Reduction of Cytotoxicity to Normal Tissues by New Regimens of Cell-Cycle Phase-Specific Drugs

Z. AGUR,* R. ARNON, AND B. SCHECHTER
Department of Applied Mathematics and Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel*

Received 11 September 1987; revised 16 December 1987

ABSTRACT

A new policy for minimizing cytotoxicity to normal tissues of cell-cycle phase-specific drugs is suggested. This policy is based on short drug pulses and on an integer relation between the period of drug application and the cell-cycle length of the host susceptible tissue. The new protocol is expected to be effective even if the cell-cycle parameters of the malignant population have the same mean as those of the host cells but their variance is larger, and as long as the variance in the duration of the drug pulse is not too large. Under these conditions protocols can be determined by the host temporal parameters alone. The method has been verified *in vitro*, treating a leukemic cell line with cytosine arabinoside (Ara-C).

1. INTRODUCTION

Many currently available chemotherapeutic agents are cell-cycle phase-specific (to be denoted CCPSD). Among these are antitumor drugs, such as cytosine arabinoside, 6-thioguanine, and hydroxyurea. These interfere with DNA synthesis while having no effect on cells that are not in the *S*-phase [1]. The effect of such drugs on proliferating normal cells can bring about bone-marrow depression and other cytotoxic side effects that are detrimental to the patient [1]. Also belonging to this group is the new anti-AIDS drug AZT (azidothymidine), a nucleoside analogue that can be incorporated into DNA and bring about chain termination. This drug is strongly cytotoxic to proliferating host cells, notably to bone marrow [2]. Consequently, it has been recently emphasized that the use of AZT for AIDS treatment may be seriously impeded by its toxicity (Research News, *Science*, 20 Mar. 1987).

*To whom correspondence should be addressed.

655 Avenue of the Americas, New York, NY 10010 0025-5564/88/$03.50

Supported by both theory and experiments, we argue here that reduction of CCPSD cytotoxicity to host tissues may be feasible by drug pulses administered so that there is an integer relation between the total chemotherapeutic period (i.e., the length of the drug pulse + the length of the drug-free interval) and the mean cell-cycle length of the limiting host population. By this method the susceptible phase in a considerable proportion of host lines will consistently coincide with the interval in which the drug is below the effective dose, and the elimination probability of these lines will be minimal. It should be stressed that this method is expected to be effective without the use of synchronizing agents. Malignant cells will be efficiently eliminated, since such a "resonance" is unlikely to occur for these cells—because their mean cycle length differs from the imposed chemotherapeutic period (either smaller or larger), and/or because their within-line variation in cycle length is relatively large.

2. THE MODEL

Our recommended strategy has been studied by means of a mathematical model, previously described in [3,4]. In this model the chemotherapeutic regimen, denoted by the function $\Delta(t)$, assumes the values 0 and 1. The value 1 for Δ corresponds to the occurrence of effective drug concentration in the system. The mean duration of this interval is δ. The value 0 for Δ corresponds to a drug dosage too low to have a meaningful effect on the population size—the drug-free interval; the average duration of this interval is denoted by ω. The cell life cycle is divided into two principal phases: a phase susceptible to the drug (e.g., the S-phase) of mean duration ξ, and a phase resistant to the drug (e.g., the $G1$-phase + $G2$-phase + M-phase for drugs that are S-phase specific), whose mean duration is ρ. The mean cell-cycle length is $\tau = \xi + \rho$. Note that each of the temporal parameters in our model may either be constant or a random variable with various distributions. It is assumed that all susceptible cells are killed upon exposure to the drug, but lower magnitudes of kill have been shown to yield similar qualitative results [5]. A Malthusian form is assumed for the intrinsic population growth [6]. Thus the number of dividing cells at time t, $N(t)$, is given by

$$N(t) = AN(t-\tau)G(t),$$
$$G(t) = \begin{cases} 1 & \text{if } \Delta(t') = 0 \text{ for } t-\xi < t' \leqslant t, \\ 0 & \text{otherwise.} \end{cases} \tag{1}$$

The parameter A in (1) is constant, denoting the intrinsic rate of increase. The function $G(t)$ that appears in Equation (1) represents the effect of the chemotherapeutic process on cells in the susceptible life phase; it assumes the value 0, so that $N(t) = 0$, if there was drug in the system at any time

during the epoch $[t-\xi, t)$ preceding cell division; otherwise $G(t)=1$, so that $N(t)=AN(t-\tau)$. Exact solution of Equation (1), for ξ negligibly short and for random drug applications, with $2\delta > \tau \geqslant \delta$, is given in the Appendix.

