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SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF BACTERIAL ATTACHMENT TO MUCOSAL SURFACES WITH PARTICULAR REFERENCE TO THE HUMAN FALLOPIAN TUBE

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Abstract

Neisseria gonorrhoeae and *Chlamydia trachomatis* are the common bacteria isolated from bacterial cervicitis and are the leading etiological agents for pelvic inflammatory disease. *Neisseria gonorrhoeae* cause infection of the mucosa of fallopian tubes in organ culture by (a) attaching to microvilli of nonciliated cells, (b) phagocytosis by these cells, (c) transport across and exocytosis from the epithelial cells. In contrast the *Chlamydia* attach to the epithelial surface without apparent ligand binding and are taken into the cytoplasm of the epithelial cell. Exocytosis of *Chlamydia* is into the tubal lumen and not into the subepithelial spaces. The ciliated epithelial cells of the fallopian tube are damaged by a gonococcal toxin but chlamydia do not exhibit such activity. These observations suggest that the mechanism of attachment to and invasion of the mucosal epithelium by gonococci and chlamydia are quite different and their potential for disease production occurs by different methods.

Key Words: Gonorrhoea, *Neisseria gonorrhoeae,* pelvic inflammatory disease, chlamydiae, *Chlamydia trachomatis*, microbial adherence, mucosal attachment.

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Introduction

The infections of the female pelvic organs have often been grouped together under the term pelvic inflammatory disease (PID). The term has been defined as "the clinical syndrome attributed to the ascending spread of microorganisms (unrelated to pregnancy or surgery) from the vagina and cervix to the endometrium, fallopian tubes and/or contiguous structures" (3). In this paper, we will consider mainly bacterial infections of the fallopian tube and accordingly use the term "salpingitis" when appropriate. The infectious agents causing salpingitis most often ascend to the fallopian tubes through the cervix and the endometrium. Evidence for this condition is shown by (a) mucosal infections which extend from the cervix via the endometrium into the fallopian tubes and which have been documented both histologically (15) and by culture (23); (b) interruption of a canicular route prevents the spread of salpingitis (10); and (c) experimental genital infections in primates (33). However, the exact mechanism by which a cervical infection ascends to the fallopian tubes to establish salpingitis is incompletely understood. In fact, it has only recently been determined, at the ultrastructural and molecular level, how mucosal pathogens such as the *Neisseria* colonize and cross mucosal barriers to cause local as well as disseminated disease.

The majority of PID is caused by bacteria representing a polymicrobial etiology. *Neisseria gonorrhoeae, Chlamydia trachomatis* as well as other aerobes and anaerobes are commonly isolated from female patients with PID. *C. trachomatis* and *N. gonorrhoeae* are the most common bacterial organisms isolated from bacterial cervicitis (34). It has been reported that 10 to 17 percent of women with gonococcal cervicitis develop clinically recognizable salpingitis (7,11). The rates may be similar for women with chlamydial cervicitis.

In this paper we review the ultrastructure of infection of human fallopian tubes by *Neisseria gonorrhoeae* and *Chlamydia trachomatis.* This review will emphasize those features of this process which may be important in the establishment of salpingitis. Many of these studies involve the human fallopian tube organ culture described by McGee and co-workers (27) and Ward et al. (44). The development of this procedure has greatly aided our understanding of the interactions between the fallopian tube mucosa and these potentially pathogenic bacteria.

Infection by Neisseria gonorrhoeae. A correlation has been observed between the stimulation of growth of virulent *N. gonorrhoeae* and the secretory phase of the menstrual cycle just prior

to initiation of menstruation. Transparent colony types of gonococci are stimulated in their growth during the secretory phase of the menstrual cycle and attach more readily to the fallopian tube epithelium than do the corresponding opaque colony types (6). However, only transparent *N. gonorrhoeae* colonies have been recovered from infected fallopian tubes. Gonococcal infection usually involves an ascending spread from the lower tract into the uterine cavity where these bacteria cause a superficial endometritis or a direct passage into the fallopian tube to cause salpingitis. The endometritis may be a common intermediate infection between cervicitis and salpingitis. It is also possible that the gonococci are carried into the fallopian tubes by their attachment to spermatozoa (18).

