Development of Spontaneous Mammary Tumors over the Life-Span of the Female Charles River (Sprague-Dawley) Rat: The Influence of Ovariectomy, Thyroidectomy, and Adrenalectomy-Ovariectomy

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Summary

Several groups of virgin female cesarean-originated, barrier-sustained Charles River CD rats were observed for incidence of mammary tumors over their life-span. Maximum life-span ranged from 885 to 1040 days. Crude MT incidence for all groups was 67.1%. When a life table method was used to correct for extraneous deaths, the mean MT incidence for all groups was 71.5 ± 5.7%. The age at onset of the median MT was 671 ± 41 days. Cumulative MT incidence agreed closely among control groups early and late in life, with a greater variability appearing from the 500th to the 600th day of age.

In normally aging rats there were at least 2 age-related changes leading to abrupt increases in the rate of development of MT—once occurring at about the 500th and the other at about the 600th day of life.

Both total MT incidence and MT incidence rate were the same for uniparous rats as for virgin controls. Thyroidectomy did not reduce the life-span incidence of MT, but did postpone slightly those MT's arising late in life. Ovariectomy nearly eliminated MT development; only 6.7% of ovariectomized rats developed MT over a maximum life-span of 1295 days. No MT developed in adrenalectomized-ovariectomized rats although they lived long enough (maximum life-span 1110 days) to have permitted observation of late-appearing MT's.

The proportion of MT's diagnosed as carcinomas in the individual control groups was highly variable, 18.6 ± 12.1%. In these studies carcinomas did not appear to arise earlier than benign fibroadenomata. Other superficial tumors arising in tissues different from the breast are tabulated.

The results of these studies are compared with those from other laboratories using the same or a related strain of rat. The relationships between development of spontaneous MT and the age-related changes in the ovary and pituitary gland are discussed.

Introduction

Mammary tumors of the rat have been studied for more than 60 years (15, 21, 30, 40). Noble and Cutts (36) and Clifton (6) recently reviewed the literature on murine breast tumors, their artificial induction, and their dependence on hormonal status.

MT's occur spontaneously in old rats (36). They can also be induced in young females by various means: (a) by external application (22, 31), feeding (26, 41), or injection of aromatic polynuclear hydrocarbons (25) or aromatic amines (53); (b) by injection (5) or implantation of estrogen (9) or injection of pituitary extracts (15); and (c) by ionizing radiation—single exposures of x-rays (17, 29, 32, 44), γ-rays (43), or proton beams (46) or multiple exposures of γ-rays (52) or neutrons (30, 52) and internal irradiation with the α-particle emitter,²¹⁰At (12).

Histologically, both benign and malignant MT's occur spontaneously, the majority being benign fibroadenomata (36). The proportions of malignant and benign MT induced by radiation are similar to those encountered with spontaneous MT (17). When the inducing agent is estrogen or a hydrocarbon, the proportion of malignancies is increased (9, 26).

MT's of the rat are highly sensitive to hormone manipulation. Ovariectomy drastically reduces (4, 14, 23) and hypophysectomy abolishes (23) MT development in hydrocarbon-treated or irradiated females. Neither agent is particularly effective in inducing MT in intact males (42, 45). Established MT's frequently regress when the ovaries are removed (58) or the estrogen supplement is withdrawn (9); and implants of fibroadenomata take poorly in ovariectomized female or in male hosts (34).

While there appear to be differences among strains, significant numbers of MT's arise spontaneously in aging rats of most strains (1, 7, 8, 10, 11, 27, 28, 33, 39, 50, 51, 56). The similarity of the chronic effects of radiation and the physiologic changes accompanying natural aging (of which MT development is one manifestation) led Bond et al. (3) to comment:

"When neoplasms of the types under study (i.e. mammary tumors) increase in time in control animals, the question arises as to whether the radiation is inducing a process, or merely accelerating a process that would normally occur in time. This question is difficult, if not impossible, to answer satisfactorily...."
since a meaningful value for the 'normal' incidence is difficult to obtain. In general, the likelihood of neoplasia increases with age, and it is not possible to state whether neoplasia of a given type would have appeared in decedents."

Investigation of MT induction by radiation or other carcinogens has been hampered by lack of information on MT incidence in aging controls (43). Except in 1 instance, when heroic measures were taken to reduce pulmonary infections (1), MT and the other lesions common to the latter half of the life-span of the rat were obscured by a high mortality from infectious diseases. Adequate study of the MT incidence, and its age distribution over the life-span of the rat, was possible only when essentially disease-free rats became available in large quantities.

