ラットにおける大腸菌性化膿性子宮内膜炎の発病におよぼすエストラジオールの阻止効果:

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Preventive Effect of Estradiol on Manifestation of Purulent Endometritis in Rat Uteri Infected with *Escherichia coli*: with Special Reference to Morphological Changes of the Endometrial Epithelium

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ABSTRACT. *Escherichia coli* inoculated into the uterus under the influence of estradiol causes asymptomatic infection, whereas under the influence of other hormones, it induces purulent endometritis. The present study was undertaken to explain how estradiol prevents *E. coli*-caused purulent endometritis in rats. Since accumulation of uterine luminal fluid caused by estrogen has been well established, attempts were made to see if it prevents endometritis. Neither retained nor transferred luminal fluid inhibited *E. coli* from causing purulent endometritis in ovariectomized rats. Then, the relationship between hormone-induced histological changes and uterine susceptibility to *E. coli* was examined. Mast cells and eosinophils were counted and luminal epithelial cell proliferation and the height of endometrial epithelial cells were examined in rats treated with progesterone and/or estradiol. The luminal epithelial cells were tall when *E. coli* failed to induce purulent endometritis. It was suggested that estradiol modifies luminal epithelial cells, histologically expressed as greater cell heights, making the uterus resistant to *E. coli*. —KEY WORDS: endometritis, *Escherichia coli*, estradiol, pathogenesis, rat.

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In rabbits [9], sheep [10], and cattle [8], uteri are more vulnerable to infection in the luteal phase than in the follicular phase. In humans, chlamydial [12], candida [5], and gonococcus infections [15] are associated with the use of oral contraceptives or the phase of the menstrual cycle. Although these reports suggest implication of ovarian hormones in alteration of the course of genital infections, hormonal influence has been demonstrated in only limited laboratory animal models [3, 14, 22].

Rat uteri serve as a useful model for investigating the relationship between ovarian hormones and genital infection [20]. *Escherichia coli* inoculated into uteri under the influence of estradiol caused asymptomatic infection, whereas it induced purulent endometritis in those under no its influence [18]. The mechanism of prevention of endometritis is still unknown.

Accumulation of uterine luminal fluid with estrogens is well known [1]. Antibodies play an important role against infection [4. 6. 26]; immunoglobulins A and G in the uterine luminal fluid increase under the influence of estradiol [28].

Mast cells contain a variety of biologically active substances and release them by exocytosis. These cells participate in inflammatory reactions with these substances as mediators [24]. In addition, mast cell degranulation may increase mucous release and be involved in host-defence mechanisms on mucosal surfaces [2]; mucin has long been credited with a protective agent, inhibiting the microbial access to the epithelial surface [21]. On the other hand, eosinophils modu-

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late the ongoing mast cell reaction by inhibiting the release of chemical mediators and inactivating some of the released mediators [27]. Ovarian hormones seem to change such cell population in the uterus [7, 16, 23]. Moreover, estrdiol enhances cell division of endometrial epithelials, and modifies the cells morphologically [13].

Estradiol may control these host factors and alter the course of uterine infection in rats. We investigated therefore to find whether the uterine luminal fluid of rats under the influence of estradiol protects purulent endometritis. The uteri in various phases of the estrous cycle and those treated with progesterone and/or estradiol were examined for mast cell and eosinophil populations and epithelial cell division and epithelial cell height. The relationship between hormone-induced histological changes and uterine susceptibility to *E. coli* will also be discussed.

MATERIALS AND METHODS

Animals: Virgin female Wistar rats at 6 to 7 weeks of age were purchased from the Shizuoka Agricultural Cooperative (Hamamatsu-shi, Shizuoka) and housed in groups of six. Feed and water were available ad libitum. The room temperature was approximately 23°C, and a lighting cycle from 7AM to 7PM was maintained. Rats were preconditioned in the room for at least two weeks, then allotted four estrous stages, determined individually by daily checking of vaginal smear. Other rats were ovariectomized through lateral incisions under pentobarbital anesthesia (3–4 mg/100 g body weight) and administered with ovarian hormones.

