THE INFLUENCE OF OVARIECTOMY ON THE SPONTANEOUS OCCURRENCE OF MAMMARY CARCINOMAS IN MICE.*

By CARL F. CORI, M.D.

(*From the State Institute for the Study of Malignant Disease, Buffalo.*)

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Lathrop and Loeb (1), working on strains of mice with a high percentage of spontaneously developing tumors, have shown that the castration of female mice below the age of 6 months leads to a marked decrease in the tumor incidence. In a later report Loeb (2) was able to confirm and enlarge his previous results.

According to Loeb an ovarian hormone regulates those tissue changes which lead to the development of breast tumors in mice. The relationship between this hormone and the development of tumors is quantitatively graded. If the quantity of hormone acting on the breast tissue exceeds a certain limit, cancer appears as frequently in castrated as in non-castrated animals. This is the case when the mice are operated at 8 to 10 months of age. If an intermediate amount of hormone has been allowed to act on the mammary gland, that is, if castration has been performed at an age of 5 to 7 months, the cancer rate is noticeably diminished and the tumors appear at a later age period than in non-operated controls. If, finally, the quantity of the hormone has been restricted still further by operating on the animals at a still younger age, tumors either do not appear at all or only in exceptional cases. Loeb concludes that the hereditary factors which determine the development of breast carcinomas in mice need the cooperation of a definite quantity of ovarian hormone, if cancer is to develop.

As far as the writer is aware, the experiments of Loeb have not been repeated. This may be due to the fact that strains of mice with a

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high tumor incidence are rather difficult to obtain. Several years ago, Mr. Marsh, the biologist of this Institute, succeeded in evolving such a strain by selective crossing. Reports concerning the tumor incidence of this strain have been made on several occasions (3, 4). In eight inbred generations, covering a period of 5 years, the cancer rate and cancer age varied but little. Close to 94 per cent of all the breeding females that reached the cancer age developed breast carcinomas. Tumors appeared as early as 5 months and reached a maximum incidence at the age of 9 months.

It is necessary to use non-breeding females as controls for the castrated animals, since prevention of breeding decreases the percentage of tumors developing and increases the age at which tumors occur. Table I[1] indicates that of 42 non-breeding females living to tumor age, 33 developed spontaneous breast carcinoma. This corresponds to a tumor incidence of 78.5 per cent. In a previous experiment, 14 out of 18 non-breeding females developed tumors, which corresponds to an incidence of 77.7 per cent. The first tumors appeared later than in breeding females, namely at 10 to 11 months of age, while the highest tumor incidence occurred between 14 and 15 months of age.

Two series of experiments were made, but for reasons to be stated later only the second series is reported here in detail. In the first series groups of 20 to 30 female mice were castrated at 2, 3, 4, 5, and 6 months of age. The operation was performed by the abdominal route, consisting of the removal of the entire capsule containing the ovary, the Fallopian tube, and the tip of the uterus horn. Males were kept in the same cages as the operated females, but a pregnancy was never observed. An autopsy was performed on most animals that died spontaneously and on all animals that developed a tumor.

Surprisingly enough, it was found that about 15 per cent of the castrated animals showed one and in a few cases even two ovaries at the site of the previous operation. Upon microscopic examination some of these ovaries were in a state of degeneration or even calcified, others, however, showed follicles and corpora lutea. The possibility, that the ovaries had been incompletely removed, seemed very remote. It was, therefore, concluded that a regeneration of ovarial tissue had taken place. This was amply confirmed by a paper by Davenport (5) that appeared later.

[1] Compiled from the statistics kindly furnished to us by Mr. Marsh.

This author made a detailed study of the conditions under which ovaries regenerate in mice. Regeneration is less apt to occur when the capsule, tube, and part of the fat body surrounding the ovary are removed than when the ovary alone is removed by cutting its stalk and picking it out of the capsule. With the former method of re-

TABLE I.

Monthly Expectancy of Tumor Incidence in Non-Breeding Female Mice (Strain 3).

Based on 42 mice living to tumor age, of which 33 developed tumors. Incidence: 78.5 per cent. Females isolated at 1 month of age, one in each cage. Remained isolated throughout life. The food consisted of dog bread.

Age	No. of tumor mice	Per cent of 42 tumored during each month	Total per cent tumor mice at end of each month
mos.			
1–10	0	0	0
11	2	4.76	4.76
12	2	4.76	9.52
12½	2	4.76	14.28
13	1	2.38	16.66
13½	1	2.38	19.04
14	4	9.52	28.56
14½	1	2.38	30.94
15	6	14.28	45.22
15½	1	2.38	47.60
16	2	4.76	52.36
18	2	4.76	57.12
19	2	4.76	61.88
20	5	11.90	73.78
20½	1	2.38	76.16
21	1	2.38	78.54
	33	78.54	

The 9 mice without mammary tumors lived 7 to 18 months, average 12.5 months. One of these mice developed a spindle cell sarcoma. Reckoned as a clean mouse above.

moval, regeneration took place in 18 per cent of the cases, but when the ovary alone was removed, regeneration was observed in 64 per cent of the operated animals. Regeneration may take place as early as 1 and as late as 6 months after the operation. The age had no demonstrable effect upon the chance of regeneration, except when the mice

were very young. In several cases two ovaries regenerated in the place of one or the new ovary arose at a point ½ cm. distant from the position of the former ovary.