Once in the fallopian tube, virulent gonococci are capable of attaching to the tubal epithelial cells and overwhelming the local defense mechanisms producing tubal infections. Chlamydial infection may be limited, at this point, to the mucosal surface of the fallopian tube. In contrast, *N. gonorrhoeae* and other facultative and anaerobic bacteria may cause infection below the basement membrane. Thus, if the subepithelial tissues as well as the serosal surfaces become involved in the inflammatory process, subsequent permanent fallopian tube damage becomes more likely.

Invasion of the Human Fallopian Tube by *Neisseria gonorrhoeae*. The gonococcus has a high degree of species specificity which precludes its study in a wide variety of animal models (12,19,20,45). The gonococcus only attaches to, invades and damages the fallopian tubes of humans while fallopian tubes of rabbits, pigs and cows are not invaded. Once the gonococci reach the fallopian tube there is a predictable series of events which occur between the gonococci and the epithelial cells of the fallopian tube. These interactions have only recently been described at the ultrastructural and molecular level (24,25,29,30).

Several surface components or products elaborated by the gonococcus may have potential roles in the early events of disease production in the fallopian tube. These include (a) pili, (b) outer membrane proteins I and II, (c) lipopolysaccharide, (d) peptidoglycan and (e) IgAl protease.

Virulent gonococci have surface hair-like structures called pili radiating from their surfaces. The presence of pili has long been associated with an increased affinity to attach to human cells (28,37,39,40,42). Unfortunately, the antigenic heterogeneity of pili is great and single isolates are known to carry multiple serologically distinct pilus types (21). This heterogeneity decreases the potential of using pili as a vaccine as well as lessening the ability of antipili antibody to block attachment of multiple types of gonococci to human cells.

Both lipopolysaccharide and peptidoglycan are released from actively growing gonococci and are capable of causing toxic damage to human fallopian tubes (13,31,32). These toxic gonococcal membrane components have an affinity for receptors on the ciliated cells of the fallopian tube epithelium causing extensive sloughing of the ciliated cells. Neither of these molecules appears to cause a disruption of the integrity of the nonciliated cells of the mucosal epithelium.

The outer membrane proteins I and II appear to be functionally different. Protein I is the major outer membrane protein of the gonococcus, is present in all gonococci, and functions as a porin protein. This protein functions to regulate macromolecular transport. In addition they have the ability to leave the gonococcal membrane and insert into artificial membranes and membranes of human erythrocytes (1,27). Protein II is a heat modifiable protein whose presence is associated with the colonial morphology of opaque colonies (41). Strains of gonococci which possess protein II tend to have increased adherence both to themselves and to human cells when compared to gonococci deficient in protein II.

An extracellular enzyme, IgAl protease, produced by gonococci and other pathogenic bacteria has been implicated as a potentially important virulence factor. This enzyme cleaves IgAl into immunologically inactive fragments (38). We have investigated the potential role that IgAl protease may have in the attachment to and invasion of the human fallopian tube mucosa (4,5). Using an IgAl protease deficient mutant and its wild type parent, we infected human fallopian tubes in organ culture using the techniques described by McGee et al. (27). The rate and extent of attachment, damage and invasion of the fallopian tube mucosa by the mutant was indistinguishable from the infection caused by the wild type parent clone. These data indicate, that in the initial encounter with previously uninfected fallopian tube mucosa, the production of IgAl protease is not critical to the ability of the gonococcus to act as a mucosal pathogen.

In the course of these studies, gonococcal attachment and invasion of the fallopian tube epithelium was observed by both light and electron microscopic examination of the tissue at various times after infection. As diagrammed in Figure 1, four main stages of the pathogenic process have been established (26): (i) attachment of gonococci to microvilli, (ii) engulfment or phagocytosis, (iii) transport of phagocytic vacuoles to the base of the cell, and (iv) rupture or exocytosis of the phagocytic vacuole contents into the subepithelial space.

