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Received March 6, 1961. P.S.E.B.M., 1961, v108.

Bacterial Flora of the Mouse Uterus.* (26876)

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While it is usually assumed that the uterus, like most internal organs, is sterile in the absence of manifest disease, the studies here reported indicate that microorganisms are present in the uterine cavity of a certain percentage of mice which, on surgical examination and routine histologic study, reveal no uterine disorder. A similar prevalence of endouterine organisms exists in immature mice with closed vaginas, indicating that the infection is not an ascending one.

Methods. One thousand and eighty mice of the Swiss, C57BR/cd, C57BL/6 and BALB/c strains underwent laparotomy under nembutal anesthesia. The uterus was stretched out on the tips of a forceps and the cervix was ligated. Each horn was pierced by a 25-gauge needle attached to a tuberculin syringe containing sterile broth or Ringer's Solution. One to 2 drops were injected, reaspirated and inoculated into sterile broth. In some cases, a dilution of 1:1000 of the aspirate was placed on blood agar plates. The cultures were incubated for from 24 to 48 hours and the bacteria examined on gram stained smears. In addition, a separate group of 222 C57BR/cd uteri were cultured in broth and the bacteria present in 25 of the 48 infected cultures were identified by routine bacteriological methods.† Two to 3 months later, a second laparotomy was performed and, in the BALB/c, Swiss and C57BR/cd mice, the uterine fluid which had collected was aspirated, examined by Gram stains and cultured in broth. After each laparotomy, penicillin was sprinkled into the peritoneum and achromycin was given for 3 days in the drinking water‡. The prevalence of bacteria found on broth cultures of the uteri at the first and second examinations is shown in Table I.

On the blood agar plates, the most prevalent organisms were identified§ as *E. coli, B. aerogenes, Proteus vulgaris* and *Streptococcus fecalis,* and numbers of the cultures per plate varied from 8 to innumerable. In the 25 broth cultures from C57BR/cd mice, the following bacteria were identified: *Corynebacterium hoffmannii* in 68%, *Proteus vulgaris* in 16%, *Streptococcus fecalis* in 4%, *Staphylococcus albus* in 4%, *E. coli* in 4%, and mixed infections of *Proteus vulgaris* and *Corynebacterium hoffmannii* in 4%.

All of the uteri tested showed no anomaly on clinical examination. Histologic sections were prepared on 50 random uteri and in some of them Gram stains revealed the presence of bacteria in the mucoid material adhering to the endometrium.‖

The uterine fluid (Uterone) which accumulated after cervical ligation(1) in from 2 to

* Supported by grants from Nat. Inst. Health, Bethesda, Md. and Am. Cancer Soc., Inc., New York.

† The cooperation of Sumner M. Joress, of New England Center Hospital, is gratefully acknowledged.

‡ Achromycin was graciously provided by the Lederle Co., Division of American Cyanamid, Pearl River, N. Y.

§ The cooperation of Dr. E. Kass, of Boston City Hospital, is gratefully acknowledged.

‖ The histologic sections were prepared by John R. Baker, and interpreted by Dr. A. B. Russfield.

TABLE I. Prevalence of Microorganisms in Mouse Uteri and in Uterone of Hydrouteri.

Strain	Bio Swiss adults	Bio Swiss immature	BALB/c	C57BR/cd	C57BL/6
No. animals	193	75	546	181	85
No. infected (%)	14	17.3	12.6	27	35.2
Gram + cocci (%)*	74.2	38.5	55.2	44.6	23.3
Gram − cocci (%)	12.9	23	15.6	21.6	0
Gram + rods (%)	3.2	7.7	10.4	8	13.4
Gram − rods (%)	9.7	23.0	15.6	21.6	63.3
Others (%)	0	7.7	3.2	4.1	0
% Uterone containing bacteria	32	—	17.5	49	—
Gram + cocci (%)†	23	—	22	39	—
Gram − cocci (%)	23	—	18	22	—
Gram + rods (%)	19	—	9	4	—
Gram − rods (%)	27	—	51	35	—
Others (%)	7.6	—	0	0	—

* On smear made from broth culture (% refers to No. of uteri).
† On smear made from uterine secretions (% refers to No. of uteri).

3 months contained bacteria in a higher percentage of cases than were found at the first laparotomy. The nature of the bacterial flora in Uterone was different from that found at first exploration, in that the prevalence of Gram-positive cocci was markedly reduced and that of Gram-negative cocci was significantly increased. Whether this change in bacterial population is due to the brief postoperative antibiotic therapy or to other factors such as, possibly, lysozyme which has been demonstrated in Uterone(2) remains to be determined. The majority of the uteri which contained bacteria at time of uterine ligation remained contaminated, showing bacteria in the accumulated Uterone (79% of the 202 BALB/c; 69% of the 54 Bio Swiss; and 50% of the 20 C57BR/cd mice). Only 24 animals out of a total of 274 showed a change from an initially contaminated uterus to sterile luminal fluid; on the other hand, 44 mice shifted from initially sterile uteri to contaminated Uterone. This higher rate of bacterial contamination in hydrouteri, while possibly due to contamination at the time of puncture, may be analogous to the well-known high frequency of clinically manifest uterine infection in mice receiving estrogen.