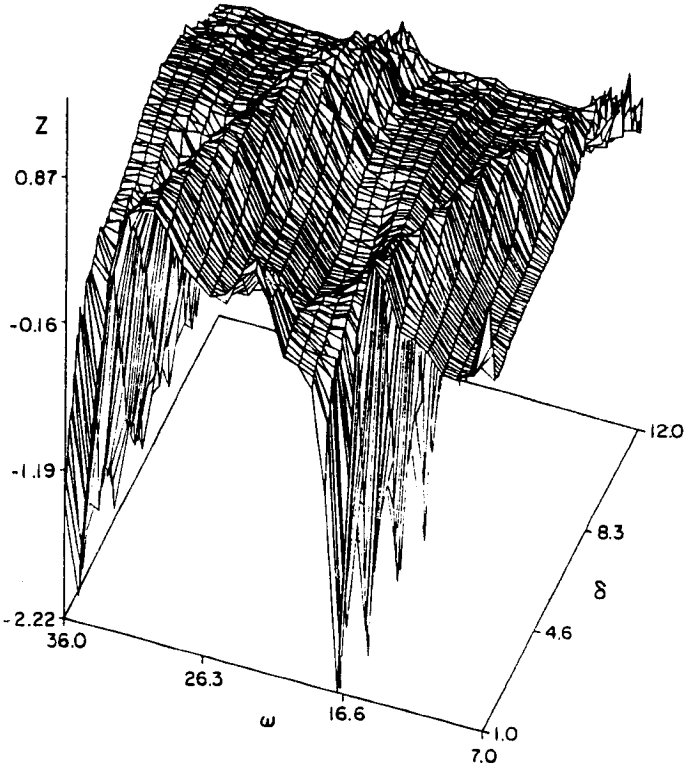
For comparing the efficacy of different protocols we approach the following optimization problem: minimize the ratio between the average elimination time of the malignant population and that of the host limiting population. Thus we define an *efficacy coefficient*, Z, such that

$$Z = 1 - \frac{T_M}{T_H}. \tag{2}$$

Here T_M and T_H denote the mean elimination time of the malignant and the host limiting population, respectively. Z will tend to unity for treatments that cause fast elimination of the malignant population but have little effect on the limiting population, will be close to zero for nondiscriminative treatments, and will be less than zero for treatments that "kill the patient before they kill the disease." It should be stressed that when we use a different formulation of the optimization problem (minimize average extinction time of the malignant population and maximize average population size of host cells) we get results that are similar to those described below for the optimization problem specified in (2). For a somewhat different measure of treatment efficacy see [7].

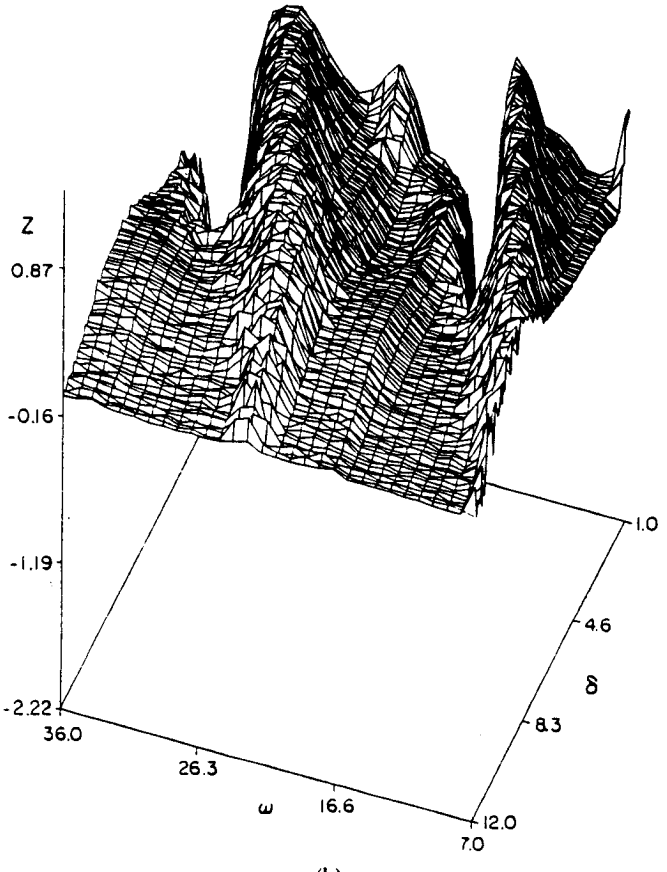
The efficacy coefficient Z has been calculated under various chemotherapeutic regimens, characterized by the distribution of the interval in which the drug appears in effective concentration in the system, and the distribution of the drug-free interval. In our simulations the initial malignant and host populations were each composed of 100 cells whose resistant life phase was a normally distributed random variable (we employed conventional Monte Carlo methods to get the actual value of the resistant life phase for each cell in the population, as well as for all other distributions described in the present work). The variation in the duration of the susceptible S-phase was assumed to be negligibly small (see below). No additional mother-daughter variation in cell-cycle parameters was assumed. The number of dividing cells under each set for chemotherapeutic parameters was calculated according to Equation (1), independently in the host and malignant populations. The population is taken to be eliminated at time $t=T$ if $N(t)=0$ for $T-\tau < t \leqslant T$. The obtained elimination times T_M and T_H were then used to calculate Z according to Equation (2) for each drug regimen.