There seems no doubt that the ovary of the mouse may regenerate from the peritoneum under favorable conditions. Prior to Davenport, Castle and Phillips (6) observed ovarian regeneration in 11 and pregnancy in 3 out of 141 operated guinea pigs.

Interesting as it may seem that ovaries regenerate in mice, it was a rather unfortunate occurrence from the point of view from which these experiments were undertaken. Animals that showed ovaries upon postmortem examination, whether they were normal or tumor mice, had to be ruled out. When these were omitted, there remained 49 animals that were still alive at an age of 19 months. 44 of these mice remained free from tumors until they died of old age or of intercurrent diseases. 5 animals out of these 49 developed tumors, 1 at 18½ months, 1 at 19 months, 2 at 20 months, and 1 at 21 months. This gives a tumor incidence of 10 per cent, as compared with 78.5 per cent for the non-breeding controls. There seems no doubt that ovariectomy performed between 2 to 6 months of age reduces the tumor incidence and increases the tumor age.

In the second series of experiments the control of the experimental conditions was very satisfactory. The underlying idea was to see if the occurrence of tumors could be prevented altogether by operating on the animals at a very young age. 100 mice were castrated between 15 and 22 days of age, that is, immediately after weaning. The ovaries were exposed by the lumbar route and the entire fat body surrounding the ovary together with a portion of the uterus horn were removed. Beginning with 6 months, the mice were examined for tumors regularly once a week throughout their life time. Great care was taken to autopsy as many mice as possible.

In no case did the postmortem examination reveal the presence of ovarian tissue. However, from the experience gained in the first series of experiments it seemed unwise to rely on the autopsy alone. The absence of ovaries at the time of death was not considered proof enough that no regeneration had taken place, since most of the animals were very old when they were examined. A regenerated ovary might have disappeared again through absorption. Use was made of the vaginal smear method of Stockard and Papanicolaou (7), which indi-

cates in a definite way whether or not ovarian activity is present. In the mouse the estrus cycle lasts from 4 to 6 days. At the height of the estrus the vaginal smear is characterized by the presence of large cornified cells without a nucleus and by the complete absence of leucocytes. In the resting stage of the estrus cycle a large number of leucocytes are present and some small, nucleated epithelial cells, while the characteristic cornified cells are missing. For 1 consecutive

TABLE II.

Absence of Spontaneous Mammary Carcinomas in Mice Ovariectomized between 15 and 22 Days of Age.

Most of the operated mice belonged to Strain 3, which has a tumor incidence of 78.5 per cent. A few mice were from Hybrid Strain 1, which has a tumor incidence of 50 per cent.

Age	No. of mice alive	Remarks
mos.		
6	94	
10	86	
12	84	
14	79	
16	73	1 killed at 15 mos. Spindle cell sarcoma of fore leg.
		1 killed at 15½ mos. Spindle cell sarcoma of tail.
18	72	
20	60	
22	37	1 killed at 21 mos. Spindle cell sarcoma of abdominal wall.
24	14	
26	5	
28	1	

week vaginal smears were made on each mouse 2, 4, and 6 months after the operation. Only one mouse with a positive vaginal smear was picked out in this way. This animal through an oversight proved not to have been operated upon. The fact that the vaginal smears were all negative makes it certain that the mice of this series were entirely free from ovarian activity throughout their life time.

The result was that none of these mice developed a spontaneous

adenocarcinoma of the breast. Table II contains a detailed record of this series of experiments. From 86 mice that were still alive at 10 months of age, that is, at the time that the first tumors appeared in the non-breeding controls, 60 mice reached an age of 20 months. At 20 months of age 73.8 per cent of the control mice had developed tumors, while none of the operated animals had shown a breast tumor so far. At 21 months the last control mouse had died, while a larger number of operated mice continued to live up to 24 months and more. The chance to develop a tumor was, therefore, greater in the operated mice than in the control mice. Nevertheless, all the operated animals remained free from breast carcinomas. This remarkable effect of the ovarian hormone on the development of spontaneous breast tumors in mice seems to be a specific one, since the absence of the ovaries did not prevent the occurrence of other types of tumors. It will be noted in Table II that 3 of the castrated mice developed spindle cell sarcomas. A low incidence of spontaneous sarcomas has been observed previously in this strain of mice and is, therefore, nothing abnormal. The conclusion seems justified that the lack of breast tumors in spayed mice is not due to some indirect effect of castration. The body changes produced by castration are not such that they lead to a resistance against spontaneous tumors in general. Only the tumors arising from the mammary gland, a tissue that is directly influenced by the sex hormone, are prevented from developing. Another point of interest is that the prevention of breeding prolongs the life time of the mouse and that mice spayed at an early age live still longer than the non-breeding controls.

