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Growth analysis of pulmonary metastases in malignant melanoma

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ABSTRACT

Growth rates of pulmonary metastases in thirty patients with malignant melanoma were characterized by calculating Gompertz growth constants and tumor doubling time. No correlation between growth parameters, tumor doubling time, presence of tumor regression, extent of parenchymal involvement at time of diagnosis, or response to chemotherapy as indicated by radiographic appearance was found. Possible explanations for these findings are discussed. The use of the Gompertz growth curve and the exponential growth curve to describe tumor behavior is studied and discussed.

INTRODUCTION

Malignant melanoma is a relatively uncommon disease, but its incidence worldwide is increasing. For 1983, there will be an estimated 17,400 new cases in the United States, about 2% of the estimated 855,000 new cases of noncutaneous cancer expected. There will also be 5,200 deaths due to this disease.[66] Despite the rarity of melanomas, two to fifteen percent of all cases of pulmonary metastasis are due to this neoplasm.[17, 32, 37, 72]

Nearly all patients with disseminated melanoma will have pulmonary involvement and in some reports the major cause of death has been respiratory failure, presumably due to parenchymal metastases.[18] The chest radiograph often provides the first sign of disseminated disease[29, 76] and periodic x-rays are often an important part of the follow-up in patients judged to be at high risk for metastases. The chest x-ray is also used as an objective measure of a patient's response to chemotherapy once pulmonary metastases are diagnosed.

This study was undertaken to investigate the relationship between growth rate and patient survival after the diagnosis of pulmonary metastases. The significance of tumor regression and response to chemotherapy will be examined. In addition some aspects of the Gompertz growth curve and the exponential growth curve will be compared.

PATTERNS OF METASTASIS

Malignant melanoma may metastasize to all organs of the human body and it is almost impossible to predict which organ system will be invaded from a given primary site. Multiple organ involvement is common in advanced melanoma. Patel et al.[55] reported that only 1-4% of their cases had single organ metastases at the time of death. Tables 1-4 summarize the findings of several investigators.

Gromet et al.[29] published a prospective study of 324 patients with malignant melanoma over a twenty-four month period. Thirteen of the 324 patients experienced dissemination and of these the thorax was the initial site of metastasis in twelve (92%). Webb and Gamsu[76] retrospectively evaluated 65 patients with thoracic metastases. Chest radiographs revealed abnormalities in 63 of the 65 patients and in 42 patients the chest film provided the first objective evidence of metastases beyond regional lymph nodes. Table 5 compares the radiographic findings of Webb and Gamsu and of Chen et al.[9]

MATERIALS AND METHODS

Patients for this study were obtained from two sources. The Melanoma Clinic at Yale-New Haven Hospital provided a population of fifty patients with known thoracic metastases seen since 1980. In addition, the Connecticut Tumor Registry provided data on all patients who had been diagnosed as having malignant melanoma in Connecticut from 1970 through 1982, a total of 502 cases. There was considerable overlap in the two sources except for patients seen in the Melanoma Clinic who did not have their melanoma diagnosed in Connecticut and for patients who were diagnosed prior to 1970.

From this population a total of 30 patients were found who fulfilled the following criteria:

1. Histologically proven malignant melanoma
2. Serial chest films of adequate technical quality which demonstrated pulmonary metastases.
3. Adequate medical records to allow accurate dating of diagnosis of metastasis and subsequent follow-up.

Only upright PA chest films were used; x-rays which employed other techniques, e.g. portable or supine films, were not included. Films with marked differences in rotation or inspiratory effort were also excluded. Single and multiple parenchymal nodules were the objects of major interest. Those roentgenograms with only extra-parenchymal evidence of metastases were excluded as well as those which demonstrated the "mili ary" or "snowstorm" pattern of metastases. Films

in which nodules were obscured (by pleural effusion, for example) were also not used.

Individual pulmonary nodules were measured using calipers and a centimeter rule. Measurements to the nearest millimeter were taken along the axes of greatest and least diameter unless the lesion was circular, in which case only one measurement was made. Lesions in which the borders were subjectively judged to be too indistinct for accurate mensuration were omitted. Measurements from the chest films of a given patient were taken by the same viewer so that inter-observer variation would not be introduced. Intra-observer variation cannot be eliminated. This variation was not calculated although an attempt was made to estimate the error inherent in the measurement process (see results). Clear overlays or masks made by developing unexposed radiographic film were used as an aid in identifying and following the nodules. The mask was placed over an x-ray and the nodules outlined and identified. The mask made it easier to identify each nodule for measurement, to judge if there had been any gross change in the size of the lesions, and to more readily determine if a nodule had been seen previously or was a new metastases.

The films of thirty patients, sixteen males and fourteen females, were studied. There were twenty-nine whites and one black. Average age at time of initial diagnosis was 49.3 years for all patients, 53.2 for males and 44.8 for females. The ages ranged from 19 years to 69 years. Average age at

diagnosis of pulmonary metastases was 51.9 years overall, 55 years for males and 48.4 years for females, with an age range of 24 to 70 years. At time of diagnosis eighteen patients were in clinical Stage I, five in Stage II, six in Stage IV and one was unspecified.

Of the thirty patients in the study, nine were alive according to the most recently available medical records. The rest had succumbed with evidence of active disease at death. The mean survival time of the twenty-one deceased patients was 9.8 months from diagnosis of pulmonary metastases. Table provides further information on each patient.

The time encompassed by the series of chest films used to evaluate pulmonary metastases in these patients ranged from 25 days to 1251 days, with a mean of 260 days.

Growth parameters were calculated from the acquired data by applying the equations of the following section. Patients were separated into prognostic categories and Kaplan-Meier[34] estimates of survivorship were determined for each category and then compared by use of the log-rank test.[56] Survival time in each patient was defined as the period from diagnosis of pulmonary metastases to death or to end of observation.

Multiple nodules were treated as the summation of independently behaving single nodules. The value of doubling time or of the Gompertz growth parameters attributed to a patient was, unless otherwise indicated, the mean of those parameters as calculated for each individual nodule.

MATHEMATICS USED IN GROWTH ANALYSIS

The mathematical characterization of the growth of tumors has interested investigators for many years. In 1956, Collins et al.,[12] presented a graphical method for the estimation of tumor doubling time which was based on the hypothesis that the simplest view of malignant cell growth was to assume that each cell divided into two cells at a constant rate, i.e., one cell became two, each of the resulting two divided so that there were now four cells, then eight, then sixteen, etc. This would result in the growth curve seen in Figure 1. From the measured diameter of a nodule at two different times the doubling time could be calculated (Figure 3) with the aid of semilogarithmic graph paper.

Subsequently, Schwartz[63] derived an approximation formula for doubling time which could be substituted for graphical methods on semilog paper. The equation was:

$$t_D = \frac{t}{10 \log (D_t/D_o)} \qquad (1)$$

where t_D = doubling time

t = time interval between first and second measurements

D_t = diameter at second measurement

D_o = diameter at first measurement.

As data of the actual growth patterns observed in tumors accumulated, the validity of the assumption of exponential growth at a constant rate was questioned. Laird,[39] after surveying the available literature, pointed out that exponential

growth of tumors had been observed only rarely and then for only brief periods. When most tumors are observed over a sufficiently extensive range of growth, they are found to expand at a progressively slower rate as the tumor enlarges with no appreciable period of growth at a constant rate as sould be expected for simple exponential growth. In most cases, tumor growth is smoothly curvilinear on a semi-log plot throughout growth. This implies that the specific growth rate of tumors is usually not constant even for a short period of time, but decreases steadily.

This pattern of growth has been found in many biological systems in addition to malignancies and is expressed by the Gompertz function:

$$S(t) = S(0)e^{\frac{\beta}{a}(1-e^{-\alpha t})} \qquad (2)$$

where $S(t)$ = tumor size at time t (size may be defined as weight, volume, or number of cells)

$S(0)$ = initial tumor size

are constants.

If in equation 2, $e^{-\alpha t}$ is expressed as a power series in αt, then for the case where αt is small, the growth function reduces to:

$$S(t)/S(o) = e^{\beta t} \qquad (3)$$

that is, the growth function describes simple exponential growth.

It is also evident from equation 2 that as the value of becomes large, the Gompertz function approaches a horizontal asymptote whose value is defined by

$$S_{Max}/S(o) = e^{\beta/\alpha} \tag{4}$$

where S_{Max} = theoretical upper limit of tumor size.

If equation 4 is substituted into equation 1, the result is:

$$S(t) = S_{Max} e^{-\frac{\beta}{\alpha} e^{-\alpha t}} \tag{5}$$

or

$$S(t)/S_{Max} = e^{-\frac{\beta}{\alpha} e^{-\alpha t}} \tag{6}$$

$$S_{Max}/S(t) = e^{\frac{\beta}{\alpha} e^{-\alpha t}} \tag{7}$$

$$\ln(S_{Max}/S(t)) = \frac{\beta}{\alpha} e^{-\alpha t} \tag{8}$$

$$\ln \ln(S_{Max}/S(t)) = \ln \frac{\beta}{\alpha} - \alpha t \tag{9}$$

Thus growth can be depicted rectilinearly with slope of if $\ln \ln [S_{Max}/S(t)]$ is plotted as a function of time.

