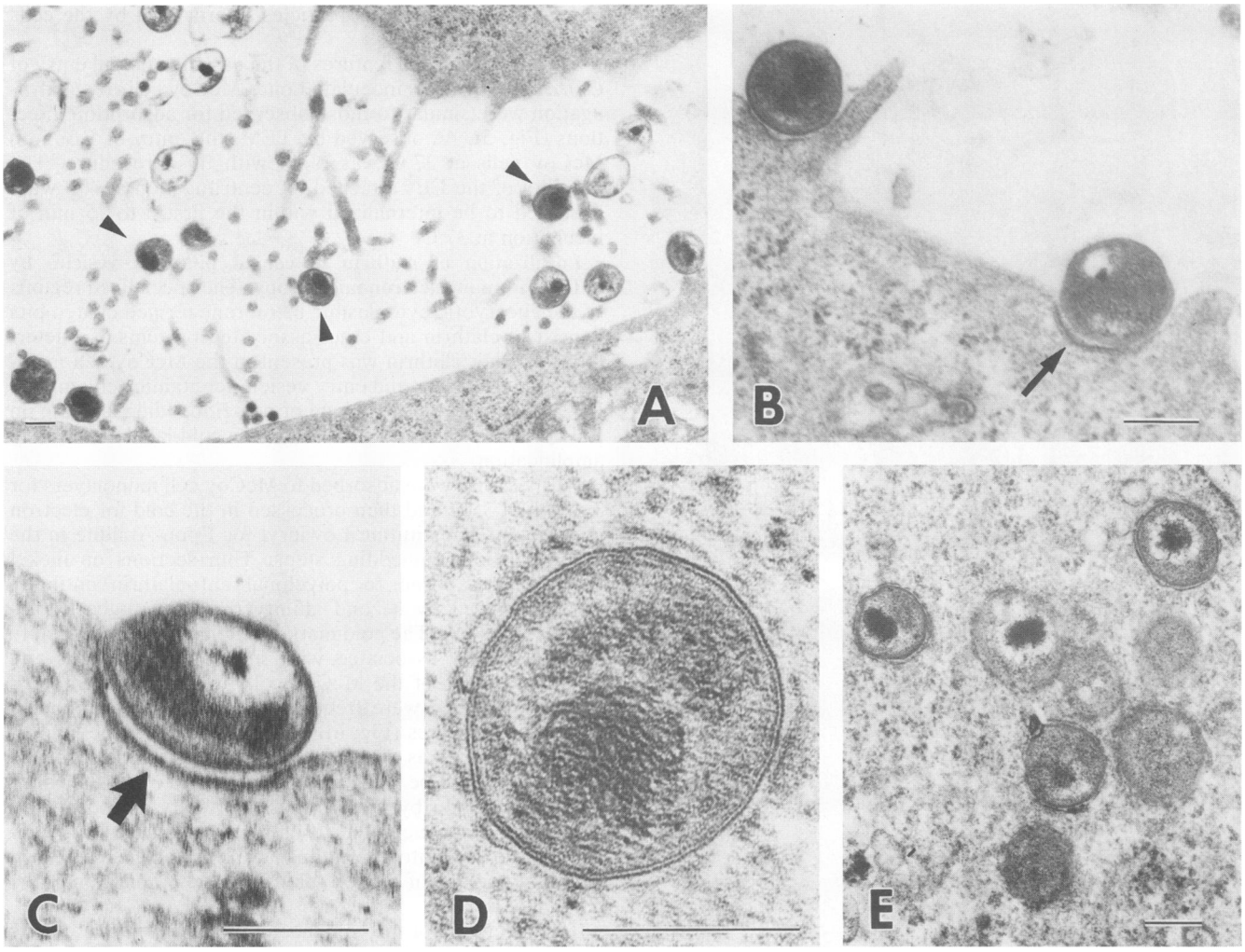

FIG. 5. Ultrastructural features of the attachment and entry of *C. trachomatis* EBs inoculated onto McCoy cells by centrifugation at 4°C. (A) Preferential binding of EBs (arrowheads) to cell-surface microvilli at 4°C. No EBs were observed to be internalized at this temperature. (B) Entry of EBs at the bases of the microvilli upon warming the infected cells to 37°C. Note the coated pit (arrow) beneath the EB entering on the right. (C) Association of an EB with an electron-dense, coated pit of the McCoy cell at 37°C. (D) Within 5 to 15 min at 37°C, EBs were observed by TEM to be internalized within tightly bound vesicles. (E) After 60 min of incubation at 37°C, numerous EBs clustered in proximity to one another and fusion events were observed. Bars, 0.2 μm.

