Influence of Sex of Hypophyseal Gland on Mammary Cancer Development in Mice.* (24064)

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It has been previously demonstrated that castrate male mice bearing transplanted ovaries have a higher incidence of mammary cancer than intact females(1). This observation has been confirmed in mice of the A stock and in AZ F1 hybrids, although in mice of this latter stock the increased susceptibility in castrate males with ovaries was indicated only by an earlier cancer age(2,3). The fact that castrate males with transplanted ovaries and a piece of vaginal tissue exhibited signs of continuous estrogenization led these investigators to postulate that the greater susceptibility to mammary cancer in these animals might be due to a continuous ovarian stimulation from the male pituitary "in such a way that at all times sufficient ovarian estrogen is produced by the grafted ovaries"(2). Recently(4) taking advantage of a surgical technic allowing transplantation of functional hypophyseal gland tissue in hypophysectomized mice. studies on the estrous behavior of castrate male mice with grafted ovaries and either female or male pituitaries, demonstrated that under these conditions these animals exhibited continuous ovarian stimulation regardless of the sex of the pituitary gland they carried. Therefore it was concluded that the continuous non-cycling type of gonadotropic stimulation observed in males was not dependent on the sex of the pituitary *per se* but rather in some other controlling structure. On the basis of these observations it was considered of interest to determine mammary cancer incidence and average cancer age in castrate male mice with transplanted ovaries and either male or female pituitaries. If the conclusions drawn from our previous experiments were correct, the same incidence of breast cancer and average cancer age would be expected to occur in castrate male mice with transplanted ovaries and either male or female pituitaries. In addition, the higher cancer susceptibility in males as compared to that in females would be maintained regardless of the sex of the pituitary glands the males might carry. Data supporting these hypotheses are here presented.

Method. Female and male F1 hybrid mice resulting from the cross between A ♀ x Z ♂ were used. When approximately 6 weeks of age the females were divided into 2 groups one of which was submitted to bilateral ovariectomy followed by the subcutaneous graft of their own ovaries into the axillary region and the other kept as sham ovariectomized controls. All males were castrated and subsequently transplanted with 2 ovaries taken from donors of the same strain and age. One week after operation they were divided into 2 groups. One was submitted to total hypophysectomy followed by transplantation of male hypophyseal glands taken from isologous male donors of the same age. The remainder group was also subjected to total hypophysectomy followed by transplantation of female hypophyseal glands taken from isologous female donors of the same age. Hypophysectomy was performed according to the technic described by Thomas(5) modified by D. Ferguson (personal communication) and the grafting of the hypophyseal gland by the method described previously(4) consisting essentially in placing the entire gland to be grafted into the emptied *sella turcica* of the hypophysectomized host. By this procedure the over-all incidence of successful takes obtained in this experiment was 75% out of a total of 60 operated animals. Mice bearing successful hypophyseal grafts showed a sustained increase in body weight throughout their lives, reaching a mean value of 27 g 12 months after operation, whereas in those failing to accept the graft the body weight remained the same or decreased, with a mean terminal weight of 19 g. Following surgery

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mice were kept on the standard laboratory chow and examined twice weekly for appearance of palpable mammary tumors which were subsequently diagnosed histologically.

Results. The results can be summarized as follows: Of 41 sham ovariectomized non-breeding females, 34 or 83% developed mammary cancer at an average age of 479 ± 21 days. Of 22 ovariectomized females bearing their own ovaries grafted, all or 100% developed cancer at 404 ± 20 days. The difference between these 2 groups in regard to cancer incidence is not statistically significant whereas the difference in average cancer age is significant at the 5% level. Cancer incidence in hypophysectomized-castrate male mice with transplanted ovaries and male pituitaries was also 100% and average cancer age 342 ± 18 days. Roughly the same results were obtained in hypophysectomized-castrate male mice with transplanted ovaries and female pituitaries since also in this group all mice developed mammary cancer (100%) at an average cancer age of 327 ± 15 days. The slight difference observed in cancer age between both groups of males is not significant. However by comparing cancer age figures between males and females it is clear that the former developed cancer significantly earlier than the latter.

Discussion. The results listed confirm the observations made by Murray(1) and by Huseby and Bittner(2,3) in regard to the higher susceptibility to mammary cancer in castrate male mice with transplanted ovaries as compared to that in females. However the interpretation of this phenomenon by the latter investigators assuming that the greater susceptibility in males could be explained as due to the continuous non-cyclic ovarian stimulation by the male hypophysis does not seem to agree with our findings. The fact that castrate males with ovaries are more susceptible to cancer than the females regardless of the sex of the pituitary gland they carry, gives evidence that the sex of this gland does not play a role in this phenomenon. This concept is also supported by previous observations from this laboratory(4) showing that the continuous non-cycling ovarian stimulation in castrate males with transplanted ovaries as opposed the cyclic type in females does not depend on the sex of the pituitary gland *per se* but upon a sex difference residing in some other controlling structure.

In the light of the data presented it is difficult to decide as to where and how the sex difference in ovarian stimulation resides and operated. The fact that the "sex" of the hypophysis does not determine the sex pattern of activity of the ovaries indicates that there is a sexually differentiated structure somewhere else in the body which determines the sex pattern of gonadotrophic hypophyseal activity. This structure might be a part of the central nervous system, perhaps the anatomically contiguous hypothalamus. However at present no facts are available which would allow one to localize the site of the mechanism.

Summary. The incidence of mammary cancer and average cancer age have been determined in the following groups of AZ F1 hybrid mice: 1) sham ovariectomized non-breeding females; 2) ovariectomized females bearing their own ovaries transplanted; 3) hypophysectomized - castrate males bearing transplanted ovaries and female pituitaries; 4) hypophysectomized-castrate males bearing transplanted ovaries and male pituitaries. The results showed no significant differences in cancer incidence between any of the groups studied. However castrate males with transplanted ovaries developed cancer significantly earlier than did the females regardless of the sex of the pituitary gland they carried. Therefore it was tentatively concluded that the greater susceptibility to breast cancer observed in males is not dependent upon the sex of the hypophyseal gland *per se* but rather on a sex difference in some other structure controlling the hypophysis.

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