Attachment of Gonococci to Microvilli. As seen in Figure 2, the mucosa of the fallopian tube consists of two types of epithelial cells; ciliated and nonciliated. The latter possess a uniform distribution of microvilli on their surface. The distribution of these cell types is very much dependent on the anatomical location. Approximately 75–80% of epithelial cells in the fimbriated region of the fallopian tube are ciliated, whereas only 60–65% of those cells in the isthmic region have cilia (30). Beginning shortly after infection and extending for approximately 30 h, gonococci begin to attach to the tips of the microvilli of the nonciliated cells (Figure 3). Occasionally, pili can be seen extending from gonococci to the surface of the microvilli (26). The gonococci are rarely seen on or within ciliated cells. Piliated gonococci attach in far greater numbers to the microvilli than do nonpiliated gonococci. However, piliated commensal *Neisseria* do not attach to the microvilli of nonciliated mucosal cells (27). These observations suggest that a secondary attachment mechanism may be involved in the attachment to the microvilli (e.g., protein II). This is supported by preliminary experiments in which piliated opaque gonococci (protein II bearing) attach in greater numbers than do piliated transparent gonococci. During this period of attachment there is also sloughing of ciliated epithelial cells (Figure 4) which can be attributed to the toxic activities of gonococcal lipopolysaccharide and/or monomeric peptidoglycan fragments.

Engulfment or Phagocytosis of Gonococci. Approximately 24 h after infection, the microvilli appear to retract and the gonococci appear to be pulled toward the surface. This brings the gonococcus into contact with the surrounding microvilli which appear to trap or enclose them against the cell surface (Figures 5 and 6). Subsequently, the gonococci are enclosed by

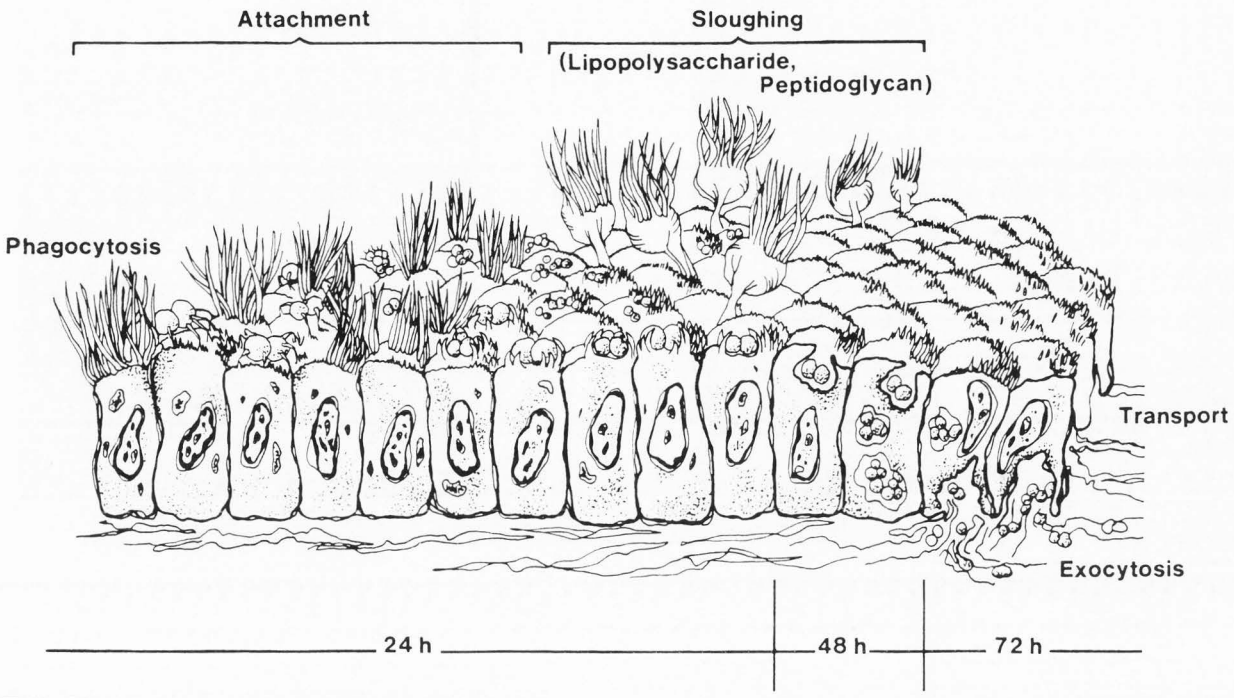


Fig. 1. The stages of gonococcal attachment and invasion of human fallopian tubes mucosa are shown here. Attachment of gonococci by pili to the microvilli on nonciliated cells, phagocytosis into the cell and the sloughing of ciliated cells due to the toxic activity of lipopolysaccharide and peptidoglycan is seen during the first 24 h period of infection. Transport of the gonococci through the epithelial cell occurs by 48 h post infection. By 72 h the gonococci are being deposited by exocytosis into the subepithelial tissue.

a membrane bound vesicle. The resulting gonococci containing vesicle appears to migrate across the cell to its base.

