Cell Kinetics and Growth of Squamous Cell Carcinomas in Man

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SUMMARY

Five squamous cell carcinomas of the head (skin, lip, or gum) were investigated just before therapy. Two of the tumors were reinvestigated after therapy treatment with either methotrexate or a combination of methotrexate and X-radiation. Pulse labeling of DNA was achieved by intracarotid injection of 2 mCi thymidine-H, via a permanent catheter applied for regional infusion therapy. The time-course changes in tumors of the fraction of labeled mitoses were assessed by autoradiography of small biopsic specimens. After subtraction of photographic background (applying an iterative method), the autoradiographic data were analyzed using the single-population model of Barrett. Analyses by multipopulation models were also tried, but the use of these more complex hypotheses was found to be unjustified. Results are as follows. In the set of tumors, mean duration of DNA synthesis ranges from 18 to 34 hr; mean intermitotic time ranges from 52 to 88 hr; initial labeling index ranges from 11 to 36%; growth fraction ranges from 31 to 84%; cell birth rate ranges from 43 to 138 cells/hr and per 10^4 cells in tissue; cell loss rate ranges from 40 to 114 cells/hr and per 10^4 cells in tissue; cell loss factor ranges from 78 to 93% of cell birth rate. The distribution of intermitotic times is broad in most but not all tumors and ranges from almost total symmetry to pronounced skewness. Intermitotic time distributions of recurrences after therapy may differ considerably from those of corresponding carcinomas before therapy. Doubling times of the carcinomas were also estimated. A correlation appears to exist between doubling time and cell kinetic parameters of the tumors. In general, the data suggest that a squamous cell carcinoma grows faster because of the cooperative effect of a higher cell birth rate and a smaller cell loss factor. A higher cell birth rate is found to be largely the result of a larger growth fraction, with intermitotic time playing a marginal role. In particular, modal intermitotic times discernibly decrease with increasing tumor growth rate. The above correlations between cell kinetic parameters and rate of tumor growth also apply to faster-growing recurrences as compared to corresponding tumors before either methotrexate or methotrexate plus X-radiation.

INTRODUCTION

During the last 15 years the study of cell kinetics in experimental tumor research has produced considerable progress in our understanding of tumors at the cellular level, and this progress has implications for cancer therapy (2). However, clinical exploitation of new concepts resulting from cell kinetic studies requires specific understanding of cell cycle parameters in human neoplastic and normal tissues, both untreated and perturbed by application of therapeutic measures (10, 15, 19, 27, 32).

Human data are scarce because the requirements of reliable methodology for cell cycle measurement are only rarely applicable to man. Cells must be sufficiently labeled and this is generally achieved by systemic administration of large doses of tritiated thymidine. Multiple sequential biopsies of the relevant tissue must be performed to follow the passage of labeled cells through mitosis according to the method of Quastler and Sherman (24). In addition to the pioneer studies by Clarkson et al. (8) on neoplastic effusions in man, cell cycle data by the Quastler and Sherman method have been obtained for the following solid tumors in man: 5 epitheliomas of skin (12); 2 invasive squamous cell carcinomas of cervix (4); metastatic skin nodules in 3 patients with advanced breast cancer and 3 patients with malignant melanomas (34); s.c. metastases of melanoma in 2 patients (26); a massive metastasis of neuroblastoma (33); and a superficial reticulum cell sarcoma (23). Not only was a minimal number of tumors studied, but for most of those examined, a 2nd wave of labeled mitoses was either not seen or was considered questionable. In several cases, this apparent lack of a 2nd wave may be attributed to unavoidably insufficient sampling. Therefore, a direct estimate of the duration of the cell cycle is not available for most of the tumors studied.

To improve our understanding of cell population kinetics in solid human tumors, 19 patients bearing squamous cell carcinomas of the head were studied by the Quastler and Sherman method. However, data sufficient for estimating cycle parameters with a reasonable degree of confidence were obtained for only 4 primary tumors and 3 tumor recurrences in 5 patients. Contrary to previous investigators, we have (a) achieved labeling of tumor cell DNA by intracarotid injection of a relatively low amount of thymi-
dine-^3H; (b) estimated the doubling time of the tumors under study in order to find a possible correlation with cell kinetic data; and (c) studied 2 tumors before and shortly after a course of therapy, when the tumors were regrowing at a faster rate after nearly complete destruction. A preliminary account of this research has been given (7).