This report covers a 6-year study of the incidence of spontaneous MT in a colony of female Charles River CD rats (descendants of the Sprague-Dawley line) whose maximum life-span was close to 3 years. We have determined the total MT incidence, the age-specific incidence of spontaneous MT, and the variability of both among different shipments from the same supplier. The effect on spontaneous MT incidence of removal of the ovaries, adrenals, or thyroid glands was also investigated.

The proportions of benign and malignant tumors and the occurrence of superficial tumors originating from tissues other than breast are briefly discussed.

Methods and Materials

ANIMALS AND ANIMAL CARE. The rats used in these studies were COBS (cesarean-originated, barrier-sustained) females of the Charles River CD strain purchased over a 4-year period from the Charles River Breeding Laboratories, North Wilmington, Massachusetts. The number of rats in each shipment, their birth date, and any special treatment are shown in Table 1. One group, Lot A, was born at this Laboratory—the results of a backcross of Charles River CD females and Sprague-Dawley males from the original colony at Madison, Wisconsin.

Rats were received as weanlings, except for the uniparous group (UP, Lot 15). On arrival they were earmarked and caged in groups of 5. As the animals grew larger they were redistributed so that the largest rats were caged in pairs. The cages were made of plastic with stainless steel tops. A layer of sterilized wood shavings served as bedding. Cages were changed, washed, and sterilized twice a week. Purina Lab Chow and tap water were fed ad lib. Adrenalectomized-ovariectomized rats were maintained on isotonic saline.

Just before receipt of the 1st shipments of COBS rats the stockroom was cleared of rats, the premises were cleaned and fumigated, and all movable equipment was sterilized. During these studies only COBS rats were kept in the colony. Deaths from pulmonary and other infections were rare, indicating that an essentially disease-free colony was being maintained with the minimal procedures described above.

The colony was weighed and examined once a month. The animal continued to deteriorate, it was sacrificed. Rats with blood in the eyes and nose and were losing weight rapidly were sacrificed. This latter combination of symptoms usually indicated the presence of a large pituitary tumor.

Cages were examined daily for sick or dead rats. Dead rats were examined to determine cause of death, if possible. Moribund rats and those that had bloody encrustations around the eyes and nose were losing weight rapidly were sacrificed. This latter combination of symptoms usually indicated the presence of a large pituitary tumor.

Only 2 end points were considered in this study: development of the primary MT and death of the animal. Unless an MT was already large when it was 1st seen, the animal was held for additional observation to make sure the palpable lump was an MT and not just hyperplastic mammary tissue or normal tissue samples. Just before receipt of the 1st shipments of COBS rats the stockroom was cleared of rats, the premises were cleaned and fumigated, and all movable equipment was sterilized. During these studies only COBS rats were kept in the colony. Deaths from pulmonary and other infections were rare, indicating that an essentially disease-free colony was being maintained with the minimal procedures described above.

The colony was weighed and examined once a month. The individual record of each rat included the monthly weight, the date an MT was 1st observed and its approximate location and notes on its subsequent growth, and comments on general health. Since the goal of the experiment was to keep a rat healthy until an MT developed, rats with simple debilitating conditions were specially treated. Rats with vaginal bleeding or skin sores were isolated to prevent cannibalism. Skin lesions were painted with gentian violet or Zephiran chloride. If the bleeding stopped or the skin sores healed, the rat was returned to the colony. If the animal continued to deteriorate, it was sacrificed. Rats with spread or broken incisors were caged separately and given powdered food. The overgrown opposite teeth were clipped periodically to facilitate eating and to prevent laceration of the jaw.

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part of other experiments, for which it was necessary to keep them alive as long as possible, or in the *Ca group, at least until the next regularly scheduled sacrifice. When one of these rats developed an MT, it was resected, and the animal was returned to the primary experiment. The resected MT was weighed, given an experimental number, and prepared for histology.

At autopsy or sacrifice the animals were examined, and the gross findings were recorded. Tumors of all kinds, endocrine glands including the pituitary, and samples of other tissues and organs were weighed and fixed for histology. The results of the collateral studies will be reported elsewhere.

Because of the lapse of time between the 1st observation of an MT and sacrifice of the rat, some other superficial tumors occasionally developed. When more than 1 superficial tumor was found at autopsy, and there was no record of the location of the primary MT, the heaviest was designated as primary. In a few instances, although rats were sacrificed as MT bearers, histologic examination of the tumor indicated an origin from a different tissue. If this was the only tumor present, the rat was removed from the MT group and recorded as having died without an MT. If a proven MT was also present, the largest was designated as the primary MT.

