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A STOCHASTIC MODEL FOR THE ORIGIN AND TREATMENT OF TUMORS CONTAINING DRUG-RESISTANT CELLS

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A stochastic model for the chemotherapy of experimental tumors is presented. The focus of this model is on the presence of drug-resistant mutants and their influence on eventual treatment outcome. Equations are derived for the joint probability-generating function for the number of chemo-sensitive and chemo-resistant cells. The model is extended to two drugs and it is shown how the model may be used to make deductions regarding the optimum scheduling of therapy.

1. Introduction. Resistance to chemotherapy represents a well-recognized barrier to the effective treatment of cancer. The cause of this resistance has been the subject of intensive investigation in both animals and man. Several mechanisms have been identified which include intrinsic resistance, pharmacologic sanctuary, locational resistance, phase resistance, G_0 or resting cell resistance and acquired resistance. Before continuing to discuss the last of these we will briefly mention the nature of each of these proposed mechanisms.

Intrinsic resistance is an ill-defined term used to indicate the *de novo* insensitivity of tumor cells to specific anticancer agents. Possible causes of such resistance would include the carry over of resistance displayed by the normal cell type to certain anticancer agents. Alternatively the tumor initiating event itself may coincidentally produce changes in cellular biochemistry which inhibits the action of the drug. Pharmacologic sanctuary occurs when the tumor arises or metastasizes to a site to which drug access is limited by the usual routes of administration, e.g. the brain. Locational resistance occurs where certain cells are not exposed to therapeutic doses because of their position within the tumor. This would be likely to arise for cells located distantly from the capillary bed when treated with larger molecular drugs which have limited perfusion capability. Phase resistance occurs when a drug with cycle-specific activity is used upon a tumor containing cells in the various phases of the cell cycle. Cells not in the appropriate phases will escape unaffected and the tumor as a whole may

exhibit resistance. Similarly cells in G_0 can escape the effects of cycle specific anticancer agents and may display a reduced sensitivity to all drugs.

Acquired resistance represents insensitivity, at the cellular level, which can develop during the lifetime of the tumor. The isolation and transplantation of cells displaying this phenomenon indicate that this process is under genetic control (Ling, 1982). A number of cellular mechanisms of resistance have been identified as arising in this way including decreased drug uptake, increased drug efflux, altered drug target and amplified drug target. These mechanisms vary in their specificity with some conferring resistance to a single drug whilst others may involve a spectrum of resistance to a variety of drugs. *In vitro* experimentation appears to indicate that these variants arise at random times when specific genetic changes occur in tumor cells which had nor previously been insensitive. *In vivo* evidence from mouse leukemias accords with the observations in *in vitro* systems although very little investigation has been done because of the great cost and complexity involved in *in vivo* experimentation (Law, 1952). Of course, evidence in man from spontaneous tumors is not available. Although the origin of acquired resistance in spontaneous human tumors has not been established, its existence has been inferred in several cases where phenotypic alterations, similar to those seen in experimental systems, have been observed in tumors not responding to chemotherapy (Carmen *et al.*, 1984; Trent *et al.*, 1984).

The relative importance in clinical disease of the different modes of tumor resistance on cancer chemotherapy failure is difficult to assess except in isolated cases. However, the observation of tumor response, followed by later regression at the same site in the face of chemotherapy represents a common clinical problem. The other mechanisms previously discussed can be amended to accord with the observation but only acquired resistance directly predicts this behaviour. Thus it seems both reasonable and desirable to model acquired drug resistance.

2. Growth Model. In order to model the development of drug resistance it is first necessary to specify a model for tumor growth. A large number of such models are available; however, we restrict attention to those which describe growth at the individual cellular level. From these we select one proposed by Till and associates (Till *et al.*, 1964; MacKillop *et al.*, 1983) in which cells are assumed to be in one of three compartments based on their proliferative potential. The structure of this model will be briefly reviewed here. The three compartments consist of stem cells, transitional cells and end cells and are defined as follows:

(i) Stem cells (C_0); cells capable of unlimited proliferation. At each division a stem cell will give rise to two stem cells with probability p,

two transitional cells with probability q and one of each type with probability $1-p-q$.