**MATERIALS AND METHODS**

**Materials**

Thymidine-methyl-^3H (specific activity, 5 Ci/mmmole) was obtained from New England Nuclear, Boston, Mass. NTB liquid nuclear track emulsion was obtained from Eastman Kodak, Rochester, N. Y.

**Thymidine Injection and Tumor Biopsy**

Patients, all consenting adult males, were prepared by the surgeon for regional infusion chemotherapy by the introduction of a permanent catheter into the external carotid artery; the catheter was lowered to a position just above bifurcation of the common carotid artery and methylene blue was injected to assure that subsequent thymidine would actually reach the tumor. Before anti-cancer treatment was started, 2 mCi of thymidine were injected and sequential biopsies were taken from the tumor.

The thymidine-^3H was diluted in 10 ml of 0.85% NaCl in water and injected at a constant rate over a 10-min period; it was followed by 10 ml of the same nonradioactive solution. Thymidine-^3H injection commenced at Time 0 of the experiments. Biopsies were taken from areas that were reached by blood and thymidine, as previously shown by methylene blue. The biopsies were taken with a punch and consisted of a few cu mm of tissue, an amount more than sufficient for measurement of total radioactivity or for squash preparation.

**Assays on Biopsy Specimens**

In the experiment carried out to investigate duration of the thymidine pulse in tumors, the biopsy specimens were treated with ice-cold 5% trichloroacetic acid, and radioactivity in the acid-soluble extracts was assayed by liquid scintillation.

In the autoradiographic studies, bulk Feulgen coloration of the biopsy specimen, squash preparations, and the method of autoradiography with liquid emulsion were carried out as previously described (5). Exposure of slides was about 4 weeks at 4°. All slides from the same tumor were processed together. The squash preparations of each specimen were scanned under oil, and the number of silver grains (grain count) up to 100 mitoses (63 on the average) and 100 labeled interphase nuclei were recorded. Only epithelial cells were considered.

**Subtraction of Autoradiographic Background**

Assessment of background grain-count distributions and their elimination to obtain corrected grain-count data were carried out according to theoretical requirements and by applying an iterative method by means of an IBM 1130 computer, as described elsewhere (3). Preliminary data (7, 11) could thus be improved. The computer was programmed not only to eliminate the background but also to furnish corrected labeling indices of mitoses and interphase cell nuclei. Background measurements for the tumors in this paper are shown in Chart 1.

**Glossary and Computation of Kinetic Parameters**

The cell replication cycle is typically subdivided into the following phases: M, mitotic phase; S, DNA-synthetic phase, during which the DNA replicates; G1, postmitotic presynthetic phase; G2, postsynthetic, premiotic phase. C (for cycle) = M + S + G1 + G2. Cells outside the cycle may be either in a reversible condition of nonproliferation, the G0 state, or irreversibly unable to replicate, the Z state (6).

**Transit Times.** $T_c$ is duration of cell replication cycle; it is usually equated to intermitotic time ($T_i$), *i.e.*, the time interval between 2 successive passages of cells through mitosis. $T_{S1}$, $T_M$, etc., represent duration of S phase, etc. Unlike preliminary studies (7, 11), the time parameters were extracted from FLM curves (24) using the simulation method of Barrett (1) and achieving the best fit by means of the algorithm of Marquardt (18) developed for least-squares estimation of non-linear parameters. Transit times derived by the manual method of Quastler and Sherman (24) were used as starting parameters.

**Indices.** $I_M$, $I_S$, $I_Z$ are the fractions (indices) of tissue cells in the given phase or state. $I_p$ (= $I_M + I_G + I_S + I_G_0$)
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is the fraction of tissue cells in the replication cycle or the "growth fraction" of Mendelsohn (19). \( I_s \) was measured by the thymidine-\(^3\)H LI, i.e., the fraction of nuclei that became DNA labeled within a time \( T_A = T_G \), from a pulse of the radioactive DNA precursor. When more than 1 assessment of labeling index within \( T_A \) was available, we used the average value for LI, a procedure that increases accuracy. In one of the tumors (F.M./R-6) a LI could not be assessed directly. An indirect estimate-in-excess of LI, for this tumor was obtained by averaging all available interphase cell-labelling indices in the 5 biopsies taken between \( T_A = T_G \) and \( T_B = T_I \) from thymidine-\(^3\)H injection and then multiplying this excessive estimate by 0.69 (25% \times 0.69 = 18%). The correction factor came from the finding that the LI in the other 6 tumors was on the average only 69% of the mean labeling index between \( T_A \) and \( T_B \).

