Population Kinetics of Carcinoma Cells, Capillary Endothelial Cells, and Fibroblasts in a Transplanted Mouse Mammary Tumor

Ian F. Tannock

SUMMARY

A combination of histological stains has been used to obtain maximum contrast between capillary endothelial cells, fibroblasts, and parenchymal cells in sections of a transplanted C3H mouse mammary tumor. Values of kinetic parameters were estimated by thymidine labeling techniques and autoradiography for each of these three cell populations. The mean thymidine labeling index of carcinoma cells was 35%. Mitotic index, labeling index, and mean grain count per labeled cell decreased with distance of carcinoma cells from a capillary; there was migration of cells from regions of rapid proliferation near blood vessels to regions of slow or non-proliferation near necrosis. The duration of the cell cycle phases was estimated by a labeled mitoses experiment, and computer methods were used to analyze these results. The median cell cycle time was 13 hr, and the growth fraction was estimated from a repeated thymidine labeling experiment to be about 50%. Therefore, the turnover time of the carcinoma cells was about 22 hr.

The mean thymidine labeling indices of capillary endothelial cells and fibroblasts were 11.4 and 9.1%, respectively. These cells rarely were recognized in mitosis, and their turnover times were estimated from a repeated labeling experiment. The results were consistent with a turnover time of 50 to 60 hr for endothelial cells and 70 to 80 hr for fibroblasts, with a wide spread in the distribution of intermitotic times.

The mean labeling index of capillary endothelial cells remained quite constant between 10 and 40 hr after a single injection of tritiated thymidine; this result implies that capillary endothelial cells are not derived from a faster-proliferating precursor population. Extension of the capillary network in the growing tumor appears to depend mainly on the division of endothelial cells within the capillary walls.

INTRODUCTION

The rate of growth of a tumor often decreases with increasing size (11, 20), probably because the supporting stroma cannot maintain a rate of growth equal to the maximum rate of proliferation of the parenchymal cells. The nutritional environment of the tumor cells becomes poorer as the spacing between blood vessels increases, and this leads to a decreased rate of cell proliferation and cell death (23, 26). Thus, the rate of proliferation of endothelial cells may limit indirectly the rate of tumor growth.

There have been several recent studies in which the rate of growth of a tumor has been related to the rates of proliferation and death of the parenchymal cells within it (8, 10, 19, 23, 24). However, there have been few attempts to study the population kinetics of vascular endothelial cells and supporting connective tissue, possibly because endothelial cells are difficult to recognize in thin tumor sections. In the present studies, values for kinetic parameters have been estimated for endothelial cells of capillary walls, for fibroblasts, and for carcinoma cells of a transplanted C3H mouse mammary tumor. Recognition of endothelial cells in tumor sections has been facilitated by the use of special staining techniques, and cell proliferation has been studied by the methods of thymidine autoradiography.

MATERIALS AND METHODS

Tumor Implantation and Growth. The C3H mammary tumor originated spontaneously in a mammary-agent-positive C3H/He mouse (17). Second-generation isotransplants of the tumor were stored in a liquid nitrogen refrigerator. Third-generation transplants were obtained by injecting clumps of cells from the liquid nitrogen store into the flanks of C3H/He mice, and these transplants were used to generate experimental tumors. A single cell suspension was obtained from them (17), and about $10^5$ cells in 10 µl Hanks' medium were injected into 1 or both legs of C3BF1 (C3H X C57BL) mice by passing the needle of a syringe through the muscle mass into the s.c. tissue of the leg. Thus, all experiments were performed on 4-generation transplants.

Tumor volume was estimated from caliper measurements of 3 principal diameters, by the formula $V = \frac{1}{6} \pi d_1 d_2 d_3$. In one experiment, tumor cells were implanted into the right...
legs of 5 mice, and serial measurements of tumor volume were made at 2- or 3-day intervals. In a 2nd experiment, serial measurements were made on tumors growing in the right flanks of 7 mice; this experiment permitted estimates of tumor volume over a wider range of tumor sizes.

**Histology and Autoradiography.** Estimates of values for kinetic parameters were based on the techniques of thymidine autoradiography. Tumor cells were injected into both legs of 30 animals. Mice were 10 to 12 weeks old and weighed 25 to 30 g. When the tumors attained a mean volume of about 0.5 ml, the experiments were started. Eighteen of the mice were killed at various intervals after a single injection of 50 μCi tritiated thymidine given i.p. (Schwarz BioResearch, Inc., Orangeberg, N. Y.; specific activity, 15 Ci/mmole). The remaining 12 mice were given injections of 25 μCi tritiated thymidine at 6-hr intervals; 2 animals were killed 1 hr after each injection.