omitting the primary anti-clathrin antibody, resulted in virtually no gold labeling, indicating there was no nonspecific adsorption of the second antibody to the cells (Fig. 6E). Finally, the Lowicryl sections were incubated with primary antibody, which had been previously adsorbed with an excess of antigen (the McCoy cell membrane sheets containing coated pits), followed by gold-labeled second antibody. This procedure resulted in little to no gold labeling, verifying again the specificity of the antigen-antibody interaction (Fig. 6F).

DISCUSSION

C. trachomatis infects only humans and is responsible for a number of syndromes, of which trachoma, inclusion conjunctivitis, urogenital tract infections, and infantile pneumonia are of major importance (29, 33, 34). In many developing countries, trachoma is a serious communicable disease, affecting some 500 million people (6). In the United States and other industrialized countries, *C. trachomatis* is considered to be the most common of the sexually transmitted disease pathogens. Approximately 3 to 5 million Americans are infected with this organism each year (33, 34). The morbidity and economic burden associated with these infections is enormous. Therefore, an understanding of how this organism invades and survives within host cells will hopefully give us some clues as to how persistent infection is accomplished. The majority of cases of tubal disease associated with chlamydial salpingitis appear to result from chronic, subclinical infection (15). Such information may also aid in the development of more effective strategies for the prevention and control of this important human pathogen.

Our electron photomicrographs of McCoy cells infected with the E serovar of *C. trachomatis* represent many of the morphological features that are characteristic of the entry mechanism of receptor-mediated endocytosis. As with our previous observations for *C. psittaci* (13, 14), the entry of *C. trachomatis* into host cells involves microvilli, coated pits,