The growth fraction \( (I_p) \) was computed from \( I_p = I_B (T_I/T_A) \), using average \( T_B \) and \( T_I \) and disregarding growth correction because the relative difference between cell loss rate and cell birth rate was generally small, i.e., the condition is only slightly different from the steady state.

Transit Rates. Cell birth rate \( (R_B) \), is defined throughout as the number of cell divisions (number of additional cells produced) in a tissue per unit time and unit number of cells in the tissue; in this paper the units were 1 hr and \( 10^4 \) cells. Cell birth rate is computed as follows: \( R_B = \frac{(I_B/T_B)}{T} \times 10^4 \), using LI as an estimate of \( I_B \) and average \( T_B \). Cell loss rate \( (R_L) \) is the number of cells lost from the tissue per unit time and unit number of cells in the tissue. Rate of loss is computed as \( R_L = R_B - \frac{[\ln 2 \times 10^4]}{T_D} \), where \( T_D (hr) \) is the tumor-doubling time. \( (R_L \times 100)/R_B \) shows the \( R_L \) as a percentage of \( R_B \) and corresponds to the cell loss factor \( (\phi) \) of Steel (28).

Tumor-doubling Time and Tumor Growth Rate

During the initial preparation of the patient, lasting about 2 weeks (work which included dental care, introduction of permanent catheter, cell kinetic study, etc.), tumor diameters were assessed at least twice. When possible, maximum width \( (d_1) \), width at right angle to the maximum \( (d_2) \), and maximum height \( (d_3) \) of the tumor were assessed either by caliper or by a 100-mm transparent lineal. The relative tumor volume was computed as \( d_1 \times d_2 \times d_3 \) (20). In some cases only \( d_1 \) and \( d_2 \) could be assessed. In this case relative volume was computed as the product of the 2 available diameters times their average. For each tumor the same criteria were applied each time a measurement was made and, therefore, relative volume increments are realistic. Doubling time of each tumor was derived from 2 or more estimates of relative tumor volume taken during the 2-week preliminary work-up, on the assumption that growth was exponential. In 2 cases, the slow-growing tumors M.C./R-2 and C.A./R-11, no apparent increase of diameters could be detected over a 2- and a 3-week observation period, respectively. Therefore, doubling times of these 2 tumors are minimum estimates, based on the maximum diameter increase that could defy detection by caliper. According to our experience, this is at most 5%, which is equivalent to an about 15% volume increase for a sphere. Tumor growth rate \( (R_G) \) is the net number of cells produced per hr and \( 10^4 \) cells in a tissue. It is derived from tumor-doubling time as follows: \( R_G = \frac{(\ln 2 \times 10^4)}{T_D} \times 24 \). Or, if cell birth rate and cell loss rate have already been computed, this can be accomplished by simply subtracting loss from birth rate.

RESULTS AND DISCUSSION

Radioactivity Pulse in Tumors after Intraarterial Injection of Thymidine-\(^3\)H. Patient F. F., a 54-year-old male, had a large squamous cell carcinoma (R-1) on the bottom of his mouth. The relative volume of the tumor was about 30 cu cm. Thymidine-\(^3\)H (2 mCi) was injected into the homolateral carotid of the tumor, via a permanent catheter applied for regional chemotherapy, at a constant rate over a 10-min period. Biopsies were taken at 5, 10, 15, 20, and 30 min after the beginning of the injection. Radioactivity, extractable by ice-cold trichloroacetic acid in the biopsy specimens, was assayed by scintillation. The results are presented in Chart 2. It is clear that the log of radioactivity concentration in specimens shows a time-dependent quasilinear decrease, with a halving time of 6 to 10 min. At 30 min after injection, i.e., 20 min after the end of injection, free radioactivity in the tumors was less than 8% of the amount at 5 min. Tumor fragments taken from this same biopsy were squashed and coated with emulsion; after a 4-week exposure, a marked specific labeling of nuclei in DNA synthesis was evident. Thus, intracarotid injection of thymidine-\(^3\)H not only is a satisfactory method for labeling DNA in squamous cell

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A Premise on the Analysis of the FLM Data. We have analyzed our data using the mathematical model of Barrett (1). This system assumes a single-cycling cell population with independent log-normal distributions of transit times. The model was simulated by the Monte Carlo method. The advantages of simulation over manual methods for extracting information from labeled mitoses data have already been pointed out (21, 22, 30).