Results of calculations for one representative coupled system are given in Figure 1, where the life-cycle parameters are those of leukemic cells— the M (malignant) population, and those of normal human diploid fibroblasts—the H (host) population. *A priori* this system may seem particularly prone to chemotherapeutic complications, owing to the relatively short cycle length of



(a)

FIG. 1. Simulation results of the computer model for two populations that vary in mean life-cycle parameters. The cell-cycle length and the resistant-phase duration of the M (malignant) population are taken as $\tau_M = 18$ hr and $\rho_M = 6$ hr respectively [8]; those of the H (host) population are $\tau_H = 16$ hr and $\rho_H = 10$ hr respectively [9]. The resistant life phases of the M and H populations were assumed to be normally distributed random variables with standard deviation $\sigma_{\rho,M} = \sigma_{\rho,H} = 1$. The variation in the duration of the susceptible S-phase is assumed to be negligibly small [12]. The effective duration of drug activity is a normally distributed random variable with standard deviation, $\sigma_\delta = \delta/10$; in the results presented here the drug-free interval ω is constant. The intrinsic birth rate is $A = 2$, the initial population size is $N_0 = 100$, and the drug dose is taken as high enough to kill all exposed susceptible cells. (a) and (b) represent the same surface and differ only by a 180° rotation.



(b)

FIG. 1. Continued.

the normal susceptible cells. The cell-cycle length and the resistant-phase duration of the M-population are $\tau_M = 18$ hr and $\rho_M = 6$ hr respectively [8]; those of the H-population are $\tau_H = 16$ hr and $\rho_H = 10$ hr respectively [9], the standard deviation in ρ being $\sigma_{\rho M} = \sigma_{\rho H} = 1$. The effective duration of drug activity was a normally distributed random variable, with standard deviation $\sigma_\delta = \delta/10$; in the results represented in Figure 1, ω is constant.

Each point on the bottom surface in Figure 1(a) represents a specific protocol. Z appears as a point on a perpendicular axis directly above the corresponding point on the bottom surface. The interconnected group of all Z points forms the top surface in the graph. Figure 1(a) shows clear peaks in Z for treatments whose period is an integer multiple of the mean cell-cycle

length of the H-population. In other words, the elimination time of the malignant population M is minimized with respect to that of the host population H when $\delta + \omega = \tau_H$, $\delta + \omega = 2\tau_H$, etc. Moreover, since $\rho_H = 10$ hr, all drug regimens whose period equals that of the H population are expected to be similarly detrimental for the M-population, but to vary considerably in their effect on the H-population. Those with a shorter δ should be less harmful, while those with $\delta > 10$ hr should be as detrimental for the H-population as they are for the M-population. Indeed, in Figure 1(b), which displays a 180° rotation of the surface in Figure 1(a), we note that increasing the duration of the individual drug pulse damps down the peaks in Z, signifying diminished ability to discriminate between the two populations. This result is further demonstrated in Figure 2, where Z is plotted as a function of δ for $\delta + \omega = \tau_H$ (i.e., 16 hr). An inflection point at $\delta = \rho_H = \epsilon$ (ϵ being small) clearly marks curves 1–4 in Figure 2. These curves represent systems in which the noise in the temporal parameters is relatively small. Figure 2 suggests that in general Z is significantly larger for $\delta < \rho_H$.

But the efficacy coefficient Z is not the only parameter to be considered when selecting the drug protocol for cytotoxic drugs. The absolute elimination time of the malignant population, T_M, may be critical in cases of acute disease. Our computations show that T_M decreases with increasing duration of each drug pulse. For example, in the simulations for curve 2 in Figure 2, T_M is about twice as large for $\delta = 3$ hr as for $\delta = 8$ hr.