Hormonal regimens: Estradiol and progesterone were purchased from Sigma Chemicals Co. (St. Louis, Mo.). Two weeks after ovariectomy, estradiol (0.1 μg/day) and/or progesterone (1 mg/day) were administered subcutaneously in 0.1 m*l* of corn oil for three consecutive days [18].

Histological study: After determining the stage of estrous cycle or the last injection of hormones, colchicine (Wako Pure Chemicals Co., Osaka) at a dose of 0.1 mg/100 g body weight (0.1 mg/m*l* aqueous solution) was given intraperitoneally to normal and ovariectomized rats. The rats were sacrificed in 4 hr. The uterus was removed, fixed in 10% buffered formalin, sectioned at 4.5 μm, and stained with hematoxylin and eosin. Cell proliferation in the uterus was quantified by counting the cells arrested in metaphase, as well as the cells composing epithelium or stroma in the focal plane of each cross section. The results were expressed in mitotic index, i.e., the number of cells in metaphase/1,000 cells. Luminal epithelial cell height was measured with a calibrated microscope eyepiece and expressed as an average of 20 epithelial cells. Mitotic cells were excluded from the measurement. For quantification of each of tissue eosinophils and mast cells, 20-sections were stained with hematoxylin-eosin or toluidine blue. The counts for each animal were recorded as gross numbers per 20 cross-sections.

Bacterial strain: A strain of *E. coli*, O:25 K: undetermined H:–, originally isolated from a case of canine pyometra was used. The organisms are piliated and cause mannose-sensitive agglutination of guinea-pig erythrocytes.

Inoculum: *E. coli* was grown on Trypto-soya agar (Nissui Pharmaceutical Co., Tokyo) at 37°C for 18–20 hr and suspended in saline at 10^6 colony forming units (CFU)/0.02 m*l*.

Uterine infection: Rats were anesthetized with pentobarbital sodium. The uterine horns were exposed and ligated at the cervical ends. Then 10^6 CFU of *E. coli* was inoculated into the lumen through the uterine wall at the utero-tubal junction. The rats were sacrificed in 24 hr.

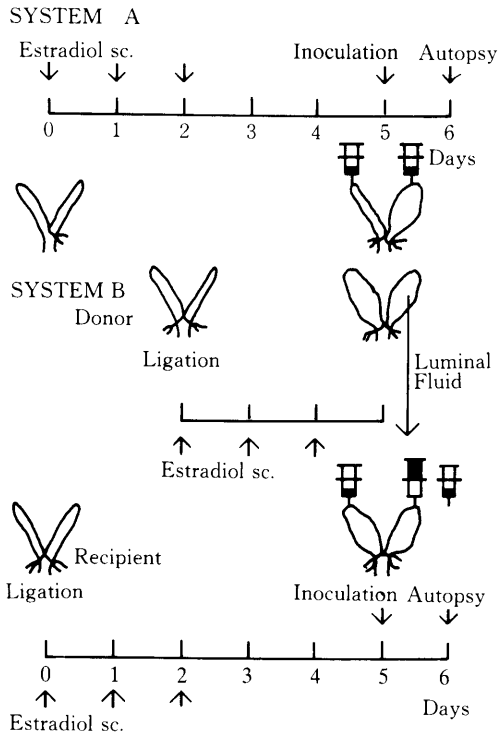

Fig. 1. Experimental design to study if uterine luminal fluid is involved in asymptomatic course of uterine infection.

E. coli and leukocyte counts of the uterine contents: The inoculated horn was flushed with 1.0 m*l* of sterile saline. Ten-fold serial dilutions of the flushings were made in saline. A 0.1-m*l* aliquot was transferred to a petridish and mixed with about 20 m*l* of melted Endo's agar. The plates were incubated at 37°C, and lactose-fermenting colonies counted as *E. coli*. No bacteria indigenous to the uterine lumen were detected. Leukocytes were counted with a bacteria-counting chamber (Erma Co., Tokyo) and expressed as a number/0.02 μl of the flushing, which represents well the magnitude of purulent inflammation of the uterus [16, 18].