The individual animal records were transferred to punched cards for processing. Each data card contained the following information: lot number, experimental group, animal number, histology reference number, age at death, weight and diagnosis of the primary MT, weight and pathologic diagnosis of the pituitary, diagnosis of other superficial tumors if any, age when the primary MT was 1st observed, age at MT resection when applicable, age at onset of the primary MT (T_o, calculated as described below), and a notation if death without an MT was classified as extraneous, i.e., accidental, deliberate sacrifice, or proven pulmonary infection.

**UNDIAGNOSED MT.** All but 29 MT’s were diagnosed microscopically. In 14 cases MT bearers died without autopsy and were inadvertently destroyed or the tumorous area had been eaten by cage mates. In 15 other cases MT specimens were lost during histlogic preparation. The total number of primary MT’s observed was 335, of which this group of 29 represented 8.6%. As discussed at the end of the section on “Results,” the chances were less than 1 in 25 that these undiagnosed superficial tumors had not originated from the breast. It was therefore assumed that such tumors were most probably MT’s and further that they were probably benign fibroadenomas.

**DETERMINATION OF THE AGE AT ONSET OF MT.** The basic end points of this study were age at death, for those animals dying without an MT, and the age at onset of the 1st MT. Age at death was an integral part of the record for each rat. Age at MT onset was more difficult to establish. During the 6-year course of the study many people were involved in the examinations. Weighing and palpating a large colony, even once a month, was sufficiently time consuming that examinations were often hasty, and those conducted by inexperienced personnel were often inadequate. As a result, some MT were 1st detected when barely palpable, but the majority were not seen until they weighed several gm.

Because of this disparity in size when the MT were 1st observed, a method was developed to normalize the age at MT onset. A growth curve as shown in Chart 1 was constructed for the “average” FA, the most common rat breast tumor. The construction of the curve, and the errors involved in its use, are described elsewhere (13).

The MT size chosen as the reference for MT onset was 1 cm diameter, or—assuming unit density for tumor tissue—a weight of 0.5 gm. The age at onset, T_o, was thus defined as the age of the animal when its 1st MT was reference size. The weight of the MT at autopsy or resection was entered into the curve in Chart 1 to determine the growth interval, the number of days the MT had been growing since it weighed 0.5 gm. T_o was then obtained from the relation:

\[ T_o = \text{age at autopsy or resection} - \text{growth interval} \pm \text{S.E.} \]

The S.E. of estimate of the growth curve (Reference 35, Chapter 16), shown as the shaded area in Chart 1, was small enough so that the uncertainty in T_o was never more than 30 days. The growth rates of a small sample of mammary carcinomas were compared with the growth of FA (13). No difference in growth rates was detectable. The age at onset of MT diagnosed as carcinoma was therefore also estimated from Chart 1. As will be shown in “Results,” the distributions of age at onset of FA and MC were alike, and no distinction was made in the analysis that follows between MT diagnosed as malignant or benign.

**PREPARATION OF LIFE TABLES.** The data cards for each control and experimental group were sorted into MT bearers and rats dying without MT. MT bearers were arranged in order of in-
creasing age at onset of the primary MT as calculated from the growth curve. Rats dying without MT were arranged in order of increasing age at death. Rats without MT were further divided into 2 groups—those whose deaths were considered extraneous and those that died of causes related to or possibly related to natural aging. The life table of the combined control group is shown in Table 6.

Separate life tables were prepared for the 4Ca group and for Lot 1, the undisturbed portion of the 1st shipment. These 2 parts of the same basic group were examined separately to determine the ability of actuarial methods to correct for the substantial early losses of rats demanded by the predetermined sacrifice schedule of the 4Ca study. Lot 14, which was essentially undisturbed, was divided for analytical purposes into 2 groups at the central rat number. A separate life table was prepared for each half, 14-1 and 14-2; these were to serve as controls for the separately maintained portions of Lot 1, and also to examine how closely part of a group represented the behavior of the whole group.

ACTUARIAL CORRECTION FOR EXTRANEOUS DEATHS. The actuarial interval used throughout this analysis was 30 days. Use of a longer interval would have obscured some age-dependent phenomena.

