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Mammary Cancer in Castrate Male Mice Receiving Ovarian and Hypophyseal Grafts at Various Ages.*

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In young virgin mice of susceptible strains, grafts of anterior hypophysis stimulate the growth of the mammary gland and increase the incidence of breast cancer.[1] Anterior hypophyses grafted together with ovaries are ineffective in young males with intact testicles; however, these transplants induce mammary cancer in young castrate males. This carcinogenic effect has been attributed to the gonadotropin given off by the transplanted hypophyses and stimulating the output of estrogenic hormone by the animal's own or by the grafted ovaries.[1,2] This view is based on the fact that in the absence of ovaries hypophyseal grafts are ineffective. The reaction of the breast tissue of male castrates to transplanted ovaries alone is definite, and the number of mammary tumors appearing in these animals may even exceed the number of spontaneous breast cancers found in virgin females of the same strain.[2-5] Moreover, the response of the male breast to injections of estrogenic hormone depends upon the age of the animal at the beginning of the treatment. A maximum susceptibility was noted if the treatment was started about the onset of sexual maturity. Loeb[6] attributed this age effect to the more marked growth reactivity of the breast of younger animals and possibly—since in females such an age factor could not be demonstrated—to an inhibiting action exerted by the male sex hormone. We found this age effect to be due only partly to the action of the testicle, since in castrates of various ages it was also present, though to a lesser degree.[7]

The present experiments were carried out in order to determine whether or not an age factor operates also in the production of breast cancer in male castrates bearing grafts of ovaries alone or in combination with anterior hypophyses.

Material and Methods. One hundred sixty-four male mice of the closely inbred strain A raised in our laboratory and originating in the Bar Harbor colony were used. The animals were castrated at 3 to 4 weeks of age and received the following grafts at the age of 1 or 7 months respectively: (a) 4 anterior hypophyses (27 young and 28 adult animals); (b) 2 or 4 ovaries (28 young and 26 adult animals); (c) 4 anterior hypophyses and 2 or 4 ovaries (26 young and 29 adult animals). The organs to be grafted were usually obtained from mice 2 to 3 months old and closely related to the host, that is, from brothers, sisters, and cousins (syngenesiotransplants)[1]; in 38 instances, non-related donors (homoiotransplants) were used. At the time of transplantation, the hypophyses and ovaries were removed from the donor animals under sterile precautions and kept in sterilized Ringer's solution for a few minutes until two subcutaneous pockets had been prepared on either side of the chest wall of the hosts. The grafts were then transferred into these pockets, half of them to the right and the other half to the left side.

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TABLE I.
Incidence of Breast Tumors and the Time of Appearance of the Neoplasms in the Various Experimental Groups.

Group	Castrates bearing	Age at transplantation	No. of mice	Age at death of all mice (mo.)		Animals with breast cancer			
				Mean	Range	No. of cases	%	Age (mo.) of appearance	
								Mean	Range
I.	Hypophyseal transplants	1 mo.	28	16.1	8-21	0	0	—	—
II.	Ovarian transplants	1 "	27*	16.9	6½-22	1	5	6.5	—
III.	Hypophyseal and ovarian transplants	1 "	26	17.1	8-22	8	32.5	12.9	8-20
IV.	Hypophyseal transplants	7 "	28	19.1	8-25	0	0	—	—
V.	Ovarian transplants	7 "	26†	15.9	7½-20	0	0	—	—
VI.	Hypophyseal and ovarian transplants	7 "	29	15.8	9-24	0	0	—	—

*7 animals still alive without tumor } 21-24 mo. old
†4 " " " }

The wounds were subsequently closed with silk sutures and the animals caged in groups of 4.

The mice were fed a stock diet of Purina Laboratory Chow and water available at all times; they were examined at regular intervals for the appearance of breast tumors or evidence of leukemia. Animals that had developed tumors or were suspected of having leukemia or any other disease were killed. At necropsy, special care was taken to recover the transplants, and definite grafts, as well as tissues suggestive of being remnants of grafted tissue were preserved for microscopic examination. In addition, mammary glands, pieces of liver, spleen, kidneys, lung, heart, thyroid, adrenals, hypophysis, prostate and some bones were removed as well as any pathological structures that were found. The histological findings and the data on leukemia will be reported elsewhere.

Observations. The results of our experiments are presented in Table I.

Age group A. Animals castrated at the age of 3 to 4 weeks and receiving grafts at the age of one month:

I. *Castrates bearing hypophyseal transplants* (28 animals) died or were killed at a mean age of 16.1 months with a range from 8 to 21 months. None of these animals showed a breast cancer. II. *Castrates bearing ovarian transplants* (27 animals) reached a mean age of 16.9 months and a range from 6½ to 22 months. One of these animals (5%) developed a breast cancer at 6½ months of age. At the time of this report 7 animals of this group, 22 to 24 months old, are still alive and without tumor. III. *Castrates bearing ovarian and hypophyseal transplants* (26 animals): The mean age at death was 17.1 months with a range from 8 to 22 months. In 8 mice (32.5%), breast cancers were found. The mean age at death of tumor-bearing animals was 12.9 months, the earliest tumor appearing at 8, the latest at 20 months of age.

