THE EFFECT OF ESTROGENIC HORMONES ON THE BACTERIAL CONTENT OF THE UTERUS*

L. WEINSTEIN, W. U. GARDNER, AND E. ALLEN

The impression is general that the uterus under normal conditions does not harbor bacteria. This is supposedly true of both man and laboratory animals. Following parturition, however, serious uterine infections may occur. In laboratory animals short periods of treatment with estrogenic hormones have usually provided very little evidence of uterine infection.[1] Extensive leukocytic infiltration, presumably aseptic, has appeared after the action of estrogenic hormones has ceased. This regressive phase was quite similar to the post-ovulatory stage, late metestrum, in the normal animal.

When rats[6, 8, 11] or mice[2, 4, 5, 7] were treated with estrogens for long periods of time in attempts to produce mammary and cervical cancers, inflammatory or suppurative uterine lesions or pyometra appeared. In some animals these lesions were extreme; the horns of the uteri were distended with pus, sometimes the gut was constricted by adhesions, and at times a generalized peritonitis occurred. If uteri were transplanted in a subcutaneous position or ligated from the cervical canal, pyometra did not occur.[9] In order to test further the possibility that treatment with estrogenic hormones might induce a condition favoring infection, the following series of experiments were undertaken.

Materials and methods

Mice of several strains and of different ages were used. Forty-six mice which had not received injections of estrogenic hormones served as controls. These mice were immature, young virgins, castrates, or multiparous individuals (Table 2). Steroid hormones, alone or in combinations, or triphenylethylene or stilbestrol† were injected subcutaneously in solution in sesame oil into 152 other mice. The mice were maintained on a diet of fox chow and water.

* From the Departments of Bacteriology and of Anatomy, Yale University School of Medicine. These investigations were supported by the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund for Medical Research, and the Fluid Research Fund of the Yale University School of Medicine.

† The stilbestrol and triphenylethylene were supplied by Dr. J. A. Morrell of E. R. Squibb & Sons.

Fifty-seven virgin female mice received 16.6 μg. of estradiol benzoate* and were killed after intervals of 2, 4, 7, 10, or 14 days (Table 2).

In another group of 13 mice the left uterine horns were cut from the cervices and ligated. One month later these mice received 16.6 μg. of estradiol benzoate subcutaneously and were killed 7 days later. The intact and ligated horns were both removed for bacteriological study (Table 5).

Each of 15 mice received 3.75 mg. of testosterone propionate in 3 injections and were killed 7 days after the first and on the third day after the last injection. Eight and 10 mice received 20 mg. of triphenylethylene and 0.4 mg. of stilbestrol, respectively, in 4 injections on alternate days and were killed one day after the last injection. Three mice were given 0.5 mg. of progesterone on alternate days and were killed on the day following the last injection. Ten other mice received testosterone propionate and estradiol benzoate simultaneously (Table 3).

Other mice, which had been ovariectomized 2 months before, and which had received one or more weekly injections of 16.6 μg. of estradiol benzoate, were killed either 3 or 4 weeks after a single injection, or one week after 3 to 8 injections (Table 4).

The mice were killed with illuminating gas and the abdomens opened under the strictest aseptic conditions. The uterine horns were removed well above the cervix, one being placed in Bouin's solution, and later sectioned and studied histologically after staining with hematoxylin and eosin or Gram's tissue stain. The other horn was ground in 0.5 cc. of sterile physiological saline and one loopful of the resulting suspension was streaked on heart infusion agar and the remaining fluid was inoculated into heart infusion broth. All cultures were incubated for 48 hours at 37° C. and the plates and fluid medium were examined for growth. Where no colonies appeared on the agar but the broth medium became cloudy, it was always plated in order to determine the type of colony. Negative cultures were not discarded until after 5 days of incubation.

Vaginal smears were taken after the uterine horns had been removed so that the stage of the estrous cycle or the existence of estrogenic stimulation could be determined.

Results

Of the untreated control mice the agar plates and broth tubes inoculated with preparations from the uteri showed the presence of organisms in 2 of the 41 cases. One positive culture was obtained

* The estradiol benzoate, testosterone propionate, and progesterone were supplied by Drs. E. Schwenk and M. Gilbert of the Schering Corporation.

from an immature mouse and one from a multiparous mouse (Table 1). None of the 6 immature mice were over 29 days of age, the genital tracts were infantile and the vaginas had not yet opened. The one positive culture probably represents a contamination rather than the presence of intra-uterine organisms. The young virgins ranged from 37 to 157 days of age. The vaginal smears of 7 of these mice were of the diestrous type and the other smears showed post- or pro-estrous stages. None of the animals of this group showed the full vaginal cornification of estrus and none yielded intra-uterine bacteria.