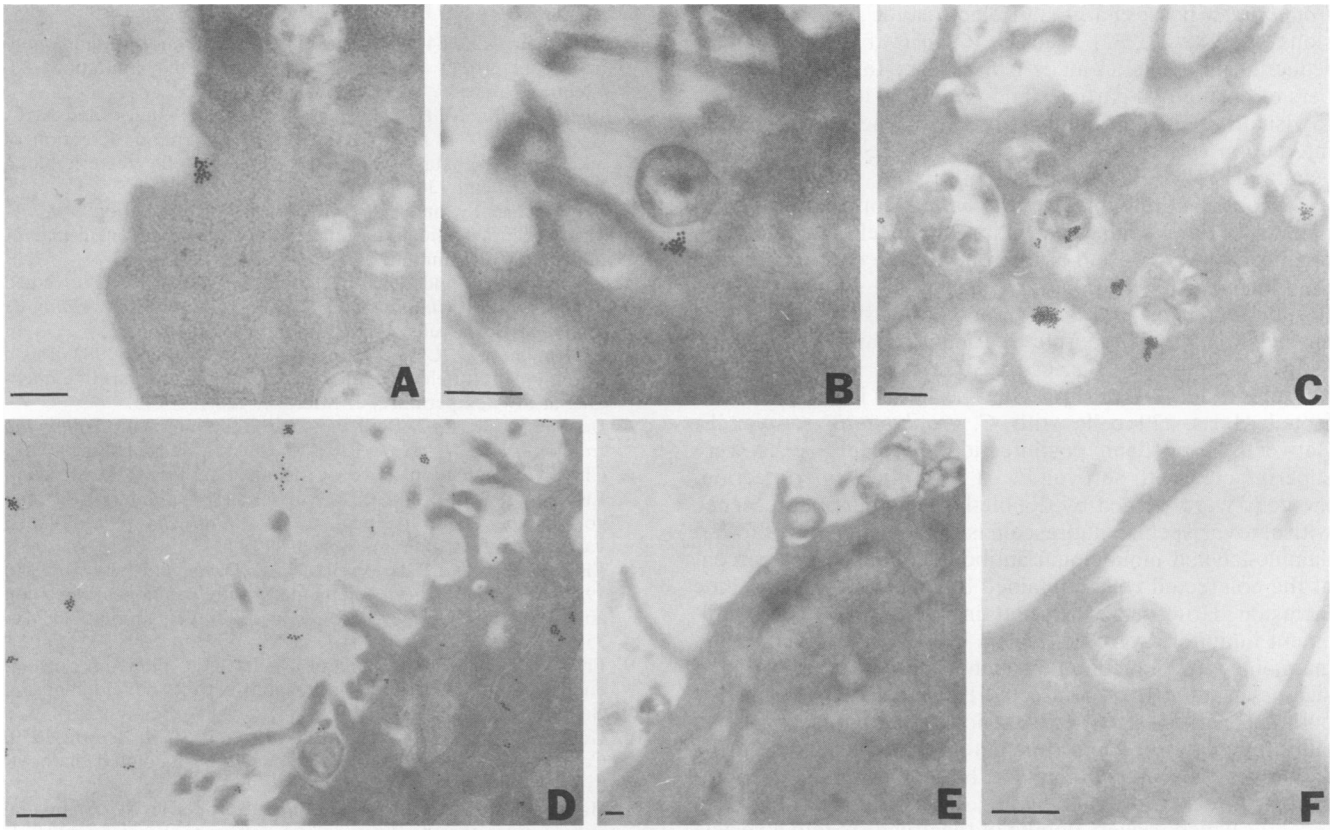

FIG. 6. TEMs of McCoy cells exposed to *C. trachomatis* EB for 3 h at 37°C. Thin sections of Lowicryl-embedded samples were incubated with polyclonal anti-clathrin antibody followed by second affinity antibody labeled with 15-nm colloidal gold. (A) McCoy cell pit. (B) *C. trachomatis* EB with a labeled pit. (C) *C. trachomatis*-containing endosomes associated with labeled α-clathrin antibody. Controls were examined for (D) both specific and nonspecific labeling, by omitting the ovalbumin blocking step, (E) nonspecific adsorption of the gold-labeled second antibody to the sections, by incubation of the Lowicryl sections directly in ovalbumin and gold-labeled second antibody (omitting the primary anti-clathrin antibody), and (F) specificity of the antigen-antibody reaction, by incubation with primary antibody, which had been preadsorbed with excess antigen, followed by gold-labeled second antibody. Bars, 0.1 μm.

coated vesicles, and endosomes. Apparently, both species of *Chlamydia* can utilize this specialized pathway to gain entry into eucaryotic cells. However, much remains to be known about the internalization process. The possibility for multiple mechanisms of entry exists (27, 38), since both species can be found associated with invaginations of the host-cell membrane which are seemingly devoid of coat material. Explanations for divergent findings among different laboratories include the techniques used to observe coated regions of the host cells or the particular plane of section examined by TEM. For example, it could reflect the inherent high contrast of the resin used for embedding our samples for TEM. Ward and Murray (38) did not find the densely stained fuzzy coat material lining pits and vesicles of HeLa cells containing the LGV biovar. These latter investigators used Spurr as an embedding resin, whose contrast is compromised in lieu of other physiochemical technological advantages. When our samples were embedded in Spurr resin there was little evidence of clathrinlike coats in the invaginations of host-cell membranes (data not shown), but when Epon-Araldite was used EBs were regularly observed within coated pits and coated vesicles.