Influence of uterine luminal fluid on uterine infection: Two systems were introduced to examine the influence of uterine luminal fluid on uterine infection (Fig. 1).

System A: One uterine horn of each ovariectomized rat was ligated at the cervical end, and the rat reveived 0.1 μg estradiol for three days. Three days later, vaginal smear was examined to confirm that influence of estradiol on genital tract was no longer present, although the ligated horns were still retaining uterine luminal fluid. An *E. coli* suspension was infused into the lumen of both horns after ligation of the other horn.

System B: Both uterine horns of each ovariectomized rat were ligated at the cervical ends; the rats were administered with estradiol for three days. Three days later, the retained luminal fluid was aspirated from one horn and replaced with fresh luminal fluid recovered from recipients just under the influence of estradiol. Both horns were inoculated with *E. coli*.

Statistical analysis: Student's *t* test was used.

RESULTS

Effects of uterine luminal fluid on E. coli infection: Retained or transferred uterine luminal fluid did not inhibit purulent endometritis. Large numbers of *E. coli* and leukocytes were recovered from the uterine lumen irrespective of uterine luminal fluid (Figs. 2 and 3). Few bacteria and leukocytes were recovered from the horns inoculated with formalin-killed *E. coli* (data not shown).

Histology of uteri on each stage of estrous cycle: Increased numbers of eosinophils were observed during proestrus and estrus (Table 1). Endometrial epithelial cells on these stages were taller than those in diestrus and pseudopregnancy. At proestrus, a marked increase in mitotic index of the epithelium was also observed.

Histology of uteri of ovariectomized rats administered with ovarian hormones: Increased mitotic index and height of epithe-

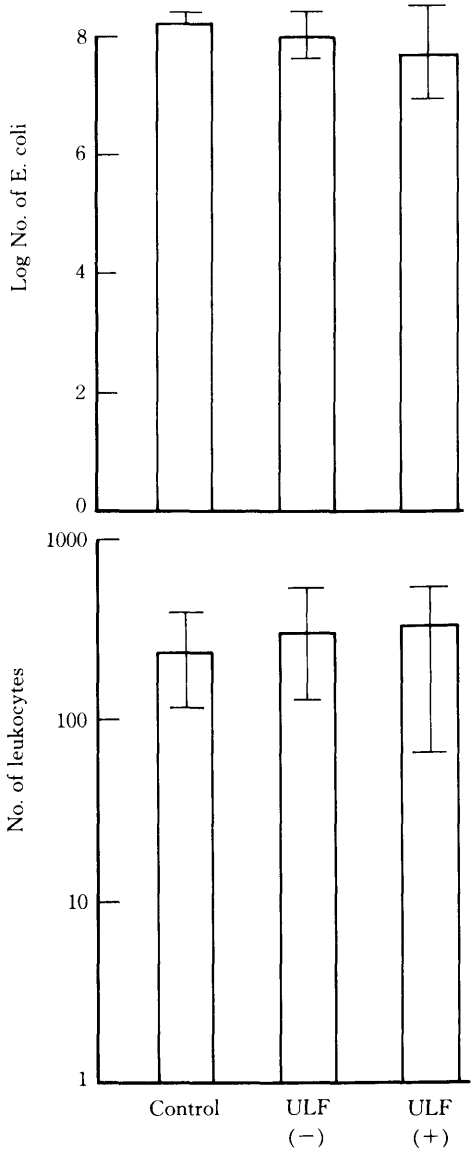

Fig. 2. The numbers of *E. coli* and leukocytes recovered from the horn retaining uterine luminal fluid (ULF +), and the horn retaining no fluid (ULF −). The results from uteri of ovariectomized rats administered with corn oil are shown as a control. For details see Fig. 1 system A. Bars and brackets reflect the mean±SE.