Tumors were excised immediately after death of an animal, bisected, and fixed in neutral formol-0.9% NaCl solution. Paraffin sections, 4 μ, were cut and stained with the Luxol fast blue, periodic acid-Schiff, and hematoxylin stains (26). This staining procedure facilitates recognition of red-stained endothelial cytoplasm and gives maximum contrast with blue-stained erythrocytes and hematoxylin-stained nuclei.

For preparation of autoradiographs, stained slides were dipped in Ilford K5 nuclear emulsion, exposed for 1 to 2 months, developed in Kodak D-19 developer, and fixed in Kodak Fixer. Slides were mounted with Permount. The distributions of grain counts over labeled nuclei were obtained for 1 or 2 sample slides of each set, and a labeling criterion of 5 grains/nucleus was chosen from these distributions. Slides were assigned a random numerical code to prevent bias in scoring the autoradiographs. Counts of at least 1000 carcinoma cells or 500 fibroblasts from a number of representative fields of each tumor section were used to determine the mean proportions of labeled cells and their standard errors. Endothelial cells were less numerous. Their labeling indices and the proportions of labeled mitoses for carcinoma cells were evaluated from counts of at least 100 cell nuclei. Prophases were excluded from mitotic counts because they were difficult to recognize when heavily labeled.

**RESULTS**

**Tumor Growth.** Tumor growth curves are shown in Chart 1. The rate of growth decreased with increasing tumor size, and for tumors growing in the flanks, the volume-doubling time was about 1 day for small tumors (approximately 0.001 ml) and about 10 days for large tumors (approximately 3 ml). Tumors in the leg grew more rapidly; for the range of volumes 0.25 to 1.0 ml, the mean volume-doubling time was 2.5 days.

**Morphology.** The tumors were anaplastic and contained widespread regions of necrosis; this necrotic tissue often extended to the periphery of the tumor and in larger tumors caused ulceration through the skin. Viable tissue was distributed throughout the tumor mass and consisted of the carcinoma cells and small regions of connective tissue composed mainly of fibroblasts. Large blood vessels often were surrounded by fibrous tissue, but carcinoma cells usually were adjacent to capillaries.

In some regions of the tumors, there were cylindrical "cords" of carcinoma cells surrounded by necrotic tissue, each with a rather straight and dilated capillary along its axis. This morphological structure resembles that of some other mouse and human tumors (23, 25, 27). The mean radius (+S.D.) of 243 cords in 14 tumors was 100 ± 20 μ; this range is close to a calculated range of oxygen diffusion distances in mouse tumors (22).

The proportions of parenchyma, connective tissue, and necrotic tissue in tumors weighing between 0.2 and 1.2 g were estimated from tumor sections by the method of Chalkley (7, 23). These data are shown in Table 1. There was a tendency for the proportion of necrotic tissue to increase in larger tumors.

**Proliferation of Carcinoma Cells.** The mean labeling index of carcinoma cells 1 hr after thymidine injection was estimated from several tumors to be 35.1 ± 3.9% (±S.D.). The proportion of labeled cells was not uniform throughout the tumor volume: there were many labeled cells near blood vessels and very few near regions of necrosis. The labeling index, mitotic index, and mean grain count per labeled nucleus therefore were estimated in 3 regions of tumor cords, defined by approximate trisection of the cord radius (Table 2). The labeling and mitotic indices varied by a factor of 5 to 7 between central and peripheral regions. There were almost no labeled cells and no mitoses immediately adjacent to a region of necrosis.

The mean proportion of labeled carcinoma cells at progressively greater intervals after a single injection of tritiated thymidine increased steadily from 35% at 1 hr to about 60% in the time interval 30 to 40 hr (Chart 2); this value is

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**Chart 1.** Tumor growth curves for transplants into the right thigh or into the right flank. Mean volumes and their standard errors are indicated (vertical lines) for groups of 5 to 7 tumors.

