The Development of Mammary Cancer in Castrate A Strain Male Mice Bearing Ovarian Grafts

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The early studies of Murray (11) suggested that male mice that had been castrated and transplanted with ovaries developed mammary cancer with approximately the same frequency as did virgin female mice of the same strain. Later, Loeb et al. (10), in a preliminary report in which no data were presented, stated that "after transplantation of ovaries into castrate male mice, the cancer rate and proliferative as well as secretory activities of the mammary gland were greater than after transplantation of these organs into normal female mice." With male mice of a hybrid cross, in which a high percentage of the virgin female mice develop mammary cancer, it was observed in this laboratory that castrate males bearing ovarian grafts developed mammary cancer with great frequency and at a significantly younger average age than did the virgin female mice of this particular cross (8). The present investigation was undertaken to study the rate and time of mammary carcinogenesis in male mice of a stock in which the virgin female mice have a low incidence of cancer, although breeding females have a high incidence.

MATERIALS AND METHODS

Male mice of the A stock maintained in this laboratory were castrated shortly after being weaned (4–6 weeks of age), and at the same time two ovaries that had been freed from the encompassing ovarian sac were implanted deep in the axillary tissue. The animals were then housed in wooden boxes, fed Purina Fox Chow ad libitum, and inspected once a week for the appearance of subcutaneous tumors. All animals developing palpable subcutaneous tumors were examined at autopsy, and the tumor mass as well as the ovarian transplant was prepared for microscopic examination. In addition, nine of the nontumorous mice were sacrificed for histological examination when they appeared to be approaching exitus and the development of mammary cancer appeared unlikely.

Approximately half the animals received ovaries from donor A females, while the others received transplants of ovaries from donor A x females. The A x strain was originated in 1934 (3) by foster nursing one litter of strain A mice of the 41st inbred generation on a female of the CBA strain (designated as the X stock) that lacked the milk agent. Since that time, the A and A x stocks have been maintained entirely independent of one another by brother to sister matings. The transplantations reported in this study were carried out between October, 1945, and March, 1948, at which time the A strain mice, both donors and recipients, were of the F73 through F80 generations, while the A x donor animals were of the F25 through F32 generations (the original fostered litter has been considered as the F1 generation). It should, therefore, be stressed that there had been no interchange of genetic material between these two separate lines of mice for from 57 to 71 generations prior to their use in this experiment.

RESULTS

Tumor development.—Approximately the same percentage of males bearing A x ovaries developed mammary cancer as did males bearing A ovaries, so that, regarding tumor development, these two groups of animals may be considered as one (Table 1). The incidence of tumor development in these castrate male mice bearing ovarian grafts is significantly higher than the 4 per cent incidence noted in virgin female mice of this strain maintained in this laboratory (4) or the 5 per cent incidence reported in a small group of ovarietomized A mice bearing subcutaneous ovarian grafts (7). However, it is well below the 80.5 per cent incidence observed in breeding A strain mice during
this period, and the average age of tumor development is considerably (approximately 3 months) later.  

Ovarian histology.—In all mice seen at autopsy (22 bearing A ovaries and 15 bearing Ax ovaries), viable ovarian grafts were found. Among the younger animals follicles were numerous, a few small corpora lutea were seen, and the interstitial tissue was generally hypertrophied and contained large, agranular interstitial cells. In short, the histology of these ovaries was essentially the same as that previously described (8) for hybrid ovaries transplanted to castrate F1 hybrid males (A × Z), in spite of the fact that the ovarian histology of normal female mice of A strain differs considerably from that of the normal AZF1 females (7).

It is also of interest to record that in four of the ovarian transplants studied histologically (three from A donors and one from an Ax donor) there was an overgrowth of granulosa cells to form typical, small granulosa-cell tumors similar to those described previously in both male and female animals of the A × Z cross bearing transplanted ovaries (8). These occurred in animals 16—20 months of age, and concurrent mammary tumors were present in three of the four animals. No attempt was made to evaluate the hormone production of these ovarian tumors.