Bacteria were found in the cultures prepared from the uterus of one of the 11 untreated multiparous mice. These mice ranged from 3½ to over 10 months of age. One mouse was killed during estrus, three during postestrum, and the others during diestrum. One of the mice at early postestrum gave the one positive culture. The uterus of this mouse was definitely abnormal in that it showed cystic glands. Eggs were found in the uterine tubes. The uterus showed no gross evidence of inflammation and bacteria were not observed in sections prepared with Gram's stain.

The uteri of the 7 ovariectomized mice did not show bacteria. The genital tissues were atrophic. Four adult mice which had received injection of sesame oil subcutaneously also showed no bacteria in their uteri.

TABLE 1

THE INCIDENCE OF POSITIVE BACTERIAL CULTURES FROM THE UTERI OF NON-TREATED MICE

Condition of mice	*Number of mice*	*Number of positive cultures*	*Number of negative cultures*
Immature	6	1	5
Virgins	13	0	13
Adults	11	1	10
Castrate	7	0	7
Virgins*	4	0	4

* Received sesame oil subcutaneously.

The uteri of mice which had received one or more injections of estradiol benzoate frequently contained bacteria. Forty of 57 mice killed from 2 to 10 days after one or two injections had organisms

in their uteri (Table 2). Bacteria appeared in the uteri of some mice within 2 days after a single subcutaneous injection of 16.6 μg. of estradiol benzoate. The number of mice furnishing positive cultures was relatively greater when they were killed 4 to 7 days after a single injection, or after two weekly injections. The mice killed after 4 or more days after a single injection usually showed hyperemic and dilated uteri. The intra-uterine fluid usually escaped during the process of removal of the tissues.

Histologically, the uteri rarely showed inflammatory foci in the areas studied. In several uteri organisms were found in short chains or in colonies in the lumina or glands of the estrogen-treated mice. Evidence of mural extension was lacking. Organisms were found infrequently in the sections prepared with Gram's stain from most of the animals, although they were present in the cultures. The uteri of some mice killed 7 to 10 days after an injection of estrogen contained leukocytes, some macrophages, and a few sloughed epithelial cells in addition to some colonies of bacteria.

TABLE 2

THE NUMBER OF ESTRADIOL BENZOATE TREATED MICE OF SEVERAL GROUPS IN WHICH THE UTERI CONTAINED BACTERIA

Number of mice	*Days after first injection*	*Number of positive cultures*	*Number of negative cultures*
8	2	5	3
13	4	8	5
10	7	8	2
2	10	1	1
15	14-15	14	1
9	2-7	4	5

Bacteria were found also in the uteri of 8 mice which received 4 injections of triphenylethylene, a total dose of 20 mg., and were killed on the day following the last injection (Table 3). The uteri of all of the 10 mice which had received 4 injections of 0.1 mg. of stilbestrol on alternate days, and which were killed one day after the last injection yielded positive cultures.

Nine mice received small doses of estrogen in order to determine whether or not the large amount of hormone administered might especially predispose the uteri to harbor organisms. They received

0.07 μg. of estradiol benzoate every other day and were killed from 2 to 7 days after the first injection. Organisms were present in the uteri of 4 of the 9 mice. All of the mice giving positive uterine bacterial cultures had estrous or early postestrous vaginal smears, whereas 3 of the 5 mice in which no uterine organisms were detected gave diestrous smears.

All of the 4 mice which had received weekly injections of 16.6 or 50 μg. of estradiol benzoate for 4 to 10 months showed uterine organisms. Two of these had pyometra. The others showed no striking evidence of septic uterine inflammation.

Organisms were found in cultures from the uteri of one-third of the mice which had received 3 injections of 1.25 mg. of testosterone propionate (Table 3), and in all of the mice which had received combinations of testosterone propionate and estradiol benzoate (Table 3). None of 3 mice which received 4 injections of 0.5 mg. of progesterone had uteri which yielded bacteria.