Our simulations suggest, then, that treatment efficacy can increase using protocols in which $\tau/(\delta + \omega) = m/n$ (m, n integers), and in such protocols, for δ somewhat smaller than ρ_H. Additional simulations show similar results for systems in which $\rho_M > \rho_H$. Similar qualitative results are obtained in additional simulations, where δ and ω are either constant or exponentially distributed, as long as their variance is not too large. These results lead to the following assertion (see also below):

ASSERTION 1

When mean life-cycle parameters of the malignant population differ from those of the limiting host tissue, the efficacy coefficient of cytotoxic drugs is maximized if there exists an integer relation between the frequency of drug application and the mean cell-cycle length of the limiting population. For increasing treatment efficacy the duration of each drug pulse should be a little shorter than the duration of the resistant life phase in the limiting host population.

A good chemotherapeutic strategy should be one that does not require estimation of many parameters in each individual patient. However, in contrast to life-cycle parameters of the host tissues, those of the malignant

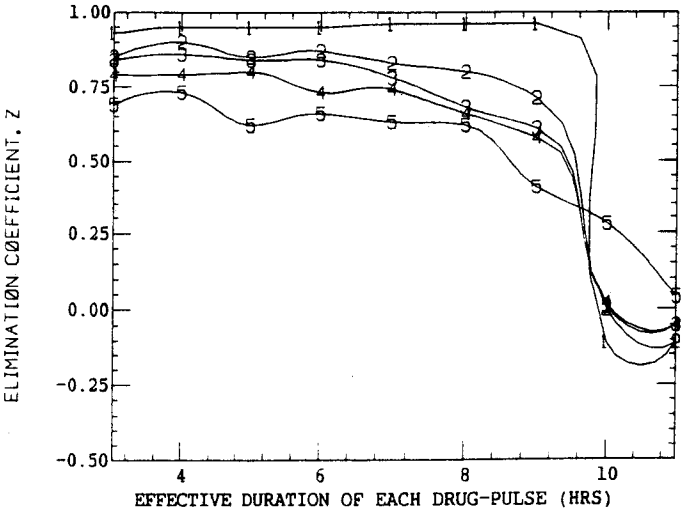


FIG. 2. Efficacy coefficient Z plotted as a function of the effective duration of each drug pulse, δ, for $\delta + \omega = \tau_H = 16$ hr, $\tau_M = 18$ hr, $\rho_H = 10$ hr, $\rho_M = 6$ hr. The temporal parameters are constant or normally distributed as follows: (1) τ, δ, ω = const; (2) $\sigma_\rho = 0.5$, δ, ω = const; (3) $\sigma_\rho = 0.5$, $\sigma_\delta = \delta/10$, ω = const; (4) $\sigma_\rho = 1$, $\sigma_\delta = \delta/10$, ω = const; (5) $\sigma_\rho = 1$, $\sigma_\delta = \delta/10$, $\sigma_\omega = 1$. Other parameters as in Figure 1.

population may vary considerably. The cell-cycle length of neoplastic tissues is known to vary among and within patients [10–12]. Below we show that the policy of drug application can be determined with no need to estimate the life-cycle parameters of the malignant population in each patient.

In general, cell-cycle length has been shown to follow a normal distribution; most of the variability occurs in the nonreplicative life phases, while the replicative phase remains relatively constant [11]. In further simulation experiments we assume that the different susceptible populations vary in the standard deviation of the distribution of their resistant life-phase duration σ_ρ. Simulations show that increasing σ_ρ reduces the effect of "resonance" between treatment and cell periods, resulting in a smaller mean elimination time. Figure 3 shows the computations of Z, according to the procedure described above, for a coupled system in which $\sigma_{\rho H} < \sigma_{\rho M}$, but mean life-cycle parameters are the same in the two populations. Maxima in Z appear in this graph for $\delta + \omega = \tau$, and $\delta + \omega = 2\tau$.

Our next assertion will therefore be

ASSERTION 2

Owing to their greater variation in cell-cycle length, neoplastic populations with mean cell-cycle parameters similar to those of the host susceptible cells can