Mammary gland histology.—The mammae of sixteen animals (thirteen tumorous and three nontumorous) were studied histologically, by use of both the whole mount and section techniques. The degree and type of development varied considerably from one animal to another and even within different glands of the same animal. This variation was much greater than that seen in virgin or breeding female mice of this stock. In general, it can be said that the glands of these male mice exhibited greater development than do those of virgin female mice of this stock. Lateral budding, which is rather minimal in the glands of A strain virgin females (6) (Fig. 1), was prominent in most of the glands studied and was frequently very extensive (Fig. 2). In the glands of several of the animals there were areas of normal lobular development resembling that seen in female mice late in pregnancy or on the first post partum day. These areas varied considerably in size, from small clusters of alveolae to areas measuring several mm. in diameter, but the histology of all was essentially the same (Figs. 3, 4). The alveolar lumina were rather large and generally contained secretion; many of the epithelial cells had secretory vacuoles within their cytoplasm; and there was less connective tissue about the alveolae than is usually encountered in the typical precancerous hyperplastic nodules seen in the resting glands of female mice of high tumor strains (6). In addition, however, typical precancerous hyperplastic nodules, which are but rarely encountered in the glands of virgin A strain mice, were moderately frequent in these male glands.

In several instances, the larger ducts were moderately to greatly distended with secretion (Fig. 5). This was most marked in one gland in which there were no lateral buds or “alveolar” formation, so that at least in this instance the secretion was formed by the duct epithelium itself. In general, the pattern of duct development seen in the glands of these male mice was rather similar to that encountered in female mice (Fig. 6). In a few instances, however, a peculiar and extensive development of very fine ducts was observed. This differed from the localized, precancerous “fine duct nodule” (5) occasionally seen in the glands of some stocks of female mice, in that in these males the small ducts occupied a large part of the fat pad and presented no particular orientation as is the case of the “nodules” seen in female glands. All in all, the glands of these male mice were considerably more developed than are the glands of virgin female mice of this stock, and in certain areas their development simulated that seen in the gland of female mice during the latter part of pregnancy. In addition, they presented some atypical features not encountered in the mammae of normal female mice.

DISCUSSION

From more recent evidence obtained in this and other laboratories, it would appear that castrated male mice bearing ovarian grafts develop mammary cancer with greater frequency than do virgin female mice of the same strain. This increased frequency of cancer development, as well as the more extensive development of the mammary glands in these male mice, may be, at least in part, the result of the essentially non-cycling male pituitary. Thus, when both vaginal fragments and ovarian tissue were transplanted to castrate male mice, the

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1 J. J. Bittner, unpublished data.
vaginal epithelium was found to be continuously cornified, suggesting a rather constant stimulation of the ovarian graft by the male hypophysis (8). In view of this, it is interesting to note that Loeb et al. (10) and Silberberg and Silberberg (12) all have observed that if additional pituitary tissue is transplanted to castrate male mice along with the ovarian tissue, a higher incidence of mammary cancer results than if ovarian tissue alone is transplanted. The rather pronounced tendency for the transplanted to castrate male mice along with the cornified, suggesting a rather constant stimulation In view of this, it is interesting to note that Loeb mammæ of castrated, ovarian-transplanted male et al. (10) and Silberberg and Silberberg (12) all of the ovarian graft by the male hypophysis (8). netic material between these lines for from 57 to 71 generations would seem to indicate a great deal of genetic principles and that, in general, eight to fourteen factors are involved in the maintenance of grafted normal tissue. The fact that ovarian tissue of the Ax stock could be 100 per cent successfully grafted into the subcutaneous tissue of A strain mice when there had been no interchange of ge has shown that the successful growth or maintenance of transplanted tissue is governed by genetic and that, in general, eight to fourteen factors are involved in the maintenance of normal tissue. The fact that ovarian tissue of the Ax stock could be 100 per cent successfully grafted into the subcutaneous tissue of A strain mice when there had been no interchange of genetic material between these lines for from 57 to 71 generations would seem to indicate a great deal of genetic stability, once a genetically pure strain has been obtained.

SUMMARY

Forty per cent of a group of castrate A strain male mice bearing subcutaneous ovarian grafts developed mammary carcinoma at an average age of 14.8 months. Only 4 per cent of virgin female mice of this strain developed mammary cancer at an average age of 15.0 months, while during the period of this experiment 80.5 per cent of breeding females were afflicted at an average age of 11.7 months. It is, therefore, evident from these and other experiments that the tendency for castrate male mice bearing transplanted ovaries to have mammary cancer is intermediate between that noted in virgin and in breeding mice of the same stock. A study of the mammæ of these male mice revealed them to be considerably more developed than those of virgin A strain mice, in that they possessed much more lateral budding, frequently contained considerable secretion in their ducts, and had areas of normal-appearing alveolae as well as typical areas of precancerous nodular hyperplasia.
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