(ii) Transitional cells (C); cells capable of limited proliferation. This class is comprised of disjoint subclasses, $C_1, \ldots, C_n$ where n is referred to as the clonal expansion number. Transitional cells which are the immediate result of a stem cell division are entered into subclass C_1. Upon division a single C_1 cell gives rise to two C_2 cells. This is analogously repeated for cells in $C_2, \ldots, C_{n+1}$.

(iii) End cells (C_{n+1}); these are functionally dead cells incapable of further proliferation. Two end cells are formed by the division of a single C_n transitional cell.

Dividing cells ($C_0, \ldots, C_n$) are assumed to divide with a fixed and common interdivision time. All cells are assumed to behave independently. We will assume that cells may be lost from each compartment (representing migration to metastasis, cell death, etc.) at a rate l_i, for $i=0, \ldots, n+1$. Let $C_i(t)$, $i=0, \ldots, n+1$ be the number of cells in class C_i at time t.

The behaviour of the model is quite complex because of the non-independence of the various classes $C_i(t)$. However, much research has been done in the asymptotic behaviour of the branching processes of this type and it can be shown using known results (Mode, 1971) that

$$\frac{\mathbf{C}(t)}{\sum_{i=0}^{n+1} C_i(t)} \to v \tag{1}$$

where $\mathbf{C}=[C_0(t), \ldots, C_{n+1}(t)]$ and v is the left eigenvector associated with the maximal eigenvalue of the matrix, M, where $M=(\mathbf{M}_0, \ldots, \mathbf{M}_{n+1})'$

$$\mathbf{M}_i=E[\mathbf{C}(1) \mid \mathbf{C}(0)=\mathbf{e}_i]$$

$$\sum_{i=0}^{n+1} v_i=1$$

and $\mathbf{e}_i$ is the vector with 1 in the ith column and 0 elsewhere. The maximum eigenvalue ρ of M is also the asymptotic growth rate of the tumor which can be explicitly shown to be:

$$\rho=(1-l_0)(1+p-q)=\lim_{t\to\infty} \frac{\sum C_i(t+1)}{\sum C_i(t)} . \tag{2}$$

We require that $\rho>1$ so that the tumor represents a growing system.

As we are usually concerned with large collection of cells in mature tumors, we will have, for a range of parameter values, that

$$\frac{\mathbf{C}(t)}{\sum_{i=0}^{n+1} C_i(t)} \text{ will be approximated by } \nu.$$

The deductions we wish to draw from this are that for a clinical tumor (with biologically plausible values for p, q, n, etc.) that growth will be approximately exponential with approximately fixed proportions of the various cell types and that elimination of the stem cell compartment is necessary and sufficient for cure of the tumor.

This suggests that if we are only concerned in the long run with behaviour of the tumor, i.e. whether it is curable or not, we need only consider the behaviour of the stem cell compartment C_0. The number of stem cells prior to treatment can be estimated by

$$\nu_0 \sum_{i=0}^{n+1} C_i(t), \text{ where } \sum_{i=0}^{n+1} C_i(t)$$

is the total tumor size. Here we will restrict attention to the curability of the tumor and thus only consider the stem cell compartment.

Now for the simplified case $l_1=l_2= \ldots =l_n=l$ we have:

$$\nu_0=\left\{\frac{(1-l_0)(1+q-p)+2(l_0-l)}{2(l_0-l)+(1-l_0)\left[\frac{2(1-l)}{\rho}\right]^n\left[\frac{1+l_{n+1}-2l}{\rho-1+l_{n+1}}\right](1+q-p)}\right\}$$

where we assume

$$\frac{2(1-l)}{\rho} \neq 1.$$

We propose to develop a model for acquired resistance among stem cells in a continuous time framework since this permits somewhat simpler mathematical development and also should better reflect the growth process of tumor cells which do not have fixed interdivision times. From their perspective of the stem cell compartment, a division of a stem cell which results in two stem cells is a birth of a new stem cell and will be referred to as a birth. A stem cell division which results in a single stem cell and a single transitional cell is a situation in which the total number of stem cells is unchanged and will be termed a renewal. Similarly, either the migration, death or the division of a stem cell to form two transitional cells,

is a loss and will be referred to as a death. If we assume that these events occur with probability $b\Delta t$, $c\Delta t$ and $d\Delta t$, respectively, to a single cell in the interval $[t, t+\Delta t]$ then

$$\begin{aligned} b &\propto p(1-l_0) \\ c &\propto (1-p-q)\,(1-l_0) \\ d &\propto l_0+q(1-l_0) \end{aligned} \tag{3}$$

and by requiring that the expected growth rate be the same for both the continuous and the discrete models of tumor growth we have:

$$b-d=\ln(1-l_0)+\ln(1+p-q). \tag{4}$$

The relationships represented in (3) and (4) can then determine b, c and d for given p, q and l_0. Here t is measured in units of mean interdivision times. All subsequent discussion will relate to stem cells unless otherwise indicated.