Similar to EBs of *C. psittaci* (13, 14), EBs of *C. trachomatis* were regularly observed within coated pits. Quantitation of this association was not possible, since we were unable to synchronize the adsorption infections and, therefore, not able to examine the process of attachment and entry as separate entities. It might be possible by TEM to quantitate the entry of EBs at 37°C after centrifugation-assisted inoculation at 4°C, but this approach assumes that chlamydial entry after this mechanical manipulation would be identical to that after normal random adhesion. Although the morphological aspects of entry seemed to be identical for EBs after either adsorption or centrifugation to the host cell surface, in both our study and that of Ward and Murray (38), this assumption was not made and quantitation was not accomplished. Recent studies by Pearce (27) have suggested that centrifugation does affect both entry and intracellular fate of the guinea pig inclusion conjunctivitis strain of *C. psittaci* in McCoy cells. Unaided inoculation results in a pinocytic entry and routing of the infectious EB to lysosomes, the end product being an unproductive state; centrifugation promotes a phagocytic entry and avoidance of phagosome-lysosome fusion, resulting in a productive infection.

The repeated finding that both species of *Chlamydia* attach to preexisting cell-surface microvilli suggests a common structure of adherence for these organisms. However, the poor efficiency of binding for *C. trachomatis* at both 37 and 4°C, compared with that previously observed for *C. psittaci*, indicates a difference in the initial interaction. This can be attributed to the fact that certain biovars of *C.*

trachomatis possess a greater electrostatic barrier to adhesion (16, 18, 20, 32) than do *C. psittaci* biovars. This reduction in adhesion made it necessary to use large numbers of chlamydial particles and prolonged adsorption times to visualize sufficient numbers of EBs in the initial stages of interaction with the McCoy cells. Thus the results may not accurately reflect in vivo conditions.

Our observations that *C. trachomatis*-containing vesicles fuse with one another early in the development of this organism may represent a difference in the entry process at the molecular level for the two chlamydia species. Vesicle fusion for *C. trachomatis* was first reported by Ward (37) and has been recently supported by Ridderhof and Barnes (J. C. Ridderhof and R. C. Barnes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, D156, p. 98). The latter investigators infected HeLa 229 cells with *C. trachomatis* serovar E. Between 1 and 24 h postinfection, the HeLa cells were superinfected with serovar F. At 48 h after superinfection, the cells were stained by double-label immunofluorescence with serovar-specific fluorescein isothiocyanate- or sulforhodamine-labeled monoclonal antibodies. Ninety-five percent of the coinfected cells contained both serovars in the same inclusion. However, when certain microtubule or microfilament inhibitors were added 1 h before superinfection and maintained in the medium throughout the infection period, a significant number of coinfected HeLa cells now contained both serovars in separate inclusions. As a consequence of entering cells by receptor-mediated endocytosis, the interaction between chlamydiae and host cells should theoretically be a receptor-ligand binding. Does *C. psittaci* bind to a receptor which triggers uptake of the organism into a vesicle lacking the molecular signals required to initiate fusion with other vesicles or lysosomes? Is *C. trachomatis* bound to a different cell receptor and subsequently internalized into a vesicle with a different intracellular destination? *C. trachomatis* EBs possess two proteins, with molecular masses of 18 and 32 kilodaltons, which bind to HeLa cell surface components (11, 39). Examination of two *C. psittaci* strains by the same techniques revealed only a single binding protein in the range of 17 to 19 kilodaltons (11). It would be premature to suggest that the differences in the binding proteins between the two species could account for the apparent differences in early intracellular fate. However, it is interesting that the mechanisms by which chlamydiae are released from infected cells also differ among chlamydiae and that these differences generally correlate with the in vivo pathogenesis of these organisms (36).

In conclusion, our electron microscopic observations reveal that both species of *Chlamydia* can enter nonprofessional phagocytic cells by a process resembling receptor-mediated endocytosis, although their subsequent intracellular destinations may differ. These findings may be important to our understanding of the early stages of chlamydial pathogenesis and should provide some insight into future studies involving the specific receptor-ligand interaction between *Chlamydia* spp. and host cells.

ACKNOWLEDGMENTS

We gratefully acknowledge Johnny Carson for the use of an electron microscope and David Moorman for technical expertise. We also thank Joyce Bradshaw for skillful secretarial assistance.

