SPECIAL ARTICLES

FUNCTIONAL ACTIVITY OF THE MAMMARY GLAND IN RELATION TO EXTRA-CHROMOSOMAL INFLUENCE IN THE INCIDENCE OF MAMMARY TUMORS

IN the experiments reported by Little[1] and Murray and Little[2,3] reciprocal crosses were made between "low-tumor" and "high-tumor" strains of mice. The F_1 generation produced by "high-tumor" females × "low-tumor" males gave mammary tumors in 39.82 per cent. of 113 virgin females that lived to reach "cancer age." Similarly, the reciprocal F_1 generation derived from "low-tumor" females × "high-tumor" males gave only 6.06 per cent. mammary tumors in 379 virgin females. The authors concluded that the incidence of mammary tumors in mice depends primarily upon the direct transmission of extra-chromosomal influences and that in the cross where the mother was derived from the "low-tumor" strain an "extra-chromosomal influence non-mammary tumor" was associated with a low percentage of spontaneous mammary tumors in the F_1 virgin females that reached the "cancer age." This general conclusion was consistent with results from back-cross and F_2 matings.

The writer has studied the effects of functional activity of the mammary gland on the incidence of spontaneous mammary tumors in various strains of mice and two strains were kindly supplied by Dr. Little's laboratory. Crosses were made between two "low-tumor" strains, one the C57 black, which was the "low-tumor" strain used by Murray and Little in the experiment described above, and another closely inbred "low-tumor" strain CBA.

The following results are based on nine sets of crosses, in each of which the female parent was derived from the C57 "low-tumor" strain. The male parent was derived from the CBA "low-tumor" strain.

According to Murray and Little the F_1 females from this cross, if kept virgin, would be expected to show only a small percentage of mammary tumors. The following results show that when such F_1 females are bred rapidly and not allowed to nurse their young they develop adenocarcinoma of the mammary gland at a relatively early age and in 100 per cent. of the nine experiments herein recorded. The tumors may be of multiple origin and one metastasized to the lung.

The results are shown in Table I. Nine females were tested. They averaged 93 days of age when their first litters were born. The average number of litters per female was approximately eight, the average number of days between litters 25, the average number of young per litter 7.3 and the average age of the females at which the tumors were first noted was approximately nine months. In each of four females three spontaneous tumors were noted, one female had two tumors and in four there were solitary growths. Histological data were obtained for all but one female, No. 294. Two tumors, one in the right axilla and one in the left inguinal region in this animal, were typical of the series, but on the death of the female the body was destroyed by cage mates.

TABLE I

Female No.	Age at first litter in days	No. litters	Ave. No. days between litters	Av. No. of young per litter	Age of female when tumor first noted	Hist. diag. mammary tumor	No. spont. mammary gland tumors
293	118	7	28	8.0	268	Adenocarcinoma	3
294	118	10	27	5.5	383	Adenocarcinoma?*	2
318	72	11	26	7.3	397	Adenocarcinoma	1
319	58	10	22	7.9	239	"	1
320	63	8	29	7.1	244	"	1
380	93	7	27	7.9	254	" (Lung metast.)	3
381	101	5	22	6.0	182	Adenocarcinoma	3
382	104	6	26	8.3	258	"	3
383	111	7	23	8.0	230	"	1
Av.	= 93	Av. = 7.9	Av. = 25	Av. = 7.3	Av. = 274	Av.	= 2

* See text.

These results are not necessarily incompatible with those of Murray and Little. They show, however, that the threshold for tumor incidence can be lowered by intensifying the functional activity of the glands. The procedure subjected the mammary glands to abnormal ovarian stimulation, they were unable to reach a normal resting condition and the lack of drainage of retained secretions may have in turn resulted in chemical irritation of the glandular epithelium. Rapid breeding was made possible by providing an adequate high protein diet. A further item of interest is that all the females in the F_1 generation produced mammary tumors and yet their parents were derived from "low-" or "non-tumor" strains. If both strains are free from cancer then one would not expect to find neoplasms in 100 per cent. of their F_1 daughters, unless more than one factor difference is involved and then the results would have to be explained on the basis of the action of complementary factors.

It is possible that the parent stocks I used were not entirely free from mammary gland cancer. This has since been further indicated by reports from Dr.

[1] Little, SCIENCE, 79: 465, 1933.
[2] Murray and Little, SCIENCE, 82: 228, 1935.
[3] Murray and Little, *Genetics*, 20: 466, 1935.