3. Development of Resistance. If a cell is sensitive to a particular drug we will say it is in state R_0, if resistant we will say it is in state R_1. By resistant we mean that a cell is less likely to be killed by exposure to a fixed dose of the drug than the parent (sensitive) line of cells. We will assume that transitions from R_0 to R_1 will occur at frequencies determined by the following parameters:

$\alpha=$ probability that a single resistant cell will be produced during a birth occurring in a sensitive stem cell

$\beta=$ probability that a single resistant cell will be produced during a renewal occurring in a sensitive stem cell

$\gamma=$ rate (in time) at which cells convert spontaneously from sensitivity to resistance.

All progeny of resistant cells will be assumed to be resistant. Let $R_0(t)$ and $R_1(t)$ be the number of sensitive and resistant cells respectively at time t, and define $P_{ij}(t)$ as follows:

$$P_{ij}(t)=P\{R_0(t)=i,\ R_1(t)=j\}.$$

Assuming that the probability of two events in time Δt, is of $o(\Delta t)$, then we may use the Kolmogorov forward equations to give the following series of differential equations for $P_{ij}(t)$.

$$\begin{aligned} \frac{dP_{ij}(t)}{dt} &= -[(b+d)j+(b+d+\eta)i]P_{ij}(t)+b(1-\alpha)(i-1)P_{i-1j}(t) \\ &\quad +[b(j-1)+\alpha bi]P_{ij-1}(t)+d(i+1)P_{i+1j}+d(j+1)P_{ij+1}(t) \\ &\quad +(\eta)(i+1)P_{i+1j-1}(t) \end{aligned} \tag{5}$$

where $P_{ij}(t)=0$ for $i<0$ or $j<0$ and $\eta=\beta c+\gamma$.

Now if we let $\phi(s_1,s_2;t)$ be the probability-generating function of the process, i.e.

$$\phi(s_1,s_2;t) = \sum_{i=0}^{\infty} \sum_{j=0}^{\infty} P_{ij}(t) s_1^i s_2^j$$

then using equation (5) we can obtain the following differential equation for $\phi(s_1,s_2;t)$ (by multiplying $s_1^i s_2^j$ and summing over i and j)

$$\frac{\partial \phi}{\partial t} = \sum_{i=1}^{2} [bs_i - d][s_i - 1] \frac{\partial \phi}{\partial s_i} + (\alpha b s_1 + \eta)(s_2 - s_1) \frac{\partial \phi}{\partial s_1} . \tag{6}$$

This equation may be solved by the method of characteristics (John, 1982) and yields the following solution:

$$\phi(s_1,s_2;t) = \psi(x(t),y(t)) \tag{7}$$

where

$$y(t) = \frac{d(1-s_2) + (bs_2 - d) \exp(-\delta t)}{b(1-s_2) + (bs_2 - d) \exp(-\delta t)} \tag{8}$$

and

$$x(t) = y(t) + \frac{f(t)}{[\delta^{2-\alpha}(s_1 - s_2)]^{-1} - b(1-\alpha) \int_0^t f(v)\, dv}$$

$$f(t) = e^{-(\delta + \alpha d + \eta)t} [b(1-s_2) + (bs_2 - d)e^{-\delta t}]^{-2+\alpha}$$

$$\delta = b - d \text{ and } \psi(s_1,s_2) = \phi(s_1,s_2;0).$$

From this relationship we may obtain directly expressions for

$$m_0(t) = E[R_0(t)] \text{ and } m_1(t) = E[R_1(t)]$$

i.e.

$$m_0(t) = m_0(0) e^{(\delta - \alpha b - \nu)t}$$

$$m_1(t) = [m_1(0) + m_0(0)\ (1 - e^{-(\alpha b + \eta)t})] e^{\delta t}.$$

Let $N(t) = R_0(t) + R_1(t)$, then we have the well-known relationship

$$\lim_{t \to \infty} P\{N(t) = 0\} = \lim_{t \to \infty} \phi(s_1,s_2;t) = \psi(\varepsilon, \varepsilon)$$

where $\varepsilon = d/b$.

