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CONSTRUCTION OF A GROWTH CURVE FOR MAMMARY FIBROADENOMA IN THE FEMALE RAT*

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June 3, 1965

Abstract

An empirical growth curve for mammary fibroadenomata of the female Sprague-Dawley rat has been constructed. Fibroadenomata used to construct the growth curve included 25 spontaneous tumors arising in normal controls and 65 radiation-induced tumors. Growth data were obtained both by measurement of external dimensions and from autopsy tumor weight. Tumor growth from 0.42 to 200 g could be represented by three (and possibly four) positive exponentials, each defined over certain limits.

The growth pattern of fibroadenomata was highly variable, reflecting the degree of morphological variation both among individual fibroadenomata and within each individual tumor. Growth rates varied so widely that it was not possible to distinguish differences, if such exist, between (a) the growth rate of carcinomata and the "average" fibroadenoma; (b) the growth of secretory and nonsecretory fibroadenomata; or (c) the growth of spontaneous tumors arising in old rats and radiation-induced tumors in young rats. Neither was it possible to estimate the general growth behavior of a fibroadenoma from scoring tests on a single histological section.

Suitability of other mathematical descriptions of tumor growth, particularly the Gompertzian function, to the growth of mammary fibroadenomata is discussed.

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Introduction

Spontaneous mammary tumors of the female rat have been under investigation for more than 60 years.[1-5] Wistar rats and their derivatives, particularly the Sprague-Dawley strain, have been the subject of most of these investigations.[6-11] The morphology of these tumors has been described--they are largely benign fibroadenomata.[2,3,7-9]

While most reports indicate that mammary tumors usually arise in "older" rats no study of the life span incidence of these tumors has been possible until recently. Unless heroic measures were employed to reduce infections,[12] rats in most colonies had an average life expectancy of less than 2 years, because of early deaths from parasite and bacterial infections, particularly pulmonary infections.[13-14] When Cesarean-Originated Barrier Sustained "COBS" rats became available in large quantities from commercial breeders, it was possible to study spontaneous tumor incidence in an essentially disease-free rat colony with a potential life span of nearly 3 years.

Such a life-span study of the incidence of tumors and other lesions in the aging female rat has been completed in this Laboratory, and the results will be reported elsewhere.[15] In the preparation of actuarial tables of tumor morbidity[16,17] required for analysis of the tumor incidence data, it became apparent that a large error was being introduced when the age at which a mammary tumor was first seen grossly was recorded as the time of tumor onset. Visual observation and palpation on a once-a-month basis had evidently failed to disclose the early presence of many tumors. Some tumors were not seen for the first time until they were several centimeters in diameter and had obviously been present for more than 30 days.

This uncertainty in the time of tumor onset meant that the time interval over which tumor incidence probabilities were to be calculated had to be of the order of 3 months to meet the requirement that all tumors recorded during the interval actually developed during that interval and not at some earlier time.[17] If a growth curve for the "average" fibroadenoma could be constructed, then the time of appearance of a tumor could be determined from the age of the animal and autopsy tumor weight, thus allowing all tumors to be referred back to some common "starting" point. The error in time of appearance could then be made small enough (hopefully, less than 30 days) to meet another actuarial requirement, i.e., there would be a low probability that animals dying of extraneous causes during the selected actuarial interval would have developed a tumor during the interval.[17]

This paper describes the construction of an empirical growth curve for the "average" fibroadenoma of the rat. If the age of the animal at autopsy and the final weight of tumor (or the dimensions of the tumor) were known, then the age of the animal at tumor onset could be estimated.

Methods

The mammary tumors included in this growth study arose spontaneously or were artificially induced by ionizing radiation. All together 160 rats were observed: 50 normal controls and 110 rats that had received a single injection of the α-particle emitter At^{211}.[10,18] The At^{211}-injected rats were females of the Sprague-Dawley strain purchased before 1959 from the original colony at Madison, Wisconsin. The normal controls were "COBS" Sprague-Dawley females purchased after late 1959 from the Charles River Breeding Laboratories, North Wilmington, Massachusetts.