Exocytosis of Gonococci into the Submucosa. After approximately 48 h of infection there is an orderly separation of the membrane of the vacuole containing gonococci along the base of the epithelial cell (26,29,30). The gonococci are then exocytosed into the subepithelial tissues. It should be pointed out that the gonococci which are exocytosed are uniform in their structure and appear intact (Figure 5b). If there is a similar process of exocytosis occurring in the *in vivo* infection of humans, this offers an explanation of how gonococci enter the submucosal tissue to cause disease (i.e., salpingitis) or enter the blood stream to cause disseminated gonococcal disease.

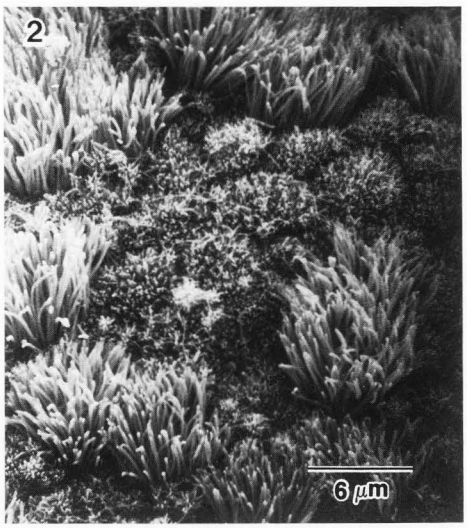
Infection by *Chlamydia trachomatis*. Unlike *N. gonorrhoeae*, *C. trachomatis* is not species specific and has been shown to infect not only human fallopian tube epithelial cells but also the epithelium of bovine, murine, rabbit, and non-human primate oviducts (17,24,35,36,43). With the exception of the work of Hutchinson et al. (17) these observations have been of *in vivo* infection occurring days and weeks after the initial infection. Although these are important observations they do not address the immediate and initial events that initiate the interaction between the elementary body of the chlamydia and the epithelial cell of the mucosa of the fallopian tube.

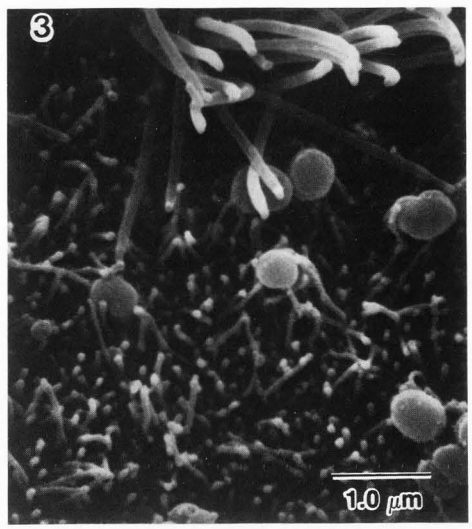
Swanson *et al.* (42) and Evans (9) both using cervical biopsy specimens from patients infected with *Chlamydia trachomatis* studied the reproductive cycle of this organism *in vivo*. It is apparent from these studies that inclusion containing cells were found only in columnar epithelium and were localized in their distribution. There was no evidence of attachment to or invasion of stratified squamous epithelium.

Most stages of the chlamydia reproductive cycle were present in these clinical specimens. Elementary bodies were found in close approximation to the epithelial surface. There is no attachment to the tips of microvilli of the cervical epithelium as there is in gonococcal attachment in the same area (8) nor is there an orderly process of invagination around microvilli to form a host surface membrane found vesicle. There is a direct association between the elementary body and the epithelial cell surface. This event is followed by a membrane bound vesicle which progresses to an inclusion body and is ill-defined and discontinuous (Figure 7). Internal progression of the elementary body to the reticulate body takes place in the cytoplasm (Figure 8a). Inclusions containing reticulate bodies undergoing binary fission were seen in the cytoplasm (Figure 8b).

