THE ESTRous CYCLES OF MICE DURING GROWTH OF
SPONTANEOUS MAMMARY TUMORS AND THE
EFFECTS OF OVARIAN FOLLICULAR
AND ANTERIOR PITUITARY
HORMONES

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During the later stages of tumor growth in extra-uterine sites in
women, cessation of menstruation has frequently been recorded. This
is often dismissed as a probable consequence of the cachectic condition
of the patient. A similar cessation of the estrous cycle may occur in
mice coincident with the growth of spontaneous mammary tumors. In
both instances the condition seems to be similar to cessation of cyclic
activity as sexual life wanes.

For several years one of us has concentrated on inbreeding mice
that develop spontaneous tumors. A number of strains have been
established with an extremely high incidence of atypical growths of
the mammary glands and with the onset of this condition relatively
early in life (Strong, 1934, 1935). The hormone control of the genital
organs during the estrous and menstrual cycles has been the senior
author's major interest (Allen, 1922, 1927), while the reaction of the
mammary glands to ovarian and pituitary hormones has been studied
intensively by one of us (Gardner, 1933, 1935). It seemed a good op-
portunity for a cooperative study of the effect of growth of these mam-
mary tumors on the pituitary-ovarian mechanism of estrous rhythm in
the genital tract.

It has been definitely established that both the estrous and men-
strual cycles are dependent primarily upon secretion of threshold
amounts of follicular hormone (theelin, estrin) by the ovaries (Allen,
Doisy, et al., 1924; Allen, 1927). Reviews of the experimental data
may be found in "Sex and Internal Secretions" (Allen, 1932). Ova-
rian endocrine function in turn requires a definite amount of gonado-
tropic substance from the anterior pituitary (Smith, 1932, 1935). De-
ficiency of either of these hormones might therefore be responsible for
the extended acyclic condition of the tumor mice.

Both hormones stimulate growth processes in the organs or tissues
upon which they act. The mammary glands are especially reactive to
theelin and require a certain amount of stimulation by this hormone
for their primary growth. This has been demonstrated recently in
males of the same strains of mice in which the mammary rudiments

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2 National Research Council Fellow.

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have been stimulated to growth equivalent to that of virgin females by injections of theelin over proper intervals of time (Gardner et al., 1934). In fact, stimulation of growth can be carried to the stage where atypical growths appear in these rudiments (Lacassagne, 1932; Gardner, et al., 1934). Since the secretion of theelin is dependent upon an anterior pituitary hormone, the atypical mammary growths might also be considered as requiring this necessary substance, which is so essential for growth of follicles in the ovaries and indirectly for growth of the genital organs.

The problem under investigation may be stated as follows: (1) As control observations, to what extent do these mice with growing mammary tumors experience interruption of rhythmic sexual functions? (2) In acyclic animals will (a) injections of ovarian follicular hormone (theelin, estrin, amniotin) or (b) implants of anterior pituitary stimulate growth of the genital tract to the estrous condition?

The criterion of response to both experimental procedures is the same, i.e., the full estrous growth of the vaginal epithelium with the formation of a superficial cornified layer, as indicated in smear preparations from the vaginal lumen. In the case of the ovarian follicular hormone, this is probably a direct effect upon the genital tract, while with the anterior pituitary hormone the response involves the ovaries as intermediaries.

A preliminary report of this work has been published (Allen, Strong, Diddle, and Burford, 1934).

**Animals and Methods**

A total of 59 mice have been under observation in these experiments, including 11 used as controls. Forty-eight animals in which spontaneous mammary tumors were readily palpable ranged in age from 170 to 528 days. They included animals from three inbred tumor-susceptible strains, A, C3H, and D (Strong). As controls, representatives of a non-susceptible strain, CBA, were included. As a routine procedure these mice were mated early in reproductive life. The number of pregnancies varied from one to six.