The method used to correct for deaths presumed to be unrelated to aging (Column 4 in Table 6) was that described by Berkson and Gage (2). This correction assumes that rats dying during a 30-day interval are, on the average, under observation for half the interval; thereafter they are eliminated from the population at risk. Pilgrim and Dowd (38) recently pointed out some errors in this method and have devised a more precise way of taking extraneous deaths into account. However the correction used in this report was considered adequate for the following reasons: (a) the number of extraneous deaths was relatively small; (b) many of the rats that died without an MT succumbed to pituitary tumors, and these have been shown to be intimately related to MT development (6, 18, 19); (c) we wished to describe MT morbidity in the presence of other lesions and diseases associated with aging rather than MT morbidity as an independent entity.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Control groups</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Mammary tumors (crude)</td>
<td>% Mammary tumors (corrected)</td>
</tr>
<tr>
<td>Life-span</td>
<td>Life-span</td>
</tr>
<tr>
<td>660 days</td>
<td>900 days</td>
</tr>
<tr>
<td>I</td>
<td>4Ca</td>
</tr>
<tr>
<td>65.2</td>
<td>47.5</td>
</tr>
<tr>
<td>Life-span</td>
<td>78.9</td>
</tr>
<tr>
<td>% Mammary tumors, corrected</td>
<td>26.3</td>
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</table>

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**Results**

MT incidence over the life-span of the normally aging female rat. The crude MT incidence—i.e., the proportion of the starting population that eventually developed 1 MT—and the actuarially corrected MT incidence are shown in Table 2 for the various control and experimental groups. There were no extraneous deaths in Lot 12, or the TX or OX groups; therefore, the crude MT incidences of these groups are the same as the corrected values. Actuarial correction substantially reduced the spread among the virgin control groups; MT incidence over the life-span (1020 days) of 4 groups of Charles River rats (Lots I, 11, 12, and 14), hereafter referred to as the combined control group, was 71.5 ± 5.7%.

The maximum life-spans of the individual control groups varied from 885 to 1010 days; therefore the MT incidence at 900 days—an age late in life that was attained by all the groups—is also shown in Table 2. The MT incidence at 660 days is included so that the early behavior of Lot 17 (23 of these rats are still alive on June 30, 1965) might be compared with the other control groups.

Table 3 shows some of the gross features of the age dependence of MT development in the normally aging female rat: the age at onset of the 1st MT in the group; the age at which 25% of the group's MTs had developed (25th percentile); the age at onset of the median MT; the age at which 75% of the group's MTs had developed (75th percentile); and the age at onset of the last MT in the group. Among the virgin control groups there was a range of nearly 200 days in the age at onset of the 1st MT. The span of age at development of the median MT was narrower, 120 days, as was the age span when the last MT appeared, 125 days. The average age of a control group at the onset of the median MT was 671 ± 41 days, and for development of the last MT, 945 ± 41 days.

CUMULATIVE MT INCIDENCE. The life-span MT incidences of the 5 virgin Charles River control groups (Lots I, 11, 12, 14, and 17) are shown in Chart 2 as linear plots of cumulative MT incidence vs. age. The shapes of the curves were similar at the younger ages, and they tended to converge to a common value late in life. However, there was somewhat greater variation in the curves shapes from the 500th to the 850th day of age. Evans and Simpson (15) and Furth and Clifton (18, 19) have suggested that MT in rodents arises as the result of complex interactions.
TABLE 3
AGE DISTRIBUTION (DAYS) BY QUARTILE OF THE APPEARANCE OF MAMMARY TUMORS (1ST MAMMARY TUMOR/RAT), MAXIMUM LIFE-SPAN, AND MEDIAN AGE TO MAMMARY TUMOR OR DEATH IN CONTROL AND EXPERIMENTAL GROUPS

<table>
<thead>
<tr>
<th></th>
<th>CONTROL GROUPS</th>
<th>EXPERIMENTAL GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>1st mammary tumor</td>
<td>317</td>
<td>305</td>
</tr>
<tr>
<td>25th percentile</td>
<td>584</td>
<td>577</td>
</tr>
<tr>
<td>50th percentile</td>
<td>709</td>
<td>630</td>
</tr>
<tr>
<td>(Median mammary tumor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75th percentile</td>
<td>736</td>
<td>821</td>
</tr>
<tr>
<td>100th percentile (last mammary tumor)</td>
<td>937</td>
<td>1002</td>
</tr>
<tr>
<td>Maximum life-span</td>
<td>975</td>
<td>1008</td>
</tr>
<tr>
<td>Median life-span (mammary tumor or death)</td>
<td>682</td>
<td>691</td>
</tr>
</tbody>
</table>

CHART 2. Cumulative incidence of spontaneous mammary tumors (MT) in 5 groups of virgin female rats obtained from the Charles River Laboratories between September 1959 and June 1963.

CHART 3. Influence of the sacrifice of healthy nontumorous rats on the cumulative life-span incidence of MT. All rats were received in the same shipment (Lot 1). The 45Ca group suffered periodic losses to meet a predetermined sacrifice schedule; their controls (Lot I) were undisturbed.