Evans (9) presented interesting evidence regarding the association of polymorphonuclear leucocytes (PMN) and chlamydial elementary bodies (EB) in cervical infections. Resistance to digestion by lysosomal enzymes and or the inhibition of phagolysosome fusion should result in PMNs laden with elementary bodies. However, this situation was not found. Instead there was evidence of lysosomal degranulation and lysis of the EB within the PMN (Figure 9).

Hutchinson et al. (17) used the fallopian tube in organ culture to demonstrate chlamydial infection in human epithelial cells. The direct isolation of chlamydia from fallopian tubes of patients with acute salpingitis (24) suggests that these organisms are pathogens of the upper genital tract. The fallopian tube organ culture has promise in aiding the understanding of the initial pathogenicity of this organism and attempts to produce infection in human fallopian tubes were successful using *C. trachomatis*.





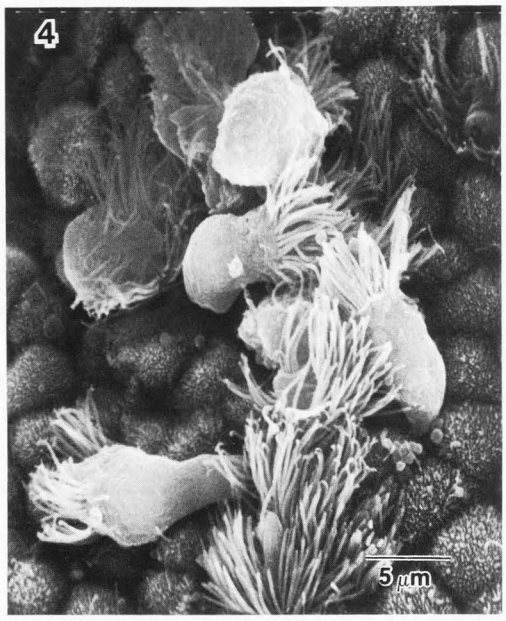
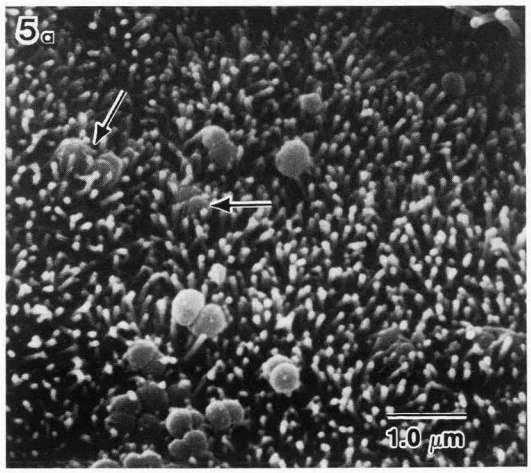


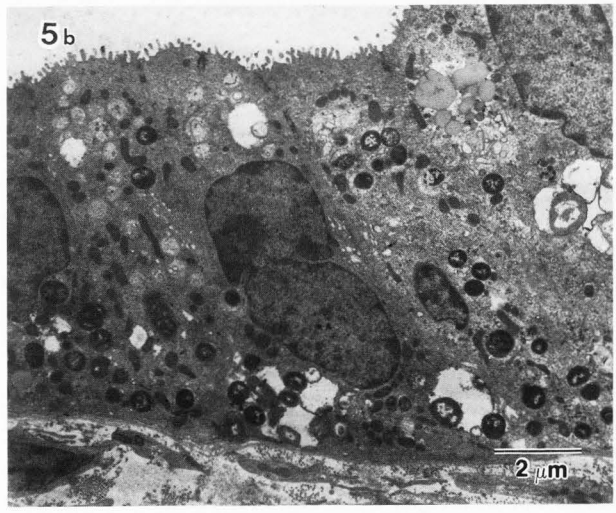
Fig. 2. Scanning electron micrograph of normal uninfected human fallopian tube mucosa. Note the distribution of ciliated and nonciliated epithelial cells. The nonciliated mucosal epithelial cells are covered with microvilli. (4) Reprinted with permission from Scanning Electron Microscopy.