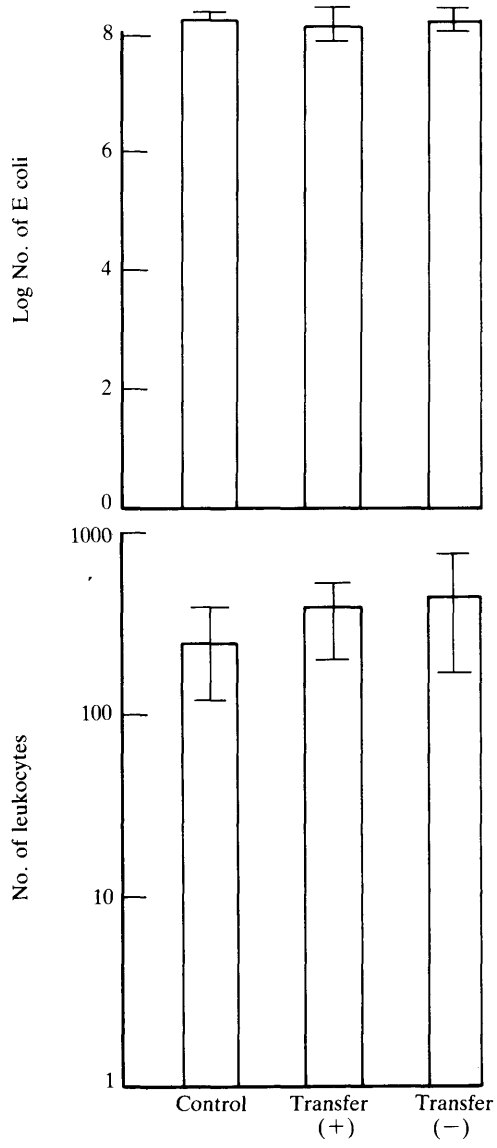

Fig. 3. The numbers of *E. coli* and leukocytes recovered from the horn (transfer +) receiving uterine luminal fluid from a horn under the influence of estradiol, and those of the other horn (transfer −). The results from uteri of ovariectomized rats treated with corn oil are shown as a control. For details see Fig. 1 system B. Bars and brackets reflect the mean±SE.

lial cells were observed in rats having received estradiol (Table 2). No eosinophils were found in uteri of ovariectomized rats in spite of various hormonal regimens.

Time course of the effect of estradiol on histology of uteri and E. coli infection: Estradiol-induced mitoses stopped within 36 hr from the last injection. However, epithe-

Table 1. The effects of estrous cycle on histological features of the rat uterus[a]

Estrous Cycle	Mitotic Index (Mitoses/1000 Cells)		Luminal Epithelial Cell Height (μm)	Mast cells per 20 Cross-Section	Eosinophils per 20 Cross-Section	Manifestation[f] of Purulent Endometritis
	Luminal Epithelium	Stroma				
Proestrus	173.4±51.0[b]	ND[g]	25.8±4.3[c]	20.4± 8.0	272.4±132.3[d]	NO
Estrus	7.8±10.6	ND	28.8±5.7[c]	17.8± 5.9	389.4± 94.8[c]	NO
Diestrus	11.2±10.3	ND	17.3±2.5	13.2± 8.3	105.8± 34.2[e]	YES
Pseudopregnancy	4.0± 6.2	ND	16.5±2.8	7.2±10.1	45.0± 19.3	YES

a) The values represent the mean±SD for a group of 5 rats.
b) Significantly different from the other stages of estrous cycle at $p<0.01$.
c) Significantly different from diestrus and pseudopregnancy at $p<0.01$.
d) Significantly different from diestrus and pseudopregnancy at $p<0.05$.
e) Significantly different from pseudopregnancy at $p<0.01$.
f) Data from the reference [20].
g) Not detected.