The cumulative MT incidence of the 45Ca group from which healthy nontumorous rats were deliberately sacrificed to meet a predetermined schedule is compared with their undisturbed control group (Lot I) in Chart 3. The curves coincided until the 700th day and diverged thereafter. This divergence was statistically significant after the 750th day [the χ² test for goodness of fit (35) yielded a P value of 0.01]. In contrast, the cumulative MT incidence curves (not shown) of the 2 parts of Lot 14 were nearly identical. It was concluded that the early loss of close to 30% of the starting population had so altered the behavior of the 45Ca group that even actuarial corrections were grossly inadequate to reconstruct the MT incidence of an undisturbed population.

EFFECT OF UNIPARITY, THYROIDECTOMY, OVARIECTOMY, AND ADRENALECTOMY-OVARIECTOMY ON LIFE-SPAN MT INCIDENCE. It is apparent from Table 2 that neither uniparity nor thyroid deficiency influenced the percentage of rats that eventually developed MT. Examination of the quartile distribution of age at
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(37, 38) have described a simple conversion of cumulative incidence data which permits determination of the rate at which a colony is developing a specific lesion, in this case MT.

Percent survival without MT is calculated from the relation,

\[
\% \text{ survival without MT} = (100 - \% \text{ cumulative MT})
\]

The properties of the curves obtained by plotting \( \% \) survival without an MT, semilogarithmically, as a function of age have been described by Pilgrim and Dowd and are as follows: (a) similarities or differences in tumor morbidity are often revealed that were not apparent either from life tables or curves of cumulative incidence; (b) any straight-line portion indicates a constant rate of tumor morbidity over that portion; (c) curves that are concave upward indicate a rate of tumor morbidity that decreases with age; (d) curves that are concave downward indicate a rate of tumor morbidity that increases with age; (e) curves are parallel when the only effect is to delay the age at onset; (f) sharp changes in slope indicate age-dependent changes in the population; (g) the logarithm of the slope of the survival curve plotted as a function of age is the familiar Gompertz curve of force of mortality.

Survival curves for 5 control groups are shown in Chart 5. The data points are not given; however, the discontinuous straight lines shown fitted closely to the best smooth curves that could be drawn through the points. The survival curves of 4 of the 5 control groups (Lot 12 was exceptional) had several common features: (a) during the period from 300 to 500 days of age MT’s were bearing slowly at an average rate of 1.8%/month; (b) between the 480th and 520th day the rate of MT development increased sharply to 4.3%/month; (c) a 2nd increase in MT development rate to 9.5%/month occurred between the 630th and 690th day; (d) after the 800th day the rate of MT development decreased to 4%/month or less.

There were then at least 3 age-related changes in these rats—2 leading to abrupt increases in the rate of development of MT and 1 late in life leading to a decrease in the rate of MT morbidity.

In Chart 6 the %’s survival without MT of the UP, TX, and Lot A groups are compared with the survival of the combined

MT onset in Table 3 indicated that uniparous rats not only developed the same percentage of MT’s over the life-span, but also developed them at the same ages as virgin animals. Table 3 and Chart 4 indicated that while thyroid deficiency from the 84th day of life did not change the total life-span MT incidence, there was some difference in the age distribution of the tumors. From the 600th to the 850th day of life the TX group was developing fewer MT than the intact controls. Thyroid deficiency evidently delayed MT development during this period and in so doing prolonged the life of the animals by about 200 days.

As expected, ovariectomy (at 77 days of age) nearly eliminated MT. Only 3 MT that contained epithelial components were found in the 45 OX rats. Appearance of the 1st MT in the OX group was 200 days later than the 1st MT found in Lot A, the control group in which MT developed most slowly. The OX rats were very long lived; the maximum life-span of the group was 1295 days, nearly 25% longer than intact controls, and more than 1/2 the group lived longer than 1000 days. The life-span of a sufficiently large proportion of this group was certainly long enough to have permitted observation of late-appearing MT, if the effect of ovariectomy had been postponed development rather than prevention.

Removal of both the adrenal glands and the ovaries on or before the 110th day of life abolished MT of epithelial origin. Both the median and the maximum life-spans of the ADX-OX group were substantially greater than intact controls, as shown in Table 3. The maximum life-span was 1110 days, and 29% of these rats lived more than 1000 days.

RELATIONSHIP OF MT DEVELOPMENT TO AGE. The data in Table 3 and the curves of % cumulative MT incidence in Charts 2 and 4 bear out the oft repeated statement that MT occur most commonly in older rats (36). One of the aims of this study was to examine in detail the relationship between the age of the rat and the development of spontaneous MT. Pilgrim and Dowd

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CHART 5. Survival without an MT in 5 groups of virgin controls. Arrows indicate the range in age at which sharp changes in slope occurred.
CHART 6. Survival without a MT of the combined group of Charles River controls compared to MT morbidity of uniparous, thyroidectomized, and outbred (Lot A) rats. Arrows indicate the control range of changes in slope of the survival curves. These infections are labeled 1 and 2 on the control curve.