Spang-Thomsen et al, further transformed equation 9 by showing experimentally that the measurements of the three diameters of a tumor are in proportion during growth, and as $d_1(t) = d_2(t)$ on average

$$d_3(t) = kd_1(t) = kd_2(t). \tag{10}$$

If two-dimensional tumor size, A(t), and volume V(t), are given respectively by

$$A(t) = d_1(t)\ d_2(t) \tag{11}$$

$$V(t) = \frac{4\pi}{3} \frac{d_1(t)}{2} \frac{d_2(t)}{2} \frac{d_3(t)}{2}$$

then

$$V(t) = \frac{4\pi}{3}\left(\frac{A(t)}{4}\right)^{3/2}\left(\frac{d(t)}{2}\right)^{3/2} = \frac{\pi}{6} k\, A(t)^{3/2} \tag{12}$$

Since the square of V(t) is proportional to the cube of A(t), equations 1 and 9 can be transformed to

$$A(t) = A(o)e^{\frac{\beta_A}{\alpha}(1-e^{-\alpha t})} \tag{13}$$

$$\ln \ln(A_{Max}/A(t)) = \ln \frac{\beta_A}{\alpha} - \alpha t \tag{14}$$

where $$\beta_A = \frac{2}{3}\beta \tag{15}$$

and $$A_{Max} = A(o)\, e^{\frac{\beta_A}{\alpha}} \tag{16}$$

The value of may be calculated if the two-dimensional tumor size is known at two different points in time by utilizing equation 14. For example, if the tumor was measured at times t_1 and t_2 with resulting values of $A(t_1)$ and $A(t_2)$ respectively, then

$$\ln \ln \frac{A_{Max}}{A(t_1)} - \ln \ln \frac{A_{Max}}{A(t_2)} = (t_2 - t_1). \tag{17}$$

One difficulty with using the various forms of the Gompertz function is that the values of S(0), S_{Max}, A(0), A_{Max}, $\frac{\beta}{\alpha}$ or of $\frac{\beta_A}{\alpha}$ are not known. There are several methods by which estimates of these values may be obtained but the one used by Lloyd[40] is perhaps the simplest. An initial estimate of A_{Max} is made and then the observed values of A(t) are plotted using equation 14. If the estimate of A_{Max} is high, the plotted points will be displaced upwards from a straight

line at larger values of t. Conversely, if the estimate is low, the points will exhibit a downward curvature. Spang-Thomsen et al., used the method of Lloyd to determine values of A_{Max} for three human malignant tumors, including a malignant melanoma, transplanted to nude mice. The estimates of $\ln A_{Max}$ ranged from 6.2 to 8.5 with a mean value of 7 corresponding to maximum two-dimensional tumor sizes of 492 mm^2 to 4915 mm^2 with a mean of 1097 mm^2. The deviation from linearity of equation 14 was found to be quite small for a wide range of values of $\ln A_{Max}$. It then becomes convenient to choose a common maximum size in order to place the variation of growth into the single parameter α. While wide variations of $\ln A_{Max}$ does not significantly affect the linearity of equation it should be remembered that the estimation of α is closely correlated with the estimation of $\ln A_{Max}$ so that growth parameters cannot be compared unless the same value of $\ln A_{Max}$ is used in all calculations.

The Gompertz function is not usually considered in terms of doublings and of doubling times and the relationship of the Gompertz constants β and α to the doubling process is not readily evident. Such a relationship has been derived by several authors[39, 41] and is given below.

$$t = \frac{1}{\alpha} \ln \left[\frac{\beta}{\alpha(\beta/\alpha \quad \ln(S/S_o)} \right] \qquad (18)$$

with $S = S(t)$
$S_o = S(0)$

for the sake of simplicity in the notation. The doubling time

is expressed as:

$$t_D = -\frac{1}{\alpha}\ln\left(1 - \frac{\alpha \ln 2}{\beta} e^{\alpha t}\right) > 0 \qquad (19)$$

Substituting equation 18 into equation 19

(20)

$$t_D = -\frac{1}{\alpha}\ln\left[1 - \frac{\ln 2}{\beta/\alpha - \ln(S/S_o)}\right]$$

or, equivalently

(21)

$$t_D = -\frac{1}{\alpha}\ln\left[1 - \frac{\ln 2}{\ln(S_{Max}/S)}\right]$$

In terms of A(t)

$$t_D = -\frac{1}{\alpha}\ln\left[1 - \frac{2\ln 2}{3\ln(A_{Max}/A)}\right] \qquad (22)$$

RESULTS

I. Estimation of A_{Max}

The modification of Spang-Thomsen on the method of Lloyd was used to obtain estimates of A_{Max}. Identical observed values of A(t) were plotted using equation 14 with each of the estimates of A_{Max}. Linearity of the transformed growth function was checked by calculating correlation coefficients of the individual regression lines. The smallest value of ln A_{Max} compatible with the observed data was 8, thus the various estimates of ln A_{Max} were set at 8, 8.5, 9, and 10. The correlation coefficient obtained with each value of ln A_{Max} was greater than -0.95 although the slope of the line of best fit (equal to $-\alpha$) varied from-0.0032 with ln A_{Max} = 8 to -0.0022 with ln A_{Max} = 10. This demonstrates that wide variations in the value of ln A_{Max} have little effect on the linearity of the growth function but that the estimation of α is closely tied to the estimation of ln A_{Max}. Figure 5 shows the growth curves obtained using the various estimates of A_{Max}.

II. Estimation of Measurement Error

An attempt was made to estimate the magnitude of the error to be expected from the measurement of pulmonary nodules from radiographic films. Brenner et al.[5], found that by re-measuring the same shadow and comparing similar radiographs of the same neoplasm taken within a short period of time there may be an error of 20-30% in the volume or 7-10% in the diameter of the tumor.

Measurements of pulmonary nodules from a series of radiographs from two patients, each with multiple lesions, were made by the same observer on two separate occasions several months apart. The mean absolute difference between each of sixty-eight corresponding measurements was 1.3 mm with a standard deviation of 1.1 mm. Since measurements were made to the nearest millimeter, only a change greater than or equal to two millimeters in the size of the nodule was considered significant. While this condition was applied to all measurements, it has a much greater influence in the calculations based on the smaller nodules where even a 1 mm change would have significant effect on the computed volume and the two-dimensional tumor size.

III. Calculation of Gompertz Constant

For ln A_{Max} equal to 8, the mean value of α for all measured lesions was 0.0044 with a standard deviation of 0.0034. The value of α ranged from -0.00042 (indicating contraction of the lesion) to 0.019. For each nodule, α was computed by using measurements from the first and the last films in which the lesion was noted and the applying equation 17 with $A_{Max} = e^{8} = 2981\ mm^2$.

The patients were separated into two prognostic categories. The first comprised those patients in whom the mean value of α was greater than one standard deviation below the sample mean, i.e., less than 0.001, while the second contained the remaining patients. The composition of the two groups is given in Table 6. Survivorship for both groups was

estimated using the Kaplan-Meier method and survival curves (Figure 6) were obtained by plotting the cumulative proportion surviving, P(t), versus time. The two survival distributions were then compared by the log-rank test. No significant difference between the survival distributions was detected.

For ln A_{Max} equal to 8.5 (A_{Max} = 4915 mm^2) the mean value of α for all lesions was 0.0033 with a standard deviation of 0.0023. The range of values was from -0.00027 to 0.01. Patients were again separated into prognostic categories according to the criteria used previously, i.e., patients in whom the mean value of α was less than the sample mean minus the standard deviation. Since that cut-off was again 0.001 the composition of each group was identical to those obtained using the value of eight for ln A_{Max}.

It may be helpful to translate the above variations of α into more familiar terms. From equation 5

$$S(t) = S_{Max} e^{-\frac{\beta}{\alpha} e^{-\alpha t}} \qquad (5)$$

The values of S(t) after an arbitrary period, e.g., 100 days, may be calculated for α_1 =0.001 and α_2 =0.0044 (derived when ln A_{Max} = 8). If a common maximum size and a common initial size are selected for both values of α, then the ratio (β/α) remains constant for both α. (Equation 4). Thus:

$$\frac{\beta_1}{\alpha_1} = \frac{\beta_2}{\alpha_2} = k$$

and

$$\frac{S_1(t)}{S_2(t)} = \frac{S\, e^{-ke^{-\alpha_1 t}}}{S\, e^{-ke^{-\alpha_2 t}}}$$

$$= e^{-k(e^{-\alpha_1 t} - e^{-\alpha_2 t})}$$

$$= e^{-k(e^{-0.1} - e^{-0.44})}$$

$$= e^{-0.26k} \qquad (23)$$

If the maximum size were 1000 times the initial size, k would equal ln 1000, or

$$\frac{S_{1(t)}}{S_{2(t)}} = e^{-0.26 \ln 1000}$$

$$= 0.166.$$

After 100 days, a tumor characterized by $\alpha_1 = 0.001$ would have only 16.6% of the volume of a tumor characterized by $\alpha_2 = 0.0044$.

IV. Comparison Using Tumor Doubling Time

Doubling times were calculated using the approximation formula given by Schwartz to determine if results differed significantly when assuming an exponential growth curve rather than a Gompertz growth curve. The mean doubling time was 58.6 days with a standard deviation of 39.9 days. Patients with doubling times greater than 100 days were compared to those with doubling times of less than 100 days. Composition of the groups is given in Table 7. No significant difference could be detected in the two groups. (Figure 7).

Plesnicar, et al.[56], obtained a mean doubling time of 49.1 days in patients receiving no anti-cancer therapy. It was also noted that the mean doubling time for nodules 3.0 mm to 14.9 mm in diameter was 24.9 days while the mean doubling time of nodules 15.0 mm to 59.9 mm in diameter was 51.0 days. The observed dependence of the growth rate on tumor size is not accounted for simple exponential growth is assumed. Equation 22 will allow a determination of the variation in doubling time expected when a Gompertz growth curve is assume. According to the data presented by Plesnicar, et al., the mean diameter of lesions in the size range of 3.0 mm to 14.9 mm was approximately 8 mm and the mean diameter of the group ranging in size from 15.0 mm to 59.9 mm was approximately 26 mm. The ratio of the doubling time of the larger nodule versus the doubling time for the smaller nodule was calculated for values of A_{Max} of 2981 mm^2, 4915 mm^2, and 8103 mm^2 (corresponding to ln A_{Max} equal to 8, 8.5, and 9). The resulting ratios were 2.9, 2.35, and 2.05 which compare favorably with the observed ratio of 2.05 (51.0/24.9).

V. Comparison Using Tumor Regression

A decrease in tumor size of greater than fifty percent is often taken to indicate favorable response to therapy. Total estimated tumor volume at the time of each chest radiograph was determined for all thirty patients. The patients were categorized on the basis of whether a fifty percent or greater decrease in tumor volume had occurred sometime during the period of observation (Table 8). Volume was calculated

using each of the two diameters measured on the frontal film with the third diameter taken to be the average of the first two. It should be noted that no patients had complete remission, i.e., 100% regression of tumor.

One-third of the patients demonstrated a greater than fifty percent regression of the volume of their pulmonary lesions, a proportion comparable to that seen at other medical centers.[9] However, no significant difference in survival was detected between those who did and those who did not display tumor volume decrease.

Patients were then categorized according to whether the total calculated tumor volume continuously increased through the observation period or whether the volume both increased and decreased during the interval (Table 9). No significant difference between the survival distributions was observed (Table 9). This is in agreement with the findings of Webb and Gamsu.

VI. Homogeneous vs. Mixed Growth Patterns

It is a common observation that in some patients all pulmonary metastases will display continuous growth while in other patients there may co-exist populations of both growing and shrinking lesions. Patients were separated into these categories (Table 10), and the survival curves plotted (Figure 10). No evidence of a significant difference between the two survival distributions was found.