However, we have also developed models of tumors consisting of 2 (or more) subpopulations with different modal cycle times and have tested these models against the data in this paper in an attempt to carry out an objective comparison with the classical single-population model with regard to their abilities to fit the data and furnish reliable results. This has been done largely in consideration of cases like the R-11 tumor, in which hand-drawing of a curve strictly following the experimental points gives a pattern compatible with the existence of a bimodal distribution of cycle times. The models tested were basically of 2 types: (a) 2 independent subpopulations; (b) a progenitor population feeding 1 or more dependent subpopulations. Several variations of these 2 basic models have been developed, but they will not be detailed here.

As compared to the 1-population model of Barrett, multipopulation models of the above type (1) fit better tumors like R-11 but fit most of the other tumors either worse or about the same as the simpler Barrett’s model. The overall improvement in analyzing the data in this paper according to multipopulation models is therefore doubtful and certainly does not justify choosing such complex models for the general analysis of results. In short, the conclusion drawn from this theoretical study is that the available data do not furnish evidence for a plurimodal distribution of cycle times in squamous cell carcinomas, i.e., they do not prove that the tumor consists of cell populations with distinctly different modal cycle times. However, the limited precision of FLM data also does not disprove such a possibility.

Gross Properties and Treatments of Tumors under Study. Some of the properties of tumors in the cell kinetics study are summarized in Table 1. Four of the carcinomas (R-6, R-11, R-12, and R-13) were previously untreated primaries, 2 of which (R-6 and R-12) regressed completely after prolonged, continuous intraarterial injection of MTX (3 mg/day for 10 to 12 days) followed by radiotherapy. The other 2 (R-11 and R-13), 1 of which had been treated with MTX only (R-13), showed incomplete response to therapy and, after more than 80% shrinkage of tumor mass, started to grow again. These recurrences (R-19 and R-15) were submitted to a 2nd cell kinetic study, 104 (R-19) and 21 days (R-15) after the end of the course of therapy, when they were regrowing at a faster rate than the corresponding primaries before therapy. Response of these 2 recurrences to another course of combined radiotherapy + MTX therapy after the 2nd kinetic study ranged from virtually absent (R-19) to incomplete (R-15). The last carcinoma (M.C./R-2r) was a local recurrence of a primary submitted to radiotherapy 5 to 6 months earlier; radiation was followed by apparently complete disappearance of tumor mass. The primary tumor was treated before this study began, and therefore information on its cell kinetics properties is lacking.

Doubling times were estimated by following the increase in tumor diameter in the period immediately before the cell kinetic study and assuming the existence of exponential growth, as described in “Materials and Methods.” For the very slow growing tumors C.A./R-11 and M.C./R-2r, the doubling times were minimum estimates. Table 1 shows that doubling time and tumor volume are not correlated in this group of carcinomas. None of the tumors in this study showed signs of gross necrosis.

FLM Curves, Interphase LI, and Derived Cell Kinetic Parameters. FLM data are shown in the upper parts of Charts 1 to 5, with the solid curves demonstrating the computer’s best fit to the experimental points of the single-population model of Barrett. The lower parts of Charts 3 to 7 show the frequency distribution of intermitotic times derived by the computer from the FLM curve above.

Charts 3 and 4 present data for the 2 untreated primary carcinomas (F.M./R-6 and L.F./R-12) that disappeared following therapy after the kinetic study. Charts 5 and 6 show, side by side, the data of primary carcinomas and corresponding recurrences growing at a faster rate shortly after more than 80% destruction of the primary mass by therapy with either MTX + radiotherapy (C.A./R-11) or MTX alone (M.R./R-13). Chart 7 shows the data for a slow-growing recurrence (M.C./R-2r), 5 to 6 months after radiotherapy of the primary. There are no data for the primary.

Mean, median, and modal transit times of the tumors, extracted by the computer from FLM curves, as well as tumor growth rates, are summarized in Table 2. Also LI,’s for the various tumors are shown in Table 2 and, using mean transit times and LI, growth fraction, cell birth rate, cell loss rate, and cell loss factor, were computed for each carcinoma.

