SOME EFFECTS OF OVARIECTOMY DURING THE PERIOD OF DECLINING REPRODUCTIVE POWERS IN MICE

By WILLIAM S. MURRAY, Sc.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor)

(Received for publication, February 13, 1936)

While cancer of the breast is known to occur in all age periods following the prepubertal development of the mammary gland tissue, the most striking thing about its appearance in mice is that the lower range of standard deviation on the incidence curve is almost exactly coincident with the average age at which the breeding females begin to show signs of those involutory changes which mark the beginning of the breaking down of sexual function.

In mice of the dilute brown (Little dba) stock this process first indicates itself by the fact that following a period of uniformity and predictability in breeding behavior, during the 120 to 220 day period, the mice show ovarian changes which are reflected in the size of the litters born and in the inability of the mothers to nurse such young as are born alive. Both of these conditions grow progressively worse as the mice grow older. Since it is recognized that the ova are generated in the ovary, and since the mammary glands have been demonstrated to be largely dependent upon the ovary and its secretions for their normal functioning, it is not unreasonable to conclude that the reason, or at least one of the reasons, for the decline in fertility and the ability to lactate is attributable to some change or changes which are occurring in the ovaries.

That these changes are slow and that the part of them which affects the mammary gland tissue is in advance of the part which affects fecundity is indicated by the fact that the mice remain fertile until old age but that mammary function is lost before that time.

It is, therefore, reasonable to infer that the absence of or change in the ovarian secretions which in normal health stimulate the mammae

is important in bringing about the change to malignancy in the mammary tissue.

With this in mind, the following experiment was attempted in an effort to demonstrate the effects upon tumor incidence of complete absence of the ovaries after a period of normal secretion.

Material and Methods

The mice used in this experiment were from the inbred dilute brown strain (Little dba). The records of the breeding females were taken from the tables published in 1934.[1] The ovariectomized females (364 in number) were mice which had been used in the breeding colony until they began to show signs of losing their reproductivity, as indicated by their increasing inability to give birth to normal sized litters, their inability to give birth to a normal proportion of living young and their failure to nurse a normal percentage of those young which were born alive.

These mice were separated from the males during the 8th, 9th, 10th, 11th and 12th months of life and were ovariectomized. After the operation they were segregated in pens containing five mice each and allowed to grow old. During this period they were examined once a week for tumors. As fast as they developed tumors or died from other causes they were autopsied and records taken.

The control group consisted of 551 females from the breeding stock. These were segregated in the same manner as the operated females and were kept under the same conditions of housing, examinations and care. In the tables and charts these mice are designated as old females.

The method of operating was as follows: The mice were anesthetized with pentabarbital sodium, 0.15 cc. of the solution obtained by dissolving 1½ grains of the salt in 15 cc. of distilled water, administered intraperitoneally.

The ovaries were then extirpated through bilateral incisions in the lumbar region, care being taken to remove all hair before cutting. Using this method, the glands are readily found caudal to the lower pole of the kidneys in young or thin mice. In those animals with some abdominal fat, the ovary is usually incorporated in a fat pad which seems to be characteristic of the locality in which the ovary is situated. In such cases, care must be taken in making the excision due to the large amount of blood which may be lost if the fat pad is cut deeply. No attempt was made to peel the ovaries from their capsules. The gland itself, the covering and the distal end of the oviduct were all removed. The incisions were closed by taking single stitches in the body wall and in the skin. With reasonable care there is little trouble from infection. The mice recover very quickly, becoming active as soon as they emerge from the anesthesia. Internal bleeding, in those animals in which the fat pad was damaged, is the largest cause of mortality.

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[1] Murray, W. S., *Am. J. Cancer*, 1934, **20**, 573.

Methods of Tabulation

In this paper three criteria of change in the cancer and death rates are used: (*a*) Percentage of cancer in the various age groupings. (*b*) Average life remaining to an individual alive at the beginning of each age class. (*c*) Deaths from cancer per hundred alive at the beginning of each age class.

The first of these differs from the third in that it records the percentage of those alive at the beginning of any age period which died of cancer in that, plus all following, age groups. The third records those dying of cancer during a single period among one hundred alive at the beginning of the period.