Thus the probability-generating function in equation (7) includes tumor growth patterns destined to become extinct. In wishing to examine the development of resistance in tumors of clinical dimension we more reasonably require $P\{R_0(t),R_1(t) \mid N(t)=N\}$ where N is the observed number of stem cells. This distribution is difficult to obtain and a number of approximations are possible (Day, 1984; Birkhead and Gregory, 1984). As a first approximation to conditioning upon $N(t)=N$, we will consider the distribution $P\{R_0(t),R_1(t) \mid N(\infty)>0\}$ which excludes spontaneously extinct tumors (Coldman and Goldie, 1985). It is possible to show that the probability-generating function of this distribution $\phi'(s_1,s_2;\ t)$ (Coldman and Goldie, 1985) is given by

$$\phi'(s_1,s_2;t)=[1-\psi(\varepsilon,\ \varepsilon)]^{-1}\ [\phi(s_1,s_2;t)-\phi(\varepsilon s_1,\varepsilon s_2;t)]. \tag{9}$$

The distribution of the number of stem cells, at time t, is then approximately geometric with mean $(1-\varepsilon)^{-1}e^{\delta t}$ (Coldman and Goldie, 1985). Thus fixing the mean number of stem cells uniquely specifies this distribution and we may compare the probability-generating function of resistant cells for various parameter values and for fixed distribution of tumor sizes using equation (9). Equation (9) may not be easily inverted to give the distribution of the number of resistant cells, however, we can use it to calculate several quantities of interest.

When the tumor has grown from a unicellular origin, i.e. $\psi(s_1,s_2)=s_1$ we have the following mean values

$$\begin{aligned} m_0'(t) &\simeq (1-\varepsilon)^{-1}m_0(t) \\ m_1'(t) &\simeq (1-\varepsilon)^{-1}m_1(t) \end{aligned} \tag{10}$$

where $m_0(t)$ and $m_1(t)$ are as before.

Let

$$m'(t)=m_0'(t)+m_1'(t)\simeq(1-\varepsilon)^{-1}e^{\delta t} \tag{11}$$

that is $m'(t)$ is the expected number of stem cells at time t. In most cases of human cancer the age t of the tumor is unknown. If the observed mean number of stem cells is N, then we may use (11) to estimate the age of the tumor (if t is unknown). That is

$$t=\delta^{-1}\ln[N(1-\varepsilon)]. \tag{12}$$

Consider the expected fraction of resistant stem cells, F, say where

$$F=\frac{m_1'(t)}{m'(t)}\ .$$

Using (10) and (12) we have

$$F=(1-e^{-(\alpha b+\eta)t})=1-[N(1-\varepsilon)]^{-(\alpha b+\eta)/\delta} \tag{13}$$

Examination of (13) shows that as d increases F increases. That is, the more rapid the rate of natural cell death the larger is the accumulation of resistant stem cells for a given overall number of stem cells. This result is not surprising since as d increases, the growth rate of the tumor slows, thus giving a longer time for resistant cells to emerge. Equation (13) may be used in experimental cases to estimate the mean proportion of resistant cells, however, it is quite variable and observing this quantity in experiments does not permit accurate estimation of the parameter $(\alpha b+\eta)$.

An important quantity, which is of clinical interest, is the likelihood that the tumor may be eliminated by chemotherapy. The probability is, of course, influenced by a large number of factors other than intrinsic resistance. We will assume here that other forms of treatment failure can be ignored, so that we may use the equations developed to estimate the curability of the tumor. Under circumstances where the drug-mediated kill of sensitive cells is high, and the kill of resistant cells zero, this likelihood is equal to the probability that, at the time of application of the drug, any existing resistant cells go spontaneously extinct (due to their inherent death rate). Let this probability be P_t, where t is the time of application of therapy, then one can show (Coldman and Goldie, 1985)

$$P_t=\phi'(1,\varepsilon;t). \tag{14}$$

Now using (12) and (9) we may consider P_t as a function of N where N is the size of the stem cell compartment when the treatment is first applied. Equation (14) is plotted as a function of N in Fig. 1, where we see that P_t declines as the tumor increases in size. However P_t is not strongly dependent on d, in contrast to what we found for F [equation (13)]. In fact for given values of the other parameters the curves P_t are essentially coincident for all d. Thus the increase in the number of resistant cells with increasing d is balanced by their increasing death rate so that P_t is unchanged. To summarize we can see that if all parameters are fixed except d, then those tumors with higher death rates will have a higher proportion of resistant stem cells, but their curability will not be affected.