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Table 1

Percentages of parenchyma, connective tissue, and necrotic tissue in sections of tumors of different sizes

<table>
<thead>
<tr>
<th>Tumor weight (g)</th>
<th>Parenchyma (%)</th>
<th>Connective tissue (%)</th>
<th>Necrotic tissue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.24</td>
<td>70.8</td>
<td>20.9</td>
<td>8.3</td>
</tr>
<tr>
<td>0.29</td>
<td>61.4</td>
<td>12.2</td>
<td>25.4</td>
</tr>
<tr>
<td>0.31</td>
<td>77.4</td>
<td>7.8</td>
<td>14.8</td>
</tr>
<tr>
<td>0.44</td>
<td>68.7</td>
<td>14.8</td>
<td>16.5</td>
</tr>
<tr>
<td>0.49</td>
<td>73.8</td>
<td>5.8</td>
<td>20.4</td>
</tr>
<tr>
<td>0.56</td>
<td>58.6</td>
<td>9.1</td>
<td>32.3</td>
</tr>
<tr>
<td>0.61</td>
<td>60.3</td>
<td>10.7</td>
<td>29.0</td>
</tr>
<tr>
<td>0.76</td>
<td>64.4</td>
<td>5.4</td>
<td>30.2</td>
</tr>
<tr>
<td>0.77</td>
<td>69.9</td>
<td>14.5</td>
<td>15.6</td>
</tr>
<tr>
<td>0.77</td>
<td>69.2</td>
<td>8.8</td>
<td>22.0</td>
</tr>
<tr>
<td>0.85</td>
<td>50.8</td>
<td>12.9</td>
<td>36.3</td>
</tr>
<tr>
<td>0.93</td>
<td>63.5</td>
<td>11.6</td>
<td>24.9</td>
</tr>
<tr>
<td>0.95</td>
<td>54.3</td>
<td>7.3</td>
<td>38.4</td>
</tr>
<tr>
<td>1.12</td>
<td>65.5</td>
<td>6.2</td>
<td>28.3</td>
</tr>
<tr>
<td>1.16</td>
<td>37.9</td>
<td>6.4</td>
<td>55.7</td>
</tr>
</tbody>
</table>

Mean ± S.D. 63 ± 10 10 ± 4 27 ± 12

Table 2

Mitotic index, labeling index, and median grain count per labeled cell in 3 regions of the tumor cords

<table>
<thead>
<tr>
<th>Region</th>
<th>Mitotic index (%)</th>
<th>Labeling index (%)</th>
<th>Median grain count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region near blood</td>
<td>4.3 ± 0.4</td>
<td>50.0 ± 2.5</td>
<td>40</td>
</tr>
<tr>
<td>Intermediate region</td>
<td>2.5 ± 0.3</td>
<td>29.6 ± 4.8</td>
<td>27</td>
</tr>
<tr>
<td>Region near necrosis</td>
<td>0.6 ± 0.2</td>
<td>10.3 ± 3.4</td>
<td>10</td>
</tr>
</tbody>
</table>

*Mean ± S.D.

close to the initial mean labeling index of the cells which were adjacent to capillaries (56%). The increase in labeling index probably occurs because of the proliferation of labeled cells and movement of their progeny towards the necrotic tissue. The rapid increase in labeling index (Chart 2) suggests a rather short lifetime of cells near necrotic regions.

The duration of phases of the cell cycle was estimated from a labeled mitoses experiment. The data are shown in Chart 3a, and a curve has been computed to the experimental points by a method devised by Barrett (1) and modified by G. G. Steel and S. Hanes (personal communication). This method assumes log normal distributions of phase time and generates labeled mitoses curves in such a way that the root mean square error between the data points and the curve is minimized. The computed means of phase duration and their standard deviations are G1 = 3.0 ± 2.0 hr, S = 7.2 ± 1.5 hr, and G2 = 3.0 ± 1.0 hr. The probable distribution of cell cycle times is shown in Chart 3b, and the median of this distribution is 12.8 hr.

The growth fraction of carcinoma cells was estimated from the repeated labeling experiment (Chart 4). An approximate estimate of growth fraction was obtained by comparing the expected labeling index for proliferating cells (calculated from the computed distribution of phase times) with the measured mean labeling index. A theoretical repeated labeling curve then was derived for this value of growth fraction by a method that has been described in detail elsewhere (19, 24). This curve was compared with the experimental points and, if necessary, the fit was improved.
Tumor Cell Kinetics

Table 3

<table>
<thead>
<tr>
<th></th>
<th>Labeling index (%)</th>
<th>Turnover time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma cells</td>
<td>35.1 ± 3.9</td>
<td>22</td>
</tr>
<tr>
<td>Capillary endothelial</td>
<td>11.4 ± 3.8</td>
<td>50–60</td>
</tr>
<tr>
<td>cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>9.1 ± 2.4</td>
<td>70–80</td>
</tr>
</tbody>
</table>

*aMean ± S.D.