Table 2. The effects of ovarian hormones on histological features of the rat uterus[a]

Hormonal Treatment[b]	Mitotic Index (Mitoses/1000 Cells)		Luminal Epithelial Cell Height (μm)	Mast cells per 20 Cross-Section	Eosinophils per 20 Cross-Section	Manifestation[e] of Purulent Endometritis
	Luminal Epithelium	Stroma				
Control	0.2± 0.4	ND[f]	12.0±2.5	15.8± 7.1	ND	YES
Estradiol	109.5±23.6[c]	ND	24.5±6.3[d]	25.7±14.1	ND	NO
Progesterone	ND	ND	14.3±2.5	17.6± 9.5	ND	YES
Estradiol & Progesterone	5.8± 7.5	ND	15.8±4.3	24.8± 7.7	ND	YES

a) The values represent the mean±SD for a group of five ovariectomized rats.
b) Animals received estradiol (0.1 μg/day) and/or progesterone (1 mg/day) daily for 3 days.
c) Significantly different from the values of other groups at $p<0.01$.
d) Significantly different from the values of other groups at $p<0.05$.
e) Data from the reference [18].
f) Not detected.

lial cells were still taller than those of control rats as well as those immediately after hormonal treatment (Table 3 and Fig. 4). The *E. coli* and leukocyte populations of the uterine lumen of rats inoculated on the last day (E0) of a three-day-treatment or 36 hr (E36) after the estradiol treatment are shown in Fig. 5. Few inflammatory cells were recovered from the uterine lumen of these rats, though large numbers of *E. coli* were present.

DISCUSSION

Accumulation of uterine luminal fluid caused by estrogens has been well established [1] and higher concentrations of immunoglobulins A and G have been found in uterine luminal fluid influenced by estradiol [28]. Since estradiol at such doses that induce accumulation of the fluid prevents purulent endometritis [18], it was assumed that the fluid protects uteri against *E. coli*.

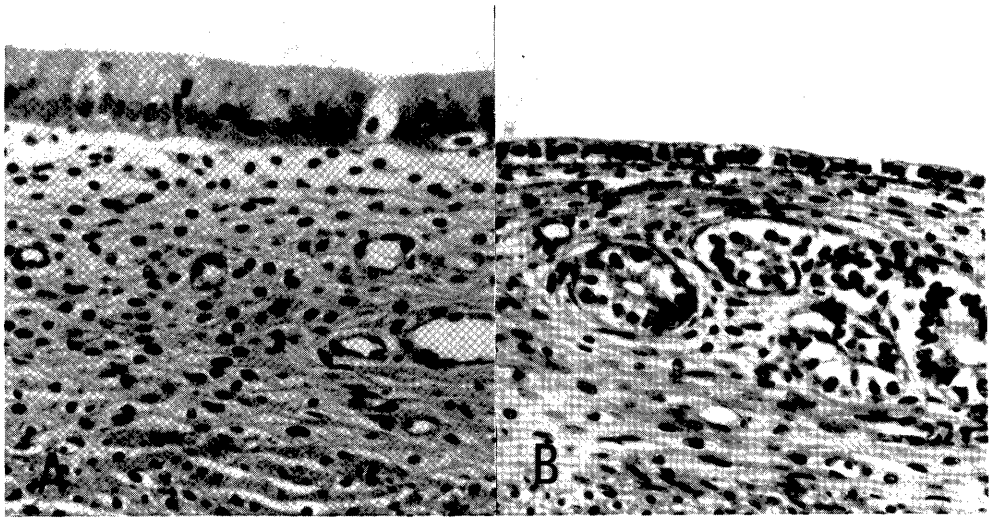

Fig. 4. Longitudinal sections of uterine horns. (A) Luminal epithelial cells of estradiol-treated rats are heightened and columnar. (B) Epithelial cells of ovariectomized rats received only corn oil are squamous. Hematoxylin and eosin staining. ×300.