This investigation was supported by Public Health Service grant AI13446 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Bendayan, M.** 1984. Protein A-gold electron microscopic immunocytochemistry: methods, applications and limitations. J. Electron Microsc. Tech. **1**:243–270.
2. **Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for detection of alkaline phosphatase-conjugated antibody on Western blots. Anal. Biochem. **136**:175–179.
3. **Brownridge, E., and P. B. Wyrick.** 1979. Interaction of *Chlamydia psittaci* reticulate bodies with mouse peritoneal macrophages. Infect. Immun. **24**:697–700.
4. **Byrne, G. I., and J. W. Moulder.** 1978. Parasite-specified phagocytosis of *Chlamydia psittaci* and *Chlamydia trachomatis* by L and HeLa cells. Infect. Immun. **19**:598–606.
5. **Carlemalm, E., R. M. Garavito, and W. Villiger.** 1982. Resin development for electron microscopy and an analysis of embedding at low temperature. J. Microsc. **126**:123–143.
6. **Dawson, C. R.** 1982. Review of eye infections with *Chlamydia trachomatis*, p. 71–81. *In* P. A. Mardh and K. K. Holmes (ed.), Chlamydial infections. Elsevier Biomedical Press, Amsterdam.
7. **Eissenberg, L. G., and P. B. Wyrick.** 1981. Inhibition of phagolysosome fusion is localized to *Chlamydia psittaci*-laden vacuoles. Infect. Immun. **32**:889–896.
8. **Eissenberg, L. G., P. B. Wyrick, C. H. Davis, and J. W. Rumpp.** 1983. *Chlamydia psittaci* elementary body envelopes: ingestion and inhibition of phagolysosome fusion. Infect. Immun. **40**:741–751.
9. **Friis, R. R.** 1972. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. J. Bacteriol. **110**:706–721.
10. **Goldstein, J. L., R. G. W. Anderson, and M. S. Brown.** 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. Nature (London) **279**:679–685.
11. **Hackstadt, T.** 1986. Identification and properties of chlamydial polypeptides that bind eucaryotic cell surface components. J. Bacteriol. **165**:13–20.
12. **Helenius, A., J. Kartenbeck, K. Simons, and E. Fries.** 1980. On the entry of Semliki Forest virus into BHK-21 cells. J. Cell Biol. **84**:404–420.
13. **Hodinka, R. L., and P. B. Wyrick.** 1986. Is the intracellular fate of *Chlamydia psittaci* governed by a specific mode of entry into host cells, p. 86–90, *In* L. Leive (ed.), Microbiology—1986. American Society for Microbiology, Washington, D.C.
14. **Hodinka, R. L., and P. B. Wyrick.** 1986. Ultrastructural study of mode of entry of *Chlamydia psittaci* into L-929 cells. Infect. Immun. **54**:855–863.
15. **Jones, R. B., J. B. Mammel, M. K. Shepard, and R. R. Fisher.** 1986. Recovery of *C. trachomatis* from the endometrium of women at risk for chlamydial infection. Am. J. Obstet. Gynecol. **155**:35–39.
16. **Kraaipoel, R. J., and A. M. van Duin.** 1979. Isoelectric focusing of *C. trachomatis*. Infect. Immun. **26**:775–778.
17. **Kuo, C. C., and J. T. Grayston.** 1976. Interaction of *Chlamydia trachomatis* organisms and HeLa 229 cells. Infect. Immun. **13**: 1103–1109.
18. **Kuo, C. C., S. P. Wang, and J. T. Grayston.** 1973. Effect of polycations, polyanions, and neuraminidase on the infectivity of trachoma-inclusion conjunctivitis and lymphogranuloma organisms in HeLa cells: sialic acid residues as possible receptors for trachoma-inclusion conjunctivitis. Infect. Immun. **8**:74–79.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lee, C. K.** 1981. Interaction between a trachoma strain of *Chlamydia trachomatis* and mouse fibroblasts (McCoy cells) in the absence of centrifugation. Infect. Immun. **31**:584–591.
21. **Matlin, K. S., H. Reggio, A. Helenius, and K. Simons.** 1981. Infectious entry pathway of influenza virus in a canine kidney cell line. J. Cell Biol. **91**:601–613.
22. **Middlebrook, J. L., and R. B. Dorland.** 1984. Bacterial toxins: cellular mechanisms of action. Microbiol. Rev. **48**:199–221.
23. **Moore, M. S., D. T. Mahaffey, F. M. Brodsky, and R. G. Anderson.** 1987. Assembly of clathrin-coated pits onto purified