Thymidine injection was 11.4 ± 3.8% (±S.D.). The mean labeling index of carcinoma cells adjacent to a capillary was 56%, so that endothelial cells have a much slower rate of proliferation than carcinoma cells in a similar nutrient environment.

Endothelial cells rarely were recognized in mitosis, and for this reason an estimate of turnover time was based on measurements of labeling index after single and multiple injections of thymidine. First, a rough estimate of $T$ was obtained from the labeling index (L.I.) by assuming a value for the duration of DNA synthesis $T_S$. The relevant equation is (18):

$$ \text{L.I.} = \lambda \frac{T_S}{T} $$

Here, $\lambda$ is a factor which depends on the position of the S phase within the cell cycle (18). Values of $T_S$ and $T$ then were tested by constructing from them a theoretical repeated labeling curve. This curve was compared with the experimental points (Chart 5), and, if necessary, the values of $T_S$ and $T$ were adjusted to improve the fit.

The duration of the S phase could not be measured in a labeled mitoses experiment. Fortunately, many of the estimates of $T_S$ that have been made for adult mouse tissue have been in the rather small range of 7 to 9 hr (3, 6, 12–14), although a notable exception ($T_S = 30$ hr) has been recorded for ear epidermis (3, 16). A value of $T_S = 8$ hr was

Proliferation of Endothelial Cells. Endothelial cells were included in the present study if they met the following criteria: (a) the cells were within the walls of blood vessels which were only 1 cell thick (i.e., endothelial cells in arterioles and venules were excluded); (b) the nuclei were long, thin, and completely surrounded by red-stained (periodic acid-Schiff-positive) cytoplasm; (c) the lumen of the blood vessel was open, and there were erythrocytes within it.

With the present staining procedure, endothelial cells could be recognized easily in tumor sections and scored as labeled or unlabeled. The concentration of grains over a labeled endothelial nucleus often was greater than over a neighboring carcinoma cell nucleus, probably because of the high thymidine concentration in the adjacent blood. The mean labeling index of capillary endothelial cells in 10 tumors 1 hr after

Chart 4. The proportions of labeled carcinoma cells after repeated injections of tritiated thymidine. Means and their standard errors for several fields of each tumor are indicated (vertical lines). Curves were derived by assuming random cell loss (---) or that nonproliferating cells were lost after a lifetime of 40 hr (——).

by repeating the calculation for a different value of growth fraction.

Both of the repeated labeling curves shown in Chart 4 were derived for a growth fraction of 50%. The broken line was generated by assuming that cell loss was random with respect to the cell cycle. The unbroken line was derived by assuming a finite lifetime of 40 hr for nonproliferating cells (19); this value is consistent with the timing of the observed increase in labeling index following a single injection of tritiated thymidine (Chart 2). The latter curve better fits the data and implies that most cell death is from the nonproliferating compartment. There is no evidence for a static, long-lived population of nonproliferating cells.

The turnover time ($T$) of the carcinoma cells (i.e., their potential doubling time in the absence of cell loss) may be estimated from the median cell cycle time and the growth fraction by the relation (19)

$$ T = T_C \frac{\log 2}{\log (1 + GF)} $$

Substitution of the values $T_C = 12.8$ hr and GF = 0.5 gives $T = 22$ hr. This value will be compared with estimates of turnover time for capillary endothelial cells and for fibroblasts (Table 3).

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Chart 5. The proportions of labeled capillary endothelial cells after repeated injections of tritiated thymidine. Curves were derived for the "constant phase model" (---) or the "G0 phase model" (—) with parameter values $T_S = 8$ hr, $T_2 = 3$ hr, $T = 53$ hr.
assumed here. If the duration of the G₂ phase (T₂) is small compared with the turnover time, λ has a value of about 0.8 (18), and Equation B gives a value of T in the range 50 to 60 hr.