Table 3. The effects of estradiol on cell division and epithelial cell height of ovariectomized rat uterus[a)]

Hormonal Treatment[b)]	Mitotic Index (Mitoses/1000 Cells)		Luminal Epithelial Cell Height (μm)
	Luminal Epithelium	Stroma	
Control	0.2± 0.4	ND)	12.0±2.5
Estradiol (E0)[c)]	109.5±23.6[e)]	ND	24.5±6.3[f)]
Estradiol (E36)[d)]	1.7± 2.7	ND	27.4±2.4[f)]

a) The values represent the mean±SD for a group of 5 rats.
b) A 0.1 μg estradiol was administered daily for 3 days in corn oil. Control animals received corn oil alone.
c) Rats were examined immediately after the last hormonal treatment.
d) Rats were examined 36 hours after the last hormonal treatment.
e) Significantly different from other groups at $p<0.001$.
f) Significantly different from control at $p<0.01$.

Although accumulated uterine luminal fluid is drained as estrogens decrease, ligation at the cervical ends disturbed the drainage and the horn retained the fluid. In such horns, however, endometritis was induced with *E. coli* as in those without the fluid. The fluid might have been inactivated after the influence of estradiol disappeared. The uterine luminal fluid just under the influence of estradiol was transferred to the horns of recipients, and the horns were inoculated with *E. coli*. This did not prevent endometritis. Thus, estradiol-induced luminal fluid does not protect uteri from the bacterial infection, but some effects given to uterine tissue does.

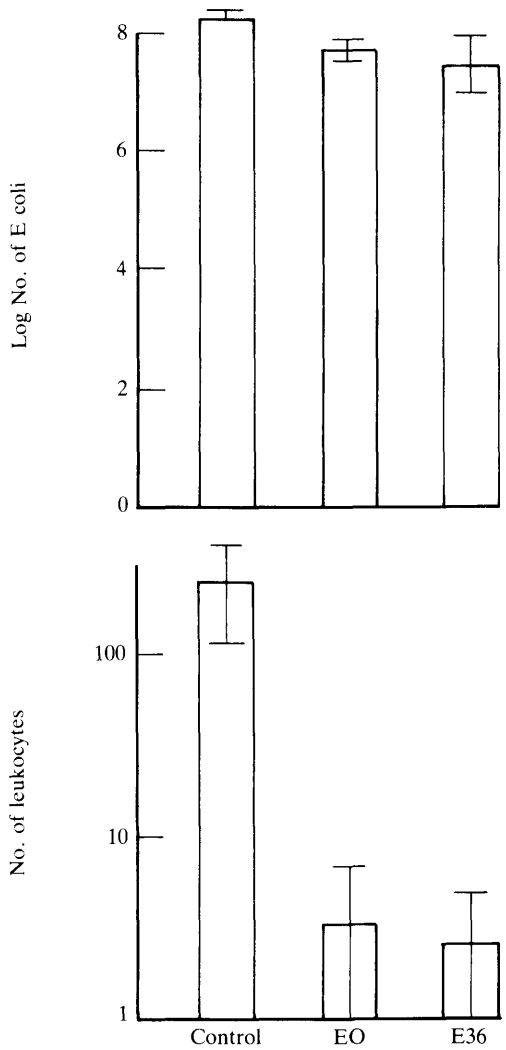

Fig. 5. The numbers of *E. coli* and leukocytes recovered from the uterine lumen of rats, inoculated on the last day (E0) of a 3-day-treatment or 36 hours (E36) after the estradiol treatment. Animals were sacrificed 24 hours after the inoculation. The results from uteri of ovariectomized rats administered with corn oil are shown as a control. Bars and bracket reflect the mean±SE.

Histological examinations of the cyclic rats revealed an increased number of eosinophils during proestrus and estrus when the organisms caused aymptomatic infection. These cells did not prevent purulent endometritis, because uterine infections in estradiol-treated rats were asymptomatic in spite of the absence of eosinophils. Since there was no correlation between the number of mast cells and the course of uterine infection, mast cells do not prevent purulent endometritis, either.

