Review of basic concepts of cell kinetics as applied to brain tumors

TAKAO HOSHINO, M.D., AND CHARLES B. WILSON, M.D.

Department of Neurological Surgery, University of California School of Medicine, San Francisco, California

The authors review and discuss the basic concepts of cell kinetics as applied to brain tumors. Uncontrolled growth of a neoplasm represents an expanding tumor cell population. Four growth parameters characterize the behavior of a neoplastic population: cell cycle time, growth fraction, tumor doubling time, and cell loss. The concept of provisionally nondividing cells explains the disparity between cell cycle time and tumor doubling time. Human gliomas, like many non-neural solid tumors, contain variable proportions of actively proliferating and nonproliferating tumor cells; this ratio is expressed by the growth fraction. The major kinetic difference between glioblastomas and differentiated astrocytomas resides in their respective growth fractions, in all likelihood an inherent biological characteristic of each tumor. Glioblastoma proliferates at a rapid rate, and only a high rate of cell loss prevents this tumor from doubling its volume in less than 1 week. The selection of drugs and design of drug schedules for treatment of glioblastomas should be made with the knowledge that 60% to 70% of the cells in this tumor are resting (nonproliferating). If experience with other solid tumors is any guide, judicious selection and combined use of drugs according to kinetically sound schedules will produce more effective chemotherapy of brain tumors.

KEY WORDS  •  cell kinetics  •  brain tumor cells

We believe chemotherapy will play a decisive role in the management of gliomas within the next decade. The striking success reported with chemotherapy of leukemia and solid non-neural tumors demonstrates that single and multiple oncolytic agents can eliminate tumor cells without irreversibly damaging the host's normal cell populations. This record indicates that more effective chemotherapy can be achieved by designing protocols based on tumor cell population kinetics. Kinetic information should influence not only the drug selected for attack on a particular tumor, but also the most appropriate schedule of drug administration.

Brain tumor chemotherapy has achieved an occasional dramatic regression; however, until recently the scientific basis for its success has not been understood. A few agents, notably the nitrosoureas, have shown unmistakable activity against some malignant gliomas. While the history of medicine contains accounts of many therapeutic advances discovered by chance, it seems unlikely that more effective chemotherapy will result from trial and error. Kinetic knowledge greatly enhances the possibility that active agents
already identified can be used in a more effective manner. Furthermore, agents previously discarded as inactive against brain tumors might prove to be effective if administered on a different schedule.

Anticipating a rapid expansion of brain tumor chemotherapy and a proportionately increased interest in the topic, we have prepared a review of tumor cell kinetics, including kinetic data obtained on human gliomas in our laboratories.

Basic Concepts of Cell Kinetics

The neoplastic process can be defined as a progressive increase, by cell division, in the cell population of a host. The proliferation kinetics of component cells define a tissue's rate of growth, and since the concept of a cell cycle was introduced in 1951, many investigators have sought to determine kinetic parameters of normally and abnormally proliferating tissues in vivo and in vitro.

Two classes of cell reproduction are recognized, namely, cell renewal systems, and expanding cell populations. Cell renewal systems are seen in many adult tissues such as bone marrow, small intestinal epithelium, and skin; in these systems the total population of cells remains constant because the rate of cell death equals the rate of cell birth. In contrast, we recognize two types of expanding cell populations in which cell birth exceeds cell death: 1) normal expanding tissues (controlled growth systems), represented by the growth of embryonal tissues, the fibroblastic response to injury, and regeneration of the liver after partial hepatectomy; and 2) uncontrolled systems, characterized by neoplastic growth.

At one time we assumed that all cells in a tumor cell population were proliferating, although at a slow rate, but newer information indicates the existence of cells residing in a nonproliferating pool where they have temporarily (and reversibly) withdrawn from the cells that are continuously dividing. This concept of a nonproliferating tumor cell population is supported by laboratory and clinical observations.

Cell loss is a feature of all tissue, whether neoplastic or normal. In normal tissue, mature cells function for their predetermined period and then die. In neoplastic tissue, cell loss, reflected by areas of focal or massive necrosis, occurs not as the inevitable fate of the mature cell but as a result of vascular or nutritional crises caused by overcrowding.