TABLE 3

NUMBER OF MICE SHOWING UTERINE BACTERIA AFTER TREATMENT WITH OTHER HORMONES

Substance	*Period treated (days)*	*Amount injected (mg.)*	*No. of mice*	*Number sterile uteri*	*Number septic uteri*
Testosterone propionate	7	3.75	15	10	5
Triphenylethylene	7	20.0	8	0	8
Stilbestrol	7	0.4	10	0	10
Progesterone	7	2.0	3	3	0
Estradiol benzoate	7	0.1	10	....	10
Testosterone propionate	7	5.0	....	....	....
Estradiol benzoate	..	0.1	10	....	10

An attempt was made to determine how long organisms would remain in the uteri after a single injection of estrogen in ovariectomized mice, and how long they might persist with continuous estrogenic treatment (Table 4). Organisms in the uteri were detected 3 weeks after a single injection of estradiol benzoate (16.6 μg.) but none after 4 weeks. The vaginal smears at autopsy were of the estrous type in 2 mice, postestrous in 4 of the mice killed at the end of 3 weeks, and uniformly diestrous from those killed at the end of the fourth week. Weekly injections of 16.6 μg. for

3 to 8 weeks resulted in septic uteri in most of the mice. All of these animals showed either typical or somewhat atypical estrous vaginal smears. A number of them showed squamous metaplasia of uterine epithelium and frank general or localized pyometra. Metaplasia of the uterine epithelium occurred in 3 of the mice treated for 8 weeks and the uteri of these mice were septic. Five mice treated 6 to 8 weeks had pyometra and gave positive uterine cultures, while one mouse with evidence of uterine inflammation showed no organisms. Several mice had cystic and distended uterine glands with no organisms.

TABLE 4

PERSISTENCE OF UTERINE ORGANISMS FOLLOWING TREATMENT WITH ESTRADIOL BENZOATE OF MICE OVARIECTOMIZED FOR 5 MONTHS

No. of mice	*No. of injections*	*Period from injection to death*	*Number septic uteri*	*Number sterile uteri*
5	1	3 weeks	2	3
5	1	4 weeks	0	5
4	3	1 week	4	..
5	5	1 week	4	1
10	6	1 week	4	6
5	8	1 week	4	1

Two possible routes of uterine infection were considered. The first and more probable was that organisms entered the uterus through the cervix. The second was that the organisms localized in the congested uterus from the circulating blood. The latter possibility was not especially indicated because organisms were not detected in the tissues microscopically, unless the uterine epithelium was largely destroyed. Organisms were seen occasionally in the uterus without evidence of mural inflammation.

The culture of preparations obtained from uterine horns ligated from the uterine cervix demonstrated that organisms very probably reached the uterus by passage through the cervix (Table 5). The preparations from 11 of the 12 intact uterine horns revealed colonies of organisms whereas only 6 of the ligated horns showed organisms and these had but few since the blood plates showed no colonies.

TABLE 5

THE NUMBER OF MICE SHOWING ORGANISMS IN THE INTACT AND LIGATED UTERINE HORNS 7 DAYS AFTER A SINGLE INJECTION OF ESTRADIOL BENZOATE

	Ligated uterine horns				*Intact uterine horns*			
No. of	*No. –*		*No. +*		*No. –*		*No. +*	
mice	*Broth*	*B.P.*	*Broth*	*B.P.*	*Broth*	*B.P.*	*Broth*	*B.P.*
13	7	12	6	1	2	2	11	11

Blood was removed from the hearts of 19 mice and broth tubes and blood plates were inoculated. All failed to show bacterial growth, indicating that hematogenous organisms were not frequently present.

Cultures were taken from the spleens of a number of mice from the different groups and uniformly failed to reveal bacteria.

Bacterium alkaligenes and an unidentified Gram-positive diplococcus were the organisms most frequently encountered and they often appeared together in cultures from the same animal. *Escherichia coli,* hemolytic and non-hemolytic streptococci, and staphylococci were also found in some mice. Similar organisms were detected in cultures from the vaginas of mice.

Discussion

The barrier preventing the organisms present in the vagina from entering the uterus is not known. In animals with mucus-secreting cervical glands a viscous and bactericidal plug may guard the uterus. Cervical glands are lacking in mice, so the presence of cervical mucus could not account for the usual aseptic uterine condition. The mechanical closure of the cervix by the sphincter-like action of the circular musculature might afford a physical barrier to the passage of organisms. The sphincter, however, does permit the passage of sperm.

It is possible that the uterine cervix might permit the passage of some of the vaginal organisms but that the uterine secretions may prevent the growth of these organisms or destroy them; that the uterine secretion might be bacteriostatic or bactericidal for the usual vaginal flora. Whether estrogenic treatment increases the entrance of organisms into the uterus or creates a more favorable environment for their growth is not known.