The function specified by equation (14) is quite complex but can be approximated by a function of simpler algebraic form (Coldman and Goldie, 1985). This function may be used in the analysis of destructive experiments where cell lines are exposed to a drug. The probability of a cell

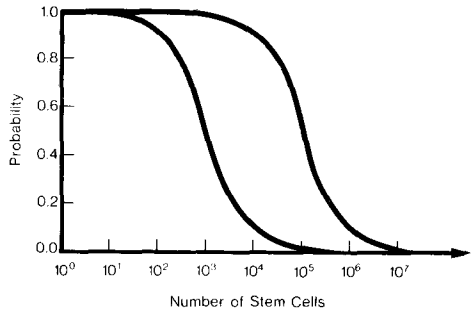

Figure 1. A plot of the probability of 'cure' P_N as a function of the number of stem cells N, that is equation (14) using the substitution for t in equation (12). The curve to the left plots the function for $\alpha=5\times10^{-4}$, $\eta=5\times10^{-4}$ and the one to the right is for $\alpha=5\times10^{-6}$, $\eta=5\times10^{-6}$. The curves are essentially coincident for all values of d.

line surviving is then given by equation (14). However, care must be taken in using this equation since it assumes that resistant cells survive administration of the drug with probability one, that the cells considered are all stem cells and that they are treated at the same fixed time after initial plating (or implantation). More complex experimental situations may be examined using the equations developed in this and the next section. However, simultaneous estimation of all the required tumor parameters would typically involve a series of different experiments, designed to estimate each parameter.

In cases where the effect of the drug on resistant cells is appreciable, then the previous considerations may be unimportant since the drug may be able to make the net growth of both the sensitive and resistant stem cells subcritical and thus extinguish the tumor. Alternatively, if the drug cannot make the net growth of the sensitive cells subcritical then the tumor will always be incurable with this drug and again the previous considerations do not apply.

4. Resistance to Two Drugs. In many clinical and experimental tumors, more than one drug is available to treat a tumor. If these drugs may be given together then as a first approximation we may treat their combination as a single drug and model the development of resistance as before. Alternatively if they cannot be given together then we must consider a more complex situation where a variety of drug-resistant states occur. We will assume that the drug-resistant cells arise in the same way as before.

Thus consider two drugs, T_1 and T_2, and define the following states R_0, R_1, R_2, R_3 where a stem cell is in R_0 if it is sensitive to T_1 and T_2, in R_1 if it is resistant to T_1 and sensitive to T_2, R_2 if it is sensitive to T_1 and resistant to T_2, and in R_3 if it is resistant to both.

Generalizing the considerations for single drug resistance we may define $R_0(t)$, $R_1(t)$, $R_2(t)$ and $R_3(t)$ as the number of stem cells in states R_0, R_1, R_2 and R_3 at time t. Letting α_i, β_i, γ_i $(i=1,2)$ be the rates at which sensitive cells become resistant to T_i (see section on single resistance), and let α_3, β_3 and γ_3 be the rates at which sensitive cells become resistant to both drugs. Define, in addition, rates $\alpha_{i,3}$, $\beta_{i,3}$ and $\gamma_{i,3}$ as the rates by which cells resistant to T_i become resistant to both drugs (that is acquire resistance to the other drug).

We may proceed as before to write down the Kolmogorov forward equations for this process. However these equations may not be simply solved. The process may be considerably simplified if we assume that the stem cells grow exponentially, i.e. $R_0(t)=A \exp\{kt\}$. We will assume that only one stem cell is present at time $t=0$ and that this cell is sensitive. In this case we will assume that $A=(1-\varepsilon)^{-1}$ and $k=\delta-a_1-a_2-a_3$ where $a_i=a_ib+\beta_ic+\gamma_i$ for $i=1,2,3$. This choice of A and k ensures that the growth rate of the stem cells is equal to the expected growth rate when they grow randomly, after growth paths which result in spontaneous extinction have been excluded [see equation (10)].