Four basic parameters define the growth characteristics of any tumor: 1) cell cycle time, or cell generation time (Tc), 2) growth fraction (GF), 3) tumor doubling time (Td), and 4) cell loss factor (CLF). Familiarity with these parameters is prerequisite to an understanding of tumor cell population kinetics.

Cell Cycle Time

Cell cycle or generation time is the period of time required for a proliferating cell to progress from one mitotic division to the next. Not all cells within a given tumor have the same cycle time. A range of variability exists, and cell cycle time as presently determined expresses the average cycle time in a specified tumor.

The cell cycle can be divided into four separate stages on the basis of nuclear deoxyribonucleic acid (DNA) content. The most readily identified stage (by light microscopy) is the mitotic phase (M), during which the previously duplicated chromosomes are shared between two daughter cells. The new daughter cells then enter the postmitotic gap or pre-DNA synthetic phase (G1), during which they synthesize ribonucleic acid (RNA), enzymes, and proteins in preparation for the beginning of DNA synthesis. From the G1 phase, cells enter the DNA synthesis

<table>
<thead>
<tr>
<th>Key to Abbreviations Used in this Review</th>
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<tbody>
<tr>
<td>CLF = cell loss factor</td>
</tr>
<tr>
<td>G0 = nonproliferating pool</td>
</tr>
<tr>
<td>G1* = presynthetic (postmitotic) gap phase</td>
</tr>
<tr>
<td>S* = DNA synthetic phase</td>
</tr>
<tr>
<td>G2* = postsynthetic (premitotic) gap phase</td>
</tr>
<tr>
<td>GF = growth fraction</td>
</tr>
<tr>
<td>LI = labeling index</td>
</tr>
<tr>
<td>M* = mitotic phase</td>
</tr>
<tr>
<td>MI = mitotic index</td>
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<tr>
<td>PLM = percent labeled mitoses</td>
</tr>
<tr>
<td>Tc = cell cycle (cell generation) time</td>
</tr>
<tr>
<td>Td = tumor doubling time</td>
</tr>
<tr>
<td>Tp = theoretical doubling time</td>
</tr>
<tr>
<td>Tg1, Tg2, Tm, Ts = duration of respective phases of the cell cycle; when added together = Ts</td>
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</tbody>
</table>

*Phases of cell activity are either in (G1, S, G2, M) or out (G0) of cell cycle.
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phase (S), in which they replicate DNA; chromosomal duplication is normally completed by the end of this phase. The end of DNA synthesis marks the beginning of another gap, the postsynthetic or premitotic phase (G₂), in which RNA and proteins are synthesized in preparation for mitosis (M phase), completing the cell cycle.

Cells in the G₁, S, and G₂ phases are called intermitotic cells, in contrast to the mitotic cells of the M phase. In G₁ phase cells, the amount of DNA (or number of chromosomes) in a nucleus is normally 2N (representing the normal diploid content); it increases during the S phase to 4N at the completion of DNA synthesis, and remains at 4N throughout the G₂ phase.

The tissue can be pulse- or flash-labeled in vitro by brief exposure to a labeled DNA precursor, radioactive thymidine (i.e., ³H-thymidine or ¹⁴C-thymidine), and a labeled mitosis curve can then be constructed by determining the percentage of labeled mitoses in repeated samples. From a percent labeled mitoses (PLM) curve the cell cycle time and duration of each phase can be calculated.

The proportion of cells labeled shortly after a flash of ³H-thymidine constitutes the labeling index (LI), or percentage of cells in S phase in relation to the total cell population. The labeling index gives a rough approximation of the proliferative activity of the tissue being studied. However, while a high labeling index reflects an actively proliferating tissue, equal labeling indices do not necessarily mean equal proliferative activity. The actual proliferative activity of a tumor depends not only on the proportion of cells in S phase, but also on the population's growth fraction (the ratio of proliferating cells to the total cell population) and on the rate of cell loss from the total tumor cell population. In other words, even if the labeling indices of two tumors are the same, their doubling times may differ dramatically because of differing growth fractions and rates of cell loss. In spite of many possible errors in estimating the rate of proliferation by means of the labeling index alone, it provides useful kinetic information when combined with other factors such as the duration of the S phase (Tₛ).¹¹

