9583 P

Bacteriology of the Uterus with Special Reference to Estrogenic Hormones and the Problem of Pyometra.*

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Mice and rabbits receiving large doses of estrogens develop pyometra.[1-6] Estrogenic hormones may induce physiological changes favoring a leucocytic invasion of genital tissues.[2, 4] It has been suggested that this inflammatory condition may be related to the leucocytic infiltration occurring during normal post-estrus. We have tried to determine whether bacteria are present in the uteri of normal mice and whether estrogenic hormones induce changes resulting in bacterial invasion of the uterus.

Mice from several strains and of varying ages were used. All that were treated with estrogens (hydroxy-estrin benzoate) were young, sexually mature, virgin females. The estrogen, in oily solution, was injected subcutaneously, usually in a single dose of 500 I.U. (1/20 cc.). The mice were killed after intervals of 2, 4, 7, 10, 14, and 15 days following the initial injection (Table II). Bacteriological studies were made on the uteri of immature, mature virgin, multiparous and castrated females; of mice receiving injections of oil alone and of the blood of injected and untreated mice (Table I). In a third series one uterine horn was cut from the cervix and

TABLE I.
Occurrence of Bacteria in Uteri or Blood of Mice Used as Controls.

Type of control	No. of mice	Age or treatment	No. with bacteria	No. without bacteria
Immature	5	30 days	0	5
Virgins	18	33 to 157	1	17
Castrated	7	Adult	0	7
Multiparous	11		1	10
Virgins	4	Oil injected	0	4
Blood bact.	7	No injections	0	7
	6	500 i.u.	0	6
	6	500 i.u. × 2	0	6

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2 Burrows, H., *J. Path. and Bact.*, 1935, **41**, 43.

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4 Zondek, B., *J. Exp. Med.*, 1936, **63**, 789.

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the cut end fixed to the abdominal wall. One month later 500 I.U. of estrogen were injected subcutaneously and 7 days later bacteriological examination of the ligated and intact horns was made.

TABLE II.
The Incidence of Uteri Showing Bacteria in Mice Receiving Estrogen.

No. of mice	Treatment		No. with bacteria	No. without bacteria
	Duration	Total I.U.		
7	2 days	500	4	3
12	4 "	500	7	5
10	7 "	500	8	2
2	10 "	500 × 2	1	1
10	14 "	500	9	1
4	15 "	500 × 2	4	0
4	4 to 10 mo.	500 i.u. wkly.	4	0
9	2 to 7 days	2 to 10 i.u.	5	4

The mice were killed with illuminating gas and opened under aseptic conditions. The uterine horns were removed well above the cervix, ground in 0.5 cc. sterile saline and one loopful of the resulting suspension was streaked on heart-infusion blood agar and another inoculated into heart-infusion broth. Growth, if any, appeared in 12 to 24 hours. Negative cultures were examined for 5 days. Frequently colonies growing on the plates were subcultured and identified.

Of 45 control mice (5 immature, 18 mature virgin, 11 multipara, 7 castrated, and 4 mature virgins receiving oil) one mature virgin and one multipara showed bacteria in the uterus (Table I). Postmortem vaginal smears revealed early post-estrous stages in these 2 mice. The heart-bloods of 19 mice, 13 of which had received estrogen, were negative. The data on animals receiving estrogens are summarized in Table II. Bacteria were present in the uteri of some mice on the second day after a single injection of estrogen and in most of the uteri at the 7- and 14-day stages. The long-term mice had pyometra. Gram-stained sections revealed bacteria in many cases. The observations on the ligated uterine horns (Table

TABLE III.
Incidence of Bacteria in Intact and Ligated Uterine Horns.

No. of mice	Treatment duration	Total i.u.	Ligated horns				Intact horns			
			No. —		No. +		No. —		No. +	
			Broth	B.P.	Broth	B.P.	Broth	B.P.	Broth	B.P.*
13	7	500	7	12	6	1	2	2	11	11

*B.P. = blood plate.

III) indicated that infection occurred by direct extension through the cervix. Histological examination showed a correlation between the response of the tissues to estrogen and the presence of bacteria.

The types of organisms isolated varied in different animals. Most common were *Bact. alkaligenes* and an unidentified Gram-positive diplococcus, usually found together. *Bact. coli,* hemolytic and non-hemolytic streptococci and staphylococci were occasionally found. Frequently a single type of organism was obtained. Cultures of the vagina yielded similar organisms.

9584 P

Production of Phage in the Absence of Bacterial Cells.*

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In recent publications[1, 2] it was shown: (a) that during one phase of the reaction between phage and bacteria in the presence of 0.25M NaCl phage is formed without concomitant bacterial growth; (b) that when pH and temperature are properly adjusted the bacterial substrate can be maintained in the resting state while [phage] rises logarithmically with time at the rate of a ten-fold increase per hour.

The obvious inference of these experiments is that bacterial growth, long held to be the prime conditioning factor for phage-formation, is actually not essential at all. Instead it would appear quite possible that resting cells may produce some sort of phage-precursor which in the presence of phage is promptly converted into more phage. This hypothesis is supported to a considerable extent by our observations during the past 3 years on the increase in phage-titer that occurs when cell-free ultrafiltrates of bacterial suspensions are added to known quantities of phage. Young cultures of susceptible organisms are washed in saline solution to free them from phage-inhibitor.[3, 4] Dense suspensions of the washed cells are

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4 Burnet, F. M., *J. Path. and Bact.*, 1934, **38**, 285.