The LI,’s are based on the following data. In F.M./R-6, interphase labeling indices were obtained only between Ta = Tg and Ta = T1 from thymidine injection; there were 5 biopsies in this period, with an average of 25% interphase cells labeled over background (single values: 17, 22, 29, 27, and 29%). A LI, of 18% was obtained from this excess estimate using an experimentally obtained correction factor, as described in “Materials and Methods.” For L.F./R-12, in 1 biopsy taken within Ta = Tg from thymidine injection, 81 of 737 nuclei were labeled (LI, = 11%). In 2 biopsies of C.A./R-11 taken within Ta = Tg, 191 of 1589
Table 1
Gross properties and treatment of squamous cell carcinomas of head (man)

<table>
<thead>
<tr>
<th>Patient/tumor</th>
<th>Primary (P) or local recurrence (R)</th>
<th>Site of lesion</th>
<th>Relative volume at study (cu cm)</th>
<th>Doubling time (days)</th>
<th>Treatment previous to cell kinetics</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.M./R-6 (32)*</td>
<td>P</td>
<td>Skin of temple</td>
<td>90</td>
<td>13</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>L.F./R-12 (64)</td>
<td>P</td>
<td>Lower lip</td>
<td>50</td>
<td>25</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>C.A./R-11 (48)</td>
<td>P</td>
<td>Lower gum</td>
<td>96</td>
<td>&gt;107</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>C.A./R-19 (48)</td>
<td>R of above carcinoma</td>
<td>Lower gum</td>
<td>132</td>
<td>18</td>
<td>MTX* + X-radiation</td>
<td>104 days after &gt; 80% destruction of primary (R-11) by MTX + X-radiation</td>
</tr>
<tr>
<td>M.R./R-13 (74)</td>
<td>P</td>
<td>Lower lip</td>
<td>28</td>
<td>34</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M.R./R-15 (74)</td>
<td>R of above carcinoma</td>
<td>Lower lip</td>
<td>17</td>
<td>12</td>
<td>MTX*</td>
<td>21 days after &gt; 80% destruction of primary (R-13) by MTX</td>
</tr>
<tr>
<td>M.C./R-2 (48)</td>
<td>R</td>
<td>Lower lip</td>
<td>3.9</td>
<td>&gt;68</td>
<td>X-radiation*</td>
<td>5–6 mo. after virtual destruction of primary by X-radiation</td>
</tr>
</tbody>
</table>

* All patients are male.
* Relative/true volume is 0.52 for a sphere.
* At time of study and assuming exponential growth; values for C.A./R-11 and M.C./R-2 are minimum estimates (see "Materials and Methods").
* Numbers in parentheses, patient age (years).
* Continuous intraarterial infusion at 3 mg/day for 10 to 12 days.
* 6000 to 7000 rads.

Chart 3. Primary, previously untreated, large squamous cell carcinomas (R-6) of the skin of temple in P.M. Upper curve, computer best fit of the Barrett's model to experimental FLM points; lower curve, intermitotic time frequency distribution derived, also by the computer, from the above best-fit FLM curve. See text for further details. Cell transit times and other properties are given in Tables 1 and 2. TdR-3H, thymidine-3H.

Chart 4. Primary, previously untreated squamous cell carcinoma (R-12) of the lower lip in Patient L.F. See legend to Chart 3 for explanation of curves and Tables 1 and 2 for data. TdR-3H, thymidine-3H.

nuclei were labeled (LI_f = 12%). For C.A./R-19, in 1 biopsy within T_A = T_DD, 72 of 403 nuclei were labeled (LI_f = 18%). In 3 biopsies of M.R./R-13 within T_A = T_DD, 274 of 2328 nuclei were labeled (LI_f = 12%). In M.R./R-15, in 1 biopsy within T_A = T_D0, 96 of 267 nuclei were labeled (LI_f = 36%); this high labeling index was confirmed by the finding that a biopsy at 5.5 hr after injection of thymidine-3H, i.e., slightly over the rather short T_A = T_DD of 4 hr in this tumor, had 155 of 378 nuclei labeled (LI_f = 41%). In 3
Chart 5. Left, primary, previously untreated, squamous cell carcinoma (R-11) of the lower gum in patient C. A. Right, local recurrence of R-11, growing faster 104 days after >80% destruction of R-11 by MTX and X-radiations. The 100% experimental point at 70 hr was excluded in assessing the best fit of Barrett's model to the set of data. See legend to chart 3 for explanations of curves and Tables 1 and 2 for data. TdR-3H, thymidine-3H.

