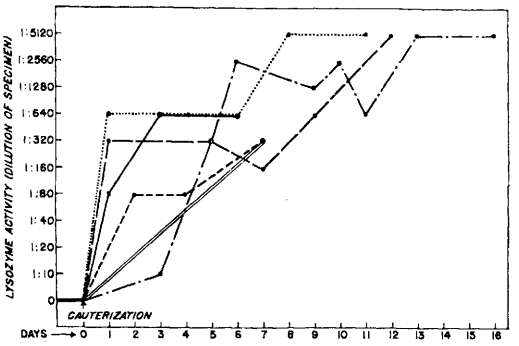

FIG. 2.

Output of lysozyme in rectum of dogs before and after cauterization.

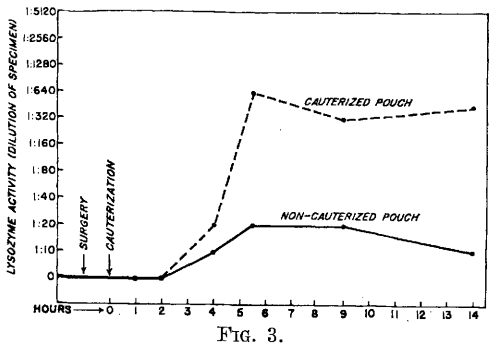

FIG. 3.

Early lysozyme activity of injured bowel.

duration of the experiment in the non-cauterized loop. Lysozyme activity of the washing from the cauterized loop continued to rise and reached a peak within 5½ hours after surgery and cautery (Fig. 3). Tissue lysozyme was detected at 1:20 dilution in a saline homogenate of the non-cauterized loop and in a 1:640 dilution of the cauterized loop homogenate. Both specimens were taken 15 hours after surgery.

Comment. The marked rise in production of lysozyme by the canine gastrointestinal tract injured by Mecholyl stimulation or by electrocautery suggests that the observed increase in lysozyme in the feces and bowel of patients with chronic ulcerative colitis may be a result of the disease process. Lysozyme seems to be produced locally in injured bowel. The marked increase in lysozyme production occurring within 5½ hours after cautery suggests that its increase is part of an early inflammatory process. Its precise biological significance remains to be determined.

Summary. 1. Lysozyme is produced in moderately large amounts by the gastrointestinal tract of dogs subjected to chronic stimulation with Mecholyl. The increase in lysozyme is demonstrable in the bloody diarrheal stools of intact animals and in material aspirated from isolated pouches of colon. 2. Cautery of canine rectum stimulates the local production of large amounts of lysozyme within 5½ hours after injury. 3. The rapid production of lysozyme by injured bowel suggests that it is an early accompaniment of the inflammatory reaction and that its presence is a reflection rather than a cause of ulcerative alimentary disease.

Received December 13, 1950. P.S.E.B.M., 1951, v76.

Susceptibility to Estrogen of Breast, Vagina, and Endometrium of Various Strains of Mice.* (18423)

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Strain differences in the susceptibility to breast cancer have been attributed by Loeb to variations in the sensitivity of the mammary gland to estrogen(1,2). According to other investigators, ovaries of mice of different strains produce different amounts of estrogen, and the larger the output of estrogen, the more readily mammary cancer will develop. Studies of vaginal smears and of whole mounts of breasts have indicated that the responses of mammary gland and vagina do not run parallel within the same animal

* This investigation was supported by the National Cancer Institute, National Institutes of Health.

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(3-7). The following investigation represents another attempt to further analyze this question.

Material and methods. Forty-two female mice—14 each of the closely inbred strains C57 black, Dba, and A—were used. The animals were raised in our laboratory and maintained on a standard diet of Purina Laboratory Chow and water available at all times. At the age of 3 to 4 weeks, the ovaries were removed, and 2 weeks later subcutaneous injections of alpha estradiol benzoate† dissolved in sesame oil were begun. The mice received 0.03 mg of the hormone once weekly for 1 or 2 weeks, 1, 2, 3, 5, or 8 months. At the end of these periods, 2 animals of each strain were killed. Of each animal 4 mammary glands, uterus and vagina were removed. All 4 mammary glands as well as uterus and vagina were cut in semiserial sections and studied microscopically. The findings were graded, as previously described in detail(8).

Microscopic Examination. Mammary gland. Resting state: The ducts are scanty, narrow, and lined by low cuboidal epithelium. Stimulation grade I: The ducts are enlarged, elongated and contain secretions; there is papillary infolding and outbudding of ductal epithelium with some mitoses. Stimulation grade II: The ducts are markedly hypertrophic and hyperplastic with numerous mitoses in the epithelium; abundant secretions and alveolar growth are present.