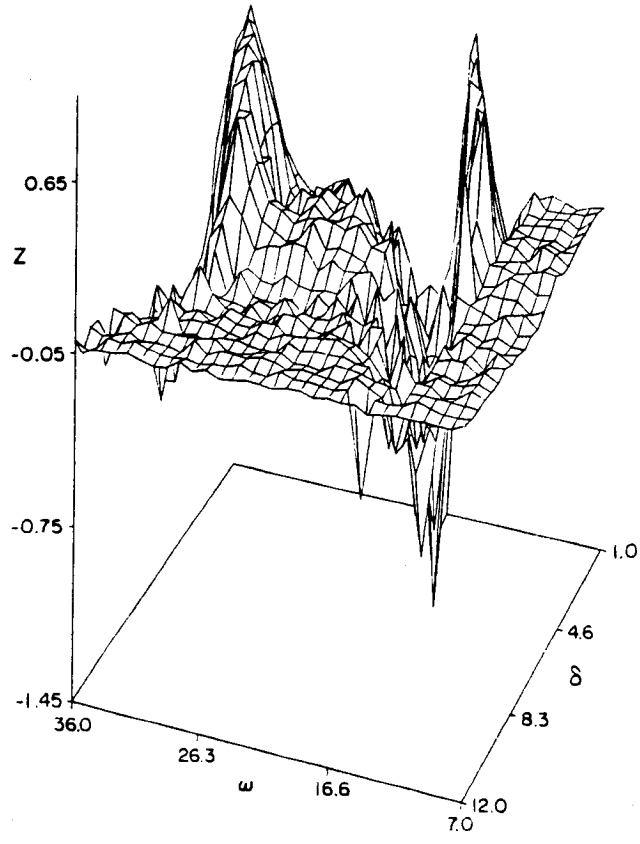


FIG. 3. Efficacy coefficient Z calculated for two populations having similar mean cell-cycle parameters but different variance in the duration of the resistant life phase: $\tau_H = \tau_M = 18$ hr; $\rho_H = \rho_M = 6$ hr; $\sigma_{\rho H} = 0.5$, $\sigma_{\rho M} = 2$. Other parameters as in Figure 1.

be eliminated faster than host cells; this can be achieved by protocols with an integer relation between the period of drug application and the mean cell-cycle length.

It is essential to verify that the method suggested here does not depend on minor details of the system, such as the exact distribution of the temporal parameters, etc. Indeed, mathematical analysis suggests that our model is robust. Using elementary mathematics one can show that in fully periodic systems (constant δ, ω, and τ), if the intrinsic growth rate is large enough, the elimination time of the population is infinite if $\tau/(\delta + \omega) = m/n$ and $m\delta < \tau$, and finite otherwise (m, n are integers). When $2\delta > \tau > \delta$ the

elimination time T is given by

$$T = \frac{\tau\omega}{|\tau - (\delta + \omega)|}. \tag{3}$$

The point $\tau = \delta + \omega$ in Equation (3) is singular, with T being infinite; T increases with δ for $\delta < \tau - \omega$ and decreases with increasing δ for $\delta > \tau - \omega$. Mathematical analysis for a system in which the drug-free intervals are exponentially distributed supports the results for the fully deterministic system; in the region $2\delta > \tau > \delta$ the mean population size is maximized when the chemotherapeutic period equals the mean cell-cycle length, i.e., when $\tau = \delta + \omega$ (see Appendix). These results corroborate our simulation results in showing that the elimination time is a nonmonotonic function of the expression $\tau/(\delta + \omega)$. Owing to this property small changes in any of the temporal parameters are expected to cause large changes in the elimination time of the population.

Further analysis, with both δ and ω exponentially distributed, shows that in the region $2\delta > \tau > \delta$, the mean population size increases with decreasing δ. That is to say that the "resonance" phenomenon disappears when variation in δ is large enough (see Appendix).

3. EXPERIMENTAL RESULTS

We turn now to experimental evidence. As it is difficult to attain normal cell proliferation *in vitro*, we verify the dependence of elimination time on the expression $\tau/(\delta + \omega)$ by varying the denominator rather than the numerator of this expression; in our experiments cells from the same population are subjected to different protocols. Two separate preliminary *in vitro* experiments were conducted, using the drug cytosine arabinoside (Ara-C) on 38C-13 cells of C3H/eB origin. The cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum, glutamine, antibiotics, and $5 \times 10^{-5} M$ 2-mercaptoethanol. The cultures were kept at 37°C in a humidified atmosphere of 5% CO_2. At a cell concentration range of 1×10^5–12×10^5 the cell-cycle length was shown to be 12 hr. In the experiments cells (0.3 ml of 2×10^5 cell/ml) were dispersed into 3 sets of 15 (12×75-mm Falcon 2003) test tubes. Each set was given 4×1-hr pulse treatments of varying concentrations of Ara-C, dissolved in 25 μl distilled water (in triplicates). Control cells were similarly treated with 25 μl water. Pulses to the different sets were given every 9, 12, or 15 hr. After each pulse the cells were washed with 2 ml medium and resuspended in 0.3 ml medium. When the control cultures reached a cell concentration of 12×10^5 cells/ml, the cells in the entire experiment were diluted twofold. Inhibition of DNA synthesis was taken as a measure of drug activity, and [^{3}H] methylthymidine

TABLE 1

Effect of Ara-C Protocol on Cell Survival[a]