Charles River controls. The general shapes of the 4 curves are similar. The displacements of the infection points of the UP and Lot A curves from the combined control curve were not significant. Both major infection points (occurring at 500 and 630 days in the controls) were delayed 100 days in the TX rats, suggesting that thyroid deficiency delayed MT onset by delaying the process or processes responsible for initiating MT.

PROBABILITY OF DEVELOPING MT AS A FUNCTION OF AGE. The age-specific MT incidence rate is shown in Column 10 of Table 6 in units of % MT/rat at risk/month. For this calculation MT bearers were removed from the population at the end of the interval in which their MT developed. All rats dying without MT (Columns 2 and 3 of Table 6) were removed from the population halfway through the interval in which they died or were lost to follow-up. It can be seen from Table 6 that there were 30-day intervals in which no new MT arose, leading to discontinuities in the curve of MT development rate. A modification due to Spicer (48) of the rate of MT development, $\mu(x)$, was used to approximate the absolute rate of MT development. By virtue of an averaging and distributing process the function $\mu(x)$ has the properties of reducing fluctuations and eliminating some discontinuities. At any interval, $T_i$, $\mu(x)$ is calculated from

$$\mu(x) = \frac{MT_i + MT_{(i-1)}}{2N_i}$$

where $MT_i$ and $MT_{(i-1)}$ are the numbers of MT developing in the interval, $T_i$, and the previous interval, $T_{(i-1)}$, and $N_i$ is the population surviving $T_i$ days without an MT.

The curves of $\mu(x)$ plotted semilogarithmically as functions of age are shown in Chart 7 for the larger individual control groups (Lots 1, 14, and 17) and for the combined Charles River controls. The sharp changes in rate of MT development that were noted in the survival curves in Charts 5 and 6 were also detectable as inflection points in all 4 of the $\mu(x)$ curves in Chart 7.

Simms (47) has pointed out that because cancer of the sex organs and accessory structures is intimately related to age, morbidity curves for these tumors should not be straight lines but should show irregularities. The survival curves in Charts 5 and 6 suggested that a plot of $\mu(x)$ should be discontinuous, because they were composed of a series of nearly straight-line segments over which the slopes [equivalent to $\mu(x)$] were constant. After the 300th day, the $\mu(x)$ curves could indeed be interpreted as rising in a stepwise manner rather than increasing continuously.

INCIDENCE OF SUPERFICIAL TUMORS OTHER THAN BREAST TUMORS. In addition to the primary MT, some animals developed other breast tumors, or tumors of other tissues. Some rats came to autopsy with only tumors of nonbreast origin. The tumors of tissues remote from the breast will be discussed in a later report. Superficial tumors of skin or subcutaneous tissues, unrelated or only questionably related to breast, will be dealt with here.

For the purposes of this study only those tumors diagnosed as adenoma and fibroadenoma or mammary carcinoma were considered to have originated from the breast. Other authors have included fibrosarcoma and fibroma (34, 36) as breast tumors. However, we have chosen to include as MT only those tumors, arising near the mammary line, that contained some epithelial components. The superficial tumors arising in tissues other than breast and those tumors whose mammary origin was questionable are shown in Table 4.

There were 335 MT bearers among the intact rats (495 virgin females and 53 uniparous animals), yielding an over-all raw MT incidence of 61.1%. Of the 30 untreated rats that developed other superficial tumors 15 died without also developing MT. In the absence of histologic confirmation, inclusion of these 15 rats in the MT group introduced only a small error—2.8% (63.9% vs. 61.1%). It was therefore considered reasonable to include

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those control animals that bore only 1 superficial tumor as MT bearers, even though the tumor was not diagnosed. However, in 2 of the smaller experimental groups, OX and ADX-OX, inclusion of all superficial tumors as MT without histologic confirmation would have substantially changed the over-all MT incidence. Fortunately, all superficial tumors in these latter groups were diagnosed.