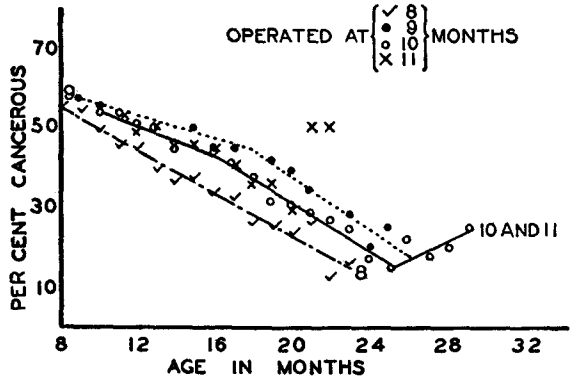

FIG. 1

The age groupings were divided into 30 day periods, which are designated in some places as months, in order to simplify the text.

In a short paper published in 1932[2] some of the effects of ovariectomy upon 195 breeding mice, operated at 7 months of age, were reported and discussed. It was found that this procedure greatly increased the average expectation of life and decreased appreciably the incidence of mammary tumors.

7 months was selected in that experiment because it marks the lower range of the standard deviation on the tumor incidence curve of this stock. This age seemed to be the most advantageous at which to

[2] Murray, W. S., *Science*, 1932, **75**, 646.

influence the incidence of mammary tumors, since two-thirds or more of those which were to become cancerous would do so in the following 4 or 5 months.

It later became apparent[3] that at 7 months of age the breeding females of this stock are entering upon a period of decline in their ability to give birth and nurse their young. Additional mice were, therefore, operated at ages varying from 8 to 12 months as they individually showed signs of loss of sexual function. In all, 364 females were operated.

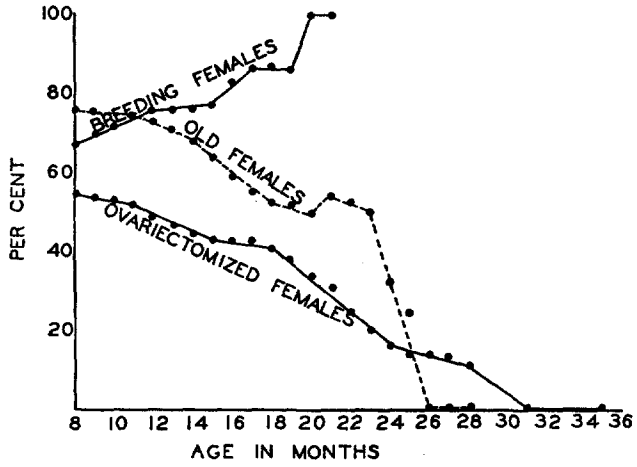

FIG. 2

That the incidence of cancer among the mice of this later experiment was approximately the same no matter at what age they were operated is shown by Fig. 1.

Inasmuch as the numbers were small when the animals were tabulated according to the month at which they were operated, especially in the older age classes to which relatively few lived, it was thought advisable to combine them, in order to show the general tendency of the effects of ovariectomy during the period when cancer of the mammary gland is most frequent in the breeding stock.

Fig. 2 which portrays the expectation of cancer in the breeding

[3] Murray, W. S., *Am. J. Cancer*, 1934, **20**, 584 (Table 14).

females, control females and operated females is illuminating since it shows the tendency in the breeding mice to rise steadily to 100 per cent in the oldest age group. This is the direct opposite of the behavior among the control and operated females which descend steadily until in the oldest age classes the mice are all free from tumors.

It will also be noticed that whereas the extreme age for breeding females was 21.5 months; in the control females it was 28 months and in the ovariectomized females 35 months. This shows an appreciable lengthening of life in both the experimental and the control classes.

