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Species Specificity of Attachment and Damage to Oviduct Mucosa by *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae colony type 1 were inoculated into organ cultures of oviducts obtained from various animal species. Gonococci rapidly attached to extensive areas of the mucosa of human oviducts (fallopian tubes), entered the mucous-secreting cells, and caused histological damage to the tissues. In addition, 2 to 4 days after infection there was complete loss of ciliary activity. In contrast, gonococci attached very scantily or not at all to the mucosa of rabbit, porcine, or bovine oviducts. However, the organisms multiplied in the medium of these organ cultures and were located sometimes in the base of mucosal cells and in large numbers in the submucosa. Despite this, there was no histological evidence of damage, and at least 7 days after infection ciliary activity was maintained equally as well as it was in uninfected control cultures. The host specificity of *N. gonorrhoeae* appears to be determined, at least in part, by a markedly diminished ability of the organisms to attach to and damage the genital mucosa of nonhuman species.

There have been several reports of using human oviducts (fallopian tubes) in organ culture to study the pathogenicity of *Neisseria gonorrhoeae* (1, 5, 7, 9). It has been shown that both pilated and nonpilated gonococci are capable of attaching to the mucosal epithelial cells of human oviduct organ cultures and of invading the tissues by penetrating into and between the cells (7, 9). This sequence of events is associated with loss of ciliary activity and shedding of epithelial cells from the mucosa. In contrast, gonococci have been observed by electron microscopy not to attach to the epithelium of rabbit oviduct tissue maintained in organ culture, and ciliary activity was unaffected (7). It was suggested that the ability of *N. gonorrhoeae* organisms to attach to the epithelial cells of human but not rabbit oviducts might be responsible, at least in part, for the host specificity exhibited by these microorganisms.

Recently, we have described a method for quantitatively assessing the loss of ciliary activity that occurs in human oviduct organ culture as a result of gonococcal infection (5). A further advance has been the development of a staining procedure that enables gonococci to be easily located in tissue sections (6). To extend our previous studies of the host specificity exhibited by *N. gonorrhoeae*, we quantitated the effects of pilated colony type 1 gonococci on the ciliary activity of organ cultures of oviduct tissue obtained from a variety of animal species. In addition, we used the new histological technique to study attachment of gonococci to the tissues, to locate them within cells, and to determine their effect on tissue integrity.

MATERIALS AND METHODS

Bacteria. *N. gonorrhoeae* 2686, colony type 1, was used in all experiments. Organisms were grown on GC agar base (Difco Laboratories, Detroit, Mich.) supplemented with 2% (vol/vol) IsoVitaleX (Baltimore Biological Laboratory, Cockeysville, Md.) (GC + Iso) in an atmosphere of 2% CO_2 in air at 37°C. The identity of the bacteria was confirmed by Gram stain, a positive oxidase reaction, and fermentation of glucose but not maltose, sucrose, or fructose. Procedures for the determination of colonial morphology and for the observation of pili were the same as those described previously (4).

Source of organs for culture. (i) Human oviducts. Oviducts were obtained from nonpregnant, premenopausal women who were undergoing hysterosalpingectomy for prolonged uterine bleeding or fibroid tumors. Patients receiving antimicrobial therapy within 48 h before operation or who had a previous history of salpingitis were excluded as donors.

(ii) Rabbit oviducts. Oviducts were obtained aseptically from rabbits weighing 3 to 4 kg as described previously (7).

(iii) Porcine and bovine oviducts. Oviducts from pigs and a cow were removed after caesarean section delivery of viable offspring. Although these tubes were not removed under strict aseptic conditions, possible

bacterial contamination was eliminated by incubating the tissue in medium containing vancomycin and colistin for 24 h after collection.

Preparation and maintenance of organ cultures. All oviducts were received in the laboratory within 30 min of removal. They were immersed in Eagle minimal essential medium (MEM) containing Earle salts, L-glutamine, and 0.05 M *N*-2-hydroxyethyl piperazine-*N*-2-ethanesulfonic acid (HEPES), the pH being adjusted to 7.45 with 1 M NaOH (HEPES-MEM). This medium was prepared by diluting a 10-fold concentrate (Grand Island Biological Co., Grand Island, N.Y.) to single strength with sterile, pyrogen-free water for injection (May and Baker Ltd., Dagenham, England) as described previously (5). At this stage in all experiments, the medium was supplemented with vancomycin hydrochloride (5 μg/ml; Eli Lilly & Co., Indianapolis, Ind.) and colistin sulfomethate sodium (3 μg/ml; Pharmax Ltd.).