plasma membranes. Science **236**:558–563.
24. **Moorman, D. R., J. W. Sixbey, and P. B. Wyrick.** 1986. Interaction of *Chlamydia trachomatis* with human genital epithelium in culture. J. Gen. Microbiol. **132**:1055–1067.
25. **Morris, R. E., A. S. Gerstein, P. F. Bonventre, and C. B. Saelinger.** 1985. Receptor-mediated entry of diphtheria toxin into monkey kidney (Vero) cells: electron microscopic evaluation. Infect. Immun. **50**:721–727.
26. **Newhall, W. J., V. B. Batteiger, and R. B. Jones.** 1982. Analysis of the human serological response to proteins of *Chlamydia trachomatis*. Infect. Immun. **38**:1181–1189.
27. **Pearce, J. H.** 1986. Early events in chlamydial infection. Ann. Microbiol. (Paris) **137A**:325–332.
28. **Salisbury, J. L., J. S. Condeelis, and P. Satir.** 1983. Receptor-mediated endocytosis: machinery and regulation of the clathrin-coated vesicle pathway. Int. Rev. Exp. Pathol. **24**:1–62.
29. **Schachter, J., and H. D. Caldwell.** 1980. Chlamydiae. Annu. Rev. Microbiol. **34**:285–309.
30. **Silverstein, S. C., R. M. Steinman, and Z. A. Cohn.** 1977. Endocytosis. Annu. Rev. Biochem. **46**:669–722.
31. **Simons, K., H. Garoff, and A. Helenius.** 1982. How an animal virus gets into and out of its host cell. Sci. Am. **247**:58–66.
32. **Soderlund, G., and E. Kihlstrom.** 1982. Physiochemical surface properties of elementary bodies from different serotypes of *Chlamydia trachomatis* and their interaction with mouse fibroblasts. Infect. Immun. **36**:893–899.
33. **Stamm, W. G., and K. K. Holmes.** 1984. *Chlamydia trachomatis* infections of the adult, p. 258–270. *In* K. K. Holmes (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York.
34. **Thompson, S. E., and A. E. Washington.** 1983. Epidemiology of sexually transmitted *Chlamydia trachomatis* infections. Epidemiol. Rev. **5**:96–123.
35. **Timms, B. G.** 1986. Post embedding immunogold labeling for electron microscopy using "LR White" resin. Am. J. Anat. **175**:267–275.
36. **Todd, W. J., and H. D. Caldwell.** 1985. The interaction of *Chlamydia trachomatis* with host cells: ultrastructure studies of the mechanisms of release of a biovar II strain from Hela 229 cells. J. Infect. Dis. **151**:1037–1043.
37. **Ward, M. E.** 1983. Chlamydial classification, development and structure. Br. Med. Bull. **39**:109–115.
38. **Ward, M. E., and A. Murray.** 1984. Control mechanisms governing the infectivity of *C. trachomatis* for HeLa cells: mechanisms of endocytosis. J. Gen. Microbiol. **130**:1765–1780.
39. **Wenman, W. M., and R. U. Meuser.** 1986. *Chlamydia trachomatis* elementary bodies possess proteins which bind to eucaryotic cell membranes. J. Bacteriol. **165**:602–607.
40. **Wyrick, P. B., and E. A. Brownridge.** 1978. Growth of *Chlamydia psittaci* in macrophages. Infect. Immun. **19**:1054–1060.
41. **Wyrick, P. B., E. A. Brownridge, and B. E. Ivins.** 1978. Interaction of *Chlamydia psittaci* with mouse peritoneal macrophages. Infect. Immun. **19**:1061–1067.
42. **Zeickhardt, H., K. Wetz, P. Willingmann, and K. Habermehl.** 1985. Entry of poliovirus type 1 and mouse Elberfeld (ME) virus into Hep-2 cells: receptor-mediated endocytosis and endosomal or lysosomal uncoating. J. Gen. Virol. **66**:483–492.