Fig. 3 shows this lengthening of life even more graphically. Whereas the average length of life remaining to each breeding female alive

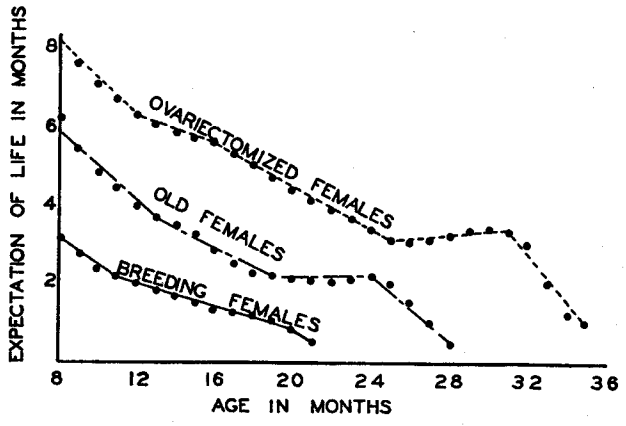

FIG. 3

at the beginning of the 8th month was 3.2 months, it was twice that for the old females and almost three times as much for the operated animals. As will be seen from the chart, these curves descend quite uniformly until at 21 months the average length of life remaining to the breeding females alive at the beginning of the period is 0.5 months; while it is 2+ months for the control females and 4+ months for the operated animals.

Fig. 4 shows the number of animals which developed tumor of the breast per hundred animals alive at the beginning of the various age periods. It will be noticed that while the tumor rate rises constantly and sharply in the breeding females, the curve for old females does not

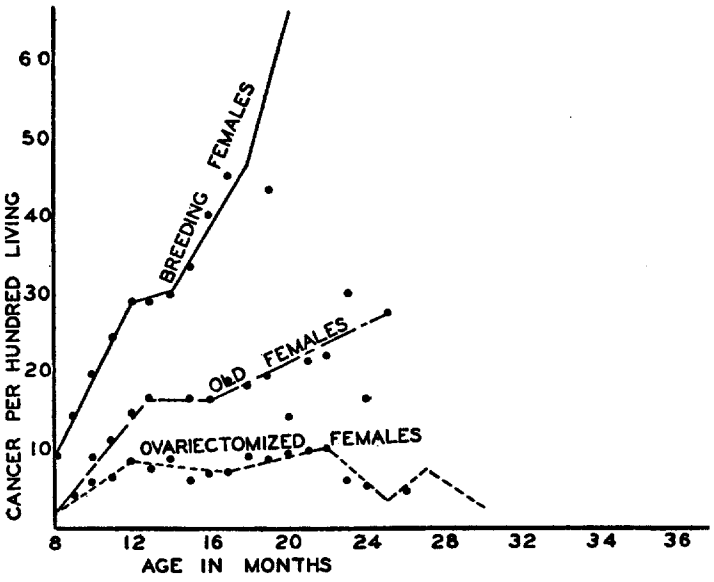

FIG. 4

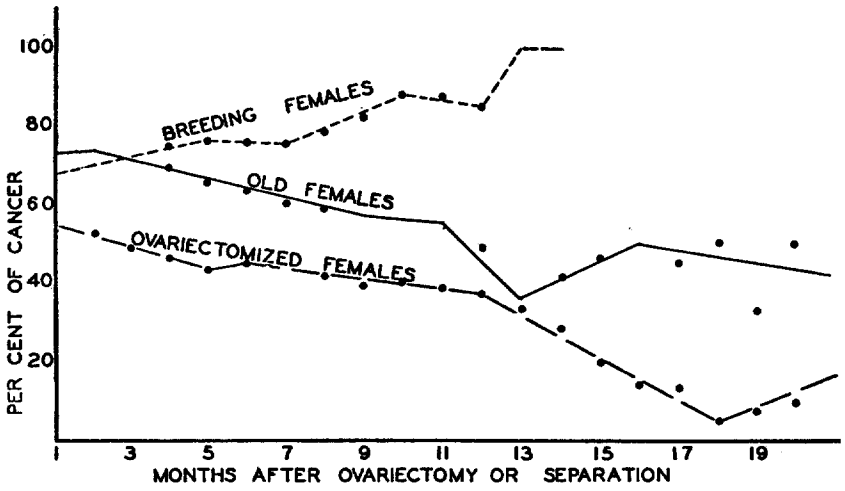

FIG. 5

rise so sharply and that the tumor rate per hundred living among the operated females is relatively constant in the various age classes. This

indicates the secretions of gestation and its sequelae in the breeding females are instrumental in the causation of cancer of the breast.