All rats were of known age. They were earmarked for identification on arrival and housed in plastic cages on sterilized wood shavings. Rats were initially grouped five to a cage, but as they grew larger, the number per cage was reduced so that the largest animals were housed in pairs. Purina Laboratory Chow and tap water were fed ad lib.

All animals were examined and palpated for mammary tumors once a month. The age at which a mammary tumor was first seen was recorded. In the course of another experiment, the external dimensions of some mammary tumors were measured with calipers to the nearest 0.1 cm. If the tumor was spherical in shape, an average diameter was recorded. For nonspherical tumors the largest and smallest diameters were recorded and the volume was calculated from the formula for an oblate spheroid.

In a previous experiment it was found that tumor volumes derived from external measurements were reliable to ±13% when the measured diameter was reduced by 10% to correct for the thickness of the double layer of skin encountered in the surface measurements.[19]

Measurements were repeated periodically until the animal died or the tumor had grown to sufficient size to warrant resection or sacrifice of the animal. At autopsy all tumors were dissected and weighed to the nearest 0.1 g. A section was prepared for microscopic examination by routine methods.

Results

Of the 160 rats originally under observation, 90 eventually developed mammary tumors. The tumors included in the growth curve are 25 from normal controls and 65 from At^{211}-injected rats. None the mammary tumors in control rats were first seen until after the animals were 400 days old, but 70% of the tumors arising in At^{211}-injected rats appeared before 400 days of age. At autopsy the tumors ranged in weight from 0.7 g to 161 g.

More than 80% of the tumors arising in these animals were fibroadenomata with varying proportions of fibrous and glandular components. The tumors that arose in animals more than 400 days of age usually contained a variable amount of milk-like secretion. Secretion was rare in tumors arising in young animals. Inasmuch as fibroadenoma was the most common mammary tumor, only tumors that were diagnosed microscopically as fibroadenoma were used to construct the growth curve.

We have chosen as the common "starting" point for all tumors that stage in development when a tumor has a diameter d_0 of 1 cm, and assuming

unit density for tumor tissue, a weight w_0 of 0.5 g. The age of the rat when a mammary tumor first appeared, T_a, was thus defined at d = 1 cm, or w = 0.5 g. This tumor size was chosen as the reference point for the practical reason that tumors smaller than 1 cm were usually overlooked in unanesthetized rats, or they were confused with normal subcutaneous structures.

Even with frequent and careful examination only 43 of the tumors (47%) included in this study were first observed when $d \leq 1$ cm. Complete descriptions of growth (as determined by successive external measurements from T_a to autopsy) were obtained only for the 12 tumors shown in Fig. 1. The highly variable growth patterns of individual fibroadenomata are apparent.

Autopsy ages and final weights were available for another 31 tumors that were first seen at T_a, but were not measured externally thereafter. The preliminary estimate of mammary tumor growth shown in Fig. 2 includes only data from these 43 tumors for which T_a was known; the 12 tumors shown in Fig. 1 provided 33 data points based on external measurements; and 43 data points that were based on autopsy tumor weights. Tumor weights were plotted on a semilog scale as a function of the tumor growth interval, GI, the number of days a tumor had been growing since T_a:

$$GI = (T_t - T_a) \text{ days}, \tag{1}$$

where T_a is the age of the animal at reference tumor size, d = 1 cm, and T_t is the age at a subsequent external measurement or at autopsy. A semilog scale was used largely for convenience. Average tumor GI were calculated over several ranges of tumor weight. A plot of these points (the open circles in Fig. 2) suggested that the best fit to the data was a pair of intersecting straight lines.

The equations of these lines fitted by least squares[20] are

$$0<GI<43 \text{ days}: \quad W = 0.52 \exp(0.693\ GI/12.5) \text{ g}; \tag{2a}$$

$$44<GI<120 \text{ days}: \quad W = 1.65 \exp(0.693\ GI/25) \text{ g}; \tag{2b}$$

standard error of estimate = ±21.2 days.

In about 70% of cases, the actual value of GI (and consequently of T_a) was within one standard error of estimate, and in 95% of cases, within 2 S. E.[21] of the regression of tumor weight on GI as given in Eqs. 2a and 2b.