Changes in the estrous cycles were followed by means of the usual vaginal smear technic, routine smears being taken daily. After a mouse with atypical mammary growth had shown a long diestrous interval, it was given injections of theelin or implants of pituitary in an attempt to induce an experimental estrous period. In these animals smears were taken three times daily at the end of the usual reaction interval.

All injections and implants were made subcutaneously. The ovarian follicular hormone used consisted of a standardized preparation of theelin. Pituitary glands were taken from gonadectomized male and female mice, since gonadectomy increases the potency of the anterior lobe as far as its gonadotropic (follicle-stimulating) secretion is concerned (Engle, 1929, and others).

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a Supplied by Parke, Davis & Co., through Dr. Oliver Kamm.
Before making the first injection of theelin or implant of pituitary, periods of diestrous smears ranging from seven to seventeen days were allowed to elapse. In case the animal failed to react to the first treatment, subsequent injections or implants were sometimes made within three to six days. This interval is, of course, not shorter than the normal diestrous interval.

It should be noted that ovaries were intact in all the experimental animals and that there is, therefore, no absolute control on the experimental results, since a spontaneous estrous period might conceivably have appeared at any time.

Observations were continued up to the time of death of the animal. In accumulated records on 101 mice of these strains the average duration of life after the mammary tumor was discovered was fifty-five days. In the animals used in this experiment the average duration of life was slightly less, perhaps influenced by the experimental procedure. At autopsy, unless post-mortem changes were too far advanced, the ovaries, vagina, uterus, uterine tubes, mammary glands, and tumor growths were studied histologically.

Observations

With continued growth of the tumor, estrous periods became infrequent and gradually disappeared. Whereas the whole estrous cycle in normal mice usually requires only four to five days, of which one or two days may be diestrus, it is quite common to find extended diestrous intervals extending up to twenty days in tumor mice. Sometimes, however, short estrous periods appeared spontaneously. Occasionally partial growth reactions appeared which did not culminate in complete estrous periods. Long diestrous periods usually preceded death. In connection with these observations indicating decreased function of the ovaries may be cited records (Strong) that mice with spontaneous tumors seldom breed. If they are pregnant when the tumor is first discovered they seldom nurse their litters. There seemed to be no consistent relationship between the size of the tumor and the acyclic condition.

A study of sections of the genital organs of the tumor mice during these extended periods of diestrus showed extremely atrophic conditions. In some animals this was equivalent to the atrophy following complete ovariectomy of long standing. The vaginal wall was extremely thin, two to three layers of cells in thickness; the uterus was very small with atrophic endometrium and no signs of secretion; the tubes, especially the ciliated parts, were decidedly atrophic. The ovaries were small, containing few follicles and no recent corpora lutea.

With one exception the mammary glands of the tumor-susceptible mice were greatly involuted. In all cases lobules or nodules of apparently functional alveoli, non-functional alveoli, or even small tumors were found scattered throughout the persisting duct system of the involuted glands. These areas were observed irrespective of hormone
treatment. In the one mouse which proved the exception, the mammary glands were as completely developed as at the end of gestation. Though this mouse had received both pituitary and theelin treatment, it is difficult definitely to ascribe the findings to the hormone treatment, which in most cases was of too brief duration for induction of decisive changes in the mammary glands.

The mammary glands of the control tumor-resistant animals were involuted to a similar degree and showed no evidence of cyclic variations. Small localized nodules may occur occasionally in the mammary glands of these mice.

All the tumors studied originated in the mammary glands. The majority were cylindrical-cell carcinomata, the epithelial cells of which were in several layers, showing an obvious origin from the alveoli. In some instances cells had broken through the basement membranes into the surrounding stroma. Different stages of mitosis were readily identifiable. A few of the tumors were adenomata. They had the appearance of normal glandular tissue which had undergone hyperplastic changes without signs of malignancy.

There follow protocols typical of the experimental procedure, and brief summaries. See also Table I, in which are listed data on some of the experimental animals.