Owing to the variability of individual females in physiological age, as shown by the divergence in the times at which they undergo the

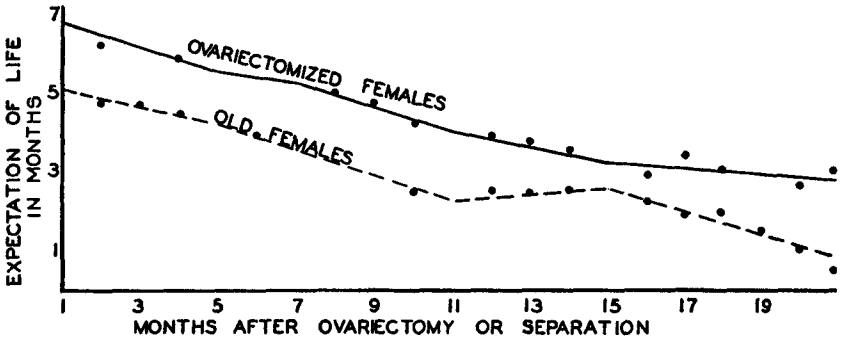

FIG. 6

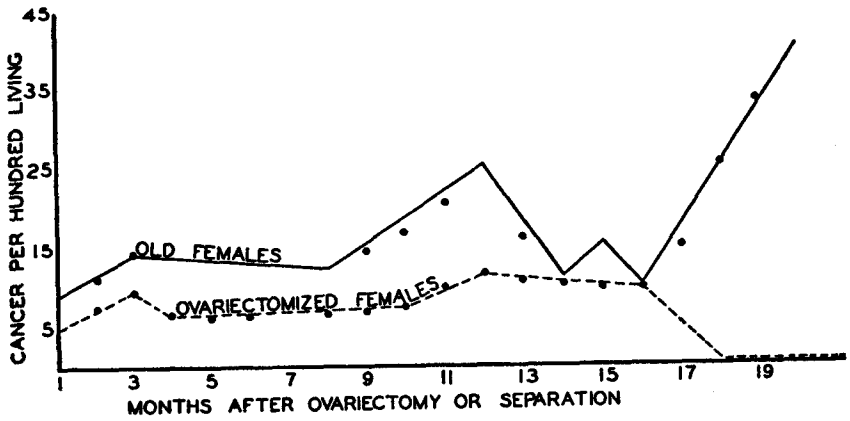

FIG. 7

decline in reproductive power, it was thought advisable to tabulate the same data using the time of separation or of operation as a starting point, thus establishing the approach of the decline in reproductive power as an index of physiological age. When this is done the expectation of tumors of the breast, in per cent, of those living at or beyond the beginning of the age period takes the form of Fig. 5.

Here as in Fig. 2, the relationship of the three classes is the same. The number of tumors in breeding females rises, whereas it gradually falls in the controls and the females operated upon.

Turning to Fig. 6, which shows the average length of life remaining to individuals living 1 to 20 months after separation, the superiority of the castrates over the control females is not so marked as when tabulated in Fig. 3. The ovariectomized females do show, however, a uniform excess of 2 months over the controls.

In Fig. 7, as in Fig. 5, the cancer rate per hundred living at the beginning of each period remains uniform in the castrated females, while it rises slowly during the first 17 to 19 months after separation in the control females.

DISCUSSION

The inference that absence of, or changes in the ovarian secretions which in normal health stimulate the mammae, are important in bringing about the change to malignancy in the mammary tissue, is shown by the above charts to have some foundation in fact. The absence of the secretions accompanying gestation is shown to be instrumental in prolonging life and in decreasing the mammary tumor incidence; while if all ovarian activity is removed as in the castrated group, the effects in the same direction are even more striking. The tables demonstrate that these effects are apparent whether the criterion of age used be chronological or physiological.

CONCLUSIONS

Cessation of breeding and castration of female mice approaching the period of declining reproductive power have at least two effects upon the subsequent life history of these animals.

1. The average expectation of life is appreciably increased.
2. The incidence of tumor is markedly decreased, especially in the older age groups.