Repeated labeling curves derived for the parameter values T_S = 8 hr, T_2 = 3 hr, and T = 53 hr are shown in Chart 5. Each of these curves was derived by assuming that all capillary endothelial cells were potentially proliferative. They differ in that the broken curve was obtained by assuming constant duration for the G₁, S, and G₂ phases of the cell cycle, while the unbroken curve was derived by assuming that cells were triggered randomly into DNA synthesis from a pool of cells in a resting (or G₀) phase. The latter model, derived by Burns and Tannock (5), is equivalent to a very wide distribution of G₁ phase duration. The curve based on this model gave a good fit to the experimental points (Chart 5); it predicts a turnover time for endothelial cells of 50 to 60 hr, with a broad distribution of intermitotic time about this range.

The relation between the proportion of labeled capillary endothelial cells and time after a single injection of tritiated thymidine is shown in Chart 6. The labeling index increased initially as labeled cells entered mitosis and thereafter stayed relatively constant; this behavior is predicted by a curve derived for the G₀ type model with T_S = 8 hr and T in the range 50 to 60 hr and is therefore consistent with the results of the repeated labeling experiment.

Proliferation of Fibroblasts. Proliferation of fibroblasts was studied in regions of connective tissue within the tumor mass; fibroblasts in the capsule were not included. The mean labeling index in 9 tumors was 9.1 ± 2.4% (±S.D.), and this increased to about 50% after 36 hr of repeated labeling (Chart 7). The difference between the labeling indices of fibroblasts and capillary endothelial cells was significant at the 10% level (0.05 < p < 0.1).

The turnover time, T, of fibroblasts was estimated in a similar way to that for capillary endothelial cells. A value of T_S = 8 hr was first assumed, for which Equation B gave a value of T in the range 70 to 80 hr. Theoretical repeated labeling curves were derived for the parameter values T_S = 8 hr, T_2 = 3 hr, and T = 75 hr for both the “constant phase duration” and “G₀” models. These curves are shown in Chart 7, and the unbroken line (derived for the G₀ model) is a reasonable fit to the experimental points. Thus, the turnover time of the fibroblasts within the tumor was probably in the range 70 to 80 hr, with a wide distribution of intermitotic times.

Cell Death and Resorption. The rate of growth of a tumor is determined not only by the rate of proliferation of the cells within it, but also by their rate of death or loss, and by the rate at which dead tissue is lysed and resorbed. Pyknotic endothelial cells or fibroblasts were not often observed in tumor sections, but the presence of large areas of necrosis (Table 1) suggest a high rate of death from the carcinoma cell population.

The rate of loss of cells from the population may be estimated as a fraction (ϕ) of the rate of cell production by the relation (18):

\[ \phi = 1 - \frac{T}{T_D} \]  

(C)

Here, T_D is the cell population-doubling time, but since the proportion of tumor volume occupied by carcinoma cells changes rather slowly (Table 1), it may be approximated by the tumor volume-doubling time. For tumors weighing between 0.5 and 1.0 g, the estimated parameter values were T = 22 hr and T_D = 60 hr; thus, the rate of loss of cells from the population was about 60% of the rate of cell production. Since carcinoma cells migrate rapidly toward a region of necrotic tissue (Chart 2), most of this cell loss was probably cell death within the tumor.

In tumors that contain large areas of necrosis, the rate of resorption of necrotic tissue can have a profound influence on the rate of tumor growth. If resorption is assumed to be an exponential process, it may be represented by a halving time, T_N (24). The halving time may be estimated in terms of the fraction of the tumor volume occupied by necrotic tissue (f_N), the turnover time of the carcinoma cells (T), and
the volume-doubling time of the tumor ($T_D$) by the relation (24):

$$\frac{1}{T_D} = \frac{(1-f_N)}{T} - \frac{f_N}{T_N}$$

(D)

This formula applies to an exponentially growing tumor containing a constant proportion of necrosis and no fibrous tissue. However, an approximate value of $T_N$ was obtained by using mean values of the parameters in Equation D and by neglecting the small amount of fibrous tissue. With the use of the value $f_N = 0.3$ (Table 1), Equation D defines a halving time for necrotic resorption of about 20 hr.

Estimates of $T_N$ are rather sensitive to values of the other parameters in Equation D, and the above value of 20 hr may be rather inaccurate. However, it does indicate a quite rapid rate of resorption from the tumor, and breakdown products from the necrosis therefore diffuse through neighboring viable tissue toward blood vessels. Some of these products may be toxic (21) and therefore might constitute one of the factors leading to inhibition of cell proliferation and cell death (25).