Growth Fraction

The growth fraction (GF) is an index of the relationship of the proliferating cell popula-

\[
GF = \frac{\text{proliferating pool cells}}{\text{total cell population}}
\]

Growth fraction is an index to the proportion of the tumor cell population susceptible to cell cycle or phase-specific drugs. Cycle- or phase-dependent drugs should not be used to treat a low-growth fraction tumor because they could not reduce the tumor size to any beneficial extent. If cells in the unaffected nonproliferating pool remained in that stage after an effective cycle-specific agent destroyed all proliferating cells, the tumor size would not subsequently increase. However, cells from the nonproliferating pool actually replace the destroyed cells, restoring and even increasing the number of cells in the proliferating pool.

Population Doubling Time

The population doubling time, usually called "doubling time" (T₅₀), should not be confused with the cell cycle or generation time (Tₖ) of tumor cells. Doubling time signifies the interval required for the whole cell population to double in number; doubling time is the same as cycle time only when all cells are proliferating (GF = 1.0) and there is no cell loss.

Growth fraction is commonly less than 1.0 in many tissues and tumors. In solid tumors, doubling time invariably exceeds cell cycle time; cells in the proliferating pool must divide more than once to double the total population because nonproliferating cells contribute nothing and cell death acts to reduce the population. If one assumes that a certain proportion of newborn cells always moves into the nonproliferating pool, the
doubling time can be derived by a mathematical equation. However, the observed doubling time is usually longer than the calculated, potential, or theoretical doubling time ($T_p$); this discrepancy is attributable to cell loss as formulated by Steel.\(^2\)

**Cell Loss**

Cell loss in a tumor is recognized by the presence of individual dead cells, massive or focal necrosis, and exfoliation of cells from the tumor mass, although quantitative estimation of the rate of loss is difficult. To express the magnitude of cell loss, Steel defined the cell loss factor (CLF) as:

$$\text{CLF} = 1 - \frac{T_p}{T_D}. \quad (2)$$

This factor represents the rate of cell loss as a fraction of the rate at which cells are added to the total population by mitosis.

Although $T_p$ and the cell loss factor are, in a sense, theoretical values, they allow comparisons among different tumor types of the importance of cell loss as a determinant of growth rate. Both $T_p$ and CLF are generally dependent on the size as well as the nature of the tumor. As a rule, an increase in tumor size is accompanied by greater cell loss, a lower growth fraction, and a longer doubling time.

**Tumor Cell Population Kinetics**

The distinction between the kinetics of non-neoplastic and that of neoplastic cells is not yet known, but three mechanisms have been proposed on the basis of studies using animal tumor models: 1) some tumor cells have a shorter cell cycle time than their normal counterparts; 2) some tumors accumulate dead cells, that is, cell loss exceeds cell removal; and 3) an increase in the proportion of cycling cells, either through the movement of nonproliferating cells into the proliferating pool or through some mechanism blocking the movement of cells into nondividing compartments, causes the population of neoplastic cells to expand.

A shorter cell cycle time relative to that of normal tissue of origin characterizes the growth kinetics of some experimental tumors.\(^2\) On the other hand, cell cycle time of certain other tumors is much longer than that of the tissues from which they originate, although present methods do not allow comparison of neoplastic with normal-tissue stem cells.\(^1\) If a simple alteration in cell kinetics cannot adequately explain actual tumor growth, a complex combination of altered population kinetics can probably offer a solution.

**Current Information on Brain Tumor Proliferation**

The brain is a unique organ from a kinetic point of view, since neurons become incapable of cell division shortly after birth. The glial cells in the supportive tissue of the brain retain their proliferative capacity, as shown by reactive and reparative gliosis,\(^4\) but so far no evidence exists that they proliferate at the rapid rate observed in other tissues. Several reports suggest that a low level of mitotic activity is continually present in the glial population of the adult rat brain,\(^10,18\) and that this very slow production of new cells is enough to balance glial cell loss. This proliferation rate is too slow for measurement of cell cycle time by current methods.