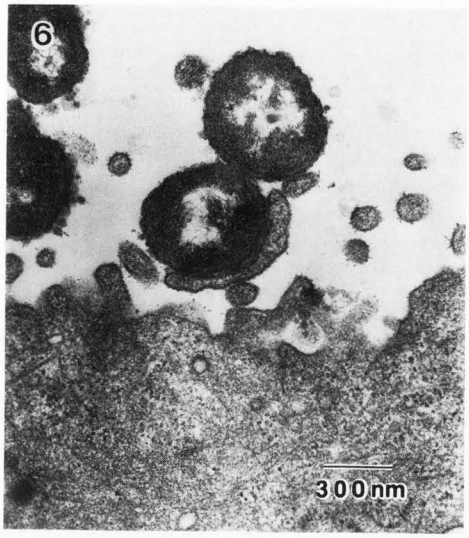
Fig. 3. Scanning electron micrograph of gonococci beginning to attach to the tips of microvilli of nonciliated cells.

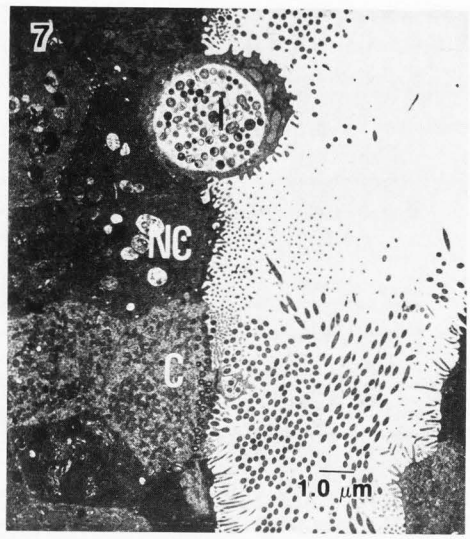
Fig. 4. Scanning electron micrograph of the sloughing of ciliated mucosal cells from the mucosal surface 24 h after infection with piliated gonococci. (4) Reprinted with permission from Scanning Electron Microscopy.

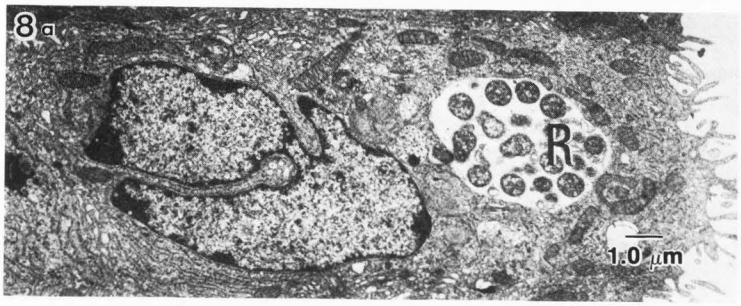
Fig. 5. (a) Scanning electron micrograph of the microvilli attaching to and entrapping (arrows) the gonococci against the surfaces of the epithelial cell. (b) Transmission electron micrograph of the invasion of the epithelial layer of the mucosa by gonococci. Note the appearance of intact gonococci at or near the basement membrane. (4) Reprinted with permission from Scanning Electron Microscopy.











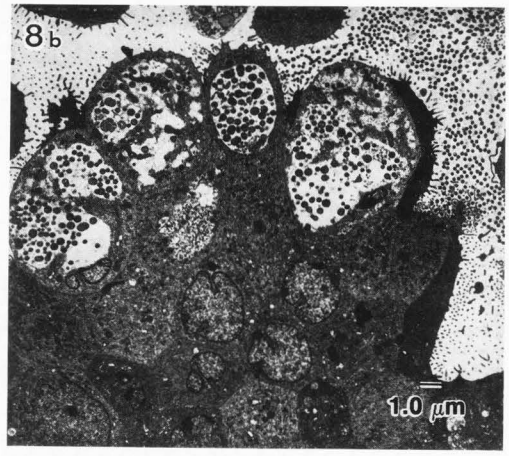


Fig. 6. Transmission electron micrograph of the microvilli attaching to and enclosing a gonococcus near the surface of the mucosal epithelial cell.

Fig. 7. Transmission electron micrograph of *C. psittaci* infected bovine oviduct organ culture seven days after inoculation (bar = 1 μm). I—inclusion; C—ciliated epithelial cell; NC—nonciliated epithelial cell (17). Reprinted with permission from The British Journal of Venereal Diseases.