Expt.	Time of [^{3}H] methyl-thymidine pulse (hr)	Time of drug pulse (hr)	DNA synthesis: Drug .19	Conc. .095	μg/ml .047		Analysis of variance
1	50	0,9,18,27	.2	.25	.51	*B*	Pr > *F* = .017
		0,12,24,36	.14	.36	.56	*A*	
		0,15,30,45	.16	.28	.46	*B*	
	55	0,9,18,27	.13	.37	.52	*B*	Pr > *F* = .0466 (.0003)[b]
		0,12,24,36	.09	.45	.7	*A*	
		0,15,30,45	.09	.31	.5	*B*	
2	49	0,9,18,27	.11	.34	.35	*B*	Pr > *F* = .0004
		0,12,24,36	.19	.55	.7	*A*	
		0,15,30,45	.12	.35	.57	*B*	
	57	0,9,18,27	.07	.19	.45	*B*	Pr > *F* = .0005
		0,12,24,36	.1	.53	.66	*A*	
		0,15,30,45	.08	.24	.57	*B*	

[a]Rates of DNA synthesis in treated cells are presented as a proportion of that of the control. Statistical results were obtained using the SAS ANOVA parametric analysis of variance on raw data (raw data not shown here); the significance probability values associated with the *F*-value, Pr > *F*, for the difference among treatments that vary in drug periods (among rows) are given; those for the difference among treatments that vary in drug concentrations (among columns) were all Pr > *F* = .0001. SAS ANOVA Duncan multiplet-range test on main-effect means is given by the letters *A*, *B*, marking decending order of means. Means marked by the same letter are not significantly different ($\alpha = .05$). Similar statistical results were attained in a nonparametric analysis of variance.

[b]Pr > *F* value for analysis of variance performed on the two lower drug concentrations only.

incorporation was used for evaluating DNA synthesis. The [^{3}H] methyl-thymidine pulses (2 hr each) were terminated using a Titertech cell harvester, and radioactivity was monitored.

Our experimental results (Table 1) show that the protocol whose period equals the cell-cycle time (12 hr) ended up in rates of DNA synthesis that were higher than under protocols of 9-hr or 15-hr period. Analysis of variance, both parametric and nonparametric, was performed on the raw [^{3}H] methylthymidine counts, as well as on the percentage of DNA synthesis in comparison with that of the control. Results of parametric analysis of variance on raw data (Table 1) show a highly significant difference between the treatment of 12-hr period and the other treatments. Similar results were

obtained in all other analyses. These experiments support our theoretical results in showing that cell survival is maximized when $\tau/(\delta + \omega) = 1$.

4. CONCLUSIONS

That differences in cell-cycle length of tumor and normal tissues should be used for minimizing drug cytotoxicity has been suggested [13, 14] and demonstrated in theoretical calculations [15]. However, the present work is new in providing a clear and simple method of exploiting these differences for reducing cytotoxicity of the drug to the host tissues. Moreover, our *in vitro* experiments support our theoretical predictions and are thus very encouraging. *In vivo* experiments are under way in our laboratory.

The main conclusion of our work is that short drug pulses at appropriate intervals may be more efficacious than a drug administered at arbitrary intervals or a continuously and slowly released drug. This conclusion is additionally supported by a clinical study of patients with refractory acute leukemia, where Ara-C toxicity to proliferating tissues was shown to be influenced by the duration of drug exposure [16]. It should be mentioned that our approach is applicable also *in vivo*, since for controlling the duration of the drug pulse one may use agents that act as antidotes, such as NB Citrovorum factor (leucovorin, folinic acid) in the case of methotrexate [17].

Another model of cell population dynamics under CCPSD has been presented elsewhere. This model suggests that optimal drug schedules should have a periodicity close to the mean cell-cycle time of the normal population; this should be so if the normal and tumor cells differ considerably in mean cell-cycle times [15]. A corollary to this conclusion is that the choice of the protocol depends on an accurate estimation of life-cycle parameters both in the host and in the malignant population, which presents a serious constraint on the usefulness of the method. In contrast to the latter work, in the present study we consider the possibility that variation in cycle length of the malignant population is more extensive than in the limiting host population—a widely documented phenomenon in cancer. Our theoretical model suggests that drug protocols can be selected so that a malignant population that has a relatively large variation in cell-cycle parameters, but whose mean cell-cycle parameters are unknown, can be eliminated markedly faster than a known host population whose variation is smaller. For this reason the frequency of drug application and the duration of each drug pulse can be determined by the cell-cycle parameters of the limiting host tissue alone. It thus appears that further intensive research is warranted concerning the possibility that appropriate scheduling policies might offer major improvements in cancer therapy.