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[20] The regression equation of GI on W, x, and y, respectively, and its standard error of estimate, S_x, were calculated from statistical equations given in reference 21. The regression equation is calculated from

$$(x - \bar{x}) = r\left(\frac{\sigma_x}{\sigma_y}\right)(y - \bar{y}),$$

where r, the correlation coefficient, is $r = \frac{1}{N}(x-\bar{x})(y-\bar{y})/\sigma_x\sigma_y$, and the variance of x and y are $\sigma_x = \sqrt{(\frac{1}{N}x^2)-\bar{x}^2}$, $\sigma_y = \sqrt{(\frac{1}{N}y^2)-\bar{y}^2}$. The standard error of estimate of the regression line (and the uncertainty in GI is given by

$$S_x = \sigma_x\sqrt{1 - r^2}.$$

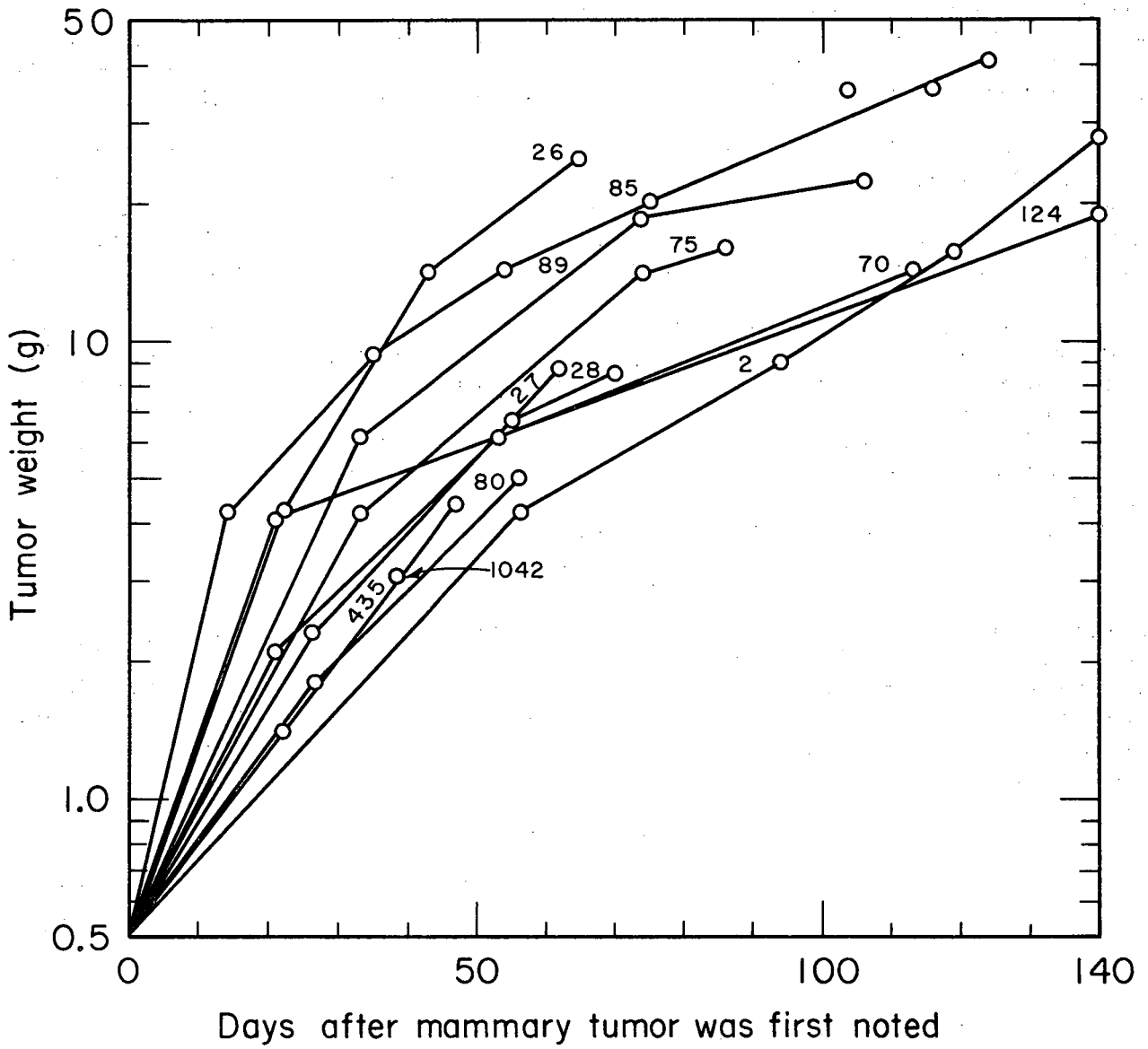


Fig. 1. Growth curves of individual fibroadenomata.

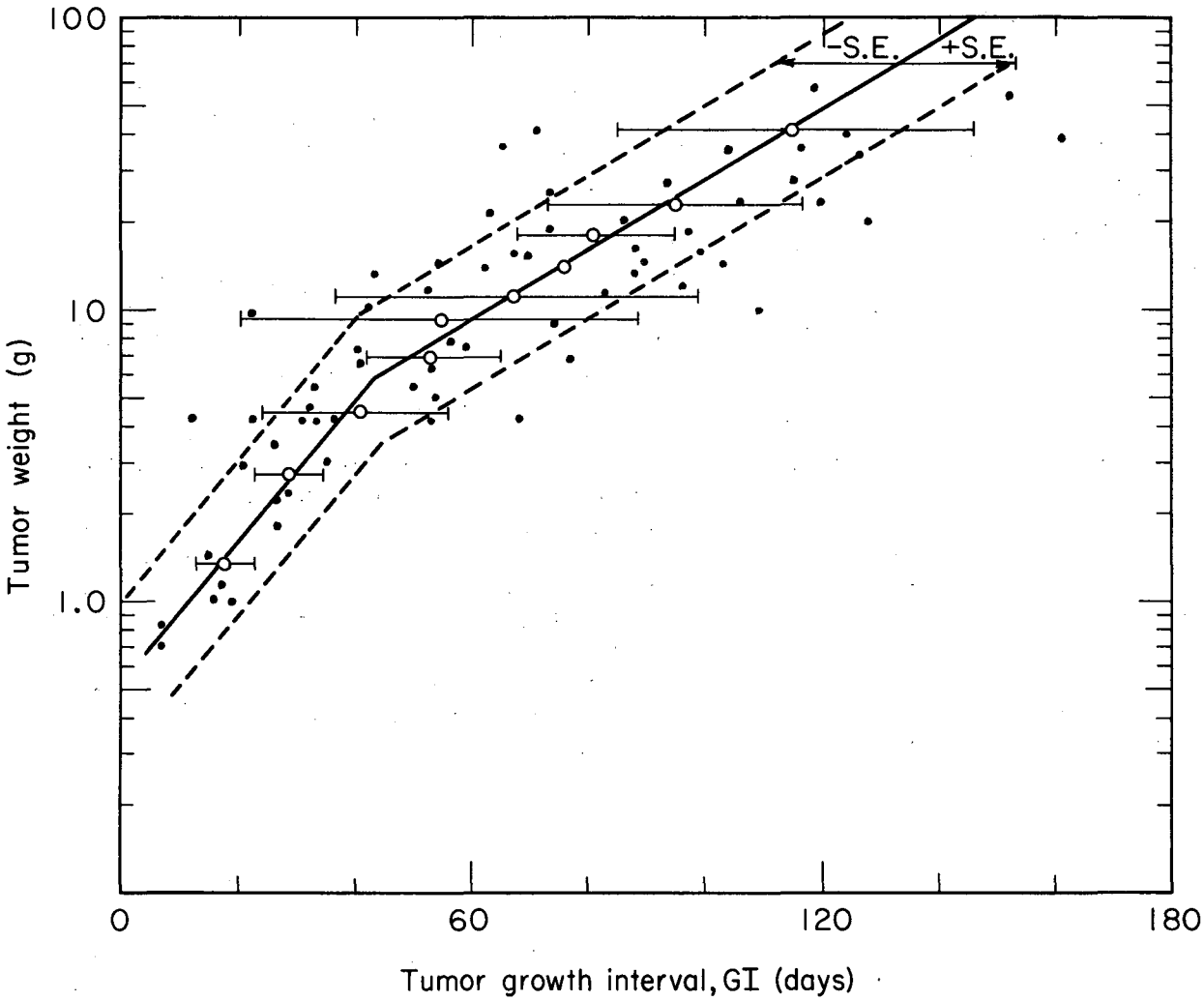


Fig. 2. Preliminary growth curve of fibroadenoma based solely on data from tumors for which T_a was known, i.e., tumors that were first seen when $W \leq 0.5$ g.