Chart 6. Left, primary, previously untreated, strongly keratinizing squamous cell carcinoma (R-13) of the lower lip in patient M. R. Right, local recurrence of R-13, regrowing faster 21 days after >80% destruction of R-13 by MTX. See legend to Chart 3 for explanations of curves and Tables 1 and 2 for data. TdR-3H, thymidine-3H.

biopsies from M.C./R-2 taken within $T_A = T_{G1}$, 185 of 1130 nuclei were labeled (LI = 16%).

**DISCUSSION**

**Transit Times.** From the data in Table 2, one can see that the mean intermitotic time ($T_I$) of squamous cell carcinomas in this study varied from 88 to 52 hr, median $T_I$ varied from 82 to 49 hr, and modal $T_I$ varied from 75 to 38 hr. Mean duration of DNA synthesis varied from 34 to 18 hr, median $T_S$ varied from 32 to 17 hr, and modal $T_S$ varied from 32 to 14 hr. Average duration of DNA synthesis accounted for 26 to 42% of average $T_I$.

In 4 cases, including 2 basal cell carcinomas and 2
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Chart 7. Locally recurrent squamous cell carcinoma (R-2) of the lower lip in patient M. C. The tumor was regrowing 5 to 6 months after total destruction of primary by X-radiations. There is no cell kinetic study of the primary. See legend to Chart 3 for explanation of curves and Tables 1 and 2 for data.

epidermoid epitheliomas, Frindel et al. (12) computed intermitotic times (varying from 1 to 4 days) and the durations of DNA synthesis (between 7 and 19 hr) using nonsimulation methods. The 2 epidermoid epitheliomas showed intermitotic periods of 25 and 48 hr and DNA synthesis times of 11 and 12 hr, respectively. These values are generally lower than those in this series of squamous cell carcinomas. The method of analysis cannot account for the difference because Steel (30), using a simulation method on the pooled data of the 2 epitheliomas described by Frindel et al. (12), computed a median $T_I$ of 38 hr and a $T_S$ of 12 hr. The $T_I$ and $T_S$ of these 2 epitheliomas are also lower than any other $T_I$ and $T_S$ computed by Steel (30), applying a simulation method to other available human tumor data.

Distribution of Transit Times. As shown by coefficients of variation in Table 2, most tumors show a very broad spread of transit times. This finding supports the results of other studies with human tumors (30, 32).

Present data, however, show a more complex picture than simply a broad, generic variation. Comparative analysis of intermitotic time distributions in Charts 3 to 7 shows that, while $T_I$ of most tumors has a broad distribution, some tumors (R-19) demonstrate a rather narrow one. Furthermore, as shown by the wide intertumor variation of intermitotic time average/mode ratio (from 1.08 to 1.6; see Table 2), the distributions range from almost perfect symmetry to pronounced skewness. Finally, the recurrences (C.A. R-19 and M.R. R-15) shortly after destruction of the primary tumors by MTX, with or without additional X-radiations, show very definite changes in the spread and symmetry of the distribution with respect to the original tumors.

Thus, the width and form of distributions of intermitotic times reflect the behavior of individual tumors, the genetic potential of the host, and the biological characteristics of the malignant cells. The variations in distribution patterns may provide some indication of the sensitivity of the tumor to therapy and its ability to respond to changes in environmental conditions. Further studies are necessary to elucidate the significance of these observations.
time may vary drastically among squamous cell carcinomas. This shows that there are differences in intermitotic times among the tumors, differences that are less evident when only average, median, or modal values are considered. In turn, this indicates that factors that influence cell cycle time, either cell inherent or environmental, must be active in squamous cell carcinomas and must vary quantitatively and/or qualitatively from tumor to tumor. In rodents, with regard to cell-inherent factors, we know that primary, well-differentiated tumors have a longer average $T_m$ and a much broader span of time than the frequently transplanted, less differentiated tumors (30) and that cell maturation in renewal tissues affects intermitotic time (6, 15). Also, environmental factors, like pH (16), immune response (25), humoral agents (32), etc., are known to influence intermitotic time and to vary from tumor to tumor. Some or all of these factors may also be operative in squamous cell carcinomas of man.