**Controls**

Mouse 94277 is typical of the controls. It was selected from strain CBA, the individuals of which, as a rule, do not develop spontaneous tumors. This mouse had had three litters of young early in her reproductive life. Estrous cycles were followed throughout the experiment from the age of 376 days to 559 days. During the entire interval the longest diestrous period did not exceed five days.

Ten other controls were followed with essentially similar results.

**Theelin Injections**

Mouse 93562: This animal was from the tumor-susceptible strain, C,H (Strong). Her earlier history included three pregnancies. A spontaneous tumor measuring \(2 \times 2\) mm. was discovered near the second left nipple at the age of 363 days. Vaginal smears were begun at 386 days of age. The estrous cycles were of normal duration up to 406 days of age. From the 406th to 415th day the vaginal reactions began to wane. For the last five of these days diestrous smears were recorded. On the 415th day the animal was injected with 1.4 mouse units of theelin, given in two equal doses, eight hours apart. The second day following injections the genital tract showed a partial reaction (+ -). On the 418th day a total of 1.4 mouse units of theelin was again administered in two equally divided doses. Sixty-four hours later a positive estrous reaction appeared. The animal died at the end of the 421st day. The tumor at death measured \(4 \times 5\) mm. Histological sections were not saved because of the post-mortem degeneration which was well advanced when death was discovered.

A total of 7 mice, after periods of diestrus ranging from five to nine days, reacted positively to total doses of theelin ranging from 1.25 to 9.00 mouse units. One of the animals gave a partial reaction after having received 1.4 mouse units of theelin. In response to second treatments with comparable doses, after two to five days of diestrous had intervened, positive estrous reactions were again obtained.
<table>
<thead>
<tr>
<th>Mouse</th>
<th>Strain</th>
<th>Age when tumor was found (days)</th>
<th>Age at death (days)</th>
<th>Location of tumor (No. of nipple)</th>
<th>No. days of diestrus preceding treatment</th>
<th>Theelin injected (m.u.)</th>
<th>Pituitaries implanted</th>
<th>Results</th>
</tr>
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<tr>
<td>94692</td>
<td>A</td>
<td>300</td>
<td>356</td>
<td>4th R.</td>
<td>6</td>
<td>(a) 6</td>
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<td>+</td>
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<tr>
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<td>217</td>
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<td>421</td>
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<td>(a) 1.4</td>
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<td>291</td>
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<td>8</td>
<td>(a) 2.2</td>
<td></td>
<td>-</td>
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<td>L. iliac</td>
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<tr>
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<td>479</td>
<td>4th R.</td>
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<td>-</td>
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<td>368</td>
<td></td>
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<td></td>
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<td>-</td>
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<td>49</td>
<td>C3H</td>
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<td>399</td>
<td></td>
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<td></td>
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<td>421</td>
<td>5th R.</td>
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<td>(a) 2</td>
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<td>1st R.</td>
<td>14</td>
<td>2</td>
<td></td>
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</tr>
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</table>
Pituitary Implants

Mouse 94023 was from the tumor-susceptible strain, A. This animal received only pituitary implants. She had previously given birth to three litters of young. At 279 days of age a tumor measuring 4 × 5 mm. was found near the second left nipple. Vaginal smears were begun for this animal at 294 days of age. On the 297th and 305th days the vaginal smear showed a partial growth reaction. For the next nine days, 306th to 314th, she remained in diestrus. Two pituitaries from mice were implanted on the 315th day, the 10th day of diestrus. Forty-eight hours later the animal was in estrus. There followed a diestrous period of five days. Implants of two pituitaries were made at the end of this time with a negative response after the expected interval. Five days later, the 323rd day, three hypophyses were implanted. No change could be observed in the smear up to the 328th day, at which date the animal was autopsied.

At autopsy the tumor measured 24 × 20 mm., or five times its original size. It was well encapsulated and contained areas of necrosis and blood in the center. Histological sections showed it to be a cylindrical-cell carcinoma. The genital organs were decidedly atrophic.