VII. Significance of Tumor Volume at the Time of Diagnosis

The extent of parenchymal involvement at the time of

diagnosis of pulmonary metastases varied widely from patient to patient. Since survival was defined as starting at the time of diagnosis of metastases, it was important to determine if the extent of disease at diagnosis was correlated to subsequent survival. The initial volume of the metastases were used to separate the patients into three groups. The first contained those in whom volume was less than 525 mm^3, the second those in whom volume was between 525 mm^3 and 2600 mm^3, and the third contained those with initial volumes greater than 2600 mm^3 (Table 11). No statistical difference in survival between any of the groups was found.

VIII. Comparison Using Response to Chemotherapy

It would seem reasonable to assume that patients who manifested a positive therapeutic response would do better than those patients who did not respond. For this analysis it was necessary to know the type and duration of chemotherapy given to the patient and to have at least two chest radiographs within the period of each anticancer regimen. Ten patients met these conditions.

Positive response to chemotherapy was defined as at least a twenty-five percent decline in the value of α or α less than zero during a given therapeutic regimen as compared to the preceding interval. Responders included those patients who demonstrated a positive chemotherapeutic effect as defined above upon the first use of chemotherapy and those who had a positive response after a change in the regimen. Non-responders included those patients in whom the values of α remained

constant or increased during therapy, and those who developed new metastases while on chemotherapy. No difference between the two survival curves was noted.

DISCUSSION

This study was unsuccessful in finding any correlation between the rate of growth of pulmonary metastases and survival time after diagnosis of metastases in 30 patients with malignant melanoma. The question which now poses itself is whether there is a correlation which has not been detected, whether there is absolutely no useful correlation between growth rates and prognosis, whether a correlation may exist but only under specific conditions not recognized in this analysis, or whether there are other explanations. One may ask even more basic questions about the validity of assuming logarithmic or Gompertzian behavior in the growth of metastases *in vivo*.

The first possibility is that a valid correlation exists between the growth of metastatic lesions and prognosis but that this was not recognized due to limitations of the protocol used. Not the least of the limitations is the small size of the sample involved. Such a small number of patients increases the likelihood of an erroneous result due to the effects of a biased sample. The sample, however, does not seem to be terribly unrepresentative of the population of melanoma patients as a whole except for the fact that by definition they all have disseminated disease. The male: female ratio was 16:14, average age at initial diagnosis was

years and average age at diagnosis of pulmonary metastases was 51.9 years. The subdivision of patients into the various categories defined by the study did not seem to

cause a sample bias which would adversely affect detection of an increased survival trend. Indeed, in some cases it appeared that the subgroup associated with the slower growth rates, and presumably better survival, would a priori be expected to do better due to a greater proportion of females (the majority pre-menopausal) and a lower mean age than the subgroup with more rapidly growing metastases. While it may be that this tendency of a group of patients with characteristics associated with a better prognosis to manifest a slower growth rate is significant, it did not translate into improved survival. It is also possible that the observation periods or distribution of chest x-rays was not optimal for accurate determination of growth parameters. The mean time of observation (defined as time between first and last chest films used in measurements) was 236 days but this ranged from 25 to 1251 days. The accuracy of the calculation of growth parameters increases as the relative ratio of the observation period to the tumor doubling time but the prognosis of patients with disseminated melanoma is generally so poor that the observation period is not much greater than the doubling time. In this instance, one could compare the mean doubling time for all measured lesions (60 days) to the mean time of observation (236 days) and see that, on average, only two doublings are seen per patient. It should be noted that the period of 236 days should not be taken to also be the mean survival time after metastases. The distribution of the chest x-rays in the observation period may also affect accuracy,

but this is less likely to be the major cause of error.

The second possibility is that there is no significant correlation between growth rate and prognosis. This is the conclusion reached by Webb and Gamsu in their study of thoracic metastases in malignant melanoma. Other experimental evidence, however, suggests that a positive correlation may exist. Zanker et al.,[73] determined that appearance of subcutaneous and pulmonary metastases in one patient was associated with decreased cell generation time. Day et al.,[21, 20] found that mitotic rate was an important prognostic variable in truncal melanomas and that mitoses/mm^2 was an important factor in lesions 1.51 to 3.99 mm thick, i.e., in those patients where metastases is most likely to occur. While these findings are interesting, it must be emphasized that the relationship between growth rate and subsequent prognosis proposed by these studies is predicated on the idea that the growth rate can be used to assess the <u>likelihood</u> that metastases will occur. It is only by extension can the question be posed if the growth rate can also be used as a prognostic variable after dissemination.

Another explanation for these results may be that the growth rate is only one of several factors determining prognosis in patients with pulmonary metastases. At the time of diagnosis of pulmonary metastases, twenty-six of thirty patients in this study had documented evidence of other metastases and/or recurrences. This introduces a variable which is often difficult to assess and to quantify yet which may be

the limiting factor in a patient's survival. Clearly a patient with simultaneous central nervous system and pulmonary metastases may die due to complications from the former even if the latter appears to have stabilized. Since it is the rule, rather than the exception, that multisystem metastases will occur, any use of the growth rate or its parameters will have to account for the influence of extrapulmonary metastases.

One solution may be to separate the patients into categories based on the sites of metastatic disease, e.g., pulmonary vs. pulmonary and central nervous system v.s pulmonary and gastro-intestinal, etc. While this may make it possible to determine a relationship between growth and survival exists in the case of metastases only to the lung, it does have a number of difficulties. First and probably foremost is that a study restricted only to patients with isolated pulmonary metastases and adequate medical and radiographic follow-up would likely have a very small sample size. Second, unless the patient comes to autopsy, there will be considerable uncertainty as to whether disseminated disease is restricted solely to the lungs throughout the period of observation as even the most thorough metastatic work-up is not foolproof and even the most enthusiastic researcher is unlikely to subject patients to periodic full-scale workups to ensure documentation of extent of disease in the event the patient's family refuses autopsy. Third, results of such a study may be of academic interest but due to the severe constraints on the design, the results are unlikely to be easily

applicable to either medical or radiologic diagnosis and treatment.

Alternately, one could develop prognostic models and analyse combinations of variables using multivariate analyses as has been done in the study of prognostic factors in Stage I melanoma. This approach is beyond the scope of this thesis.

There are many other variables not accounted for in this analysis. Recent reports[61, 65] claim that cigarette smoking is a significant risk factor in developing metastases in melanoma, possibly due to a suppression or diminution of host response. If immunologic suppression is indeed responsible for the increased metastatic rate in smokers, then patients who continued to smoke after the discovery of pulmonary metastases may also have a decreased survival time compared to non-smokers.

In addition, no attempt was made to match patients in the prognostic categories defined by the calculated growth parameters according to stage at initial diagnosis because it was assumed that the fact that all patients were now Stage IV obviated that need. However, if it is shown that stage at initial diagnosis also affects survival once dissemination has occurred, then this will have to be allowed for in subsequent analyses.

There is also the problem of what is the optimal way to assess the behavior of multiple nodules. In the past, some investigators[71] have chosen to use only the fastest growing

nodule in their analysis on the grounds that it was expressing the most malignant behavior. This does not take into account the influence of local factors such as variation in blood supply or the fact that the growth rate is volume and time dependent, as observed by Plesnicar. Thus one can evaluate only a small lesion on the exponential portion of its growth curve while a much larger lesion in its plateau phase kills the patient through respiratory embarrassment and then erroneously assert that a short doubling time is correlated with a poor prognosis. Even the use of the Gompertz constants to account for the volume and time dependence of the growth rate may not be a satisfactory solution. In the hypothetical example given previously the small lesion may have a more rapid intrinsic growth rate than the larger nodule yet may still not be the limiting factor in the patient's survival. This also demonstrates that the method used in this analysis, i.e., equal weight being given to all calculated parameters, may also be unsatisfactory. The problem is accentuated by the independent patterns of behavior expressed by the nodules.
[49]
Nathan found in his analysis of multiple nodules in a single patient that the doubling times determined were usually of the same order of magnitude. There was often a wide variation in the growth parameters calculated for the different nodules and that at times there was even co-existence of expanding and contracting lesions. The significance of this pattern is unclear but there was no difference in survival between patients with this phenomena and those without.

There is also uncertainty over whether a given volume increase in one nodule is of the same importance as an equal volume gain spread over several nodules. The assumption used was that there was no difference but there is little or no evidence in the literature one way or the other. Webb and Gamsu found no difference in survival in patients with single versus multiple nodules.

A related question is the significance of the appearance of a new nodule or the disappearance of an old nodule. The clinical scale used by many oncologists assigns the most dire rating to the situation in which the first nodule is seen but what of the appearance of subsequent nodules? What additional weight, if any, should be given to the appearance of a new nodule other than to factor its contribution into the calculation of tumor volume and mean growth parameters? Since over two-thirds of the patients in this study demonstrated the appearance of new lesions sometime in the course of their disease, this is not an idle question. Most likely some satisfactory method of weighting the appearance of new nodules will have to be developed in order to bring the experimental growth model more in line with the clinical model. Ideologically it makes sense that new nodules are poor prognostic signs for the patient. The new lesions could represent (a) continued metastases from another site, (b) reactivation of dormant tumor cells, or (c) continued growth of a small inoculum of metastatic cells which was previously of insufficient size to be detected. If the patient were

receiving therapy any of the above possibilities would indicate a poor response to treatment.

The disappearance of a nodule is also difficult to evaluate as one often cannot be certain if a nodule has disappeared due to therapy. Szamosi[74] pointed out that the radiologic appearance of any nodule can vary considerably from one film to another even to the point of approaching invisibility.

Another result of the analysis of the lesions was that the extent of disease at the time of diagnosis of pulmonary metastases did not have a detectable effect on the subsequent survival. This was a pertinent question since it was possible that patients diagnosed late in the course of their disease may have artificially low survival times simply because they went unrecognized as having disseminated lesions much longer than others in the sample. This finding is quite surprising as one would expect that fewer and smaller lesions would be a better prognostic sign than more and larger lesions. If this finding is valid it would serve to further emphasize the idea that survival is not simply related to extent and rate of growth of disease but to many variables, e.g., metastases in other organ systems.