Most, if not all, of the factors cited above as possible causes of differences in intermitotic time among tumors are also known to vary within a tumor. For example, 2 tumors of the same histogenesis may show an overall difference in the degree of differentiation. The squamous cell carcinoma is a case in point. In addition, environmental factors are known to form gradients within a tumor and, while their heterogeneity appears to have little influence on the distribution of intermitotic times in fast-growing rodent tumors (31), this may well not apply to far slower growing human tumors (15). The above biological considerations thus suggest some degree of compartmentalization of cells with respect to transit times in squamous cell carcinomas, and this in turn furnishes a possible explanation for the broad variation of intermitotic times found in most of these tumors. The considerable differences in the distributions, from virtual symmetry to sharp skewness, also concur in suggesting the presence of cells cycling with different modal speeds.

**Limitations of the Barrett Tumor Model.** The above hypothesis of compartmentalization in transit times is somewhat in conflict with the assumption of the classical model of Barrett (1), which we have used for the analysis of the present data (that being that transit times are completely independent). As pointed out in “Results,” the accuracy of the FLM data was insufficient to either show or exclude the presence of subpopulations with different modal times, and therefore the simpler Barrett model was chosen in the end, despite the biological considerations suggesting that the model gave an oversimplified interpretation of the real situation. The limitations of the model in its application to human squamous cell carcinomas could, at least in part, justify the imperfections in the fit of theoretical curves to FLM data of some tumors under study. Tumor R-11 (Chart 5) has a larger spread and fits the model much less satisfactorily than its recurrence R-19, which shows much less variance of intermitotic times and is fitted very well by the model. This supports the interpretation that variance is produced, at least in part, by compartmentalization of intermitotic times and, therefore, the smaller the variance, the closer is the tumor to the hypothetical model of Barrett.

A further implication of the poor fit resulting from limitations of the model is that some spurious spread is added to the real biological variance and, therefore, in the cases of a poor match, the variance of intermitotic time assessed by using the Barrett model is an estimate-in-excess.

**Indices and Transit Rates.** In the present set of tumors, the LI$_r$ and Transit Rates. In the present set of tumors, the LI$_r$ ranges from 11 to 36% and the growth fraction from 31 to 84%; cell birth rate ranges from 43 to 138 cells per 10^4 cells in tissue per hr; and cell loss rate ranges from 40 to 114 cells per 10^4 cells in tissue per hr. Cell loss rate ranges from 78 to 93% of cell birth rate. For their 2 epidermoid epithelioamas, Frindel et al. (12) found labeling indices of 10 and 17% and for both tumors a growth fraction of 40%.

One can see that differences in LI$_r$ and growth fraction in the present set of tumors are large and indeed account for most of the differences in cell birth rate among the tumors. This will be discussed in detail later, when birth rate will be considered in relation to growth rate. Most of the factors that are known to influence intermitotic time are also effective in affecting the growth fraction of tumors (31). The large differences in growth fraction thus corroborate the conclusion based on the above considerations on intermitotic times, i.e., that these biological factors must vary considerably, quantitatively, and/or qualitatively, among the tumors.

With regard to cell loss rate, the only previous estimate for human tumors obtained, applying the model of Barrett, was that for a malignant melanoma (30) where the cell loss factor was computed at 70%. Estimates by Frindel et al. (12) in 2 basal cell carcinomas and 1 epidermoid epithelioama of man gave values ranging from 90 to 97%, and they are therefore close to the higher values in the present series of carcinomas. Biological factors at the basis of cell loss in tumors have recently been reviewed by Cooper (9).

**Correlation between Growth Rate and Kinetic Parameters.** The availability of doubling time estimates for the present set of squamous cell carcinomas made it possible to carry out an analysis of the cell kinetic basis of difference in growth rate of squamous cell carcinomas. To this end, cell birth rate, cell loss rate, and cell loss rate as percentage of cell birth rate (cell loss factor) are plotted against tumor-doubling time in Chart 8.

From the slowest to the fastest growing squamous-cell carcinomas, the cell birth rate increases from 43 to 138 cells/hr and per 10^4 cells in tissue, i.e., by a factor of 3.2. Cell loss rate also increases, although somewhat less, from 40 to 114 cells/hr and per 10^4 cells in tissue, i.e., by a factor of 2.9. Thus, while cell loss rate was 93% of cell birth rate in the slowest growing tumor, it was only 83% in the fastest growing tumors. Is this relatively slower rate of cell loss in tumors with a higher birth rate significant? This cannot be decided by a test of significance, but consider (a) that the 2 tumors with the lowest birth rates have the highest cell loss factors, i.e., 91 and 93%; and (b) that a pattern of decreasing cell loss factor with increasing tumor growth speed is present in experimental tumors (29). Thus, it seems reasonable to conclude that a squamous cell carcinoma grows faster because of the cooperative effect of a higher cell birth rate and a smaller cell loss factor. Using data at
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face value, one may compute that the variation in growth rate by a factor of about 8 in the present set of tumors is brought about by a 3.2 times variation (138/43) in cell birth rate times a 2.4 times variation \([100-83]/(100-93)\) in net saving of cells due to change of cell loss factor. In more general terms, present data suggest that cell birth rate and cell loss rate concur with about equivalent weight in determining differences in growth rate of squamous cell carcinomas. This conclusion may not apply to differences in growth rate among human tumors of different histological origin (17).