The work was supported by the Joseph Perez grant, by the U.S.-Israel Binational Science Foundation grant, No. 1983, and by the John D. and Catherine T. MacArthur Foundation. We thank Z. Artstein, L. A. Segel, and S. Taasan for helpful discussions, and L. A. Segel for comments on the manuscript.

APPENDIX. CELL GROWTH IN REGIMES OF RANDOM DRUG APPLICATION[1]

In this note, we calculate exactly the average size for a cell population subjected to a regimen of random drug applications. The total population size is described by the following finite stochastic difference equation:

$$N(t+\tau) = A(\tau)N(t)[1-\Delta(t+\tau)]. \tag{A1}$$

Here $N(t)$ stands for the number of cells that are at the drug-susceptible life phase at time t, A is a reproduction rate, and Δ is a stochastic process assuming the values 0 and 1. The value 1 corresponds to the occurrence of drug in this system so that all susceptible cells die (this epoch will be denoted "disturbance"). The cell-cycle is τ, and in this form of the model it is assumed that the duration of the susceptible life phase is negligibly short, so that τ is also the duration of the resistant life phase.

By applying Equation (A1) recursively one finds

$$\begin{aligned} N(t+n\tau) = A^n(\tau)N(t)[1-\Delta(t+n\tau)] \\ \times\{1-\Delta[t+(n-1)\tau]\}\cdots[1-\Delta(t+\tau)]. \end{aligned} \tag{A2}$$

To calculate the average population size after n generations, we note that the probability that a susceptible population at time t, $0 < t < \tau$, has progeny at $t+n\tau$ is

$$P\{\Delta(t)=0,\ \Delta(t+\tau=0,\ldots,\Delta(t+n\tau)=0\}. \tag{A3}$$

If the process Δ is Markovian, this equals

$$P\{\Delta(t)=0\}\cdots P\{\Delta(t+n\tau)=0 \mid t+(n-1)\tau=0\}.$$

If, further, we assume that the transitions $\Delta=0 \rightarrow \Delta=1$ and $\Delta=1 \rightarrow \Delta=0$ occur at *constant* rates k_0 and k_1, then it is well known that

$$P\{\Delta(t+\tau)=0 \mid \Delta(t)=0\} = \frac{k_1}{k_0+k_1} + \frac{k_1}{k_0+k_1}e^{-(k_0+k_1)\tau} \tag{A4}$$

---

[1] By Christian Van den Broeck and Zvia Agur, Limburgs Universitair Centrum, B-3610, Diepenbeek, Belgium.

for all t. If we assume an initial equilibrium distribution

$$P\{\Delta(0)=0\}=\frac{k_1}{k_0+k_1}, \tag{A5}$$

then from (A2), (A4), and (A5) one can obtain the average number of susceptible cells after n generations:

$$\frac{1}{\tau}\int_0^{\tau} N(t+n\tau)\,dt = A^n(\tau)\times N_0\tau\frac{k_1}{k_0+k_1}\left[\frac{k_1}{k_0+k_1}+\frac{k_0}{k_0+k_1}e^{-(k_0+k_1)\tau}\right]^{n-1}. \tag{A6}$$

Here we have assumed that the population size is constant during the first generation of the process:

$$N(t)=N_0 \qquad \text{for} \quad 0\leqslant t\leqslant\tau. \tag{A7}$$

Let us denote the average durations of the states $\Delta=0$ and $\Delta=1$ as $\omega_0=1/k_0$ and $\delta_0=1/k_1$, respectively. In this model both the state $\Delta=0$ and $\Delta=1$ have a stochastic duration δ and ω respectively, whose probability densities are given by (A4). Now we can write (A6) in terms of the average durations δ_0 and ω_0:

$$\frac{1}{\tau}\int_0^{\tau} N(t+n\tau)\,dt = A^n(\tau)\,N_0\tau\frac{\omega_0}{\omega_0+\delta_0}\left[\frac{\omega_0}{\omega_0+\delta_0}+\frac{\delta_0}{\omega_0+\delta_0}e^{-(1/\omega_0+1/\delta_0)\tau}\right]^{n-1}. \tag{A8}$$

