

Adherence of *Escherichia coli* in Pathogenesis of Endometritis and Effects of Estradiol Examined by Scanning Electron Microscopy

YOSHIKAZU NISHIKAWA

Department of Veterinary Surgery and Obstetrics, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka, 591, Japan

Received 21 February 1984/Accepted 20 July 1984

***Escherichia coli* was inoculated into the uterine lumen of ovariectomized rats, and the endometrial surfaces were examined by scanning electron microscopy. Adherence of *E. coli* to the epithelium and destruction of the surface leading to purulent endometritis were noticed. When rats were treated previously with estradiol, adherence of *E. coli* was not detected.**

It has been suggested that ovarian hormones are implicated in the alteration of the course of genital infections (4, 7-10, 13, 16), but hormonal influences on the infections have not been well understood. This could be attributed to a limited number of models showing apparent effects on genital infections (2, 12, 19).

Much attention has recently been given to the concept that adherence of bacteria to mucosal surface is a determinant factor in the pathogenicity of an organism (1). *Escherichia coli* isolated from the urine of patients with symptomatic urinary tract infection has definite affinity to human urinary tract epithelial cells in vitro (21).

Earlier studies reported that rat uteruses can serve as a useful model for investigating relationships between ovarian

hormones and uterine infection, and the studies demonstrated that *E. coli* inoculated into the uteruses under the influence of estradiol caused asymptomatic infection, whereas in uteruses under the influence of other hormones, *E. coli* induced purulent endometritis (accompanying paper [14], 15).

The present study was designed, with scanning electron microscopy (SEM), to examine the adherence of *E. coli* to the endometrium and the possible influence of estradiol on *E. coli* adherence in vivo.

Virgin female Wistar rats, 10 to 12 weeks old, were used in this study: they were ovariectomized 10 to 15 days before being used in this study. A group of rats were treated daily with 0.1 µg of estradiol in 0.1 ml of corn oil for 3 days.