Even more surprising and disturbing is that patients who had a greater than 50% reduction in calculated tumor volume sometime during the period of observation did no better than those who did not. This is in agreement with Webb and Gamsu's finding that regression is not a positive prognostic sign

(although they may not have used the greater than 50% reduction as their criteria). Chen et al., found that 34% of the patients in their study had 50% or more tumor regression, which is almost identical to the 33% in this study. They do not comment on whether these patients had increased survival time but do note that in cases successfully treated by systemic chemotherapy, pulmonary nodules shrink much faster than lymph nodes (about 10% of cases). Adenopathy was not evaluated in this study because it was judged that the treatment with BCG given to many of the patients would make it difficult to distinguish nodal metastasis from BCG reactive nodal enlargement. The patients in this study responded poorly to chemotherapy. Several demonstrated measurable reduction in tumor size during treatment but tumor growth continued unabated after cessation of therapy.

Several other observations can be made. The mean doubling of all lesions as calculated by the Schwartz approximation was on the order of sixty days. Plesnicar gave a figure of 49 days but his patients were on no systemic chemotherapy. Webb and Gamsu found a mean doubling time of two months in their patients, the majority of whom received therapy. Plesnicar also noted that doubling time for lesions measuring 3 mm to 14.9 mm in diameter was 24.9 days while those metastases from 15.0 mm to 59.9 mm in size had a mean doubling time of 51.0 days. This variation in doubling time is not accounted for in models using the assumption of exponential growth but fits very well with the model using the Gompertz growth curve. The experimental data used in determination

of $\ln A_{Max}$ in this study also fit well with the Gompertz model with the correlation coefficient of the equation $\ln \ln \frac{A_{Max}}{A(t)} = \frac{\beta}{\alpha} - \alpha t$ being greater than -0.95.

It is by now evident that the evaluation of pulmonary metastases is fraught with difficulty. If further studies are done to confirm or correct the findings presented here, some suggestions may prove useful.

(a) It may prove easier if the study is a prospective one; prospective in the sense that the collection and organization of radiographic and medical data be done systematically with the study in mind. For example, a flow sheet could be kept giving the exact date of the initial diagnosis of pulmonary metastases, the exact dates and type of therapy, and the date of serial chest x-rays. Dating is especially important in a disease where progression can be rapid and survival time short. More accurate dating would allow expansion of the time scale to weeks, or even days and thus increase the possibility that differences in survival can be detected, although such a difference may not prove to be of major benefit to the patient. Many patients were excluded from all or several parts of the study because medical documentation, while more than adequate for patient care, was not accurate enough for analysis.

(b) Once a patient has been found to have metastases he should be followed with serial x-rays at regular intervals of perhaps one month. This is generally done in any case to monitor the patient's disease.

(c) Future analysis may multivariate or covariate analysis to determine important prognostic parameters.

Table 1
Incidence of Metastatic Melanoma in Various Organs
(Autopsy Series)

System	Reference	Das Gupta & Brasfield[3]	Einhorn et al.[4]	Patel et al.[6]	Meyer[7]	Nathanson et al.[8]
	Period	1935–60	1967–73	1959–74	1927–75	1947–66
	Institution	Memorial	M.D. Anderson	Roswell Park	Pondville	Harvard Hospitals
	Pt. No.	125	96	216	74	22
Respiratory	Lung	70%	87%	71%	76%	82%
	Pleura	24%	15%	—	—	—
	Diaphragm	17%	15%	—	—	—
	Upper tract	—	2%	8%	—	—
Gastrointestinal	Liver	68%	76%	58%	54%	77%
	Peritoneum	13%	26%	43%	—	—
	Pancreas	53%	38%	38%	32%	41%
	Spleen	36%	43%	31%	27%	36%
	Small bowel	58%	26%	36%	34%	36%
	Colon	22%	14%	28%	24%	
	Stomach	26%	7%	23%	26%	
	Gall bladder	15%	4%	9%	20%	
	Esophagus	4%	3%	9%	—	
Bone, soft tissue	Breast	20%	2%	—	7%	—
	Skin	75%	54%	68%	—	63%
	Subcutaneous/ muscle		50%		—	—
	Vertebra	42%	23%	49%	35%	41%
	Other bones	36%				
Lymph node	Abdomen	65%	74%	56%	—	72%
	Thorax	55%		55%	—	
	Others	—		42%	—	
CNS	Brain	39%	54%	49%	40%	36%
Cardiovascular	Heart	49%	55%	47%	40%	41%
	Pericardium	10%	11%	24%	—	—
Endocrine	Adrenals	50%	54%	47%	51%	36%
	Thyroid	39%	21%	26%	23%	27%
	Pituitary	5%	—	16%	—	—
	Parathyroid	2%	—		2%	—
Urinary	Kidney	45%	58%	35%	38%	45%
	Bladder	18%	14%	13%	16%	—
	Ureter		2%		3%	—
	Prostate	3%	5%	—	—	—
Genital	Testis	8%	7%	—	7%	14%
				13%	12%	14%

Table 2
Common Sites of Metastatic Melanoma
Overall versus Initial Involvement

Reference	Patel et al.[6] (1978)		Das Gupta & Brasfield (1964)[3]	Stehlin et al.[9] (1967)	Einhorn et al.[4] (1974)
Pt. No.	216	216	652	222	332*
Organs involved	Overall involvement at autopsy	Only site involved at autopsy	First site of extra-regional metastasis	Initial site of distant metastasis	Initial site(s) of distant metastasis
Lung	71%	2%	7%	21%	45%
Liver	58%	1%	4%	4%	13%
Lymph nodes	74%	2%	—	19%	29%
Brain & meninges	55%	4%	6%	8%	12%
Bone	49%	0	2%	5%	8%
Gastrointestinal	43%	0	2%	—	2%

*Patients were referred for chemotherapy of disseminated non-ocular melanoma. In many patients, more than one organ was involved.

Table 3
Less Frequent Sites
of Melanoma Metastasis

Site	Percent of Patients
Scalp[3]	8
Dura[3]	5
Eye[3]	1
Bile duct[3]	6
Duodenum[3]	12
Rectum[3]	5
Anus[3]	1
Uterine cervix[3]	2
Broad ligament[3]	1
Labia[3]	1
Bone marrow[4]	16
Vagina[4]	2
Major blood vessel[6]	6

Table 4
Causes of Death

Cause	Percent of 216 patients*
Respiratory failure	39
CNS complication	20
Cardiac failure	10
Liver failure	7
Infection	7
Renal failure	2
Adrenal failure	1
Miscellaneous	14

*Patients were autopsied from 1959 to 1974 (Patel et al.[6]).

Table 5

Normal chest x-ray film	2
Pulmonary metastasis	57
Solitary nodules	14
Multiple nodules	41
Miliary (snowstorm) nodules	8
Lymphangitic spread	5
Enlargement of lymph nodes	28
Pleural effusion	10
Atelectasis and bronchial obstruction	8
Lytic bone metastasis	6
Cardiomegaly	4

74

From Webb and Gamsu .

Lesion	No. Patients	(%)	Average Survival (Months)
Adenopathy	9	(7)	10.3
Pulmonary metastasis:			
Multiple nodules	52	(40)	30.9
Solitary nodules	26	(20)	50.7
Miliary infiltrate	2	(1.5)	4
Pleural effusion	3	(2)	23
Extrapleural mass	1	(0.8)	25
Bone lesion	1	(0.8)	8
Combined	36	(28)	20.5

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From Chen, et al.

Table 6 - Composition of categories with ln A_{Max} = 8, 8.5.

	< 0.001	> 0.001
No. of Patients	7	23
Male:Female	2:5	14:9
Mean age at Dx. of mets	44.7 yrs.	54.1 yrs.

Table 7 - Composition of categories using doubling time.

	t_D > 100 days	t_D < 100 days
No. of Patients	9	21
Male:Female	4:5	9:12
Mean age at Dx. of mets	47.4 yrs.	53.8 yrs.

Table 8 - Composition of categories using > 50% regression.

	>50% regression	<50% regression
No. of Patients	10	20
Male:Female	5:5	11:9
Mean age at Dx. of mets	46.8 yrs.	54.5 yrs.

Table 9 - Continuous Growth vs. Growth and Regression.

	Continuous Growth	Growth and Regression
No. of Patients	17	13
Male:Female	9:8	7:6
Mean age at Dx. of mets	53.2 yrs.	50.2 yrs.

Table 10 - Homogeneous (all growing) vs. Mixed (growing and shrinking).

	Homogeneous	Mixed
No. of Patients	17	13
Male:Female	9:8	8:5
Mean age at Dx. of mets	53.9 yrs.	50.2 yrs.

Table 11 - Initial Tumor Volume.

	$V \leq 525$ mm^3	$525 \leq V \leq 2600$ mm^3	$V \geq 2600$ mm^3
No. of Patients	12	7	11
Male:Female	5:7	4:3	7:9
Mean age at Dx. of mets	50.7 yrs.	48.6 yrs.	55.4 yrs.

Table 12 - Response to Chemotherapy.

	Response	No Response
No. of Patients	7	3
Male:Female	4:3	2:1
Mean age at Dx. of mets	57.4 yrs.	54.0 yrs.

Table 13

Pt.	Age	Race	Sex	Histology*	Stage	Site	Survival (mos.)+
F.A.	54	W	F	N,III,3.8	I	back	6
W.B.	40	W	M	SS,IV	I	ear	13
P.B.	63	B	F		IV	leg	17
L.B.	45	W	F			shoulder	3
J.C.	47	W	M	SS IV	I	foot	3+
G.C.	19	W	F	N+SS, IV	I	shoulder	9
M.C.	47	W	F	V	I	shoulder	13+
W.C.	33	W	F		II	back	3
J.C.	32	W	F	SS, IV	I	back	4
B.D.	27	W	F	III	I	forehead	48+
F.F.	68	W	M		I		58
R.F.	43	W	F	V	I	calf	11
C.G.	55	W	M	N	I	shoulder	17+
C.J.	59	W	M		II	thumb	4
J.K.	63	W	M	V	I	toe	2
C.L.	50	W	M	SS, IV	I	back	5
D.L.	47	W	M	SS, III	I	trunk	9
E.L.	69	W	F	LMM	II	forehead	6
H.L.	56	W	M	III	I	nose	24+
J.M.	55	W	M	N, IV	I	back	15+
M.N.	61	W	M	LMM	IV	shoulder, neck	15
J.O.	60	W	M		IV	knee	21+
D.P.	27	W	M	III	II	scalp	10
A.P.	63	W	M		IV	shoulder	5
G.S.	51	W	F		I		7+
J.T.	67	W	M	N, V	IV	flank	3
R.T.	31	W	M	SS, II		scalp	9
G.W.	50	W	F	SS, II	I	scapula	3
J.Y.	46	W	F		I	hand	7
H.Z.	50	W	F		II	back	11+

*Classification, Clark level, Breslow (mm.)