Thus, GI estimated from Fig. 2 for tumors weighing less than 6 g was 95% reliable within a range of ±23 days. For tumors larger than 10 g, the confidence limits of GI spread to ±42 days. Beyond a weight of 30 g the shape of the preliminary tumor growth curve was uncertain, since there were only nine data points.

Because of the highly variable growth patterns of individual tumors and the lack of direct information on growth of large tumors, it was necessary to augment the data shown in Fig. 2 by including some tumors whose growth records were not complete.

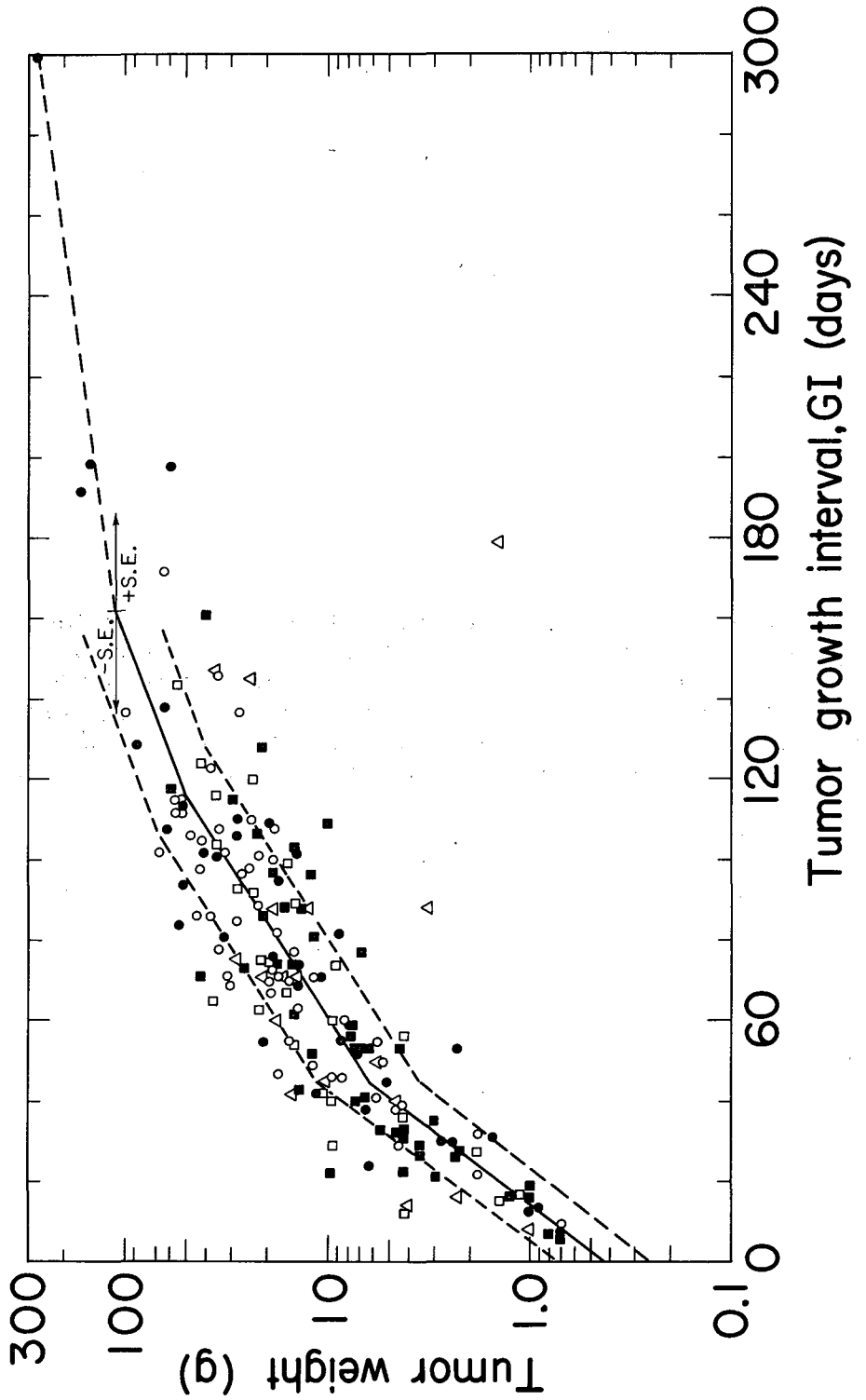
Forty-seven tumors that eventually grew quite large were first observed when their estimated weight was more than 0.5 g but still less than 10 g. Autopsy ages and final tumor weights were known. Periodic *in situ* measurements of some of these tumors provided 39 more points. The age when the tumor was first observed was corrected to the reference age T_a by using Fig. 2. The error introduced in estimating T_a for these tumors was small. For example, when GI was estimated for a 40-g tumor first seen at a weight of 4 g, the error introduced was ±11.5 days, or about 10% of the total GI of 108 days. Growth intervals were calculated for each of these 47 additional tumors, and GI was plotted against log tumor weight as shown in Fig. 3. Figure 3 includes 76 points derived from *in situ* measurements or final tumor weights or tumors for which T_a was known, and 86 points derived from measurements and final weights of tumors for which T_a could be approximated without introducing an additional error of more than 10%. Growth of the "average" fibroadenoma could be represented by the three least-square-fitted lines shown in Fig. 3. The two initial segments were not distinguishable from the preliminary curve of Fig. 2. The equations of the lines in Fig. 3 are:

$$0<GI<44 \text{ days}: \quad W = 0.42 \exp(0.693\, GI/11)\text{g}, \quad \text{S. E.} = \pm 8.8 \text{ days}; \tag{3a}$$

$$45<GI<117 \text{ days}: \quad W = 1.56 \exp(0.693\, GI/23)\text{g}, \quad \text{S. E.} = \pm 19.8 \text{ days}; \tag{3b}$$

$$118<GI<200 \text{ days}: \quad W = 7.13 \exp(0.693\, GI/41)\text{g}, \quad \text{S. E.} = \pm 27.1 \text{ days}; \tag{3c}$$

Fig. 3. Composite growth curve for the "average" fibroadenoma in the female Sprague-Dawley rat. Squares (□) are those tumors for which T_a was known; circles (O) are those tumors for which T_a was approximated; triangles (Δ) are tumors that were diagnosed as carcinoma. Open figures for which $T_a < 400$ days of age; closed figures are those tumors for which $T_a > 400$ days of age.


Tumor weight (g)
300
100
10
1.0
0.1
0
60
120
180
240
300
Tumor growth interval, GI (days)
+S.E.
-S.E.
MUB-6722

Growth data were available for four tumors that were allowed to attain a weight of more than 100 g, but these were not included in the computation of the least-squares fit of the curve shown in Fig. 3. The data points are plotted in Fig. 3, and their positions on the plot suggest that a fourth exponential term should be added. The fourth term, shown as the dashed line in Fig. 3, is

$$200<GI<300: \qquad W = 38.0 \exp(0.693\ GI/106)\text{g}. \tag{3d}$$

The three-segment curve is Fig. 3 was also plotted on a linear scale, and the best fit to the curve was a third-order polynomial equation,

$$W = 0.5 - 2.3\times10^{-3}GI + 2.2\times10^{-3}GI^2 + 1.2\times10^{-5}GI^3 \text{ grams}. \tag{4}$$

Laird recently suggested[22] that a Gompertzian function might be a suitable representation of tumor growth, because both approach a limiting value. Therefore the parameters of the Gompertzian function,

$$W = W_0 \exp[(A/a)(1 - e^{-at})], \tag{5}$$

were determined for the growth of fibroadenoma by machine fitting[23] both the curve in Fig. 3 and the original data points. The parameters determined by fitting the curve in Fig. 3 were $W_0 = 0.4$ g, $A = 0.071$, and $a = 0.010$. The point-to-point differences between Fig. 3 and the fitted Gompertzian were small, usually only a few percent, indicating that the agreement was good. The parameters obtained by fitting the original data points were similar, $W_0 = 0.3$ g., $A = 0.071$, and $a = 0.0097$.