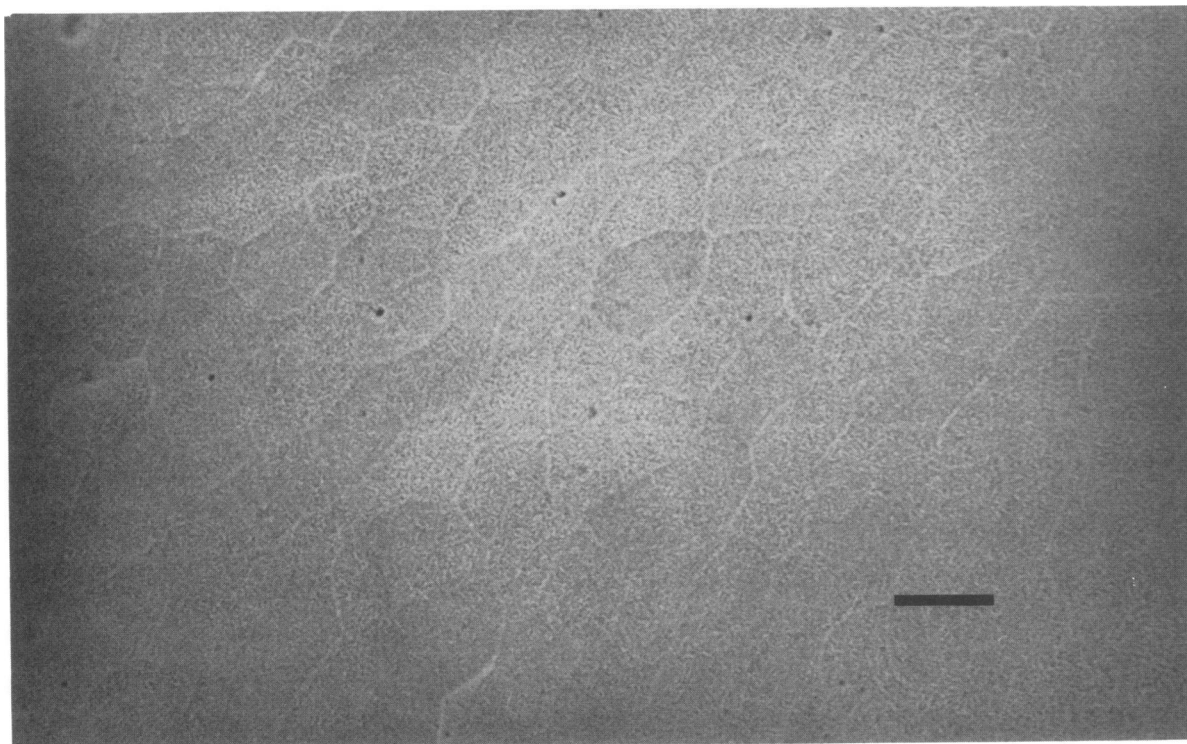


FIG. 1. Endometrial surface of estradiol-treated rats at 24 h after *E. coli* inoculation into the uterine lumen. Bar, 10 µm.