When estrogens are injected into mice the uteri become hyperemic, the mucosae thicken and become more edematous, and the

glandular lumina are distended with a serous fluid which is retained by the cervix. In such uteri bacteria were found in most cases within 2 to 7 days after the injection of estrogen. Although the sphincteric action of the cervix retained the uterine secretions, organisms were present and the bacteria persisted at least long enough to be recovered at autopsy. Since bacteria were not found in the blood, or spleens, and those found in the uteri were of the same types as those present in the vagina, and apparently first appeared in the lumina of the uteri, they apparently gained entrance through the cervical canal.

The uterine distention may be prolonged by repeated injections of estrogen and the septic state may persist without further evidence of inflammatory reaction of the surrounding endometrium. After several weeks the organisms may establish themselves in the tissues and pyometra may appear, either throughout the uterus or localized to one or more glands. The uteri of most mice must be stimulated and presumably kept in contact with the available organisms for some time before an extensive septic inflammation occurs and before invasion of the mucosa takes place.

The appearance of organisms in the uteri of mice receiving testosterone or testosterone plus estradiol benzoate indicates that uterine distention is not essential. The uteri of these animals were hyperemic but not distended.

The reactions of animals of different species indicate that rats and mice may be quite similar, since both may show pyometra after prolonged periods of estrogenic treatment. Rabbits, on the other hand, have had aseptic uteri when similarly treated although the uteri show an infarct-like necrosis.[10]

The relation of sepsis to uterine metaplasia might also be considered. Although all uteri yielding cultures of bacteria did not have a metaplastic epithelium, all uteri showing metaplasia had abundant bacteria. Metaplasia has also been described in certain of the genital glands of male mice and was accompanied by leukocytic infiltration, although sepsis was not evident at the time in most cases.[4] In the vaginal epithelium the presence or absence of leukocytes in the epithelium during the diestrous or estrous phases of the cycles has been associated with the extent of epithelial cornification and the hydrogen ion concentration. During diestrus the pH is high and leukocytes appear, and during estrous the leukocytes disappear and the pH is low.[3] It is possible that the pH of the uterine

contents might be altered after chronic uterine stimulation and that this might permit the penetration and survival of organisms.

Summary

1. Bacteria were found in cultures of 2 of 41 uteri from untreated control mice of varying ages.

2. A high percentage of mice had uterine organisms when examined 2 to 10 days after a single injection of 16.6 μg. of estradiol benzoate.

3. Uterine bacteria were also found in mice receiving stilbestrol or triphenylethylene.

4. The simultaneous administration of testosterone propionate and estradiol benzoate also resulted in uterine sepsis.

5. The uteri of a number of mice remained septic for 3 weeks after a single injection of 16.6 μg. of estradiol benzoate and sepsis persisted for 8 weeks when injections were repeated at weekly intervals. Pyometra occurred in some of these mice.

6. Bacteria were much more frequently found and they were more numerous in the intact uterine horns than in horns ligated from the cervix.

7. The flora was comparable to that occurring in the vagina. Bacteria were not found in the blood or spleens of the treated mice.

REFERENCES

1 Allen, E., Doisy, E. A., Francis, B. F., Gilbson, H. V., Robertson, L. L., Colgate, C. E., Kountz, W. B., and Johnston, C. G.: Am. J. Anat., 1924, *34*, 133.

2 Allen, E., Hisaw, F. L., and Gardner, W. U.: Chapt. VIII, *Sex and Internal Secretions.* [Allen, E., Doisy, E. A., and Danforth, C. H., editors, 2nd edition, Williams & Wilkins, Baltimore, 1939.]

3 Beilly, J. S.: Endocrinology, 1939, *25*, 775.

4 Burrows, H.: J. Path. & Bact., 1935, *41*, 43.

5 Gardner, W. U., Allen, E., and Strong, L. C.: Anat. Rec., 1936, *64*, 17 (suppl.).

6 Kaufmann, C., and Steinkamm, E.: Arch. f. Gynäk., 1936, *162*, 553.

7 Lacassagne, A.: Compt. rend. Soc. de biol., 1935, *120*, 1156.

8 McEuen, C. S.: Am. J. Cancer, 1936, *27*, 91.

9 Weinstein, L., Gardner, W. U., and Allen, E.: Proc. Soc. Exper. Biol. & Med., 1937, *73*, 391.

10 Zondek, B.: J. Exper. Med., 1936, *63*, 789.

11 Zondek, B.: Am. J. Obst. & Gynec., 1937, *33*, 979.