Effect of Secretory Activity

As noted above, most of the tumors arising in rats more than 400 days of age contained some secretory material, while secretion was rare in the tumors induced by At^{211} in the younger rats. It was of interest to test whether the growth rate of a secretory tumor was demonstrably different from the growth rate of a tumor that was nonsecretory. Accordingly the data were regrouped according to whether the rats were less than 400 days old or more than 400 days old at tumor onset. Each of the two subsets of data were fitted by least squares to three positive exponentials over the tumore weight ranges of Eqs. 3a, 3b, and 3c. The equations of these regression lines are given in Table I. The first two segments of both curves are

Table I. Regression analysis of growth of mammary tumors arising in young rats, < 400 days old, and older rats, >400 days old.

Interval	Regression equation	Standard Error of estimates
Rats less than 400 days old		
0 < GI < 50 days:	W = 0.47 exp(0.693 GI/12)g;	S. E. = ± 9.2 days
51 < GI < 126 days:	W = 2.4 exp(0.693 GI/28)g;	S. E. = ± 17.6 days
127 < GI < 200 days:	W = 2.45 exp(0. 693 GI/22)g;	S. E. = ± 21.7 days
Rats more than 400 days old		
0 < GI < 37 days:	W = 0.33 exp(0.693 GI/10)g;	S. E. = ± 8.4 days
38 < GI < 126 days:	W = 1.5 exp(0.693 GI/24)g;	S. E. = ± 20.6 days
126 < GI < 200 days:	W = 7.9 exp(0.693 GI/43)g;	S. E. = ± 30.2 days

identical up to a tumor weight of 50 g. Nonsecretory tumors larger than 50 g arising in young rats appeared to continue growing at the same rate as smaller tumors, while the large tumors in older rats (and these almost always contained secretion) appeared to be growing at a rate only half as fast as they had from 6 g to 50 g. However, this difference between the growth of tumors larger than 50 g arising in young and old rats may not be real, inasmuch as the slope of the fitted line for tumors in young rats is determined for the most part by the cluster of tumors that had reached 50 g weight in 100 to 120 days; unfortunately, only two tumors that arose in young rats were allowed to grow to more than 50 g weight.

Growth of Carcinoma

Only a small fraction of spontaneous mammary tumors were classified microscopically as pure carcinomas, as distinguished from fibroadenomata with sarcomatous change or fibroadenomata containing carcinoma in situ. Since they constituted almost 20% of the total tumors,[15] carcinomas had to be included in any analysis of mammary tumor incidence. It was necessary to determine whether the growth of the carcinomas was comparable to that of fibroadenomata. Pathological and growth records of several hundred mammary tumors were searched, and adequate growth records were found only 18 carcinomas. The observed growth intervals for these carcinomas are compared in Table II to the growth interval that would have been estimated (from Fig. 3) for a fibroadenoma of the same size. The individual data points for the carcinomas have also been plotted in Fig. 3. The growth intervals of 14 of the 18 carcinomas were within two standard errors of estimate of the curve for fibroadenoma, and three of the other four carcinomas grew much more slowly than the average fibroadenoma.

Morphology and Growth Rate

In a study of the growth of transplanted rat fibroadenomata, Millar and Noble found that a tumor line with nearly homogeneous morphology grew at the same rate over several implant generations.[24] Their results also suggested that the more fibrous implants grew more slowly than those that were largely epithelial. A blind test was devised to learn whether it was possible to predict the growth rate of a fibroadenoma--fast, average, or slow--from microscopic examination of a single central section. The labels of 45 slides were covered. The slides were scored on a numerical scale of four for each of the following characteristics: (a) proportion of fibrous or epithelial tissue, (b) overall cellularity, (c) mitotic figures, (d) secretory activity, (e) necrosis, (f) presence of cysts or hemorrhage. On the basis of these scores for each individual characteristic and for combinations thereof, an attempt was made to predict the general growth pattern. The results were inconclusive. None of the tests applied was able more than 50% of the time to predict whether a fibroadenoma would fall into a fast, average, or slow category.