**INCIDENCE OF CARCINOMA.** The total numbers of MC and FA with carcinomatous pockets (MC in situ) that were observed among MT bearers in all groups are shown in Table 5. All but 4 of the MC were primary MT, i.e., the 1st MT observed in the rat. The % of MT bearers who bore at least 1 carcinoma was highly variable within the individual control groups. In the normal virgin control groups the % of MC ranged from none (Lot A, born in this laboratory) to a high of 37.5% (Lot 17). The over-all proportion of MC encountered among the 242 MT-bearing virgin rats born at the Charles River Laboratories was 18.6 ± 12.1%. The variability of MC among normal control groups indicates that all but the most dramatic shifts in proportions of benign and malignant MT should be viewed with skepticism. The experimental groups were compared with their nearest controls—TX with Lot 1, and UP with Lots 14 and 17—by use of the $\chi^2$ test (35). Neither uniparity nor absence of thyroid hormone appeared to influence the proportion of MC developed. The apparent difference in the proportion of MC between the virgin controls born several years ago and those rats obtained more recently suggests that the dealer may not be achieving the stated goal of random breeding.

**Huggins et al.** (24) and Cutts and Noble (9) have shown that when MT are induced by either hydrocarbon feeding or implantation of estrogen pellets, respectively, MC arises sooner after treatment than does FA. Our data were examined for indications of an earlier onset of spontaneous MC. The punched data cards of all MT bearers, except those in the TX and OX groups, were sorted according to histologic diagnosis. The FA and MC groups were further subdivided by age at MT onset into succeeding 60-day intervals to obtain a frequency distribution. Because of the small number of MC—55 MC compared with 257 FA—the frequencies of MT/interval were converted into %'s of the total number of each kind of tumor. Chart 8 showed that the frequency distributions of FA and MC with
Demonstrable difference in age at onset between MC and FA.

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300 days, only 1 was an MC. It appeared that there was no demonstrable difference in age at onset between MC and FA. Combination of all MT into a single population for tumor morbidity analysis was considered a valid procedure.

Discussion

It was of interest to compare the results of this study with the MT incidences observed for female Sprague-Dawley rats or descendants of the Sprague-Dawley line in other laboratories. The data available from the literature are shown in Chart 9 along with the curves for our combined Charles River controls and the group born at this laboratory (Lot A). Single MT incidence values were given by 3 authors: Davis et al. (10) obtained an MT incidence of 54.8% in a colony whose maximum life-span was 1100 days; their observed value is shown as a single point at 1000 days. Thompson et al. (51) observed a 42% MT incidence in a colony whose mean life-span was given as 630 days; their value is shown as a single point at 900 days. Syndor et al. (49) specified both the MT incidence and the age at which their single observation was made.

The age-incidence data of Shellabarger et al. (43), Vogel and Jordan (52), and Hartwig et al. (20) were more extensive; several points were available from each paper. The laboratory routine used by Shellabarger et al. was such that their age at MT appearance and that used in this study are very close. The other 2 authors (20, 52) made no mention of tumor size in relation to the age at which an animal was scored as an MT bearer. If the tumors were already large when seen, the placement of these data on the age scale of Chart 9 may err on the high side by as much as 60 days.

Spontaneous MT incidence observed over the past 10 years in other laboratories was within the variation we observed among control groups up to the 600th day. The results of Shellabarger et al. and Vogel and Jordan agreed with this report through the 700th day.

Vogel and Jordan's colony had not died out at the time their report was written, and complete life-span data were not available for their rats. However, rats in the Argonne Laboratory colony are raised under essentially the same conditions as the COBS rats from Charles River Laboratories, and continued agreement with our results is expected.

All the other authors, except Syndor et al., whose colony contained only young rats, reported a high mortality from pulmonary infection. The high rate of early deaths from lung disease rendered the results of Hartwig et al. almost meaningless. The x² test for goodness of fit (35) indicated that our crude life-span incidence of 61.7% MT was not different from that reported by Davis et al., 54.8%. Our actuarially corrected result, 71.5%, was significantly higher than any other so far reported.

Comparison of Charts 3 and 9 suggests that the early sacrifices in the 4Ca group and early losses of rats in other colonies from infections had the same net effect—a trailing off of the MT incidence at the beginning of the 3rd year. Inasmuch as we endeavored without success to correct for the early losses suffered by the 4Ca group, it would appear that if there is as much as a 30% loss of animals after the 400th day of life an intractable error is introduced into the life-span MT incidence. Smaller losses, such as those sustained by Lot I and Lot A, evidently can be corrected for without introducing a substantial error.

Our own experience with several normally aging groups purchased from a single supplier over several years, and the agreement of our results with those from other laboratories using rats bred by other suppliers (at least until pulmonary infections supervene), lead us to conclude that the MT incidence of the female Sprague-Dawley rat and its sublines is a characteristic which has remained stable through many generations. Not only is the MT incidence over the whole life-span reproducible, but MT incidence can also be predicted at any age within narrow limits.