Fig. 8. Electron micrographs of *C. psittaci* infected bovine oviduct organ cultures seven days after inoculation (a) inclusion containing reticulate bodies (R) (bar = 1 μm); (b) inclusion containing cells being extruded from the mucosal surface (bar = 1 μm) (17). Reprinted with permission from The British Journal of Venereal Diseases.

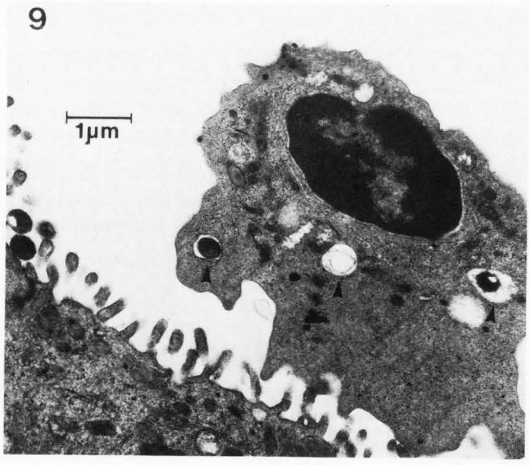


Fig. 9. Transmission electron micrograph of a polymorphonuclear leukocyte on a columnar epithelial surface. Elementary bodies in various stages of lysis (arrows) (bar = 1 μm. (9) Reprinted with permission from the Journal of Infection.

The infectivity of the organism for the fallopian tube was increased when the inoculum was centrifuged into the organ cultures. This method of increased infectivity does not have an *in vivo* clinical correlate and suggests that the infection produced in the organ cultures may be self limiting because of the inability of the liberated chlamydial elementary bodies to reinfect without further centrifugation. Further, chlamydial infection does not appear to cause a quantitative loss of ciliary activity in the organ cultures when compared to uninfected controls. However, the fact that fallopian tube organ cultures can be infected with chlamydia offers the opportunity to further refine the parameters of infection and examine the initial events in the pathogenesis of infection. Since it is obvious that the chlamydia are infecting the organ cultures of the fallopian tubes (17), the failure to demonstrate their presence by electron microscopy is surprising.

In similar studies using *C. psittaci* to infect bovine oviduct organ cultures, numerous inclusions were detected by day five of the infection. In these bovine oviduct infections all stages of the chlamydial growth cycle were seen and were similar to those seen in infected cell cultures (21,22) and in naturally infected cervical epithelium by *C. trachomatis* (42). Again, these chlamydial infections of bovine oviducts did not result in quantitative differences in loss of ciliary activity from controls.

Discussion

The breaching of the mucosal barrier of the human fallopian tube by mucosal pathogens such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* is central in their pathogenesis of disease. For *Neisseria gonorrhoeae* the initiation of pathogenesis requires a complex series of events which have only recently been elucidated. These events require the combination of ligands on the microbe, receptors on the host cell surface and responses by the host to complete this sequence of events. In the case of the gonococcus, specific ligands such as pili and the outer membrane protein II have been implicated in attachment to fallopian tube epithelial cells. However, ligands of chlamydia are more obscure. The elementary bodies lack components which are analogous to pili although they do have some structures which project from their surface (14). These structures play no apparent role in the attachment of chlamydia to host cells. Thus the mechanism by which chlamydia attach to epithelial cells to initiate infection continues to be unresolved. The literature provides little experimental data on the mechanisms of chlamydial pathogenesis in the female upper genital tract and much of the information that is available is either from natural human cervical infections or tissue culture cell models of experimental infection, or experimental animal infection.

Much of our knowledge of chlamydial interaction with cell surfaces comes from the study of chlamydial infection of cell cultures. Attachment is a discrete event in chlamydial infection and can be inhibited by trypsin treatment of mucosal cells or by incubation of the chlamydia at 56° or 60° C for 1 h. These studies suggest that the chlamydial receptor may be protein in nature while other studies suggest that N-acetyl-D- glucosamine may play a role in the chlamydial receptor.

When gonococci attach to human fallopian tube tissue there is a toxic manifestation to ciliated mucosal cells observed with a resulting ciliostasis and sloughing of intact cells. This event has not been observed in similar experiments using chlamydia to infect fallopian tube organ cultures. This may be due to a lack of a potent toxin liberated by the chlamydia. However, a lipopolysaccharide has been isolated from *C. trachomatis* and partially characterized both biochemically and biologically (16). Its role in toxic damage to ciliated human genital epithelial cells is unknown.