Kinetic data on normal human adult glia are not available, but incidental examination of unaffected brain from tumor-bearing patients to whom \(^3\)H-thymidine had been administered revealed almost no labeled cells; this suggests low proliferative activity.\(^11\) The kinetic state of normal brain clearly contrasts with that of brain tumors in which the proliferating population is obvious.

The first in vivo study of a human glioblastoma was carried out in 1960 by Johnson, et al.,\(^13\) who injected multiple doses of \(^3\)H-thymidine intravenously into a terminal patient and calculated a labeling index of 0.6%. Chigasaki\(^1\) studied the in vitro uptake of \(^3\)H-thymidine in biopsy specimens of a glioblastoma, an astrocytoma, and an oligodendroglioma, obtaining labeling indices of 0.94%, 0.44%, and 0.33% respectively. He estimated the generation time of glioblastoma to be 45 to 60 days. A few years later, Kury and Carter\(^14\) examined the labeling index of several gliomas in vitro, using a method almost identical to Chigasaki's. They reported that two glioblastomas had labeling indices of 3.6% and 6.0%, and five astrocytomas (Grade 2 or 3) had labeling indices of 2%, 2%, 2.6%, 5.5%, and 7.4%, all considerably higher than those reported by Johnson, et al., and Chigasaki. Kury and Carter
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estimated generation times (approximating potential doubling time in our terms) of glioblastomas and malignant astrocytomas to be 3 to 5 days and 2 to 10 days respectively by assuming an S phase of 6 hours. Fukuma, et al., using local injection of \(^{3}H\)-thymidine into glioma tissue at the time of craniotomy, reported labeling indices similar to those obtained by Kury and Carter.

Tyro introduced the stathomokinetic method, adding to the flash labeling technique the use of vinblastine sulfate. Vinblastine, like colchicine, facilitates kinetic analysis by arresting and holding cells in metaphase. Studying a single glioblastoma, Tym obtained a labeling index of 1.6% and calculated a G2 phase of 5 hours, an S phase of 8 hours, and a cell cycle time of 125 hours (5.4 days) to 242 hours (10 days).

In a recent study we observed that the labeling index varied among gliomas and among different areas of the same tumor. However, the labeling index of a particular area correlated reasonably well with its histological characteristics although the relationship was not a strict one. For example, the labeling index within necrotic areas was nearly zero, whereas the index within the viable part of the same tumor might approach 20%. By means of a double radioautography technique, we also estimated the duration of S phase for each tumor and calculated turnover time.

Table 1 summarizes these kinetic parameters on 12 brain tumors. By our method, glioblastomas had an average labeling index of 5% to 10% and astrocytomas 1% or less. The labeling index of anaplastic astrocytomas fell between that of well-differentiated astrocytomas and glioblastomas. Within anaplastic astrocytomas, areas containing well-differentiated astrocytes, with or without microcysts, had a labeling index close to that of more organized astrocytomas, whereas areas containing anaplastic astrocytes with neoplastic vascular hyperplasia had a labeling index approaching that of similar areas within glioblastomas. Predominantly pilocytic areas in some glioblastomas possessed a far higher labeling index than that of otherwise identical pilocytic areas within astrocytomas; this suggests that despite morphological similarities, the pilocytic astrocytes in glioblastomas are biologically different from pilocytic astrocytes in differentiated astrocytomas. In other glioblastomas and anaplastic astrocytomas we have observed large areas of well-differentiated astrocytes whose low labeling index suggests the tumor's origin from a low-grade astrocytoma.

In contrast to the variability of the labeling index in different astrocytic tumors, the duration of S (DNA synthetic) phase remained within a range of 7 to 10 hours. Several factors may influence the duration of S phase in vivo, but the uniformity of the S phase in astrocytomas of widely differing behavior indicates that degree of differentiation (or dedifferentiation) is not one of these factors.