Oviduct pieces (3- to 4-mm square) were prepared as previously described (5). Dishes, each containing three pieces of tissue, were incubated at 37°C in an atmosphere of 2% CO_2 in air. In all experiments, 24 h,ju after preparation of organ cultures and just before their infection, medium containing antibiotics was exchanged for medium free of antibiotics.

Inoculation of organ cultures. Organ cultures were inoculated 24 h after preparation as described previously (5). In brief, after incubation for 20 to 24 h on GC + Iso, gonococci were scraped from the plates and suspended in HEPES-MEM free of antibiotics. The gonococcal suspension was agitated for 30 s on a Rotamix Deluxe agitator and then centrifuged at 100 × *g* for 3 min. The optical density of the supernatant bacterial suspension was adjusted to 0.4 at 530 nm with HEPES-MEM, and the suspension was then diluted 1:40 with HEPES-MEM. When 0.22 ml of this diluted suspension was added to organ cultures containing 2 ml of medium, the final concentration of organisms was about 2×10^5 colony-forming units (CFU) per ml.

Assessment of tissue damage. Damage to the mucosa of organ culture pieces was assessed by observing, with an inverted microscope, the proportion of the periphery (expressed as percent) of the tissue pieces with ciliary activity (5).

Enumeration of gonococci in organ culture medium. In experiments with human and porcine tissues, the organ culture medium was replaced with fresh medium each day. The number of gonococci in the removed medium, expressed as CFU per milliliter, was determined by making serial 10-fold dilutions in HEPES-MEM and plating 0.05 ml of each dilution on the surface of GC + Iso medium. After incubation for 20 h at 37°C in an atmosphere of 2% CO_2 in air, the colonies were counted, and the proportion of type 1 was assessed by observing 100 colonies. In experiments with bovine tissues and in some of those with rabbit tissues, the organ culture medium was not changed. A 0.2-ml volume was withdrawn, and the number and type of gonococci was assessed as described above. An equivalent amount of medium was then added to the organ cultures to restore the culture media to their original volumes.

Histological procedures. The tissue pieces were fixed in formol sublimate for about 20 h and were then processed by routine histological methods. Sections (4-μm thick) were stained by the method of Sowter and McGee (6) by using Gram:methyl green–pyronin:light green (Gram-MGPLG).

RESULTS

Multiplication of gonococci and their effect on ciliary activity. (i) Human oviducts. Six experiments were performed in which human oviduct cultures were infected with colony type 1 gonococci. Whereas gonococci suspended in organ culture medium alone died within 24 h, those in the same medium in which organ culture pieces were present increased from 2×10^5 to about 1×10^7 CFU/ml within 24 to 48 h (Fig. 1). Most of the organisms in the human oviduct organ cultures retained colony type 1 morphology for 3 or more days (Fig. 1).

In all experiments there was a rapid decrease in the percentage of periphery with ciliary activity of human oviduct organ cultures during the first 24 to 48 h after infection with gonococci (Fig. 1), and ciliary activity was no longer detectable within 2 to 4 days after infection.

(ii) Rabbit oviducts. The results of initial experiments indicated that there was a decrease in the number of viable gonococci in the organ culture medium during the first 24 h after infection. Consequently, the medium was not changed in two subsequent experiments so as to avoid removing the remaining viable organisms. In these two experiments (the results of one of which are shown in Fig. 1), gonococci decreased from an initial concentration of 4×10^5 to about 2×10^3 CFU/ml within 24 h. However, there was no further decrease in the number of organisms, and by day 4 there was clear evidence of multiplication. On day 5 the gono-