Table II. Growth of mammary carcinoma compared to fibroadenoma of equal final tumor weight.

Rat number	Tumor diagnosis	Weight y (g)	Tumor growth interval (days)	
			Observed[a]	Estimated from Fig. 3
152	CA	13.9	71	73
71	ADCA[a]	1.4	179	20
2861	ADCA	1.0	8	14
2883	CA	2.3	16	27
913	Pap CA	3.1	88	33
923	Pap CA	18.9	88	83
953	Pap CA	4.1	14	37
930	CA	15.1	42	75
952	CA	4.5	40	39
21	CA	34.7	146	103
15	ADCA	20.2	77	85
104	CA	13.7	88	71
2893	ADCA	5.4	50	42
2863	CA	10.2	45	62
1447	CA	28.4	75	96
2278	ADCA	18.1	60	81
2305	ADCA	17.4	71	80
1169	CA	23.5	145	90

a. Ulcerated and necrotic.

Discussion

These experiments indicated and other authors have also shown[2,3,7,24] that individual fibroadenomata are morphologically highly variable. Different areas of a single tumor vary from closely packed glandular tissue, through areas of ducts dispersed in connective tissue, to areas of almost pure connective tissue. Individual tumors may show varying degrees of necrosis and (or) secretory activity, or they may be undergoing sarcomatous or carcinomatous changes, or they may show areas of carcinoma in situ. Many of these morphological variations can often be seen in a single microscopic section. It is this enormous variability in both cell type and organization that apparently underlies the great variability in growth patterns. If differences in growth rate exist between carcinoma and the "average" fibroadenoma, they were masked, both by the wide range of growth rates of the individual fibroadenomata that comprised the growth curve and by the small sampling of carcinomata. Similarly, if there were differences in the growth rates of secretory and nonsecretory fibroadenomata, or differences in the growth rates of tumors arising in old rats and young rats, or differences between the growth rates of spontaneous and radiation-induced fibroadenomata, they may also have been masked by the inherent variability of the tumor.

Morphological variation within regions of a single tumor probably also accounts for our inability to predict overall growth rate of a tumor from the microscopic appearance of a single section.

The average growth rate of fibroadenomata represented by Fig. 3 agreed as far as we were able to determine with the growth of fibroadenoma transplants reported by Millar and Noble.[24] Their method of reporting tumor growth was so different--the area of the largest cross section--that direct comparison was difficult.

We have chosen a relatively simple mathematical expression to describe the growth of the "average" fibroadenoma in the rat, namely, three (and probably four) positive exponentials defined within limits. Other mathematical expressions fit these data equally well, but they are more complicated to work with. A third-order polynomial (Eq. 4) could be fitted to the data at least for tumors weighing less than 70 g. A Gompertzian (Eq. 5) could be fitted over the entire range of available data, and the values obtained for the three parameters were within the range found by Laird for some other solid soft-tissue tumors.[22]

Since at least three different mathematical expressions could be fitted to the growth data for fibroadenomata with roughly equal precision, the limiting factor became the variability of the growth rates of individual tumors. This variability introduces a sufficiently great uncertainty in estimating tumor growth from any "average" curve that the choice becomes mostly a matter of taste. The Gompertzian although difficult to handle without a computer, is probably the best choice. It has the special property--namely, the approach to an asymptotic limit--that agrees with the observed behavior of this particular tumor.

Glenn et al.[25] observed the growth of a mammary fibroadenoma implanted in female Sprague-Dawley rats. Small pieces of the tumor weighing 50 to 90 mg were implanted intramuscularly, and final tumor weights were determined at times up to 90 days after implantation. Growth was relatively slow during the initial 30 days as the implants became established. After attaining a size of 0.5 g, the implants grew at rates within the range reported here for spontaneous fibroadenomas.

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