A total of 12 animals which had been in diestrus from three to fourteen days were given pituitary implants, 9 receiving two implants each. Five of these animals reacted positively. Two of the others gave partial reactions and 2 responded negatively. Two others, given three pituitary implants, returned positive and partial reactions respectively. A twelfth mouse, receiving one pituitary, gave a partial reaction.

Four of the above animals, which received two hypophyses each and which reacted positively, were again given two pituitary implants six to eight days following the previous treatment. Two showed no change in the vaginal smear, and two gave positive reactions. Two of the animals, one negative and one positive to the second treatment, were given a third series of pituitary implants three and five days later. The results were equivocal and positive in the order named.

Mouse 48 was from the tumor-susceptible strain, C.H. This animal received only pituitary implants. At the age of 466 days a tumor was found near the fourth left nipple. The animal was in diestrus for ten days. Beginning with the 477th day of age one hypophysis was implanted every forty-eight hours until five had been given. The animal continued in diestrus throughout the period of treatment. She died at the age of 487 days.

To 6 animals, each of which had been in diestrus for a period of ten days, one hypophysis was administered every forty-eight hours until five had been given. All of the mice remained in diestrus during the entire course of treatment except one (49), which showed a partial reaction after the fourth gland was implanted.

Alternating Pituitary Implants and Injections of Theelin

Mouse 97113 was from the tumor-susceptible strain, C.H. This animal received alternately pituitary implants and theelin injections. Early in her reproductive life she had had four litters of young. At the age of 359 days a mammary tumor was discovered near the right fifth nipple. Smears were begun and for the next sixteen days there was no clear-cut estrous period. Two pituitaries were implanted subcutaneously on the 377th day and a positive vaginal reaction resulted on the second day following. During the next eleven days there was one period of partial reaction. On the 399th day, three days later, 4 mouse units of theelin were injected in two equally divided doses. Fifty hours
later a partial reaction was obtained. A period of diestrus followed for eight days, at the end of which two pituitaries were implanted, with a partial reaction forty-eight hours later. After a diestrous interval of two days, 6 mouse units of theelin injected in one dose returned a positive estrous smear. The next treatment consisted of implantation of four pituitaries at one time, with a negative result. The animal was killed on the 62nd day after the tumor was discovered, at the age of 421 days. Histological sections of the tumor showed it to be a cylindrical-cell carcinoma. The genital tract was atrophic.

**Animal 94692**, strain A, received three successive treatments which consisted of 3 mouse units of theelin, three pituitary implants, and 2 mouse units of theelin respectively. Theelin in both instances gave positive reactions. The result of the pituitary implants was equivocal because the animal was in estrus at the time the implants were made. The treatments were spaced eleven and four days apart respectively.

**Animal 97113** received the following treatment spaced at twenty, ten, and four day intervals: 4 mouse units of theelin, two pituitary implants, 9 mouse units of theelin, and four pituitaries respectively. The first and second treatments returned partial reactions, the third was positive, and the fourth negative.

A summary of combination of pituitary and theelin treatment follows. In 6 mice which had previously received pituitary implants, doses of theelin ranging from 1.4 to 9.0 mouse units were administered in two equally divided parts. Three of these animals had previously reacted only slightly to three pituitary implants each given at the same time. Two others had given positive results to two implants of pituitary and another a partial reaction to two implants. Five of these mice gave positive vaginal smears forty to fifty-five hours after the first injection of theelin. The 6th animal (91782), which had received three pituitary implants three days before without any change in the vaginal reaction, gave a partial reaction with 2.4 mouse units of theelin. This animal had been in diestrus nineteen days before the pituitary implants had been made and twenty-two days before theelin treatment was begun.