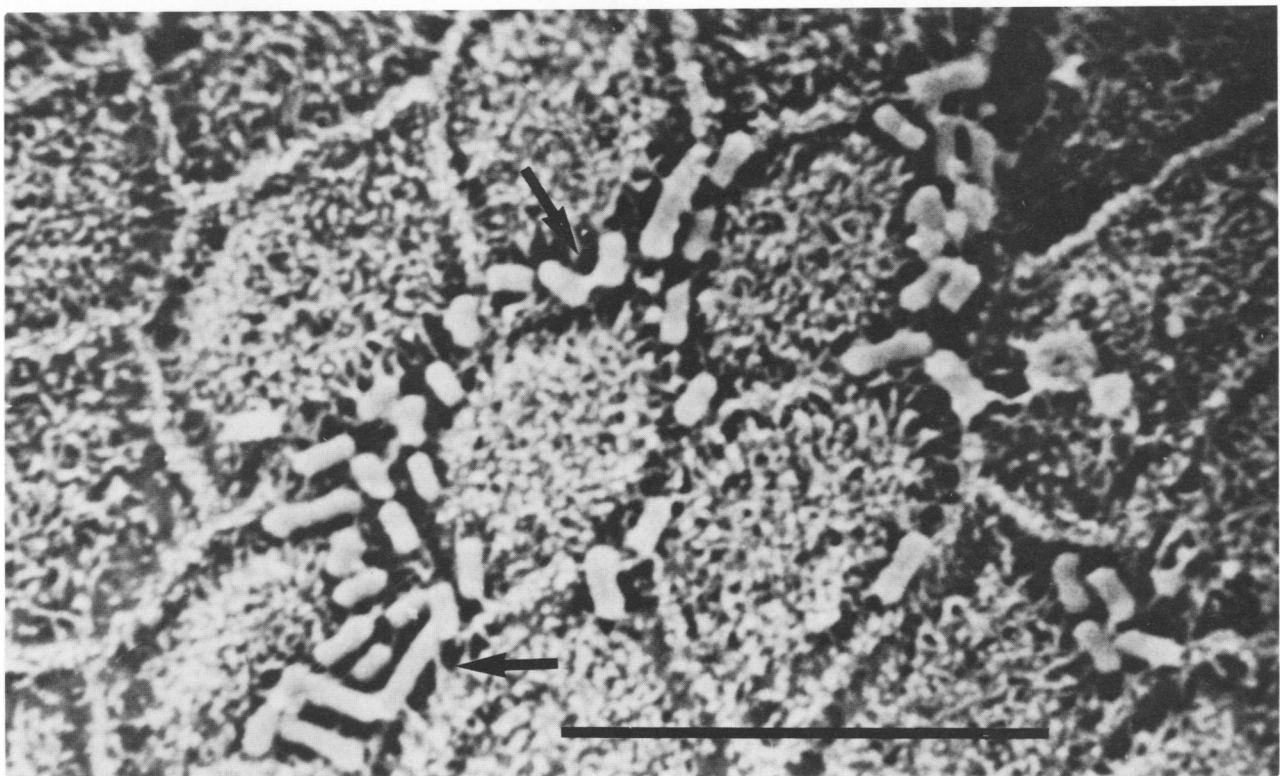
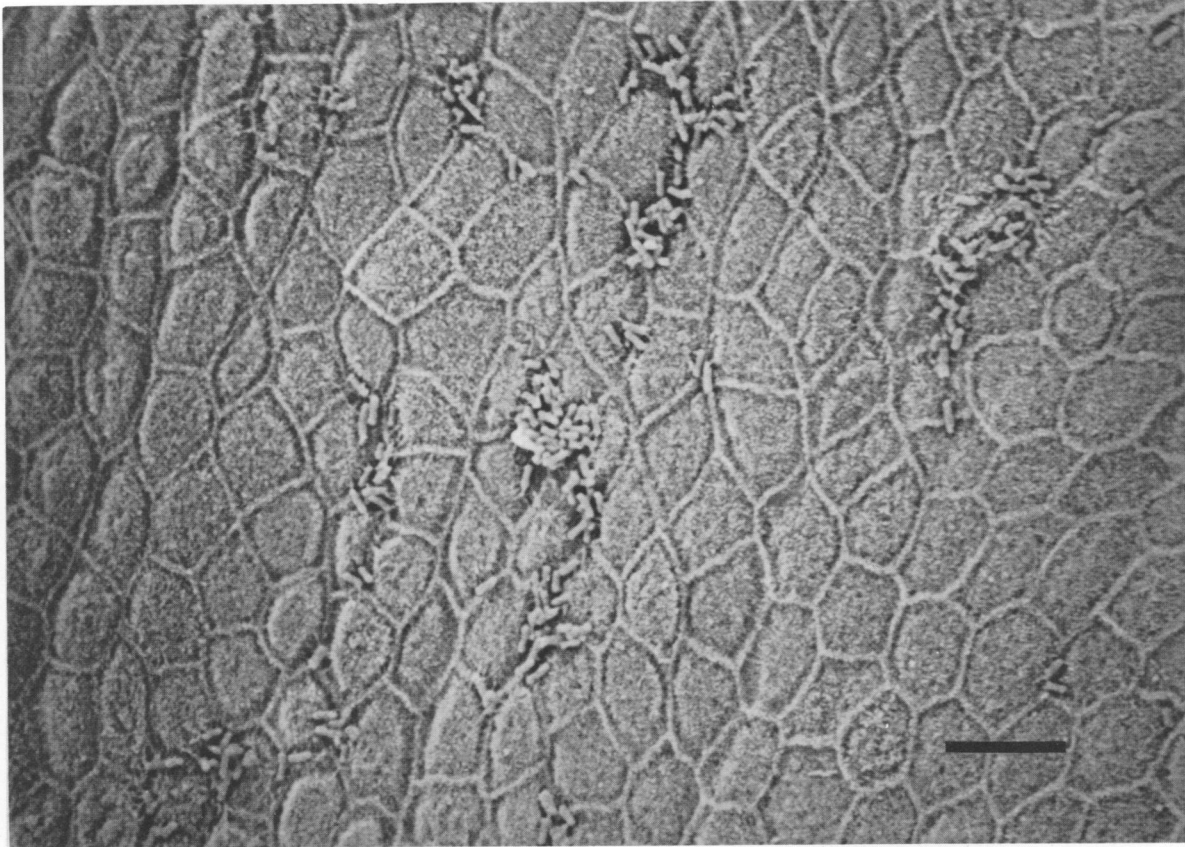


FIG. 2. Endometrial surface of ovariectomized rats at 6 h after *E. coli* inoculation. (A) Numerous bacteria are observed. (B) Erosion of the cell surface-adhering *E. coli* is apparent. Fibrin-like strands (arrows) connecting the *E. coli* organisms to each other and to epithelial surfaces are seen. Bar, 10 μ m.