It is known that inhibition of intestinal epithelial cell division by irradiation results in enteritis, diarrhea, and septicemia with the intestinal flora [11, 25]. From this and the present results, it is conceivable that decreased cell division of endometrial epithelia is responsible for the increased susceptibility to infection of the uterus. However, asymptomatic infection in the uterus inoculated at estrus and 36 hr after the three day-estradiol treatment indicates that enhanced mitosis is not essential to prevent purulent endometritis. The present results suggest that the epithelial cells heightened with estradiol are essential to prevent endometritis. This is in accordance with our previous observations that purulent inflammation was induced even in estradiol-treated rats when *E. coli* was infused into the epithelia-sloughed uterine horn [18]. Recently we have observed that *E. coli* adheres to luminal epithelials, but not to the cells of estradiol-treated rats [17, 19]. It is suggested that estradiol inhibits *E. coli* from adhering to endometrial epithelial cells by direct modification of the epithelium and protects the uterus against the organisms. The synthesis or availability of the receptor for *E. coli* adhesins may be dependent upon hormonal control. It might be hypothesized that hormonal change within physiological range is involved in opportunistic adherence and is a key to the pathogenesis of opportunistic infection in any target organs of hormones.

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REFERENCES

1. Armstrong, D. T. 1968. Hormonal control of uterine lumen fluid retention in the rat. *Am. J. Physiol.* 214: 764–777.
2. Askenase, P. W. 1980. Immunopathology of parasitic diseases: involvement of basophils and mast cells. *Springer Semin. Immunopathol.* 2: 417–442.
3. Baker, D. A., and Plotkin, S. A. 1978. Enhancement of vaginal infection in mice by herpes simplex virus type II with progesterone. *Proc. Soc. Exp. Biol. Med.* 158: 131–134.
4. Bienenstock, J., and Befus, A. D. 1980. Mucosal immunology. *Immunology* 41: 249–270.
5. Catterall, R. D. 1971. Influence of gestogenic contraceptive pills on vaginal candidiasis. *Br. J. Vener. Dis.* 47: 45–47.
6. Gibbons, R. J. 1974. Bacterial adherence to mucosal surfaces and its inhibition by secretory antibodies. *Adv. Exp. Med. Biol.* 45: 315–325.
7. Gibbons, A. F. E., and Chang, M. C. 1972. Number of mast cells in the rat uterus with special reference to its relation hormonal treatment and decidual response. *Biol. Reprod.* 6: 193–203.
8. Hawk, H. W., Brinsfield, T. H., Turner, G. D., Whitmore, G. W., and Norcross, M. A. 1964. Effect of ovarian status on acute inflammatory responses in cattle uteri. *Am. J. Vet. Res.* 25: 362–366.
9. Hawk, H. W., Turner, G. D., and Sykes, J. F. 1960. The effect of ovarian hormones on the uterine defense mechanism during the early stage of induced infection. *Am. J. Vet. Res.* 21: 644–648.
10. Hawk, H. W., Turner, G. D., and Sykes, J. F. 1961. Variation in the inflammatory response and bactericidal activity of the sheep uterus during the estrous cycle. *Am. J. Vet. Res.* 22: 689–692.
11. Hellman, S., and Botnick, L. E. 1977. Stem cell depletion: an explanation of the late effects of cytotoxins. *Int. J. Radiation Oncol. Biol. Phys.* 2: 181–184.
12. Hilton, A. L., Richmond, S. J., Kilne, J. D., Hindley, F., and Clarke, S. K. R. 1974. Chlamydia A in the female genital tract. *Br. J. Vener, Dis.* 50: 1–9.
13. Kirkland, J. L., Barrett, G. N., and Stancel, G. M. 1981. Decreased cell division of the uterine luminal epithelium of diabetic rats in response to 17β-estradiol. *Endocrinology* 109: 316–318.
14. Kita, E., Matsuura, H., and Kashiba, S. 1981. A mouse model for the study of gonococcal genital infection *J. Infect. Dis.* 143: 67–70.
15. 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.
16. Maraspin, L. E., and Bo, W. J. 1971. Effects of hormones, pregnancy and pseudopregnancy on the mast cell count in the rat uterus. *Life Sci.* 10: 111–120.
17. Nishikawa, Y. 1985. Adherence of *Escherichia coli* in pathogenesis of endometritis and effects of estradiol examined by scanning electron microscopy. *Infect. Immun.* 47: 318–321.
18. Nishikawa, Y., and Baba, T. 1985. Effects of ovarian hormones on manifestation of purulent endometritis in rat uteruses infected with *Escherichia coli. Infect. Immun.* 47: 311–317.
19. Nishikawa, Y., and Baba, T. 1985. In vitro adherence of *Escherichia coli* to endometrial epithelial cells of rats and influence of estradiol. *Infect. Immun.* 50: 506–509.
20. Nishikawa, Y., Baba, T., and Imori, T. 1984. Effect of the estrous cycle on uterine infection induced by *Escherichia coli. Infect. Immun.* 43: 678–683.
21. Parsons, C. L., Greenspan, C., Moore, S. W., and Mulholland, S. G. 1977. Role of surface mucin in primary antibacterial defense of bladder. *Urology* 9: 48–51.
22. Rank, R. G., White, H. J., Hough, A. J. Jr., Pasley, J. N., and Barron, A. L. 1982. Effect of estradiol on chlamydial genital infection of female guinea pigs. *Infect. Immun.* 38: 699–705.
23. Rytömaa, T., and Teir, H. 1961. Relationship between tissue eosinophils and peroxidase activity. *Nature* 192: 271–272.
24. Saeki, K. 1976. Role of mast cells in inflammatory responses. pp. 86–113. *In*: Handbook of inflammatology Vol. 1 (Yamamura, Y., and Hayashi, H. eds.), Igakushoin, Tokyo (in Japanese)
25. Smith, W. W., Marston, R. Q., Gonshery, L., Alderman, I. M., and Ruth, H. J. 1955. X-irradiation in hamsters, and effects of streptomycin and marrow spleen homogenate treatment. *Am. J. Physiol.* 183: 98–110.
26. Svanborg Edén, C., and Svennerholm, A. M. 1978. Secretory immunoglobulin A and G antibodies prevent adhesion of *Escherichia coli* to human urinary tract epithelial cells. *Infect. Immun.* 22: 790–797.
27. Weller, P. F., and Goetzl, E. J. 1979. The regulatory and effector roles of eosinophils. *Adv. Immunol.* 27: 339–371.
28. Wira, C. R., and Sandoe, C. P. 1980. Hormonal regulation of immunoglobulins A and G in the rat uterus. *Endocrinology* 106: 1020–1026.