Little's laboratory (personal communication) showing the occurrence of 13 recently discovered spontaneous mammary gland tumors in females of the CBA line. The "functional test" that I have described above when applied to the C57 black "low-tumor" strain has already resulted in locating four females with mammary carcinoma. Twenty-one C57 females were included in this group. Five died when under 5 months of age, seven under 10 months and one at 13 months. One is alive at 10 months and three at 4 months of age. None of these showed tumor growth. The four females showed the first evidence of a mammary gland tumor when 6½, 8, 10 and 11 months of age, respectively.

In addition another female from "low-tumor" strain CBA developed a spontaneous mammary gland tumor when 13 months of age. This animal was from a group of 17 females and the only one to show a spontaneous growth. Of the remaining animals, twelve died when under 10 months of age, two at 13 months, one at 17 months and one at 18 months of age.

Conclusion: Increased functional activity of the mammary glands, under the conditions of the experiment described above, has resulted in masking any such reaction as an "extra-chromosomal influence" on the incidence of mammary tumors in mice as reported by Murray and Little. The results show that female mice, with hybrid vigor, whose mothers are derived from a strain relatively free from spontaneous mammary adenocarcinoma, have the capacity of producing such growths spontaneously, at a relatively early age, and in a high percentage of the females so far studied. The results are preliminary and are now being tested on a larger series of animals and using the exact crosses which Murray and Little have reported on.

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DELAYED KILLING OF MAIZE SEEDLINGS WITH X-RAYS

In the course of experiments with x-ray treatments of dry seeds of maize and related species, a range of dosages has been found which causes all the plants to die in the seedling stage without reducing the percentage of germination. This phenomenon has been termed "delayed killing." In the published accounts of lethal x-ray dosages applied to dry seeds little significance has been attached to the fact that seedlings may make growth before they die. A study of the seedlings that have lost—by previous x-raying of the seeds—something essential to their continued existence should throw light on the nature of the biological changes brought about by the x-rays.

Seeds of dent corn were exposed to a source of x-rays obtained from a Coolidge x-ray tube with tungsten anode operating under a constant potential of 48 kv. This provided a continuous band of radiation with maximum intensity at approximately 0.50 Å and a short wave-length limit of 0.26 Å. The x-ray dosages were measured by the use of a standard open-air ionization chamber according to the design developed at the National Bureau of Standards.[1] The ionization current was determined by means of a circuit for amplification of direct currents using the FP-54 pliotron.

The seeds (germ side up) were always placed upon a thin layer of cheesecloth which eliminated the effect of back secondary scattering. The ionization chamber could be easily placed in the equivalent position of the seeds for measurement of the dosage. In general the maximum error in the dosage measurements was less than 5 per cent. For a majority of the treatments the seeds were placed about 13 cm from the anode. The dosage then received was approximately 500 "r" units per min. for 6 milliamperes plate current at 48 kv. d. c.

The treated seeds were germinated in one of two ways. For comparing root and shoot elongation at the very early stages the seeds were placed in moist cloths or blotters in germinating chambers. For the study of delayed killing and seedling growth, the seeds were planted in sand contained in small flats placed on a clinostat in the greenhouse. With doses of less than 1,000 r units no differences were detected between treated seed and controls. Dosages from 1,000 to 20,000 r units showed increasing reduction in the rate of elongation with an occasional plant killed at the higher dosages. Treatments above 40,000 r units resulted in delayed killing, the percentage of killed plants increasing with the dosage. From 60,000 to 100,000 r units the *percentage of germination remained unimpaired but all plants died in the seedling stage.* An attempt was made to find the dosage that would inhibit germination. One lot given 1,000,000 r units germinated 39 per cent, and another lot given 2,400,000 r units failed to germinate at all. *It is estimated, therefore, that doses of approximately 2,000,000 Roentgens completely inhibit germination.*

At the time of emergence of the plumule in plants subjected to a lethal dose of 60,000 r units the rate of elongation is only slightly less than that of plants from untreated seeds. The rate decreases rapidly and elongation ceases altogether in about a week, at which time there will usually be a thick crumpled first leaf showing. After elongation has stopped the seedling remains green for about another week before dying. The stage at which the seedlings die shows that growth involving cell division has taken place and that the observed germination has not been simply cell expansion.

[1] L. S. Taylor and G. Singer, *Radiology*, 15: 637, 1930.