Let us now consider a model with fixed duration of a disturbance, so that $\delta=\delta_0=$ constant, and with an exponential interdisturbance interval. Now Δ is no longer Markovian, since the state $\Delta=1$ has a fixed duration and the process has a "memory" during the time interval δ_0. Nevertheless the Markovian property remains valid for this process provided the time differences $t_n-t_{n-1},\ldots,t_2-t_1$ are all larger than this memory time δ_0. For $\tau>\delta_0>\tau/2$ the transition from $\Delta=0$ at $t=0$ to $\Delta=0$ at $t=\tau$ can only occur in two ways: either there is no disturbance at all in the time interval $[0,\tau]$, or there is one disturbance starting at a point t in the interval $[0,\tau-\delta_0]$ and no disturbance in the interval $[t+\delta_0,\tau]$. The probability $P\{\Delta(t+\tau)=0\,|\,\Delta(t)=0\}$ is thus the sum of the probabilities for these two possible situations:

$$P\{\Delta(t+\tau)=0|\Delta(t)=0\}=e^{-\tau/\omega_0}+\int_0^{\tau-\delta_0}e^{-t/\omega_0}\cdot e^{-[\tau-(t+\delta_0)]/\omega_0}\,dt. \tag{A9}$$

Integrating (A9), one finally obtains [assuming again that (A7) holds true]

$$\frac{1}{\tau}\int_0^{\tau} N(t+n\tau)\,dt = A^n(\tau)\,N_0\tau\frac{\omega_0}{\omega_0+\delta_0}\left\{e^{-(\tau/\omega_0)}\left[1+\frac{e^{\delta_0/\omega_0}}{\omega_0}(\tau-\delta_0)\right]\right\}^{n-1}. \tag{A10}$$

By numerical computation of (A8) and (A10) one can show the basic difference between these two models. In Equation (A8), where δ is an exponentially distributed random variable, the average population size, for $\tau > \delta_0 > \tau/2$, decreases with increasing δ_0. In Equation (A10), where δ is constant, the average population size in the same region increases with δ, and is maximized for $\tau = \delta + \omega_0$.

The distinction between these two cases may have interesting empirical applications in chemotherapy, where δ is the duration of the drug pulse. From Equation (A10) there emerges the possibility that increasing half-life of the drug may increase the persistence of the malignant population. This will be so when variance in the half-life of the drug is relatively limited and when the period of drug application remains constant [3].

REFERENCES

1 I. Tannock, Cell kinetics and chemotherapy: A critical review, *Cancer Treatment Rep.* 62:1117 (1978).
2 H. Mitsuya and S. Broder, Strategies for antiviral therapy in AIDS, *Nature* 325:773 (1987).
3 Z. Agur, Increasing half-life of cycle-specific drugs can increase elimination time, presented at 2nd Cyprus Conference on New Methods in Drug Research, Limassol, 1985, A26.
4 Z. Agur, The effect of drug schedule on responsiveness to chemotherapy, *Ann. N.Y. Acad. Sci.* 504:274–277 (1986).
5 Z. Agur and J. L. Deneubourg, The effect of environmental disturbances on the dynamics of marine intertidal populations, *Theoret. Population Biol.* 27:75 (1985).
6 M. K. Sundareshan and R. Fundakowski, On the equivalence of mathematical models for cell proliferation, *Cell & Tissue Kinet.* 17:609 (1984).
7 B. W. Brown and J. R. Thompson, in *Epidemiology* (D. Ludwig and K. L. Cooke, Eds.), SIAM, 1975, p. 38.
8 H. E. Skipper, in *The Cell Cycle and Cancer* (R. Baserga, Ed.), Marcel Dekker, New York, 1971, p. 355.
9 M. Lipkin, in *The Cell Cycle and Cancer* (R. Baserga, Ed.), Marcel Dekker, New York, 1971, p. 1.
10 M. Minden, P. Major, J. Griffen, A Wu, and D. Kufe, Generation time of leukemic blast progenitor cells, *Cell & Tissue Kinet.* 16(6):577 (1983).
11 D. A. Cantrell and K. A. Smith, The interleukin-2 *T*-cell system: A new cell growth model, *Science* 224:1312 (1984).
12 F. M. Schabel Jr., Concepts for systematic treatment of micrometastases, *Cancer* 35:15 (1975).

13 R. N. Baserga, The cell cycle, *New Eng. J. Med.* 304:453–459 (1981).
14 M. Kimmel and F. Traganos, Estimation and prediction of cell cycle specific effects of anticancer drugs, *Math. Biosci.* 80(2):187–208 (1986).
15 F. B. Dibrov, M. A. Zhabotinski, Y. A. Neyfakh, M. P. Orlova, and L. I. Churikova, Mathematical scheduling of cancer chemotherapy. Periodic schedules of phase-specific cytotoxic agent administration increasing the selectivity of therapy, *Math. Biosci.* 73:1 (1985).
16 R. H. Herzig, S. N. Wolff, H. M. Lazarus, G. L. Philips, C. Karanes, and G. P. Herzig, High-dose cytosine arabinoside therapy for refractory leukemia, *Blood* 62:361 (1983).
17 S. K. Carter, M. T. Bakowski, and K. Hellman, *Chemotherapy of Cancer*, Wiley, 1981.