Invasion of the epithelium by gonococci began approximately 24 h after infection and was associated with microvilli appearing to withdraw and encompass the gonococcus. A phagocytic vacuole was formed, transported through the cell and the gonococci were exocytosed into the subepithelial tissue around 72 h of infection. In bovine oviducts infected experimentally with *C. psittaci* inclusions were seen about four days after infection. The inclusions were observed in the cytoplasm near the mucosal surface. It is interesting to note that inclusions were observed mainly in epithelial cells which possessed only microvilli, a situation analogous to the gonococcal invasion of the mucosal epithelium. Occasionally there was a ciliated epithelial cell with inclusions but mainly the inclusions were found in nonciliated cells. Further, all stages of the developmental cycle of chlamydia are represented. In contrast to gonococcal infection, chlamydial inclusions were seen to be rupturing, releasing elementary bodies from the mucosal surface. The inclusions were not observed to be exocytosed from the base of the cell as occurs with gonococcal infected tissue.

The information provided by morphological examination of the mucosal surface of the upper genital tract infected by *N. gonorrhoeae, C. trachomatis,* or *C. psittaci* suggest that the organ culture of fallopian tube tissue provides a useful model for studying the pathogenesis of these infections. The host tissue provides cells which are capable of being infected by both microorganisms and can provide an environment for the infection to progress. The initial conditions for and mechanisms of attachment and penetration of the mucosa by chlamydia is much less well understood than are those same conditions for gonococcal infection. Detailed studies of the mechanisms of attachment and penetration by the organisms will go far in our understanding of the initiation of acute pelvic inflammatory disease (salpingitis).

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Discussion with Reviewers

S.V. Nicosia: In this review, you state that gonococci are exocytosed into the subepithelial space. However, no pictorial documentation is provided. If, indeed gonococci gain access to subepithelial tissues, does this process happen by true exocytosis (i.e., via membrane fusion-fission) or merely through partial degeneration of the infected cells?
Authors: Our pictorial representation of exocytosis is not as dramatic as we would like. However, the process has been described and documented by McGee et al. (1983; Rev. Infect. Dis. **5**, 708–714) as there being an orderly parting of membranes at the base of nonciliated cells and an exocytosis of gonococci into the subepithelial tissue.

S.V. Nicosia: How do chlamydial elementary bodies enter the oviductual cells? What is the nature of "direct association" between these bodies and the cell surface? Are the organisms entering the cells by an active or passive process?
Authors: The actual mechanism by which chlamydial elementary bodies enter mucosal epithelial cells is not known. It is assumed that it is a receptor mediated event which is similar to that seen in the uptake of elementary bodies by tissue culture cells. It is not known whether this is an active or passive process.

S.V. Nicosia: Chlamydial reinfection and loss of ciliary activity are not seen in organ culture. Is, perhaps, the absence of polymorphonuclear leukocytes a responsible factor? Also, how was ciliary activity quantitated *in vitro*?

Authors: The lack of chlamydial reinfection in organ culture is most likely due to conditions of the model itself. The loss of ciliary activity is the result of toxin production and the toxic products of chlamydial origin have been of low biological activity. Lack of polymorphonuclear leukocytes probably does not influence the loss of ciliary activity. The ciliary activity was quantitated by measuring the amount and activity of cilia of a given area over time of infected and control tissues.

J.J. Gilloteaux: Do you see any cell surface change(s) in the fallopian mucosa in the case of chlamydial infestation or have any authors described such change(s)? Does the cervical mucosa show similar changes?
Authors: I am unaware of any gross morphological changes in the surface structure of the mucosa. There is no sloughing of ciliated cells or appreciable loss of ciliary vigor. I do not have information on cervical mucosa following chlamydial infection.

W.H. Wilborn: What are the detailed ultrastructural features of the attachment between microvilli and gonococci?
Authors: There is a close initial association between the microvillar membrane and the gonococcal cell membrane and wall. However, there does not appear to be a fusion of the two membranes but a binding through gonococcal surface ligands (pili initially and Protein II later) to establish physical contact.