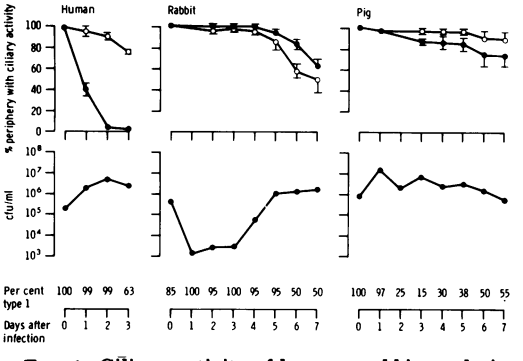


FIG. 1. *Ciliary activity of human, rabbit, and pig oviduct organ cultures infected with N. gonorrhoeae 2686 colony type 1* (●) *compared with that of uninfected organ cultures* (○). *Mean values for the percentage of periphery with ciliary activity plus or minus the standard error are recorded.*

cocci had reached a concentration of 10^6 CFU/ml, 95% of which still produced colonies of type 1 morphology.

In contrast to the rapid and complete loss of ciliary activity observed in human oviduct organ cultures, the ciliary activity of the rabbit oviduct cultures was not adversely affected by the gonococci (Fig. 1). Even 5 to 7 days after infection, when large numbers of organisms were present, the rabbit cultures maintained their ciliary activity as well as that of uninfected cultures.

(iii) Porcine oviducts. In two experiments (the results of one of which are depicted in Fig. 1), gonococci multiplied in the medium of porcine oviduct organ cultures to attain concentrations comparable to those found in human oviduct organ cultures. In both experiments with porcine tissue, the proportion of gonococci that produced type 1 colonies decreased during the first 3 days. However, after this time, the number of organisms that produced type 1 colonies appeared to increase.

In both experiments, the ciliary activity of the porcine oviduct tissues after 7 days was maintained as well as that of the control cultures (Fig. 1) despite the multiplication of gonococci and their presence in high concentration.

(iv) Bovine oviducts. One experiment was undertaken in which gonococcal-infected and uninoculated bovine oviduct organ cultures were maintained for 3 days. The concentration of gonococci increased from about 1×10^5 to 4×10^5 CFU/ml during the course of the experiment, and a majority of the organisms maintained colony type 1 morphology during this time. Although a strict quantitative assessment of the ciliary activity of these tissues was not undertaken, there was no indication that the gonococci inhibited ciliary activity.

Histopathological studies of gonococcal-infected organ cultures. (i) Human oviducts. Examination of infected human oviduct organ cultures showed that many gonococci were attached to large areas of the mucosal surface, primarily to the surface of mucus-secreting cells, by 18 h after inoculation (Fig. 2a). Tissues sectioned at subsequent time intervals were found to have gonococci within the mucosal cells, and by day 3 there were many organisms in the submucosa and the integrity of the mucosa was considerably disrupted (Fig. 3a).

(ii) Rabbit, porcine, and bovine oviducts. In contrast to the extensive attachment of gonococci to human oviduct tissue, the organisms attached scantily, if at all, to the mucosal surface of oviduct tissue from the three nonhuman species. The epithelial surfaces were virtually free of adherent bacteria, whether observed early (Fig. 2b, c, and d) or late (Fig. 3b, c, and d) after inoculation. However, gonococci were frequently found attached to and penetrating the nonmucosal surface of the tissue pieces. Moreover, from day 2 or 3 onwards, the organisms were frequently visible singly or in clumps at the junction of the mucosa and submucosa (Fig. 3c and 4a). Occasionally gonococci could be seen in the base of the mucosal cells (Fig. 4b).

Despite the presence of gonococci within them, the infected tissues of the three nonhuman animal species had no more signs of pathological change than the uninfected control tissues. The architecture of the mucosa was undisturbed, and a majority of the mucosal cells were still ciliated at least 5 days after inoculation of the cultures (Fig. 3b, c, and d). In fact, such maintenance of tissue integrity was noted in porcine oviduct cultures that were kept for as long as 8 days after infection.

DISCUSSION

Naturally occurring gonococcal infections are host specific and do not involve nonhuman species. Furthermore, experimental infections of the genital tract have been achieved only in humans and chimpanzees (2, 3). The current studies were designed to investigate whether this host specificity was expressed in genital tissues maintained in organ culture and, if so, the possible basis for the specificity.