**Discussion**

In an early study of the endocrine glands in mice bearing spontaneous tumors, Rohdenberg and Bullock (1915) observed a smaller percentage of functioning ovaries as compared to controls. This work antedated the numerous experiments which have clarified the interrelations of hormone factors in growth and function of the genital organs. The demonstration of the anterior pituitary-ovarian-genital cycle mechanism has again focused attention on these hormones so necessary for growth of the tissues involved.

An interesting report by Guilbert and Goss (1932) indicates that a restricted protein intake is followed by effects on the estrous cycle of the rat similar to those found in mice with spontaneous mammary tumors. Do these nutritional deficiencies operate through checks upon hormone secretion?

Several interesting observations have been made in connection with the possible relation between senescence and the endocrine function of the ovary and pituitary. Injections of theelin into senescent animals, after sexual function has waned, have resulted in typical waves of estrous growth in the accessory organs similar to those resulting from
theelin injections into ovariectomized test animals (Steinach, Heinlein, and Weisner, 1925; Laqueur, Hart, and de Jongh, 1926; Slonaker, 1927). These hormone effects at this time of life, however, are only transitory. There is apparently little if any stimulating effect upon the ovaries as the chief source of this hormone. The evidence indicates that theelin may temporarily rejuvenate the accessory genital organs of senescent females.

Effects of pituitary extracts and implants into old animals are not as clear-cut as the effects of theelin. It has been suggested in women who are past the menopause, and who show large amounts of the follicle-stimulating hormone in the urine, that the ovary loses its capacity to react to the pituitary hormone (Smith, Engle, and Tyndale, 1934). It has been shown, however, that old female mice, after cessation of estrous cycles, will react to pituitary treatment (Zondek and Aschheim, 1927; Hoffman, 1931).

Laassagne (1934) found that estrus was prolonged to forty-eight hours in a tumor-susceptible strain of female mice. The cycle lasted five days. In another strain which developed few, if any, tumors, estrus was usually so short that it could easily be passed unnoticed. It was renewed at irregular intervals, separated by long periods of diestrus, frequently prolonged more than one week. Administration of folliculinn rapidly provoked a prolonged estrous vaginal reaction in the susceptible group and only a slight reaction in the non-susceptible group.

Mahnert (1927) made intramuscular transplants of tumors into female mice. For controls 0.2 to 0.3 c.e. of muscle was injected into mice of similar ages. One to three weeks after transplantation of the tumors the period of estrus was lengthened from one or two to three to six days. The period of diestrus was four to five days. After two to three weeks estrous cycles became fewer in number. These mice died twenty-five to thirty days after the tumors were implanted. Ovaries of these animals in late stages of tumor growth contained no follicles and only a few old corpora lutea. Mahnert concluded that the tumor secondarily produced a proteolytic substance which caused degenerative changes in the tissues of the body. Our observations as to the irregularity of the estrous cycle in mice with spontaneous tumors are comparable to those described by Mahnert on mice with transplanted tumors. In addition, our results indicate that an estrous reaction may be obtained in the vaginal epithelium with minimal doses of theelin after the ovaries fail to respond to implants of pituitaries in the number and over the interval given.

**Summary**

Estrous cycles have been studied in 59 mice of four different strains inbred with regard to early appearance of spontaneous mammary tumors. The types of tumor involved are mammary carcinomata and adenomata. With advanced growth of the tumor estrous cycles became infrequent and long-continued periods of diestrus occurred. See-
tions of the genital organs showed them to be in an extremely atrophic condition approaching that of ovariectomized animals.

After long periods of diestrus the accessory genital organs of these tumor mice have been stimulated repeatedly to normal estrous cycles by injections of theelin and by implants of two or three pituitaries from gonadectomized mice. Several successive treatments of either theelin or anterior pituitary or combinations of both have been tried. There is a time in the degenerative phase of the ovary when pituitary treatment is no longer effective. Minimal doses of theelin produce positive vaginal reactions in such animals. These results indicate that the acyclic condition in tumor mice probably involves primarily the gonadotropic function of the pituitary.

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