A further insight into the relationship of cell birth rate to growth rate of a tumor was obtained by independent plotting of intermitotic time and growth fraction as a function of tumor-doubling time in Chart 9. One can see from Chart 9 that intermitotic times show a definite rise with increasing tumor-doubling times. This trend appears to be more pronounced for modal and median times which, over the whole range of doubling times, increases 1.6 and 1.4 times, respectively, in contrast to average intermitotic time which increases 1.2 times only. This different behavior of mode, median, and average (R-19 is an exception) can be explained by the fact that long to very long intermitotic times are also present in faster growing tumors and buffer the reflection of a shorter mode on the average intermitotic time. Because it is the average intermitotic time that concurs with the growth fraction in determining birth rate, it follows that intermitotic time plays a marginal role in the variations of birth rate, and therefore growth rate, within the present set of squamous cell carcinomas. As shown in Chart 9, it is the decrease in growth fraction that accounts for most of the variation in birth rate. Using data at their face value, 83% of the approximate 3.2 times variation in cell birth rate between the fastest and slowest growing carcinomas in the present group is brought about by a difference in growth fraction, while only the remaining 17% is accounted for by change in intermitotic time.

The estimates of tumor-doubling time that we were able to obtain are, of course, rough. Therefore it is important that the above conclusions regarding correlation between growth rate and cell kinetic parameters would still hold if our separation of tumors (determined by growth rate) would have been so basic as to simply distinguish them in (a) tumors showing no apparent growth (R-2, R-11) and (b) tumors showing visible growth (R-6, R-12, R-13, R-15, R-19) during the almost 2-week period of observation prior to therapy, or if we would have simply compared fast-growing tumors (R-6, R-15, R-19) with the remaining ones; or, as shown in Table 2, if we had compared the 2 tumors studied before a course of therapy (R-11, R-13) with their faster growing recurrences (R-19, R-15) shortly after the end of the therapeutic treatment that had resulted in almost complete destruction of the tumor mass. We can therefore conclude that, while roughness of doubling time estimates confer a certain latitude regarding degree of inclination and form of correlation curves, all evidence indicates that these correlations are basically sound. We choose to plot cell kinetic parameters against single doubling time estimates, instead of pooling tumors according to more general estimates of growth rate, because the correlations uncovered are quite smooth; this suggests that our measurements are basically sound.

A further consideration is in order with regard to
correlation between cell kinetic parameters and tumor-doubling time in the present set of tumors, i.e., that these tumors are a selected group. One of the prerogatives for which they were chosen is that they showed no gross ulceration or necrosis, as required in view of the multiple biopsies ahead. It is likely that, in cases in which there is gross ulceration with bacterial infection and necrosis, a correlation between rate of mass increase and cell kinetic parameters may not show up clearly because of these external interferences with the growth of the tumor.

It also seems worthwhile to point out that the correlations of cell kinetics with growth rate refer to the growth rate at or about the time of the cell kinetic study. Changes in growth rate during the natural life of a squamous cell carcinoma are known to occur, and possibly they result from the same basic differences in cell population kinetics as were found among different tumors growing at different speeds. Growth rate and cell kinetic parameters were shown to be independent of tumor size. Thus, factors other than size must be invoked to explain differences in growth rate and cell kinetic parameters in squamous cell carcinomas. Earlier in the paper we commented on some of them.

Finally, comparison of faster growing recurrences with their primaries before therapy has had prior attention. Tubiana (32) reported on decrease of cell cycle time in 2 human tumors after therapy, and studies with experimental tumors showed an increased cell production after either radiotherapy (14) or chemotherapy (13). It now seems clear that, within the same histological group of tumors, the case of the faster growing recurrence versus its slower growing primary is only a special case of the general behavior of cell kinetic parameters in tumors of differing growth rates.

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