The rapid decrease in the ciliary activity of human oviduct organ cultures produced by gonococci was in marked contrast to the excellent maintenance of activity observed in infected rabbit, porcine, and bovine oviduct organ cultures. In the latter group of cultures, despite the multiplication of gonococci and their presence in high concentrations in the medium, the decline in ciliary activity was no greater than in uninfected cultures. The presence or absence of attachment of gonococci to mucosal cells correlated directly with the amount of ciliary activity maintained by the organ cultures. In human mucosa, extensive attachment was accompanied by rapidly diminishing ciliary activity, even though the organisms did not seem to attach to ciliated cells. The almost total lack of observable attachment of gonococci to the mucosa of rabbit, porcine, and bovine oviducts was associated with prolonged maintenance of ciliary activity. Furthermore, histopathological examination revealed that by day 3, when gonococci had invaded and disrupted the mucosa of human oviducts, the integrity of the mucosa of nonhuman oviducts was completely maintained. Indeed, there was no disruption of the mucosa in cultures that were kept for much longer periods of time. This was so despite the occasional location of some gonococci within the basal portion of mu-

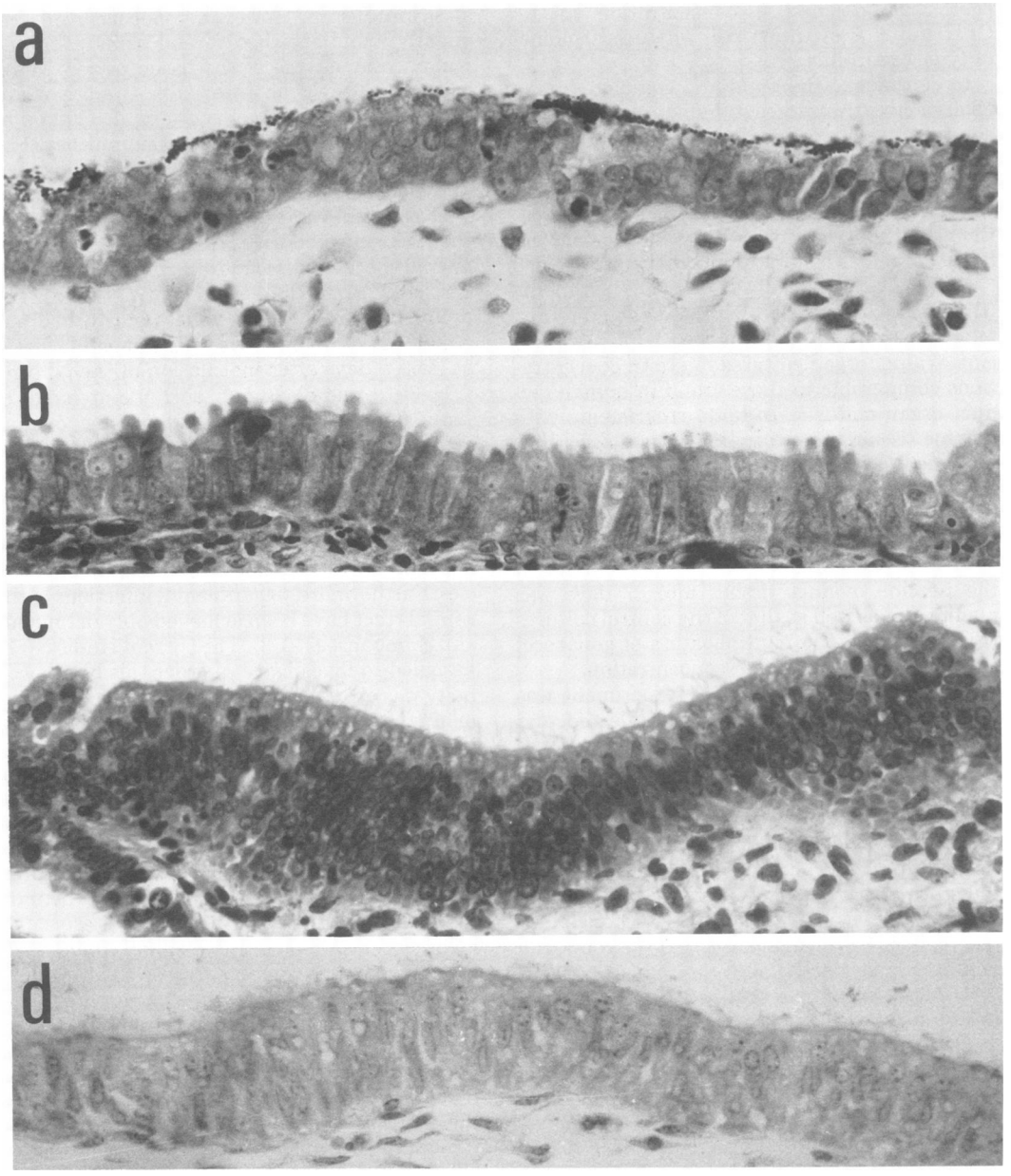
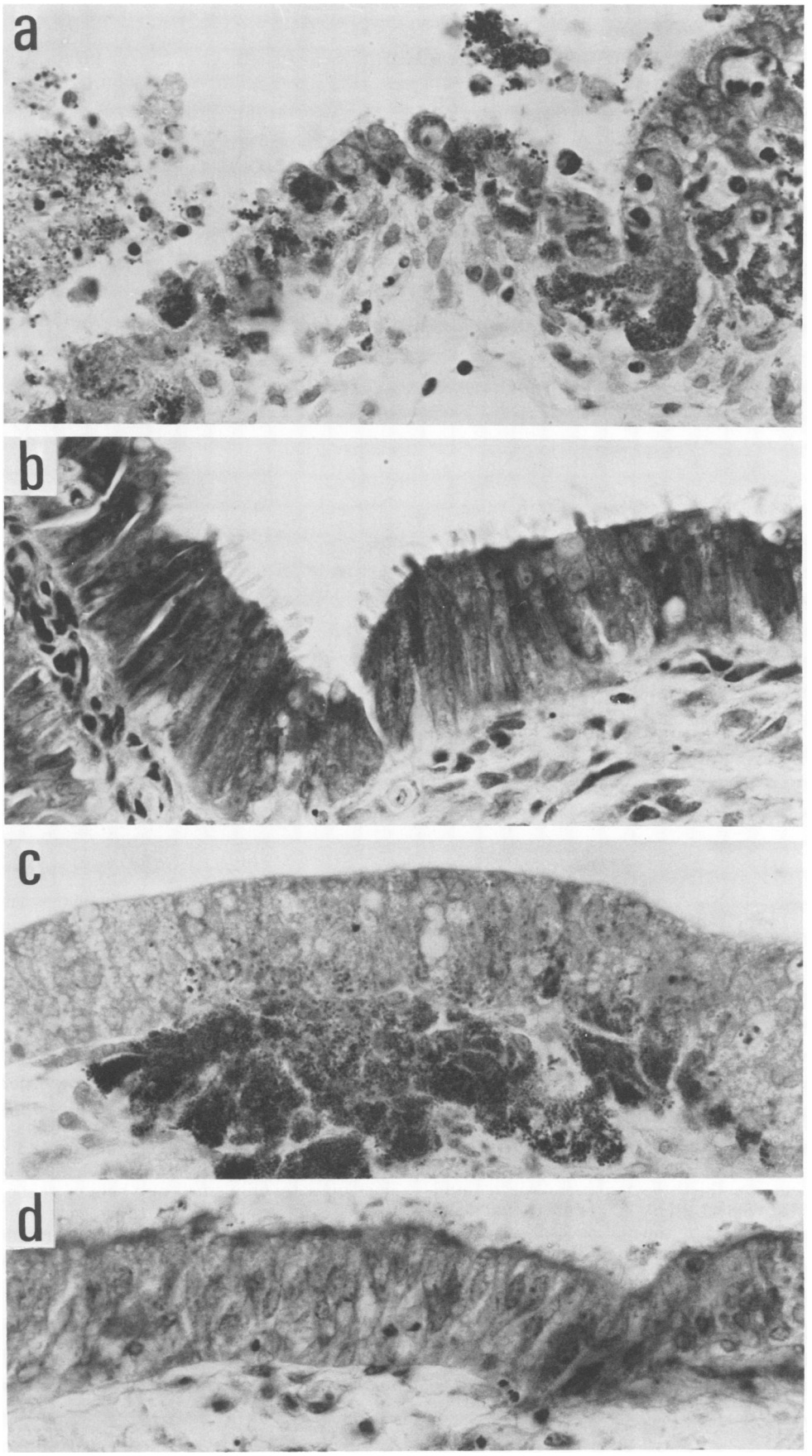