As expected, hormonal manipulations that affect induced or implanted MT in rats also affected development of spontaneous MT in the same direction and to about the same degree. Ovariectomy postponed the onset of the 1st MT and reduced over-all MT incidence almost to the vanishing point. Simultaneous removal of the ovaries and adrenal glands prevented development of MT altogether. Uniparity was without effect, while thyroid deficiency slightly postponed MT development in the 2nd half of the life-span.

Furth and others (6, 18, 19, 28, 55, 57) have implicated the estrogen-stimulated pituitary and mammotropic (lactogenic) pituitary tumors in the etiology of carcinogen-induced and estrogen-induced MT in the rat. Although a full discussion of the relationships between spontaneous MT and age-related changes in the pituitary, ovaries, and adrenals is beyond the scope of this paper, some interesting coincidences are worth noting.

Spontaneous MT began to appear in significant numbers in

CHART 9. Comparison of the cumulative incidence of spontaneous MT observed in this laboratory with published results for Sprague-Dawley rats. Circled letters refer to the source of single values: ©, Davies et al. (10), ©, Syndor et al. (49), and ©, Thompson et al. (51).

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the latter half of reproductive life. There was a sharp upturn in the rate of MT development at about the time menopause is reported to occur in the rat—450 to 540 days of age (16, 54); preliminary examination of other tissues from the rats in this study indicated that pituitary hypertrophy and microscopic adenomas of the pituitary and adrenals begin to appear at about this same age. There was a 2nd marked increase in the MT incidence rate near the end of the 2nd year; during this period of life—from 600 to 800 days of age—the great majority of rats coming to autopsy had enlarged pituitaries and adrenals and bore microscopic or gross pituitary adenomas. Regardless of whether or not an MT was present the breast tissue was almost always highly developed, ranging from hyperplastic to actively secretory.

The dominant role played by pituitary mammotropes in the etiology of induced MT, as postulated by Furth and his co-workers, appears to be repeated in the case of spontaneous MT. It is suggested that the postmenopausal ovary provides an unruptured supply of estrogen (54) sufficient to stimulate the pituitary, which in turn provides the hormonal stimulation of the breast, leading to hyperplasia and secretion, and in a genetically susceptible strain such as Sprague-Dawley to spontaneous MT.

Addendum

The life table of mammary tumor development and of mortality without tumor is shown for the combined Charles River control group in Table 6. An explanation of the column headings in the Table follows:

Column 1. Age at end of interval: the age of the colony at the end of each 30-day actuarial interval.

Column 2. MT: the number of rats developing a 1st mammary tumor in the interval.

Column 3. Deaths: deaths of rats without tumor, including deaths due to other neoplasms or degenerative diseases, and deaths for which cause was not determinable.

Column 4. Excessive deaths: deaths of rats (without tumor) known to be totally unrelated to mammary tumor development, i.e., deliberate sacrifice of rats for other experiments, accidental deaths, or deaths from proven pulmonary infection.

Column 5. Corrected population: the corrected population, which takes into account loss of rats from extraneous causes (Column 4). This actuarial correction, due to Berkson and Gage (2), assumes that rats dying in the interval lived, on the average, for \(\frac{1}{2}\) the interval; Corr. pop. = Pop. at start of interval − 0.5 (Extran. deaths).

Column 6. Cumulative MT: total number of mammary tumor bearers to the end of this interval.


Column 8. Population at start: rats surviving without tumor to start of interval: Pop. at start1 = Pop. at start1 − (MT1 + Deaths1 + Extran. deaths1), where the subscripts refer to succeeding intervals, 1, 2, 3, 4, 5.

Column 9. Rats at risk for month: number of rats available to develop a 1st mammary tumor during interval: Rats at risk = Pop. at start − 0.5 (all deaths in interval).

Column 10. % MT/rat-month: % of rats at risk developing an MT during interval.

References


41. Shay, H., Harris, C., and Gruenstein, M. Influence of Sex Hormones on the Incidence and Form of Tumors Produced in Male or Female Rats by Gastric Instillation of Methylcholanthrene. Ibid., 15: 307–32, 1952.


44. Shellabarger, C. J., Lippincott, S. W., Cronicke, E. P., and Bond, V. P. Studies on Radiation-Induced Mammary Gland Neoplasia in the Rat. II. The Response of Castrate and Intact Male Rats to 400 r of Total-Body Irradiation. Ibid., 12: 94–102, 1960.

47. Simms, H. S. Logarithmic Increase in Mortality as a Manifestation of Aging. *J. Gerontol.*, **1**: 13–26, 1946.


Development of Spontaneous Mammary Tumors over the Life-Span of the Female Charles River (Sprague-Dawley) Rat: The Influence of Ovariectomy, Thyroidectomy, and Adrenalectomy-Ovariectomy

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