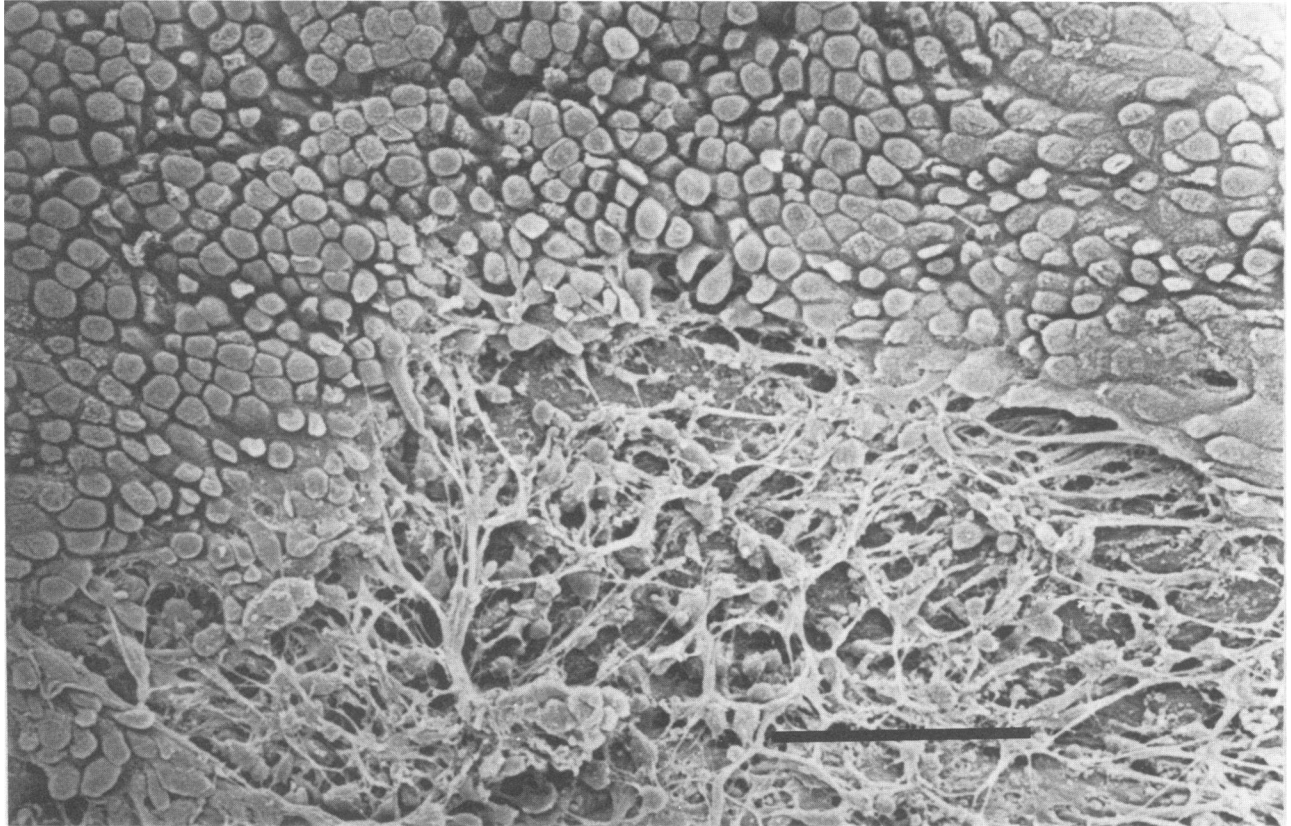


FIG. 3. Endometrial surface of ovariectomized rat at 24 h after *E. coli* inoculation. Intercellular spaces are dilated and covered with a reticulum of long, thin fibers. Bar, 50 μ m.

Another group received only corn oil. On the last day of the 3-day hormonal treatment, rats were inoculated according to the procedures described in the previous report (15). In brief, the posterior abdomen was incised, and the uterus was exposed. Uterine horns were ligated at the cervical ends to prevent possible leakage of inoculum through the cervical canal. *E. coli*, 10^6 CFU, was inoculated into the lumen of right uterine horn. Formalin-killed *E. coli* was infused into the lumen of left uterine horn. Three, five, and five rats administered corn oil alone were used and killed at 1, 6, and 24 h, respectively, after inoculation. Each of the four estradiol-treated rats were killed similarly. The uterine horns were then removed and cut open longitudinally, and the mucosal surface was exposed and gently washed in three changes of 0.1 M phosphate buffer (pH 7.4). For SEM, tissue samples were placed in 1% phosphate-buffered glutaraldehyde for fixation, then dehydrated in ascending concentrations of acetone (25 to 100%), and critical point dried in liquid CO_2 . Specimens were mounted on stubs with silver paint, sputter coated with gold palladium, and examined with a JEOL scanning electron microscope (JSM-T20) at 19 kV.