Vagina. Resting state: There are 4 to 6 rows of keratinizing epithelial cells, the basal layer showing 1 mitosis in about 500 cells. Stimulation grade I: The epithelium is conspicuously keratinized and hyperplastic, and is composed of up to 12 layers of cells with 1 mitosis in about 200 basal cells. Stimulation grade II: The epithelium has undergone further keratinization and hyperplasia, and 15 or more layers of cells were counted with 1 mitosis in about 100 basal cells.

Endometrium. Resting state: The glands are small and do not secrete. Stimulation grade I: The glands are enlarged and contain secretion; they are lined by columnar epithelium that shows mitotic proliferation; there is early epidermization of the uterine glands closest to the muco-epidermal junction. Stimulation grade II: The glands are tortuous, cystically dilated and densely packed; they are lined by hyperplastic epithelium with abundant mitoses, and often contain secretions. Epidermization of the glandular epithelium is marked.

Results. The findings are summarized in Table I.

Mammary gland. In mice of strain C57 treated with estrogenic hormone for one or 2 weeks, the ducts were resting or at best slightly dilated. In mice of strain Dba, there was growth stimulation of grade I after one, and stimulation of grade II after 2 weeks' treatment. In mice of strain A, alveolar growth was distinct already after one injection of the hormone. After one month of treatment, growth stimulation was still slight in mice of strain C57 (Fig. 1), while alveolar growth was accentuated in mice of strain Dba (Fig. 2), and numerous alveolar nodules had been formed in mice of strain A (Fig. 3). In mice of strain C57, ductal growth was marked after 2 months of observation, and only after 3 months alveolar growth was noted. After 5 or 8 months, alveolar growth was widespread in a few mammary glands of mice of strain C57; in corresponding animals of strains Dba and A, multicentric alveolar growth and adenomatous nodules were present; the epithelium showed hyperchromatic or pycnotic nuclei, and in 2 mice mammary cancer had developed.

Vagina and endocervix. In mice of strain C57, the growth stimulation was of grade II as early as after one injection of the hormone: There was abundant keratinization of the epithelium, which still increased during the later stages of the experiment. In mice of

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† We are indebted to the Schering Corporation for the generous supply of estradiol.

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TABLE I. Microscopic Findings in Mammary Glands, Vagina, and Endometrium in the Various Strains Following Treatment with Estrogen.

		Mammary gland				
Strain	Estrogen inj. for	No. ducts in one low power field: 48 mm obj., 10× ocul.	Ductal growth*	Alveolar growth†	Vagina‡	Endometrium§
C57 Black	1 week	6-12	(+)	0	++	++
	2 "	9-16	(+)	0	++	++
	1 month	7-15	(+)	0	++	+++
	2 "	12-24	+	0	++	+++
	3 "	14-35	++	+	++	+++
	5 "	16-38	++	+	++	+++
	8 "	14-40	++	++	++	+++
Dba	1 week	8-16	+	0	+	+
	2 "	8-26	++	+	+	+
	1 month	24-40	++	+	++	++
	2 "	25-35	++	++	++	+++
	3 "	22-35	++	++	++	+++
	5 "	20-38	++	++	+++	+++
	8 "	22-40	++	++ (1 cancer)	+++	+++
A	1 week	12-25	++	+	0	0
	2 "	14-40	++	++	+	+
	1 month	18-45	++	++	+	+
	2 "	15-36	++	++	++	++
	3 "	14-21	++	++	++	++
	5 "	15-28	++	++	++	++
	8 "	20-40	++	++ (1 cancer)	++	+++

* (+) minimal ductal hypertrophy and hyperplasia; + ductal hypertrophy and hyperplasia with secretion; ++ marked ductal hyperplasia with secretions.

† + alveolar budding; ++ multicentric adenomatous nodules.

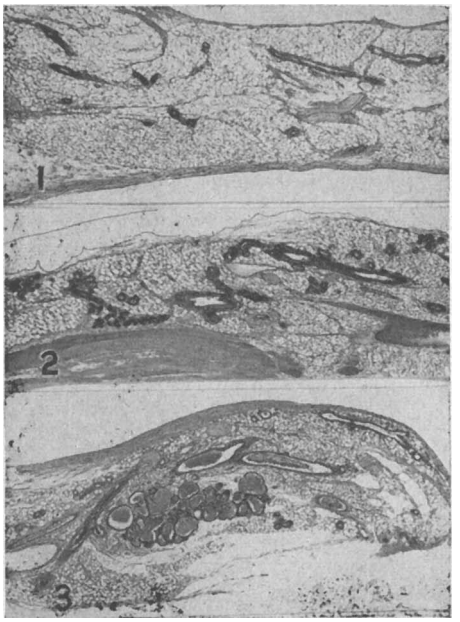
‡ + 7-12 layers of epithelium with keratinization; ++ 13 and more layers of epithelium with advanced keratinization; +++ downgrowth of epithelium into cervical stroma.