要　　約

ラットにおける大腸菌性化膿性子宮内膜炎の発病におよぼすエストラジオールの阻止効果：特に子宮内膜上皮の形態学的変化について：西川禎一・鎌田洋一・馬場　威[1)]（大阪府立大学農学部家畜外科学教室，[1)]家畜微生物学教室）――ラットの子宮内に大腸菌を接種した場合，エストラジオール（E_2）の影響下では，不顕性に経過した。しかし，E_2作用を受けていない子宮あるいはプロジェステロン優勢状態の子宮においては，化膿性子宮内膜炎が誘発された。E_2が子宮分泌液の増加と貯留をひきおこすことから，分泌液に発病阻止能力があるか否か調べたところ，卵巣摘除ラットの子宮頚管結紮によって分泌液を貯留させた場合にも，E_2投与ラットから分泌液を移入した場合にも，大腸菌接種による子宮内膜炎の発生を抑制することはできなかった。性周期各期の子宮ならびに各種ホルモン処置ラットの子宮について，肥満細胞数，好酸球数，子宮内膜上皮細胞の増殖程度，細胞の大きさを調べたところ，E_2影響下で大腸菌感染が不顕性化する子宮においては，常に内膜上皮細胞の丈が高く，E_2は子宮内膜上皮細胞の性状を変化させて，大腸菌感染に対する抵抗性を高めているものと推察された。