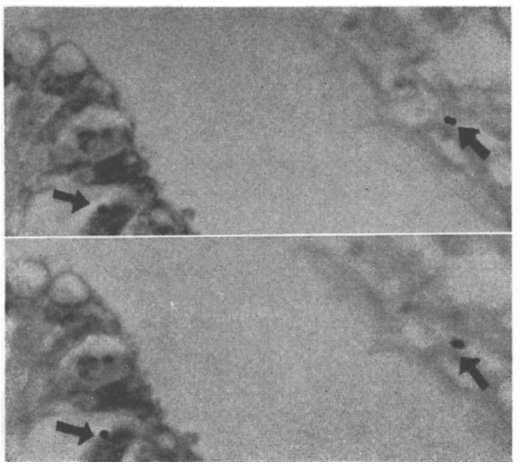

FIG. 1. Mouse uterus. Arrows point to Gram-positive cocci in intrauterine secretions (right) and in endometrial epithelial cell (left). Top and bottom photographs show the same field but in slightly different focus. Arrows correspond to about 5 μ in length (Gram stain, × 1000).

Discussion. The demonstration of bacteria in the uteri of immature and mature mice based on direct histologic examination of uteri and on culture of uterine washings may have far-reaching biologic significance. It renders necessary a critical reevaluation of studies on the chemistry and biologic activity of endometrial secretions(3) which may, in part, be properties of bacterial products or influenced by them. It raises the question whether the embryo, during even the earliest phases of pregnancy may, contrary to general belief, develop within an environment which is complicated by the presence of bacteria. This may have an important bearing on the physiology of cesarean section-derived experimental animals. It may also be a fac-

tor in causation of fetal anomalies. Responses of the uterus to estrogenic and progestational hormones may be the end result of interaction between hormones, target and bacteria. Finally, it would appear that Uterone may contain substances which favor growth of some bacteria while inhibiting others.

Summary. Microorganisms were demonstrated by direct smears and cultures of uterine fluid in immature and mature mice and in uterine tissue sections. Incidence of uterine contamination varied from strain to strain. Contamination persisted in the luminal fluid of hydrouteri. However, Gram-negative bacteria increased and Gram-positive organisms decreased during accumulation of uterine fluid.

The technical assistance of Suzanne Warner is gratefully acknowledged.

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Received May 1, 1961. P.S.E.B.M., 1961, v108.

Stimulation of Progestin Release from Rabbit Ovary *in vivo*.* (26877)

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The principal functional roles ascribed to progesterone have been related to pregnancy, and the primary site of synthesis of this steroid has been considered to be the corpus luteum of the ovary. This would imply that ovarian progesterone is not released in the absence of functioning corpora lutea. However, Forbes(1) detected elevations in progestational activity of peripheral blood drawn from female rabbits shortly after mating, several hours before ovulation could be expected; and Edgar(2) reported that the progesterone content of ewe ovarian vein blood could not be correlated with the number of functioning corpora lutea in the cannulated ovary. Zander(3) found high concentrations of progesterone in the human Graafian follicle shortly before ovulation and also isolated the progesterone metabolite, Δ^4-3-ketopregnene-20 α-ol (20α hydroxy-pregn-4-en-3-one) from the mature human follicles(4). Recently Taylor(5) has demonstrated the presence of appreciable progesterone in ovarian tissue of immature rats. Progesterone and its α 20-ol metabolite have also been found in the adrenal venous blood of calves of both sexes(6).

This paper gives further evidence that release of progesterone and Δ^4-3-ketopregnene-20 α-ol from rabbit ovaries *in vivo* is independent of the presence of corpora lutea, and that release of these steroids is rapidly activated following coitus or injection of hormonal agents which induce ovulation. Rabbits were chosen for this study since this species ordinarily ovulates only in response to coitus, and presence or absence of corpora lutea can easily be controlled.

Materials and methods. Steroid assays were made of ovarian blood of more than 60 New Zealand white rabbits. Sexually mature females (3.5-5 kg) were anesthetized with pentobarbital (approx. 25 mg/kg body wt), and 500 USP units/kg heparin were injected *i.v.* immediately before a polyethylene cannula (o.d. = 0.060″) was inserted into the ovarian vein. Blood samples were collected for specific time intervals in order that progestin content could be expressed in units

* Supported by USPHS grant.

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