In estradiol-treated rats, no pathological changes of endometrial surfaces were observed after the inoculation (Fig. 1). No morphological differences were noted as compared with the control specimens from the left horns infused with Formalin-killed *E. coli*.

In rats receiving no estradiol, infected horns exhibited numerous rod-shaped bacteria at 6 h after inoculation (Fig. 2). The organisms adhered preferably to the regions adjacent to lateral cell borders. Eroded cell surfaces were apparent

around the adhering bacteria. Fibrin-like strands connecting bacterial cells to each other and to epithelial surfaces were seen. By 24 h after bacterial inoculation, the cell surfaces appeared roughened and irregular (Fig. 3). There was prominent shrinkage of epithelial cells, and the intercellular junction was dilated. Neither pathological changes nor adhering bacteria were observed on the endometrium of the uterus injected with Formalin-killed organisms.

In vitro adherence of *Neisseria gonorrhoeae* (6), *Proteus mirabilis* (22), and group B streptococci (3) to isolated genitourinary tract epithelial cells from women has been reported to change with the stage of the menstrual cycle. It was also reported that estradiol altered bacterial adherence to HeLa cells (20). These studies suggest that estrogens enhance the attachment of bacteria to vaginal cells and urinary epithelial cells. On the other hand, the present data reveal that estradiol inhibits adherence of *E. coli* to the endometrial epithelium of rats in vivo. This difference may be attributed to the species of host cells and bacteria subjected to experimentation. These studies suggest that ovarian hormones influence bacterial adherence to the target cells of the hormones.

It has been recognized that adhesion of pathogens to host tissue is required for pathogenicity (1, 21). Earlier findings indicate that estradiol suppresses the occurrence of purulent endometritis with *E. coli* in rats (14), and the present results indicate that estradiol may decrease the susceptibility of endometrial epithelium to adhesins of *E. coli*, thereby preventing purulent endometritis.

Ramphal et al. (18) proposed that alteration of the cell surfaces or cell injury facilitates the opportunistic adherence

of *Pseudomonas aeruginosa*. My observations suggest that the hormonal changes within physiological ranges may be involved in opportunistic adherence and may be a key to the pathogenicity of opportunistic infectious diseases.

Further work is needed to determine the mechanisms involved in the susceptibility to infection of the endometrium under hormonal influence, except estradiol. Conceivable factors to be determined are microbial virulence (5), immunoglobulins (24), surface mucin (17), the hormonally-induced alteration of cell surfaces (11), and cell metabolism (23).

I am indebted to A. Arakawa of the Department of Veterinary Internal Medicine for the help with preparing the English manuscript, and T. Baba of the Department of Animal Microbiology and T. Imori of the Department of Veterinary Surgery and Obstetrics for valuable discussions.