The turnover time is derived from a formula relating the duration of S phase to labeling index:

\[
\text{turnover time} = \frac{T_s \times 100}{LI}
\]

For malignant gliomas the turnover time is on the order of a few days to a week, and in astrocytomas it approaches 2 months. Multiplying the turnover time by a conversion factor of 0.7 yields the potential doubling time (\(T_p\)) in tumors with an expected \(T_p\) over 100 hours. According to these calculations, malignant gliomas, including glioblastomas, would double their viable-area size in less than 5 days in the absence of cell loss. However, at a symptom-producing size of 50 to 100 gm, glioblastomas seem unlikely to

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Pathology</th>
<th>LI (%)</th>
<th>(T_s) (hrs)</th>
<th>(T_{over}) (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glioblastoma</td>
<td>5.9</td>
<td>5</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>glioblastoma</td>
<td>11.4</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>glioblastoma</td>
<td>8.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>glioblastoma</td>
<td>5.5</td>
<td>10</td>
<td>191</td>
</tr>
<tr>
<td>5</td>
<td>glioblastoma</td>
<td>4.4</td>
<td>9</td>
<td>203</td>
</tr>
<tr>
<td>6</td>
<td>malignant astrocytoma</td>
<td>5.4</td>
<td>11</td>
<td>198</td>
</tr>
<tr>
<td>7</td>
<td>malignant astrocytoma</td>
<td>8.2</td>
<td>13</td>
<td>156</td>
</tr>
<tr>
<td>8</td>
<td>malignant astrocytoma</td>
<td>2.3</td>
<td>7</td>
<td>307</td>
</tr>
<tr>
<td>9</td>
<td>astrocytoma</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>astrocytoma</td>
<td>0.8</td>
<td>10</td>
<td>1154</td>
</tr>
<tr>
<td>11</td>
<td>metastasis</td>
<td>6.3</td>
<td>7</td>
<td>108</td>
</tr>
<tr>
<td>12</td>
<td>metastasis</td>
<td>13.6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* LI=labeling index by a pulse of \(^{3}H\)-thymidine; \(T_s\)=duration of S (DNA synthetic) phase; \(T_{over}\)= turnover time (see text).
double their size every week or so; to explain this discrepancy we must assume extensive cell loss within the tumor. A tentative calculation, assuming a potential doubling time of 1 week and a clinically reasonable ("actual") doubling time of 5 to 6 weeks, yields a cell loss factor of 0.80 to 0.85 (80% to 85%), which, surprisingly, is not high when compared to the cell loss estimated in other tumors, such as head and neck tumors, 95%,20 and melanomas, 70%.24

Astrocytomas have the same S period as glioblastomas, but a lower labeling index and a longer turnover time. Presumably, the cell population of an astrocytoma increases at a slower rate. Histological examination of astrocytomas reveals a fairly homogeneous population of well-differentiated astrocytes supported by blood vessels exhibiting neither prominent perivascular proliferation nor endothelial thickening. As a rule, astrocytomas lack the dense cellularity and compactness characteristic of glioblastomas; this observation suggests that slow proliferation is not the consequence of crowding.

From their histological patterns, astrocytoma cells do not appear to suffer from lack of essential substrates, seemingly one of the main factors limiting the growth of malignant gliomas and other rapidly growing tumors. Tumor cells in well-differentiated astrocytomas either have a very long cell cycle time or reside mostly in the nonproliferating pool. Because necrosis is not a feature of astrocytomas, cell loss should be smaller than that in malignant gliomas, the low rate of cell loss occurring as a consequence of abortive mitoses and cell death by aging or degeneration. Thus, the observed labeling index in astrocytomas probably approximates the theoretical labeling index. Independent of size, a gradual increase in mass is maintained at the same steady rate in most well-differentiated astrocytomas, and this may reflect a biological characteristic ensuring a very low growth fraction throughout the span of tumor growth.

Cell cycle time and growth fraction are relevant to drug choice and scheduling in the chemotherapy of tumors. Growth fraction is equivalent to the ratio of the observed labeling index to the theoretical labeling index. Theoretical labeling index represents the proportion of S phase cells in the proliferating pool, and this can be calculated if one knows the durations of cell cycle time, S phase, and G2 phase, using a formula proposed by Cleaver.7 Unfortunately, we cannot obtain growth fraction in this manner, since techniques practicable for use in human intracranial tumors can only determine the S phase.