§ + glands enlarged; ++ glands cystic and hyperplastic with epidermization of cervical glands; +++ adenomatous glands.

strain Dba treated for one or 2 weeks, the Growth stimulation was of grade I; grade II changes were observed after treatment for one month (Fig. 4-6). Subsequently, epithelial growth became so active that after 5 or 8 months of injections, down growth into the stroma, closely resembling squamous carcinoma, occurred. In mice of strain A, the vaginal reaction was slowest: After one month, the growth stimulation was one of grade I, and only after 2 months, grade II stimulation had been reached.

Endometrium. In mice of strain C57, growth stimulation of grade II was seen after one injection of the estrogen, and after 2 months and later, polypoid growth was noted; however, no cancerous change was observed. In mice of strain Dba treated for one or 2 weeks, the growth stimulation was of grade I, and after one month, stimulation of grade II was observed; yet, growth was less marked than in the corresponding animals of strain C57. In mice of strain A, the endometrial reaction was even more delayed than in strain Dba, and did not reach grade II until after 2 months of treatment. At later stages of the experiment, there were no fundamental differences in the endometrial reaction, although mice of strain C57 reacted more vigorously than animals belonging to strains Dba or A.

Discussion. According to the present results and in agreement with the findings of Loeb (1,2), strain differences in the response to estrogen do not manifest themselves as a general susceptibility or non-susceptibility of the animals; instead, there are distinct differences in the sensitivity of the individual tissues, such as mammary gland, vagina and endometrium respectively. At the present time the nature of the factor or factors that determine the tissue susceptibility to estro-

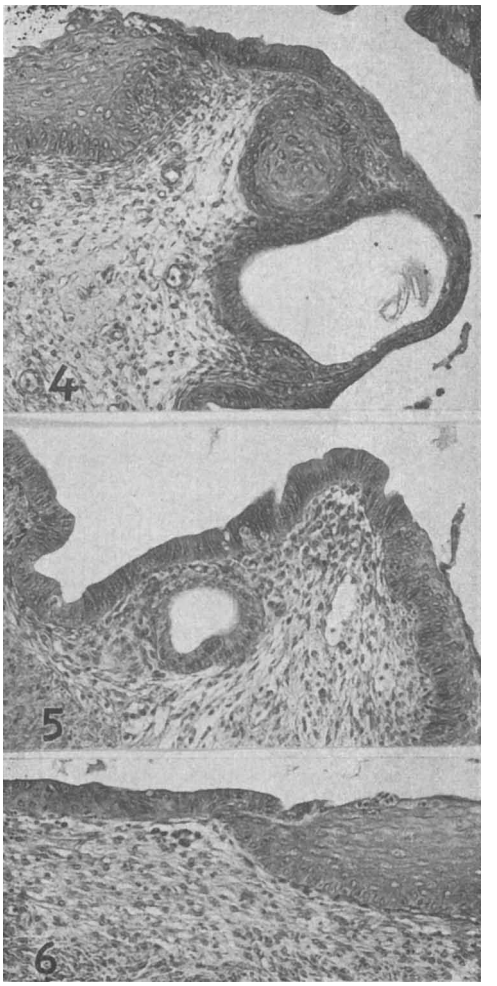


A. Sections through the mammary glands of mice ovariectomized at the age of 3 weeks and subsequently injected with 0.03 mg of alpha estradiol benzoate once weekly for one month. ×20.

FIG. 1. Mouse of strain C57 black: Ductal growth minimal; alveolar growth 0.

FIG. 2. Mouse of strain Dba: Ductal growth grade II; alveolar growth grade I.

FIG. 3. Mouse of strain A: Ductal and alveolar growth grade II.



B. Sections through the endocervix of mice ovariectomized at the age of 3 weeks and subsequently injected with 0.03 mg of alpha estradiol benzoate once weekly for one month. ×110.

FIG. 4. Mouse of strain C57 black: growth grade II with advanced epidermization.

FIG. 5. Mouse of strain Dba: growth grade II.

FIG. 6. Mouse of strain A: growth grade I.

gen is unknown. The role of the milk agent in the mammary and vaginal response to estrogen has been repeatedly investigated, but the results are not unequivocal(5,9-11). Experiments are in progress in this laboratory which should help to determine the role of the milk agent in mammary growth preceding the development of cancer.

Summary. In ovariectomized mice of strains with different mammary cancer incidence (C57, Dba, A), injected with equal amounts of alpha estradiol benzoate, the response of mammary gland, vagina and endometrium respectively varied in degree: Mice of strain C57 showed less mammary stimulation than mice of strains Dba or A. The vaginal epithelium was most sensitive in mice of strain C57, slower in responding in mice of strain Dba, and least responsive in mice of strain A. The endometrium reacted most vigorously in mice of strain C57, and less actively in strains Dba and A. Differences in the mammary cancer incidence of the strains examined are considered to be due to differences in tissue susceptibility rather than to genetic differences in the output of estrogen.

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Received December 14, 1950. P.S.E.B.M., 1951, v76.