LITERATURE CITED

1. Arbuthnott, J. P., and C. J. Smyth. 1979. Bacterial adhesion in host/pathogen interactions in animals, p. 165-198. In D. C. Elwood, J. Melling, and P. Rutter (ed.), Adhesion of microorganisms to surfaces. Academic Press, Inc., New York.
2. Baker, D. A., and S. A. Plotkin. 1978. Enhancement of vaginal infection in mice by herpes simplex virus type II with progesterone. Proc. Soc. Exp. Biol. Med. 158:131-134.
3. Botta, G. A. 1979. Hormonal and type-dependent adhesion of group B streptococci to human vaginal cells. Infect. Immun. 25:1084-1086.
4. Catterall, R. D. 1971. Influence of gestogenic contraceptive pills on vaginal candidiasis. Br. J. Vener. Dis. 47:45-47.
5. Fitzgerald, T. J., and S. A. Morse. 1976. Alteration of growth, infectivity, and viability of *Neisseria gonorrhoeae* by gonadal steroids. Can. J. Microbiol. 22:286-294.
6. Forslin, L., D. Danielsson, and V. Falk. 1979. Variations in attachment of *Neisseria gonorrhoeae* to vaginal epithelial cells during the menstrual cycle and early pregnancy. Med. Microbiol. Immun. 167:231-238.
7. Hawk, H. W., T. H. Brinsfield, G. D. Turner, G. W. Whitmore, and M. A. Norcross. 1964. Effect of ovarian status on induced acute inflammatory responses in cattle uteri. Am. J. Vet. Res. 25:362-366.
8. Hawk, H. W., G. D. Turner, and J. F. Sykes. 1960. The effect of ovarian hormones on the uterine defense mechanism during the early stages of induced infection. Am. J. Vet. Res. 21:644-648.
9. Hawk, H. W., G. D. Turner, and J. F. Sykes. 1961. Variation in the inflammatory response and bactericidal activity of the sheep uterus during the estrous cycle. Am. J. Vet. Res. 22:689-692.
10. Hilton, A. L., S. J. Richmond, J. D. Milne, F. Hindley, and S. K. R. Clarke. 1974. Chlamydia A in the female genital tract. Br. J. Vener. Dis. 50:1-9.
11. Karlson, K. A. 1976. Aspects on structure and function of sphingolipids in cell surface membrane, p. 245-274. In S. Abrahamsson and I. Pascher (ed.), Structure of biological membranes. Plenum Publishing Corp., New York.
12. Kita, E., H. Matsuura, and S. Kashiba. 1981. A mouse model for the study of gonococcal genital infection. J. Infect. Dis. 143:67-70.
13. Koch, M. L. 1947. A study of cervical cultures taken in cases of acute gonorrhea with special reference to the phases of the menstrual cycle. Am. J. Obstet. Gynecol. 54:861-866.
14. Nishikawa, Y., and T. Baba. 1985. Effects of ovarian hormones on manifestation of purulent endometritis in rat uteruses infected with *Escherichia coli*. Infect. Immun. 47:311-317.
15. Nishikawa, Y., T. Baba, and T. Imori. 1984. Effect of the estrous cycle on uterine infection induced by *Escherichia coli*. Infect. Immun. 43:678-683.
16. Oriel, J. D., B. M. Partridge, M. J. Denny, and J. C. Coleman. 1972. Genital yeast infections. Br. Med. J. 4:761-764.
17. Parsons, C. L., C. Greenspan, S. W. Moore, and S. G. Mulholland. 1977. Role of surface mucin in primary antibacterial defense of bladder. Urology 9:48-51.
18. Ramphal, R., P. M. Small, J. W. Shands, Jr., W. Fischschweiger, and P. A. Small, Jr. 1980. Adherence of *Pseudomonas aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. Infect. Immun. 27:614-619.
19. Rank, R. G., H. J. White, A. J. Hough, Jr., J. N. Pasley, and A. L. Barron. 1982. Effect of estradiol on chlamydial genital infection of female guinea pigs. Infect. Immun. 38:699-705.
20. Sugarman, B., and L. R. Epps. 1982. Effect of estrogens on bacterial adherence to HeLa cells. Infect. Immun. 35:633-638.
21. Svanborg Edén, C., L. Å. Hanson, U. Jodal, U. Lindberg, and A. Sohl Åkerlund. 1976. Variable adherence to normal human urinary-tract epithelial cells of *Escherichia coli* strains associated with various forms of urinary-tract infection. Lancet ii:490-492.
22. Svanborg Edén, C., P. Larsson, and H. Lomberg. 1980. Attachment of *Proteus mirabilis* to human urinary sediment epithelial cells in vitro is different from that of *Escherichia coli*. Infect. Immun. 27:804-807.
23. Upchurch, S., and M. G. Gabridge. 1981. Role of host cell metabolism in the pathogenesis of *Mycoplasma pneumoniae* infection. Infect. Immun. 31:174-181.
24. Wira, C. R., and C. P. Sandoe. 1980. Hormonal regulation of immunoglobulins A and G in the rat uterus. Endocrinology 106:1020-1026.