N nodular melanoma
SS superficial spreading melanoma
LMM lentigo maligna melanoma

+ "+" indicates patient alive at last follow-up.

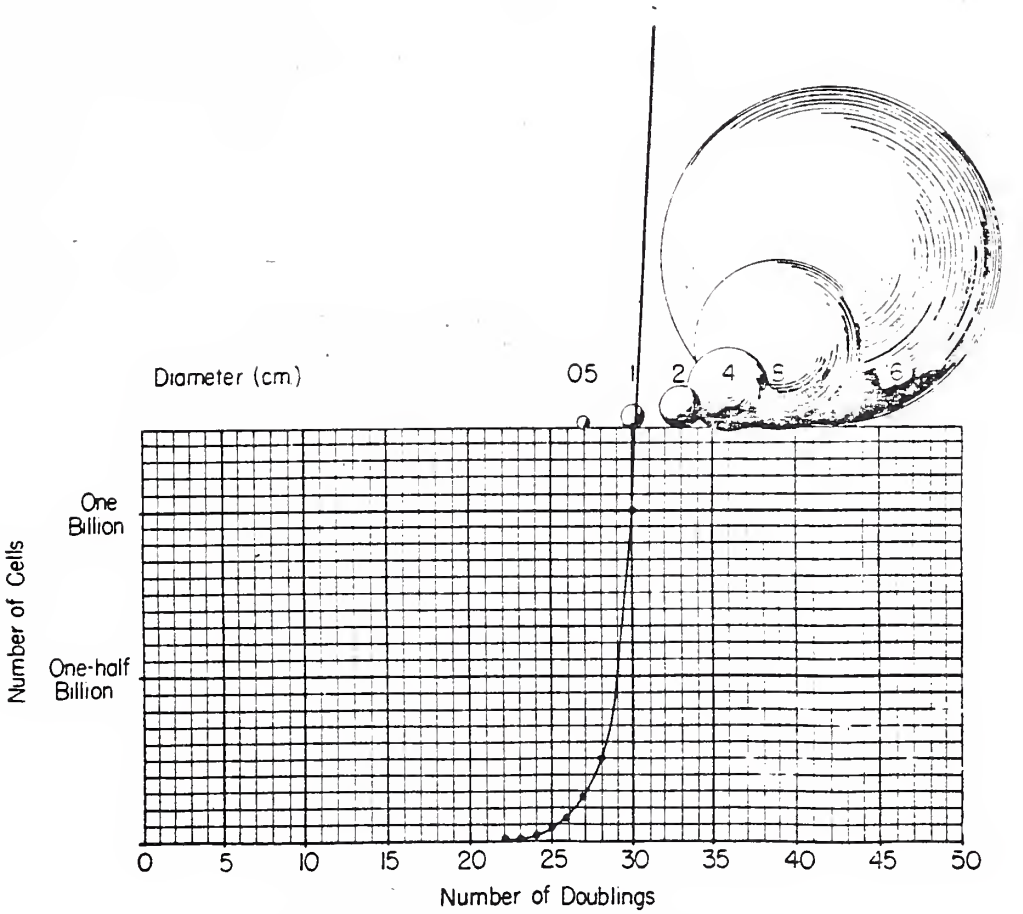

FIG. 1. Growth curve of hypothetical tumor on arithmetic coordinates.

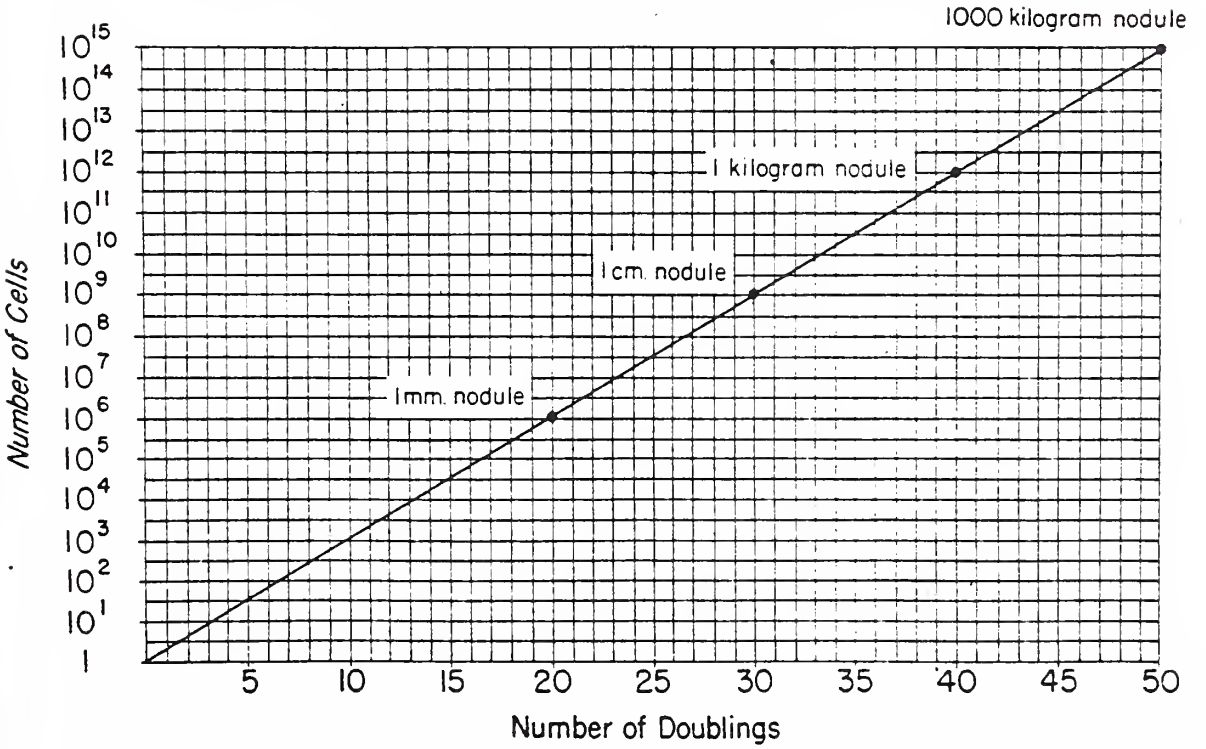

FIG. 2. Growth curve of same hypothetical tumor on semilogarithmic scale.

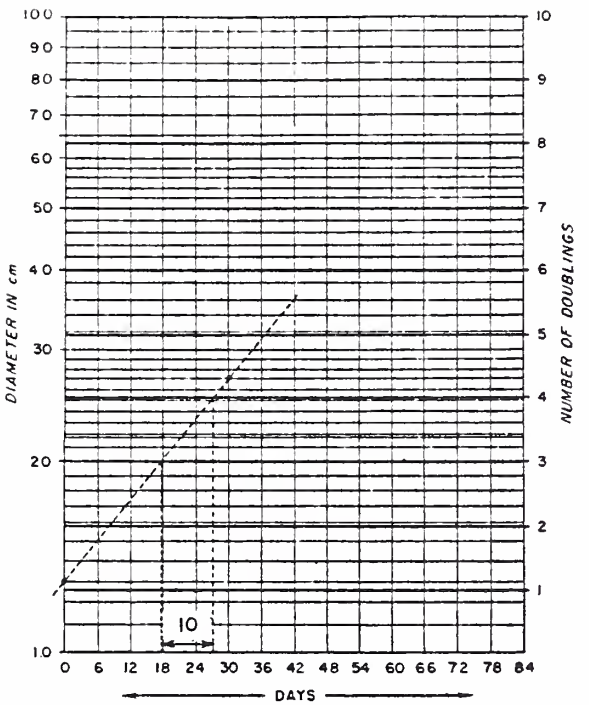

FIG. 3. Chart for estimating doubling time directly from measured diameter of nodules. This graph paper is based on the logarithm of the cube root. The heavy, evenly spaced lines represent successive doublings in volume. The procedure is as follows: (a) The diameter of the pulmonary metastasis at the first observation is plotted on *O* vertical axis. The diameter of the same nodule at the second observation is plotted on vertical line appropriate to the interval in days between the first and second observation; (b) draw straight line between plotted diameters; (c) where this line crosses any two heavy lines indicating doubling, drop two verticals to the base line; (d) the horizontal distance between these two vertical lines represents the doubling time in days.

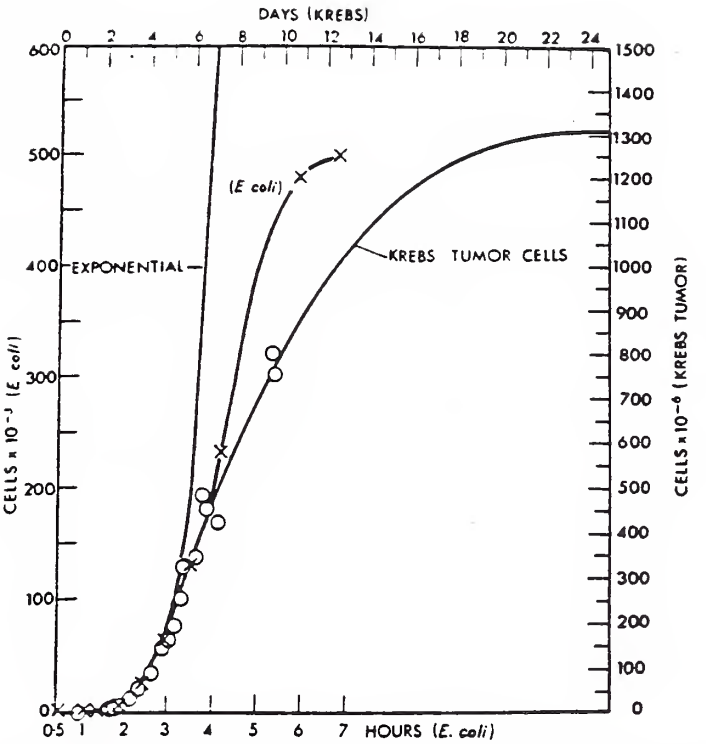

FIG. 4.—An arithmetic plot of (1) the theoretical Gompertz curve giving the best fit by the method of least squares to the experimental data, Krebs ascites carcinoma. The circles are the original experimental points. Data of Patt and Blackford (1954). (2) Growth curve of *Escherichia coli*, B.r. grown in broth culture.* (3) An exponential curve fitted to the early growth data, showing the course growth would have taken if no retardation had occurred in either the bacterial culture or the tumor. The small scale of the graph obscures the fact that the early experimental points for the tumor also deviate from the exponential curve, as would be necessary to allow us to compute an upper limit of growth on the basis of a Gompertz function.

