weight than the other, or that it will lead to total nutritive collapse sooner. Admittedly experimentation under these conditions is not wholly satisfactory, but investigators should reconcile themselves to the fact that it is difficult to demonstrate the inadequacy of a given ration in any essential dietary factor when the experimental animal will not readily partake of it. Probably in many situations of this character the use of growth experiments is contraindicated. The success of any biological investigation is undoubtedly endangered when the experimental animal will not cooperate to a certain minimum extent.

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SPECIAL ARTICLES

OVARIAN SECRETION AND TUMOR INCIDENCE

FOR several years an attempt has been made to build up, by selective inbreeding, lines of mice which should have a very high incidence of mammary cancer. This effort has been quite successful, and it has been possible to establish two lines in an inbred dilute brown stock,[1] which produce very nearly 100 per cent. cancerous females. Among 183 breeding females of this dilute brown stock, taken in linear order from our ledger, 122 were tumorous. Of the remaining sixty-one, many died before reaching tumor age.

Under normal breeding conditions the neoplasms appear in the vicinity of the mammae of the females between the ages of four and fourteen months. The tumors usually appear, however, between the beginning of the seventh and the end of the eleventh months, the mode being at nine months.

It has been known for some time that the internal secretions of the ovaries play an important part in the physiological condition of females during and after the gestation period. That the influence of these hormones has also a direct effect upon the ability of mice to combat the growth of neoplasms has been demonstrated by Dr. L. C. Strong[2] (1922) in his work upon transplanted tumors. Dr. Leo Loeb[3] has also published (1915) a brief note on the effects of castration and enforced non-breeding on tumor incidences. More recently (1927) Dr. Carl F. Cori[4] has published a very interesting paper on the results of castration and ovarian transplantation in mice.

[1] This stock has been developed from a single pair by Dr. C. C. Little, and has been inbred, for the most part, by brother to sister matings since 1909.

[2] Strong, L. C., *Jour. Expt. Zool.*, 36: 1, 1922.

[3] Loeb, L., SCIENCE, Vol. XLII, No. 1095, Dec. 24, 1915.

[4] Cori, Carl F., *Jour. Expt. Med.*, Vol. XIV, No. 6, June 1, 1927, pp. 983–991.

I. What is the effect of enforced non-breeding upon cancer incidence in female mice?

That either or both of two factors: (a) the stimulating effect of lactation and pregnancy, and (b) the effect of sex hormones, may be involved in the appearance of cancer in the stock under observation, is demonstrated by the fact that when 207 virgin females of the dilute brown stock were separated from the males before sexual maturity and allowed to grow old under exactly the same conditions as the stock mice, which in many cases were siblings of the virgin females, but twenty tumors have appeared among them, although the youngest of these animals is fifteen and a half months old. The earliest age at which cancer appeared was ten months, the average 14.7 months, and the oldest seventeen months, as contrasted with four months, nine months and fourteen months for the breeding females (see Table I, lines 1 and 2).

TABLE I

AGE IN MONTHS

	Youngest	Average	Oldest
Breeding females	4	9	14
Non-breeding females	10	14.7	17
Castrated females	9	15.6	18.8
Castrated males with ovarian implants	8	11	13

From this we may infer that enforced non-breeding delays very markedly the age of tumor appearance and may even inhibit entirely the development of cancer in mice, which would probably have had a high incidence of tumor appearance had they lived a normal sexual life.

II. Will the female mouse grow a tumor when completely castrated?

The ages at which tumors occur in the normal breeding females indicate that the appearance of the neoplasms is closely correlated with the normal[5] activity of the ovary and ovarian hormones. One possibility is that cessation of ovarian action following a period of activity is the chief stimulating factor in producing mammary cancer. If, then, females in which the ovary has functioned at a low rate for a time are completely castrated, it might be expected that these animals will develop tumors at an age which is actually *younger* than that at which normal breeding females develop cancer. With this in mind, 210 females were spayed and allowed to grow old under the same laboratory conditions as the stock animals. To date twenty-one of these females have developed

[5] Normal activity of the ovary is here taken to mean that of a breeding female rather than a non-breeding animal.

tumors. However, the ages at which these animals develop neoplasms is strikingly higher than that at which the normal breeding animals develop cancer. (See Table I.)