FIG. 2. *Mucosa of oviduct organ cultures of (a) human, (b) rabbit, (c) porcine, and (d) bovine origin 18 h after infection with N. gonorrhoeae 2686, colony type 1. Note that gonococci are attached to the mucosa of human oviduct culture but not to that of other species. Gram-MGPLG. ×430.*

FIG. 3. *Mucosa of oviduct organ cultures of various animal species at indicated days after infection with N. gonorrhoeae 2686, colony type 1. (a) Human after 3 days, (b) rabbit after 5 days, (c) porcine after 5 days, and (d) bovine after 3 days. Note gonococci in mucous-secreting cells of human mucosa and disruption of architecture. The oviduct mucosa of other animal species is undamaged despite gonococci in submucosa as indicated in (c). Gram-MGPLG. ×620.*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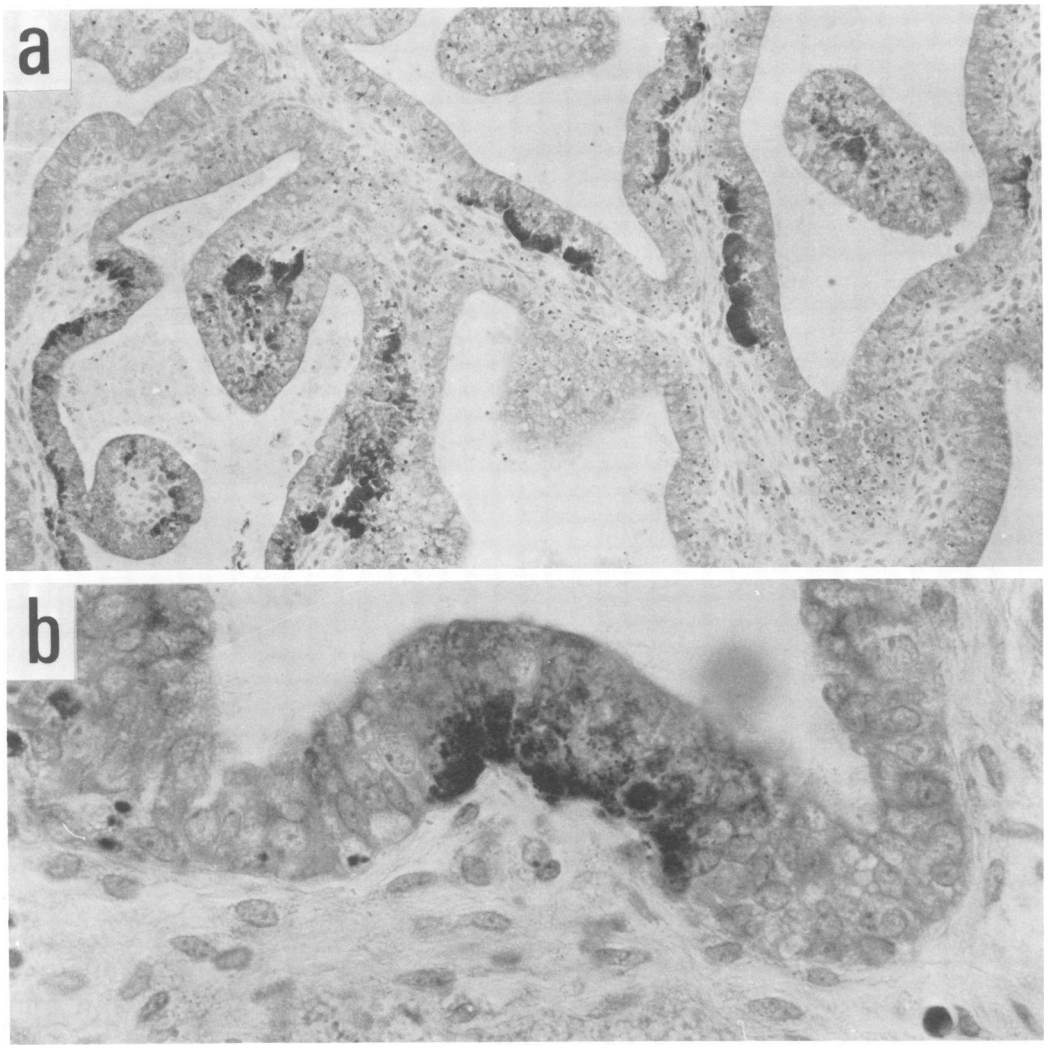