**DISCUSSION**

The labeled mitoses technique is, at present, the most sensitive method for estimating duration of cell cycle phases, particularly when analyzed by computer methods. This technique has been combined with a repeated labeling experiment to measure turnover time for carcinoma cells. The repeated labeling method used alone is less sensitive to cell cycle parameters, and much wider confidence limits must be assigned to the estimates of turnover time for capillary endothelial cells and for fibroblasts than for carcinoma cells. However, by comparing calculated repeated labeling curves with experimental data, the present method of estimating turnover time applies a more rigorous test of the assumptions than an estimate based on a single labeling index.

Because of variability in grain count over labeled carcinoma cells (Table 2) it was necessary to inject a rather high dose of tritiated thymidine (approximately 2 μCi/g body weight) and to use autoradiograph exposure times of up to 2 months. The rather low grain counts of labeled cells near regions of necrosis demonstrate either an extended duration of DNA synthesis or a lesser availability of tritiated thymidine. In an earlier study with a tumor of similar morphology, the duration of the S phase was found to remain constant in regions where the mean grain count was low (23). Probably, the variable grain count results from competition between labeled thymidine diffusing from blood vessels and a large endogenous pool of unlabeled DNA precursors diffusing from regions of necrosis. Also, hypoxia is known to inhibit uptake of tritiated thymidine by tissue pieces in vitro (15). Variability in grain count could lead to the scoring of cells in DNA synthesis as unlabeled. However, the comparable variations in mitotic and thymidine labeling indices (Table 2), and the reasonably sharp cutoff of grain counts between labeled and unlabeled cells, suggest that thymidine was available to label most of the cells in DNA synthesis.

Measurements of labeling index after multiple and single injections of tritiated thymidine (Charts 5 and 6) have been found to be consistent with a turnover time of 50 to 60 hr for capillary endothelial cells; this is considerably longer than the estimate for carcinoma cells (22 hr). The results of Chart 6 also imply that capillary endothelial cells are not derived from a faster-proliferating precursor population. If such a population existed, the labeling index of endothelial cells at intervals after a single injection of tritiated thymidine should increase to the initial labeling index of the precursor cells. This behavior was observed for the population of carcinoma cells (Chart 2): the precursor population consisted of the cells adjacent to capillaries. The absence of a steady increase in the labeling index of capillary endothelial cells (Chart 6) suggests that extension of the capillary network in the growing tumor depends mainly on the division of endothelial cells within the capillary walls.

The rather different rates of proliferation of parenchymal and stromal cells may be a major cause of slowing of tumor growth (Chart 1). In a small, well-nourished tumor, the carcinoma cells are probably all in cycle with a turnover time of 13 hr or less (Chart 3). As the tumor grows, the relatively slow proliferation of endothelial cells would lead to an increase in the mean intercapillary distance and to a decrease in the concentration of nutrients in regions far from blood vessels. This probably leads to a slower rate of proliferation of carcinoma cells, and to cell death, so that a morphological relationship with necrosis in regions remote from blood vessels is established. In the present tumor, the observation of cords of viable tissue surrounding isolated blood vessels is consistent with this hypothesis.

The concentrations of several nutrient metabolites or catabolites might influence the rate of proliferation of carcinoma cells. However, there is evidence from tissue culture experiments (2, 4) and from in vivo studies of another transplanted tumor (23, 25) that the rate of cell proliferation is particularly sensitive to the local oxygen concentration. The similarity of the measured range of tumor cord radii to a calculated range of oxygen diffusion distances for mouse carcinomas (22) suggests also that hypoxia is involved in the causation of necrosis at the periphery of a tumor cord. Tumor cells are known to be resistant to hypoxia in ascites tumors, but in solid tumors hypoxia might enhance the action of cytotoxic agents diffusing from a region of necrosis (24, 25).

Because of a high rate of cell death, the doubling time of the present tumor in the volume range of 0.5 to 1.0 ml (2.5 days) was similar to the estimated turnover time of endothelial cells. This suggests that the concentration of capillaries in larger tumors should remain constant, as reported by Gullino (9) for rat tumors. However, the doubling time of the present tumor continued to increase (Chart 1). This continued slowing of tumor growth was probably caused by vascular stasis and consequent loss of capillary function. By a technique in which $^{51}$Cr-labeled erythrocytes are injected into tumor-bearing animals and located in autoradiographs (26), it has been shown that the tumor contains a rather
high proportion of static blood (I. F. Tannock, unpublished observations). Thus, the rate of tumor growth probably is limited both by the proliferation of the endothelial cells and by loss of capillary function caused by blood stasis.

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REFERENCES

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