DISCUSSION.

Loeb interpreted his results on the basis of the interrelation between the ovary and the mammary gland. Since that time our knowledge of the ovarian function has been increased considerably, chiefly through the isolation of a potent ovarian hormone from the liquor folliculi by Allen and Doisy (8). If the ovaries are removed before sexual maturity is reached, the mammary tissue remains in an undeveloped state throughout the life of the animal, similar to the undifferentiated state of the breast tissue of the male animal. Our experiments indicate that a malignant change does not occur in this

undifferentiated tissue. Nevertheless, these animals must be regarded as potential tumor bearers, since they have inherited an organ disposition to develop tumors from previous generations. But the hereditary organ disposition alone does not lead to carcinoma of the mammary gland. The cooperation of the ovarian hormone is necessary, first, to cause the full development of the breast and second, to induce a periodic stimulation of the mammary tissue. During the life of the adult female the estrus cycle, which lasts from 4 to 6 days, provides a periodic stimulation of the breast tissue. One can calculate that a mouse has about 70 to 80 estrus cycles during life. If castration has been performed at 2 to 5 months of age, that is, after 3 to 20 estrus cycles have taken place, the occurrence of tumors is greatly reduced but not entirely suppressed. Castration after 6 to 7 months, that is, after 30 periodic enlargements of the mammary gland, has no decided influence on the tumor incidence. The tumors appear at the same rate as in the animals that had intact ovaries throughout their life. The point of interest is that a relatively short period of functional activity of the mammary gland leads to those tissue changes that result later on in the development of carcinoma. Equally striking is the long latent period between the ovarian stimulus that leads to cancer and the onset of the cancerous change itself.

When the tumor is once established, the ovarian hormone seems to be without influence on the rate of growth of the tumor. This is shown by the fact that spontaneous tumors in previously castrated animals do not grow less rapidly than tumors developing in normal animals. Even a surplus of ovarian hormone does not increase the rate of growth of mammary carcinoma, as may be judged from experiments that have been made with the ovarian hormone of Allen and Doisy. This hormone, when injected into spayed animals, produces the typical estrus changes in the genital organs and causes an enlargement of the milk ducts of the breast with proliferation of the glandular elements. It seemed of interest to investigate, whether this hormone would also have a growth-promoting effect on the adenocarcinoma which arises from the normal glandular elements of the breast. 50 normal females with either spontaneous or transplanted breast carcinoma received 4 to 10 hormone injections at 5 day intervals. The potency of the hormone had previously been tested on spayed animals.

However, no definite influence of this surplus of ovarian hormone on the rapidity of growth of the tumors could be observed.

It is a well known fact that the males belonging to inbred strains of mice with a high tumor incidence, do not show mammary carcinoma. Even though the males transmit the hereditary factors, they remain free from tumors, because the mammary tissue remains undeveloped in analogy to the female mice castrated before puberty, which also remained free from tumors. Loeb tried to produce breast tumors in males by transplanting ovaries into them, but obtained negative results. The author also obtained negative results by this procedure. Males belonging to the high tumor strain were castrated at an early age. When they were 2 and 6 months old, two ovaries of adult females were transplanted into the peritoneal cavity of each mouse. Most of the animals reached an old age, but breast tumors were not observed. It was also tried to produce breast tumors in males by the injection of the ovarian hormone. The hormone was prepared from the liquor folliculi of hogs according to the original prescription of Allen and Doisy (8). The chief difficulty was that at that time the only suitable solvent for the hormone was oil. The injections could not be extended over 10 weeks without causing infiltrations and general impairment of the health of the animals. Many months after the injections had been stopped, unabsorbed oil was still present in the subcutaneous tissue. A positive result could only be expected, if the injections could be continued throughout life. One of the animals developed a sarcoma adjacent to an oil cyst, the other animals remained free from tumors. Recently it has been possible to prepare the hormone in a water-soluble form. The experiments on male animals are being repeated, since it would be of considerable importance to produce breast tumors in male animals experimentally.

SUMMARY AND CONCLUSIONS.

1. Castration of female mice between 15 and 22 days of age entirely prevented the occurrence of spontaneous adenocarcinoma of the breast, while the non-breeding control mice of the same strain showed a tumor incidence of 78.5 per cent. 3 of the spayed animals developed a spontaneous spindle cell sarcoma. This indicates that the influence

of the ovary on the development of breast carcinomas is a specific one, since ovariectomy does not lead to a resistance against other types of spontaneous tumors.

2. Castration between 2 and 6 months of age led to a marked reduction of the tumor incidence, but did not entirely prevent the occurrence of mammary tumors.

3. It is concluded that the spontaneous mammary carcinoma of the mouse is due to a hereditary organ disposition, which remains latent in the absence of ovarian function, but which becomes manifest when a certain amount of ovarian hormone, corresponding to 5 to 30 estrus cycles, has acted on the breast tissue.

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