Age Group B. Animals castrated at the age of 3 to 4 weeks and receiving grafts at the age of 7 months:

IV. *Castrates bearing hypophyseal transplants* (28 animals) reached a mean age of 19.1 months with a range from 8 to 25

months. None of these mice had breast cancer. V. *Castrates bearing ovarian transplants* (26 animals) did not do well, the mean age at death being 15.9 months with a range from 7½ to 20 months. Again, no breast cancer appeared in any of the animals. Four mice of this group 21 months old and older are still alive and without tumor at the time of writing. VI. *Castrates bearing ovarian and hypophyseal transplants* (29 animals) reached a mean age of 15.8 months with a range from 9 to 24 months. None of these mice had a mammary cancer.

Tumor development was independent of the number of ovaries grafted inasmuch as tumors were found in animals that had received 2 or 4 ovaries in addition to the hypophyseal transplants. The presence or absence of tumor could not be correlated to the state of preservation of the grafted tissue at the time of necropsy. In a number of animals without breast cancer functioning transplants were found at the time of necropsy, whereas in some animals with breast cancer no remnants of transplanted tissue could be detected. The degree of relationship of host and donor apparently did not influence the development of tumors, since breast cancer was found in animals bearing grafts from unrelated donors. This is probably due to the fact that the strain had been closely inbred for years and that the individuals have reached a state of close genetic similarity.

Discussion. The incidence of spontaneous breast cancers in breeding females of our strain A stock was 49%. The earliest breast cancer appeared at 6½ and the latest at 20 months of age with a mean age of 11.4 months. No spontaneous breast cancers have developed in our virgin females of strain A.

No breast cancer was observed in castrate male mice of strain A bearing transplants of anterior hypophyses. This indicates that no direct stimulation was exerted on the mammary tissue by hypophyseal activity or that any existing stimulus[8,9] was too weak to induce cancerous growth.

Of 20 male castrates bearing ovarian grafts one developed a breast cancer. This incidence is considerably lower than that observed by Loeb[2] and by Huseby and Bittner,[5] but approaches more closely the results of Murray.[3] The comparatively small number of carcinomas observed in our experiments is also in agreement with the failure of our virgin female mice of this strain to develop spontaneous mammary cancer. Moreover, castrate male mice showed a maximum susceptibility to estrogen-induced cancer if the injections were begun about the age at which sexual maturity is reached in animals with intact testicles.[7] Similar conditions may exist in regard to estrogenic hormone secreted by the transplanted ovaries. The mice used in the present investigation might not have reached the age of maximum susceptibility at the time the transplants began to function. The susceptibility of the breast tissue at the onset of stimulation seems to play a more important role even than the length of time during which the stimulus is allowed to act. The only tumor found in castrate males bearing ovarian transplants occurred at the age of 6½ months, that is 5½ months after grafting and as early as the earliest cancer in our breeding females. Apparently, the breast tissue of this animal was, for reasons unknown, so highly sensitized that a comparatively slight stimulus of relatively short duration sufficed to produce cancerous growth. In castrate male mice bearing hypophyseal transplants in addition to ovarian grafts the breast cancer rate was 32.5%, that is, about 6½ times as high as in castrates bearing ovarian transplants only. This indicates a marked stimulation of ovarian activity by the grafted anterior hypophyses.

No mammary tumors were found in castrate male mice receiving endocrine transplants at the age of 7 months. This confirms our earlier observations that there is with advancing age a loss of sensitivity of the mammary tissue to hormonal stimulation independent of the testicle.[6,7]

Summary. Syngenesiotransplants of ovaries made at one month of age caused mammary cancer in 5% of castrate male mice of strain A. The tumor incidence rose to 32.5%,

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if anterior hypophyses were grafted together with ovaries. Transplantation of anterior hypophyses alone failed to produce breast cancer. No mammary tumors occurred in castrates into which ovaries, anterior hypophyses alone or anterior hypophyses in combination with ovaries were grafted at 7 months of age. Thus in male mice, an age factor which is independent of the testicle operates in the production of breast cancer by ovarian and anterior hypophyseal grafts.

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Effects of Restricted Feed Intake in Intact and Ovariectomized Rats on Pituitary Lactogen and Gonadotrophin.*

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Starvation generally has been shown to result in decreases in the weights and functions of the endocrine glands, and these decreases have been attributed to a reduction in secretory activity by the anterior pituitary.[1-6] The adrenals represent an exception, since in cases of extreme or total starvation their weight and activity are actually augmented rather than reduced.[1,7,8]

Assays of the gonadotrophin content of the pituitaries of starved rats have revealed a decrease in the hands of some workers,[4,5] while others have reported no change in pituitary content.[9,10] The lactogenic hormone content of the pituitary was found to be reduced during complete starvation in lactating rats.[11]

It was the purpose of these experiments to seek further clarification of the effects of restricted food intake on the pituitary content of these two hormones, since under other conditions the latter has served as a reliable index of the amounts of these hormones present in the blood stream.[10-13] It was also considered desirable to determine the above effects in both intact and ovariectomized rats, since in the latter the pituitary gonadotrophin content is known to be higher[14-16] and pituitary lactogen lower than in intact rats.[13,17]

Methods. Forty-eight intact female rats of about 200 g body weight and 35 rats which had been ovariectomized about a month previous and weighed about 230 g were used in

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