In 1962, Mendelsohn17 reported that the ratio of labeled mitoses to total mitoses, observed several generation times after a single pulse of 3H-thymidine, approaches the theoretical labeling index. Based on this principle we designed the following experiment. A patient with recurrent glioblastoma received an intracarotid injection of 3H-thymidine during selective angiography approximately 1 week before operation. A mitostatic agent, vinblastine sulfate, was given 4 to 6 hours before operation, not only to increase the number of cells in metaphase but also for stathomokinetic analysis of cell cycle time as originally designed by Puck and Steffen.19 Specimens of the tumor removed at operation were handled by usual autoradiographic methods. A pulse labeling index was obtained, and all mitoses, labeled and unlabeled, were scored. Growth fraction (GF) was obtained from the following formula:

\[
GF = \frac{\text{labeling index}}{\text{labeled mitoses/total mitoses}}
\]  

If one knows the growth fraction and mitotic index (MI), cell cycle time (Tc) can be obtained from the following formula:

\[
T_c = \frac{t \log 2}{\log (1 + \frac{\text{MI}}{\text{GF}})}
\]

where t is the interval between the administration of the mitostatic agent and the time of tumor excision. The derivation of this formula will be explained in a separate paper.

Table 2 shows these parameters analyzed in two glioblastomas. Growth fraction was 0.30 to 0.40 and cell cycle times were 2 to 3 days. The areas of tumors used for the labeling index, mitotic index, and labeled mitoses were confined to tumor specimens without microscopic necrosis; the growth fraction would be significantly smaller for the whole tumor, since glioblastomas characteristically contain necrotic zones. If more than half of the tumor is necrotic, a situation often observed in glioblastomas, the growth fraction would
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### TABLE 2

<table>
<thead>
<tr>
<th>Case No.</th>
<th>LI</th>
<th>Growth Fraction (± SD)</th>
<th>Cell Cycle Time (hrs ± SD)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>8.6</td>
<td>0.39 ± 0.10</td>
<td>64 ± 13</td>
</tr>
<tr>
<td>2</td>
<td>9.8</td>
<td>0.32 ± 0.10</td>
<td>55 ± 12</td>
</tr>
</tbody>
</table>

*LI= labeling index by a pulse of ³H-thymidine; SD = standard deviation.

be reduced by half. In that case, the growth fraction of glioblastomas might be 15% to 20%.

**Cell Kinetics and Chemotherapy**

The limited effectiveness of brain tumor chemotherapy has recruited few enthusiastic proponents. Even when a tumor mass regressed under chemotherapy, in most cases the remission was brief. Without a better understanding of biological differences between normal and neoplastic tissues, clinicians have attempted to identify effective chemotherapeutic agents by administering the highest tolerable dose. This approach to cancer chemotherapy has been similar to the rationale for antibiotic therapy in infectious diseases. The objective of both modes of chemotherapy is the destruction of living organisms and tissues harmful to the host. The development of anticancer agents on the basis of antimicrobial therapy was considered reasonable because the characteristic feature of a tumor is unrestricted cellular proliferation. However, the action of anticancer agents differs considerably from that of antibiotic agents. In antibiotic therapy, drug screening and sensitivity testing are valuable guides for determining the appropriate drug for a specific organism. An antibacterial antibiotic with a specific affinity may block an essential metabolic pathway that is either absent or unimportant in the host, and therapeutic effectiveness can be achieved with little or no toxicity to the host.

In contrast to antibacterial antibiotics, most anticancer agents do not have specific affinity for tumor tissue, nor is there a pronounced difference between therapeutic and toxic doses, principally because most anticancer drugs were developed to arrest or restrict proliferating tissue rather than to treat a specific neoplasm. In the past, we accepted as an article of faith that a tumor proliferated at a more rapid rate than the normal tissue from which it originated, but recent evidence clearly indicates that often the reverse is true.45 The rate of cellular proliferation in many tumors is slower than that in certain normal tissues, for example, small intestinal epithelium18 and bone marrow,1 the critical tissues most susceptible to toxic effects of cancer chemotherapeutic agents.