FIG. 4. *Mucosa of porcine oviduct organ culture 5 days after infection with N. gonorrhoeae 2686, colony type 1. (a) Gonococci in clumps, mainly at the junction of mucosa and submucosa. Gram-MGPLG. ×167. (b) Gonococci situated intracellularly in basal portion of mucous-secreting cells. Gram-MGPLG. ×684.*

cosal cells and their frequent occurrence in clumps at the junction of mucosa and submucosa in nonhuman oviduct tissue.

It is only possible to speculate on how gonococci came to be present in the submucosa of oviducts from the three nonhuman species studied. Histological study has shown that some organisms attach to the nonmucosal surface and may reach the submucosa this way. In addition, we have occasionally observed gonococci entering the tissue through breaks in the epithelial surface. However, the occurrence of gonococci in masses close to the base of, and occasionally within, mucosal cells suggests an alternative explanation, namely that the organisms were phagocytosed by the mucosal cells and transported through them to the submucosa. Our failure to detect attachment and our observation that relatively few mucosal cells contained gonococci suggest, however, that even if such a mechanism does operate, only small numbers of gonococci would be transported across the mucosal epithelium. Thus, the large aggregates of organisms observed at the base of the mucosa are probably due to their multiplication once they have reached this site.

Our observations may seem to be at variance with those of Tebbutt et al. (8), who reported that colony type 1 gonococci attached to both human and nonhuman (guinea pig) urogenital

tissue in vitro and concluded that attachment to human tissue was not the critical factor determining the host specificity of gonococcal infections. However, both the semiquantitative histological data in the current studies and the quantitative data in the studies of Tebbutt et al. indicate at least a 5- to 10-fold greater association of gonococci with human oviduct tissue than with nonhuman genital tissue. In their studies, when human and guinea pig genital tissues were incubated with colony type 1 gonococci, washed thoroughly, and homogenized, about 10% of the total viable organisms recovered remained associated with the human tissue, whereas <2% of the total viable organisms recovered remained associated with the guinea pig tissue. Further, the histological observations of Tebbutt et al. indicate that most of the organisms associated with human tissue were found on the mucosal surface. Although they do not indicate the distribution of gonococci associated with nonhuman genital tissue, in our studies a majority was found on the nonmucosal surface. Thus, the data of Tebbutt et al. are compatible with the hypothesis that the greater affinity of gonococci for human genital mucosa is a critical factor in determining the host specificity of gonococcal infections.

Our results lead us to suggest that the host specificity of *N. gonorrhoeae* is related, at least in part, to the capacity of these organisms to attach to and damage the genital mucosa of humans but not that of other species. The relative failure of gonococci to attach to the genital mucosa of the three nonhuman species studied suggests the presence of specific receptors on human mucosal cells that are absent or blocked on animal mucosal cells. If such tissue receptors exist, then corresponding bacterial attachment sites are not confined to colony type 1 gonococci or to pili, since nonpilated, type 4 organisms have also been shown to exhibit host specificity for human genital tissue in the organ culture system (7). An explanation that may account for the finding of gonococci within some mucosal cells of nonhuman tissue, despite the relative infrequency of attachment to the mucosal surface, is that the few gonococci that did attach, rather than remaining at the surface for many hours, rapidly invaded the cells or were phagocytized by them. This concept is not incompatible with the idea of species-specific receptors. Our observation that only a very small proportion of mucosal epithelial cells in nonhuman tissue contained gonococci suggests that the attachment and entry was either a random event or that specific receptors exist on only a few cells of the nonhuman genital mucosa. The absence of tissue damage to the nonhuman genital mucosa suggests either resistance of the tissues to the mechanisms by which gonococci damage human genital mucosa or a dependence of these mechanisms upon attachment.

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