* Data kindly given us by H. Kubitschek, of the Argonne National Laboratory.

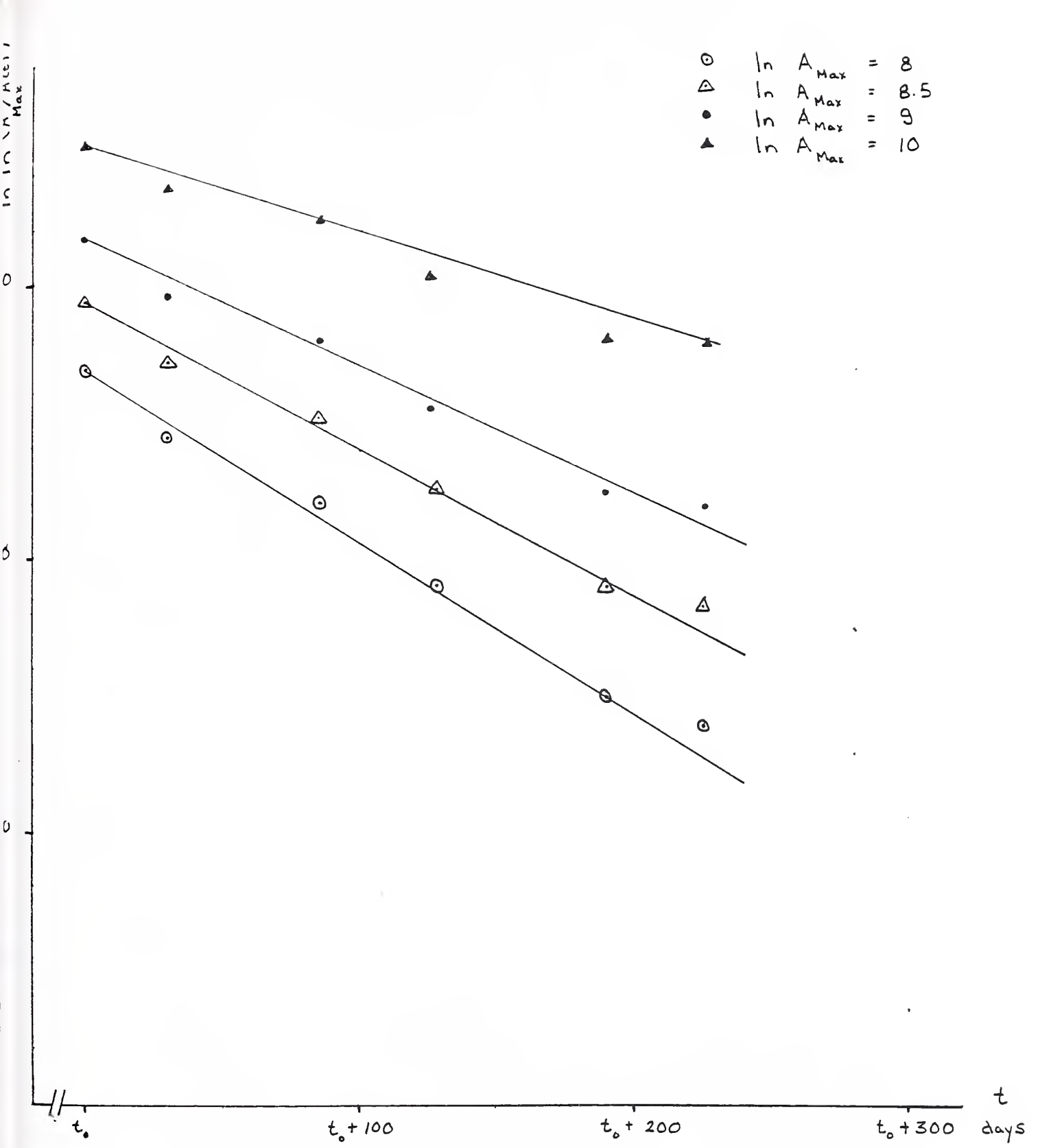
ln A_Max = 8
ln A_Max = 8.5
ln A_Max = 9
ln A_Max = 10
t
t_o
t_o + 100
t_o + 200
t_o + 300
days

Figure 5.

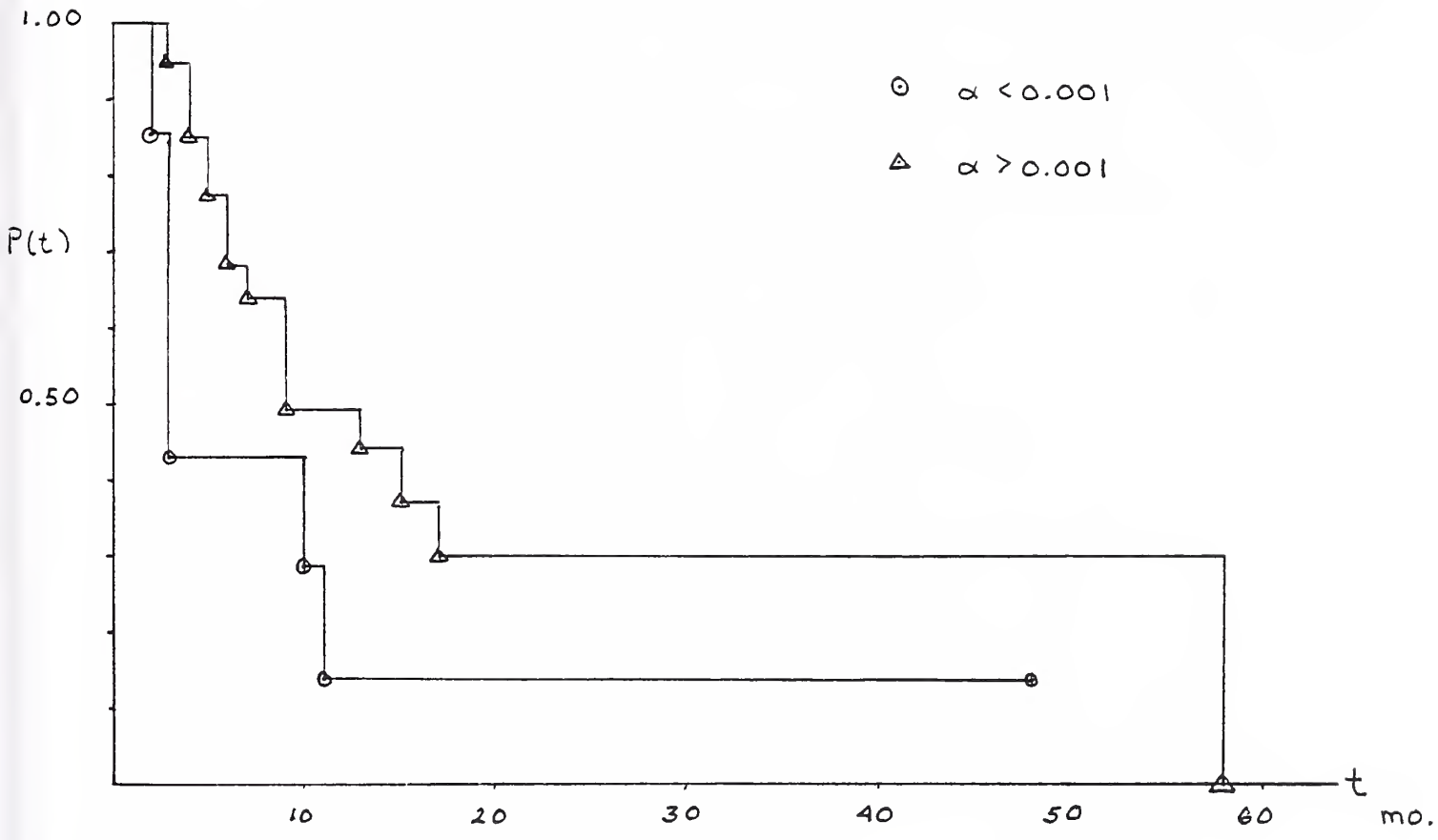

Figure 6. Survival curves for $\alpha < 0.001$ and $\alpha > 0.001$ for $\ln A_{Max} = 8$ and 8.5