It appears from these figures that the absence of hormone activity has a retarding effect on neoplasmic development, and may even inhibit tumorous growths, since practically all of the normal breeding females develop tumors if they live to 14.9 months of age, while in the castrated females only twenty in 210 have developed tumor, although they have all reached an age equal or greater than that at which the oldest breeding female developed neoplasms.

The complete absence of ovarian secretion has much the same effect on cancer incidence as does forced non-breeding.

It appears from this that the secretions of the ovaries under ordinary non-breeding conditions are not primarily the ones which stimulate tumor, but rather that it is commonly those conditions of hormone secretion in anticipation of the feeding of the embryo and young.

III. Do testicular hormones inhibit the growth of mammary tumors?

Since mammary cancer does not occur among the males of this race of mice, we might expect that possibly the testicular hormones inhibit these neoplasmic growths. If this is the case, the removal of the testes would remove the inhibitor and the operated individuals would be likely to develop tumor. In order to test this theory, 241 males of this race of mice were castrated at about four weeks of age and allowed to grow old under the same laboratory conditions as the stock animals.

The youngest of these animals lived to be fifteen months old and the oldest twenty-two months of age without developing a single tumor. The relief from inhibition caused by castration at four to five weeks of age is, therefore, not sufficient to allow the growth of mammary tumor. There is a possibility that the testes of these mice had begun to secrete in sufficient amounts to protect these mice against tumor after castration. Possibly, had they been castrated at an earlier age, different results might have been obtained. This, however, is very doubtful.

IV. Is it possible to grow mammary tumors in castrated males by transplanting ovarian tissue?

If it is the ovarian hormones which are causing the growth of tumors, ovarian tissue transplanted to castrated males encourages tumor development. With this in mind, 210 males were castrated and a whole ovary implanted subcutaneously in the abdominal region. The animals were then allowed to grow old. At the time of writing, when the youngest of these animals is ten months of age, *four have developed mammary tumors*—a thing never seen in the thousands of normal male mice of this inbred stock.

It would seem that the ages of cancer appearance in these animals should approach the curve of the virgin females, although the numbers are very small.

DISCUSSION

These results are interesting in that they correspond very closely with those reported by Dr. Carl F. Cori (1927). Our data, however, differ in several ways from his. Whereas he castrated his females at 15–22 days of age, we castrated ours at 28–35 days of age. Tumor was completely inhibited in his mice, while it was only partially inhibited in ours. This somewhat supports his conclusion that spontaneous cancer in the mouse is due to the lack of ovarian hormones after having had the use of it for a time.

Dr. Cori reports that in his castrated males, to which he transplanted two whole ovaries, no tumors appeared. Our experiment differed from his in that we transplanted but one ovary and obtained four males with mammary tumor from 210 animals treated in this manner. It is of interest to note that pathological diagnosis shows these tumors to be of the same type as those developed by the females in this strain of mice, namely, adenocarcinoma.

It is well known that in order to make successful transplantations of tissue, the animals involved must be very closely related. Possibly Dr. Cori's failure to feminize males was due to the fact that his stock was not sufficiently inbred, and the ovarian implant therefore degenerated.

CONCLUSIONS

(1) Non-breeding reduces tumor incidence in mice and delays the time of tumor appearance (207 mice used).

(2) Two hundred and ten female mice castrated at 28–35 days behave much the same as non-breeding females.

(3) Two hundred and forty-one males castrated at 28–35 days did not develop tumor, thus resembling non-castrated males.

(4) Spontaneous tumors, never obtained in thousands of normal males of the stock used, may develop in castrated males which have received subcutaneous transplants of ovarian tissue (210 operated—four tumors).

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THE CHROMOSOMES OF THE RAT

AN attempt to use the chromosomes of the rat as confirmatory evidence in certain phases of work on