Chemotherapy protocols have emphasized drug selection, dosage, and mode of administration; unfortunately, drug schedules have received less attention. Kinetic data on tumor proliferation offer information relevant to chemotherapy scheduling; in addition, recent experimental and clinical trials designed in the light of proliferation kinetics indicate many effective agents are schedule-dependent. In most animal tumor systems relatively high doses of alkylating agents spaced at appropriate intervals achieve greater oncolytic effect and less host toxicity than long term daily dose schedules. By contrast, cell cycle specific agents, such as antimetabolites, require sustained periods of adequate drug levels which must be matched with the cell cycle characteristics and the proliferative state of the specific tumor.28

Since patients harboring well-differentiated gliomas may survive for several years after incomplete surgical excision, we assume that the movement of predominantly Go or non-proliferating pool cells into the very small proliferating pool is insignificant; this may be an inherent characteristic of slow-growing tumors. Incomplete surgical removal of such a tumor gives the residual tumor space for expansion and effectively reduces the number of both proliferating and nonproliferating pool cells. This situation does not apply to clinical experience with malignant gliomas; rapid regrowth indicates that they possess a higher growth fraction and that nonproliferating cells have the capacity rapidly to enter the proliferating pool. Growth in malignant gliomas appears to be depressed by crowding, and partial removal of the tumor stimulates cells in the nonproliferating pool to move into the proliferating pool, rapidly repopulating the tumor. Thus, removal does not significantly reduce the proliferating population, and only temporarily reduces tumor volume. Solid tumors such as glioblastomas
respond poorly to cycle-specific or phase-specific anticancer drugs because the susceptible fraction (proliferating or S phase cells) is not large, and the regrowth of unaffected (nonproliferating) cells soon replaces affected cells. The objective, therefore, must be to kill not only the proliferating cells in the residual mass, but also nonproliferating cells that have the capacity to re-enter the proliferating pool.

A patient harboring a symptomatic malignant glioma has a tumor mass of 50 to 100 gm containing approximately 0.5 to 1.0 \( \times 10^{11} \) cells. Our goal is elimination of the last cell.

The success recently achieved in the treatment of childhood leukemia involved three therapeutic steps; namely, the induction of a complete remission, continued treatment during remission, and eradication of the final small number of neoplastic cells. With successful first-phase therapy, the tumor cell population is reduced to 10^9 cells (1 gm); this is remission induction treatment. In leukemia, many effective chemotherapeutic agents are available; for example, the combination of prednisolone and 6-mercaptopurine or prednisolone and vincristine will induce complete remissions in more than 80% of treated cases. Obviously, agents of comparable activity are not available to treat gliomas and we must intensify the search for drugs and drug combinations capable of inducing an initial remission. Other methods applicable at this initial phase are extensive surgical resection of tumor and intensive radiotherapy with or without a radiosensitizer such as bromouridine.

The second phase of treatment has received little attention. At least 10^9 tumor cells remain in a viable state, and without additional therapy they will repopulate the tumor. The small quantity (1 gm) of tumor remaining after a complete clinical remission may have kinetic characteristics and drug sensitivities unlike those of the original tumor. An aggressive attack on this residual population of cells has been a major factor in the successful treatment of leukemia. Selection of appropriate drugs and drug schedules can accomplish further reduction of the tumor cell population.

Therapy must continue until the tumor cell population is reduced to the point that host defense mechanisms can eliminate the remaining cells. This final phase, dependent upon the host's immunological competence, may involve the elimination of 1 mg (10^6 cells) of tumor, possibly less. This final phase can be seriously compromised by the immunosuppressive effects of chemotherapy; because of this possibility the selection of drugs and the choice of drug schedules assumes critical importance.

References

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This work was supported in part by NIH Cancer Center Grant CA-13525, NINDS Training Grant 5593, and a gift from Phi Beta Psi Sorority.

Address reprint requests to: Charles B. Wilson, M.D., Department of Neurological Surgery, University of California School of Medicine, San Francisco, California 94143.