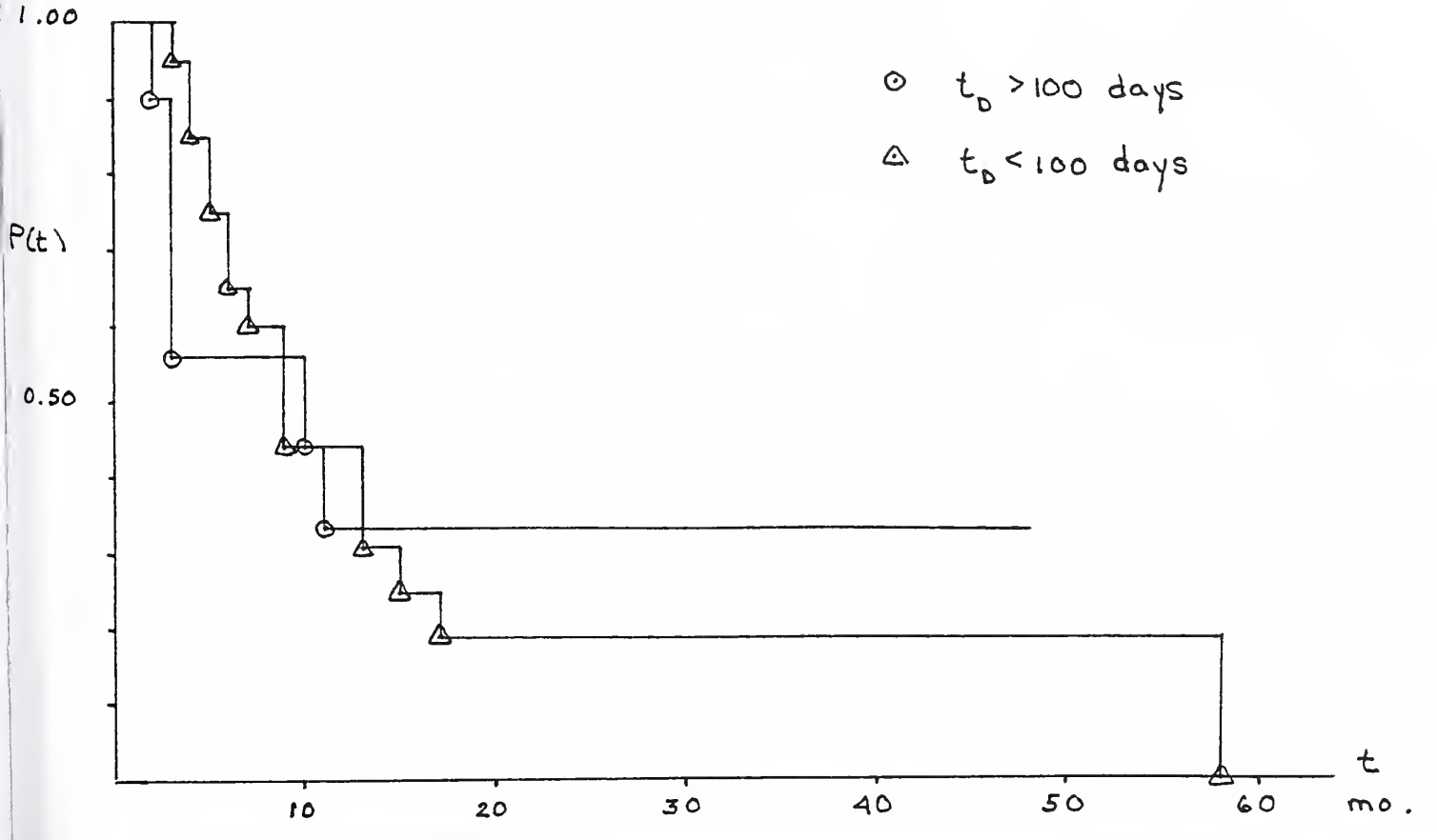

Figure 7. Survival curves for t_D >100 days and t_D <100 days.

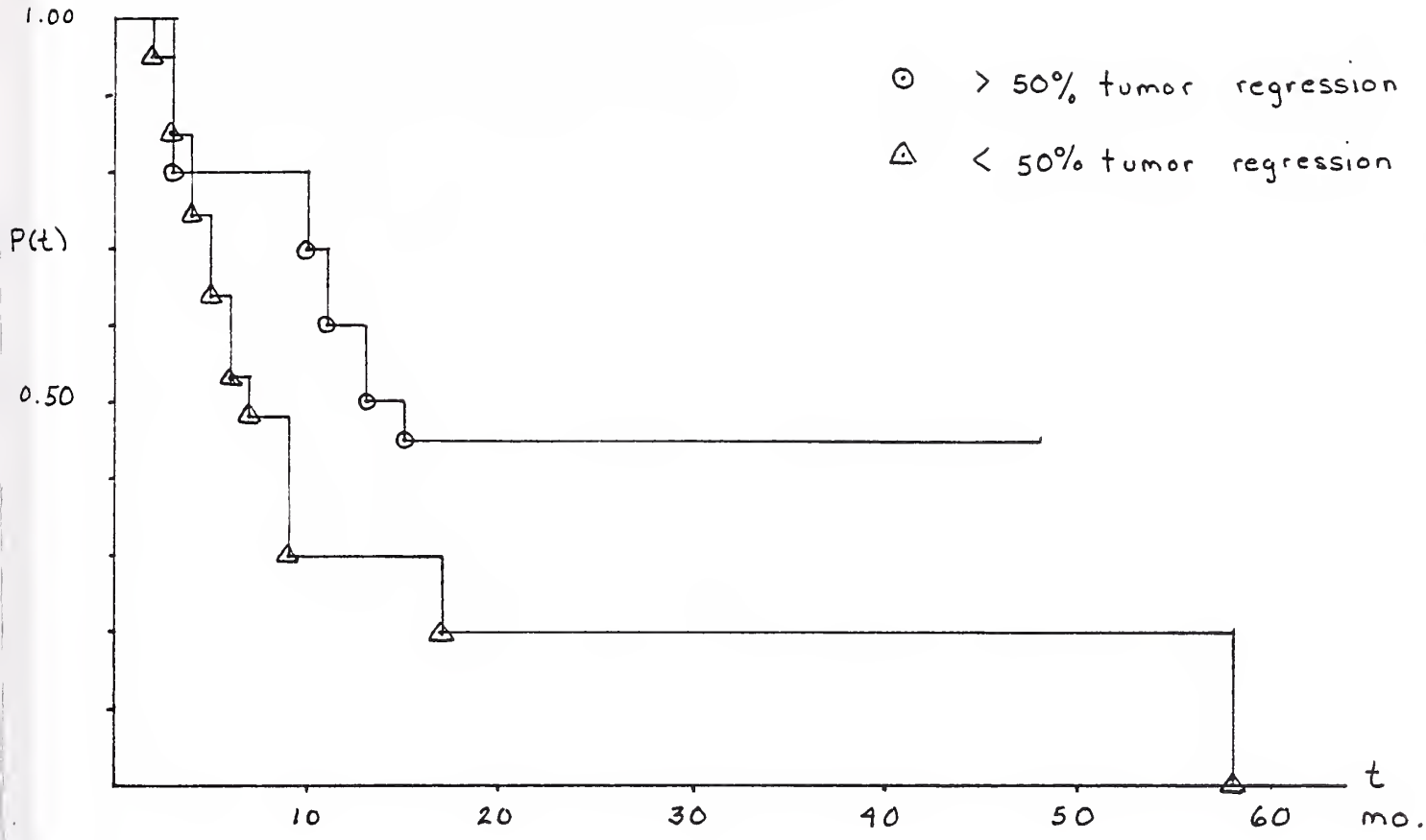

Figure 8. Survival curves based on tumor regression.

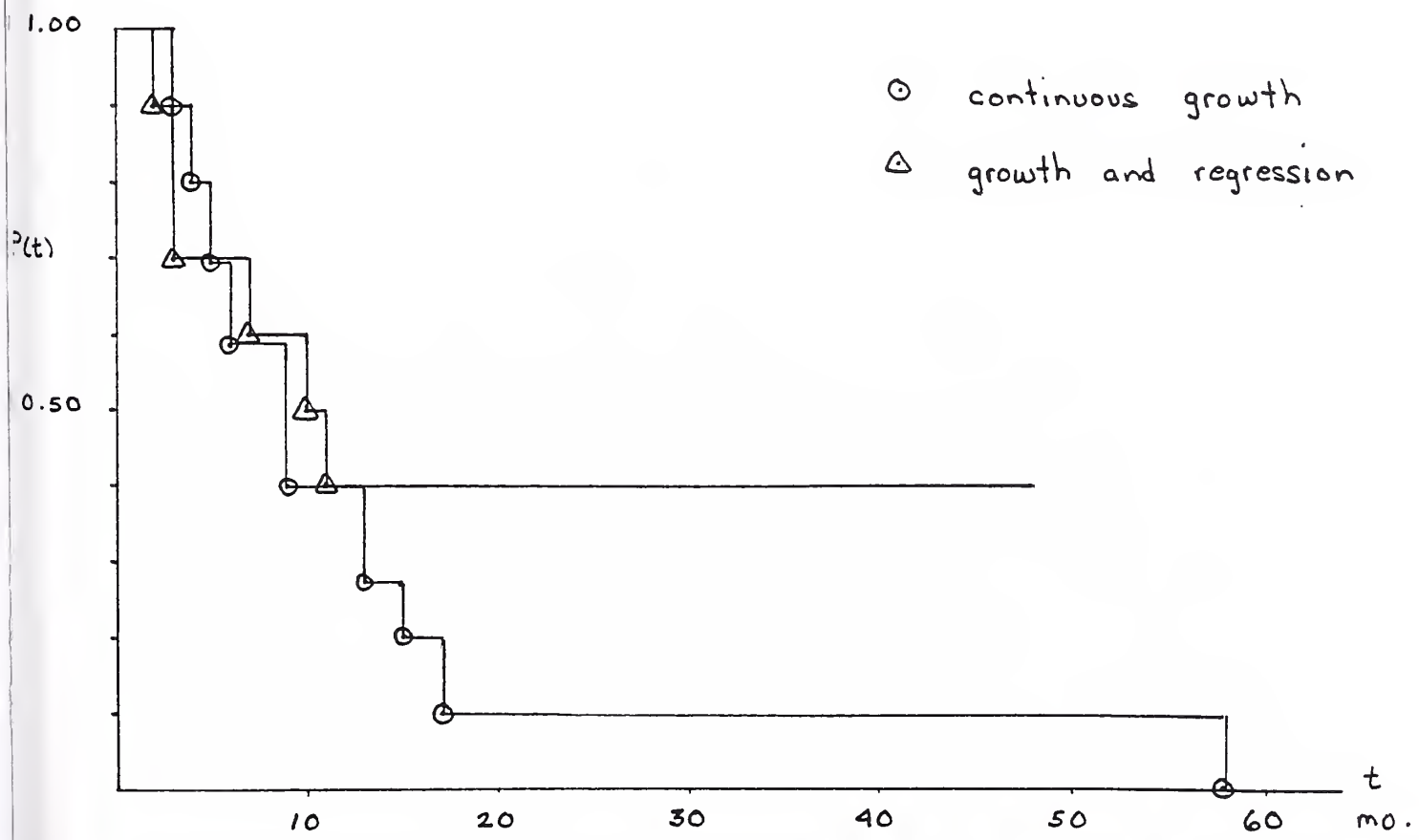

Figure 9. Survival curves based on aggregate growth pattern.

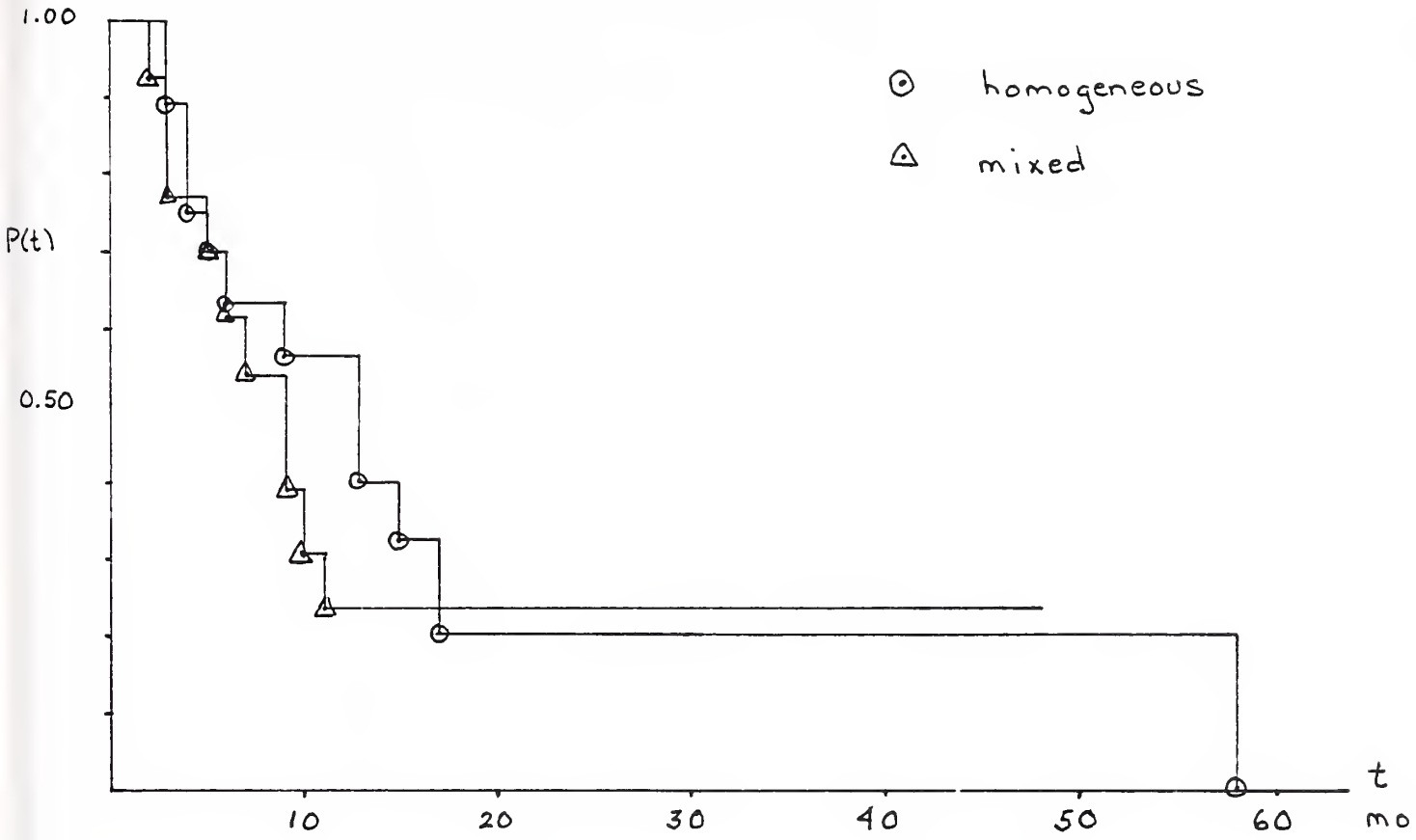

Figure 10. Survival curves based on nodule populations : homogeneous (all nodules growing) vs. mixed (growing and regressing nodules).

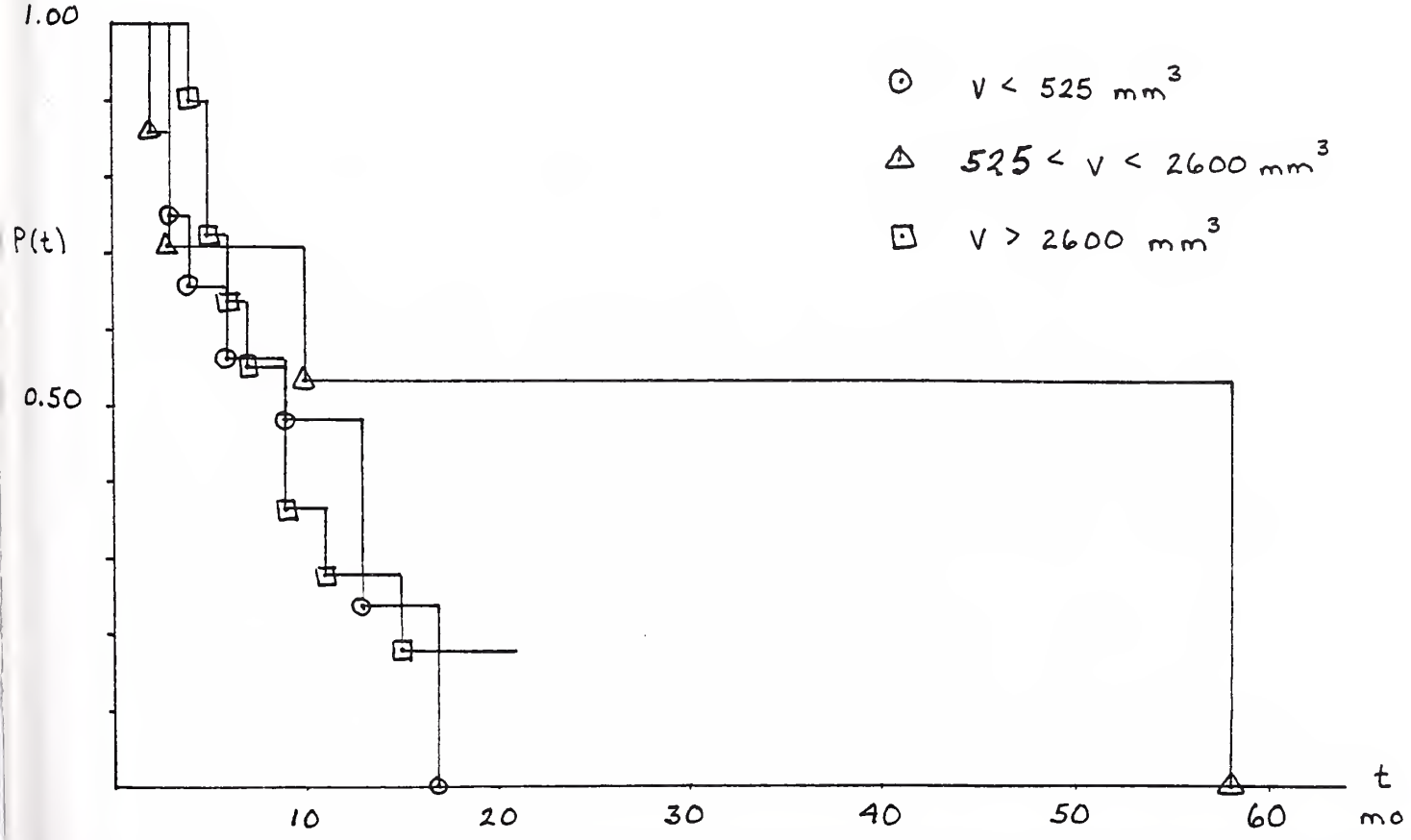

Figure 11. Survival curves based on initial tumor volume.

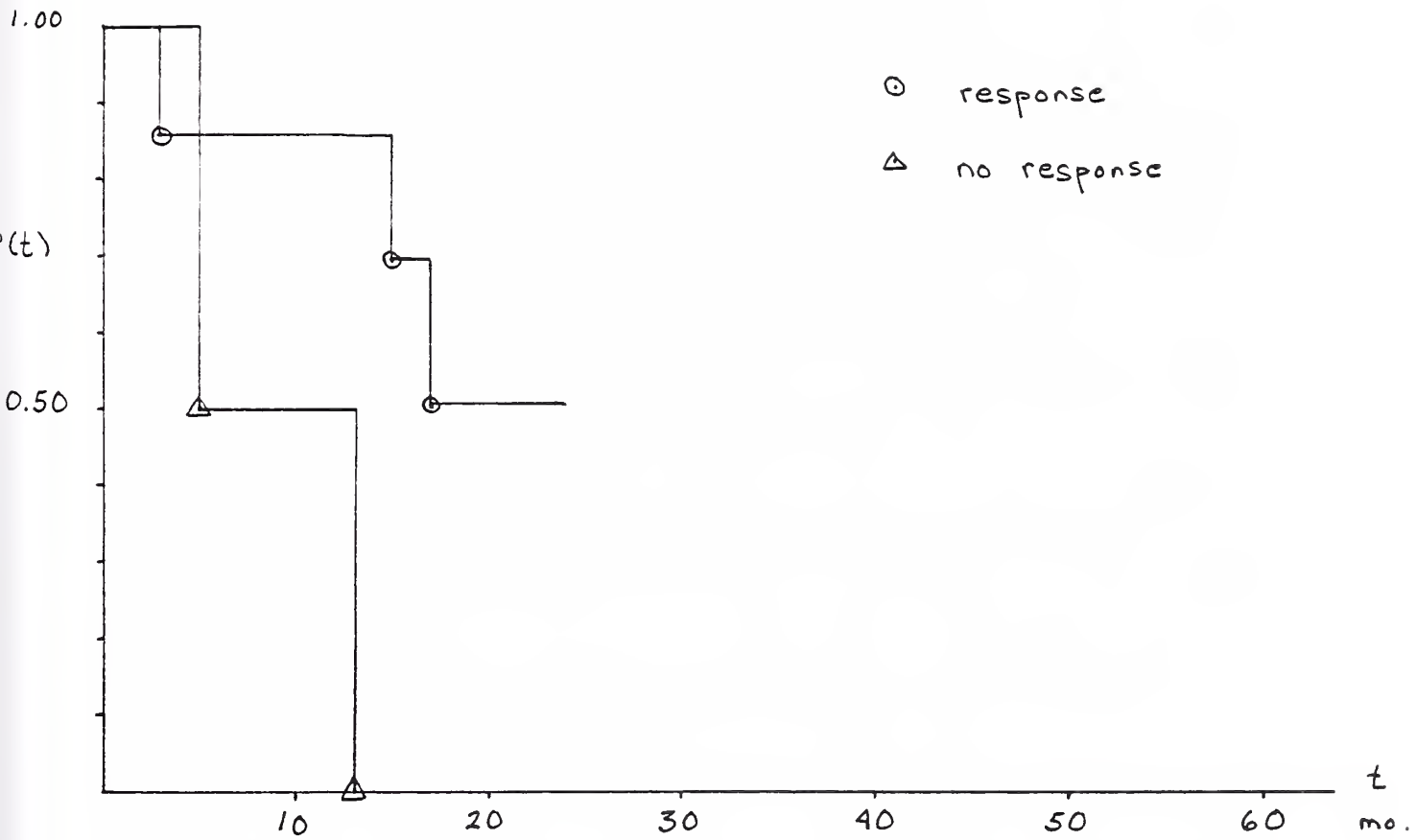

Figure 12. Survival curves based on response to chemotherapy

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