Let $P_{ijk}(t)=P\{R_1(t)=i, R_2(t)=j, R_3(t)=k\}$ be the distribution function for resistant cells which have arisen from sensitive cells, and

$$\Phi(\mathbf{s};t)=\Phi(s_1,s_2,s_3;t) = \sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\sum_{k=0}^{\infty} P_{ijk}(t)\, s_1^i\, s_2^j\, s_3^k$$

then we may use the Kolmogorov forward equations to obtain a series of linked ordinary differential equations for $P_{ijk}(t)$. Using these equations we may derive the following differential equation for $\Phi(\mathbf{s};t)$ [in the same way as (6)].

$$\frac{\partial\Phi}{\partial t} = \sum_{i=1}^{3}[bs_i-d][s_i-1]\frac{\partial\Phi}{\partial t}+a_3(s_3-1)\, R_0(t)\Phi$$

$$+\sum_{i=1}^{2}\left\{a_i\,(s_i-1)\, R_0(t)\Phi+(s_3-s_i)\,(\alpha_{i,3}bs_i+\eta_{i,3})\frac{\partial\Phi}{\partial s_i}\right\}$$

where $\eta_{i,3}=\beta_{i,3}c+\gamma_{i,3}$ for $i=1,2$, $a_i=\alpha_ib+\eta_i=1,2,3$.

This equation may be solved using the method of characteristics to yield:

$$\ln\Phi\,(\mathbf{s};t)=I_0A+A\sum_{i=1}^{2}a_iI_i(s_i) \tag{15}$$

where

$$I_0=(a_1+a_2+a_3)\delta(s_3-1)\int_0^t \frac{e^{k(t-v)}\,dv}{b(1-s_3)+(bs_3-d)e^{-\delta v}}$$

$$I_i(s)=\int_0^t \frac{e^{k(t-u)}\,g_i(u)\,du}{[\delta^{2-\alpha_{i,3}}(s-s_3)]^{-1}-b(1-\alpha_{i,3})\int_0^u g_i(v)dv}$$

$$g_i(v)=e^{-(\delta+d\alpha_{i,3}+\eta_{i,3})v}\,[b(1-s_3)+(bs_3-d)e^{-\delta v}]^{-2+\alpha_{i,3}}.$$

This represents a rather complex function and it is not easy to deduce the underlying joint distribution.

Evidence from experimental tumors indicates that a constant proportion of homogeneous cells survive administration of a drug at fixed dose (Skipper, 1978). Assuming that cells respond to therapy independently this implies that there is a probability, $\pi(D)$, that a cell will survive administration of the drug at dose D. Thus define

$\pi_{i,j}(D)$=probability a cell in state R_j (j=0,1,2,3) will survive a course of drug T_i at dose D.

If we let $X_{i,j}$ be a random variable which takes on the value 1 if a cell in R_j survives administration of the T_i and 0 if is does not survive then the probability-generating function for $X_{i,j}$, $\xi_{i,j}(\mathbf{s})$, is

$$\xi_{i,j}(\mathbf{s})=1-\pi_{i,j}(D)+\pi_{i,j}(D)s_j.$$

Thus if we let t_k be the time of the kth treatment and $\phi(\mathbf{s};t)$ be the probability-generating function for $\{R_1(t),R_2(t),R_3(t)\}$ then we have

$$\begin{aligned}\phi(\mathbf{s};t_k)&=\phi(\xi_i(\mathbf{s});\bar{t_k})\\ \xi_i(\mathbf{s})&=(\xi_{i,1}(\mathbf{s}),\xi_{i,2}(\mathbf{s}),\xi_{i,3}(\mathbf{s}))\end{aligned}\tag{16}$$

and

$$R_0(t_k)=\pi_{i,0}R_0(\bar{t_k}).$$

where drug T_i is given at time t_k.

Between treatments the existing tumor cells will divide and new resistant cells will be produced. If we take a single cell in R_i (i=1,2) at time 0 then the probability-generating function of the number of cells in R_i and R_3 at time t derived from this cell is $\zeta_i(\mathbf{s};t)$ where $\zeta_i(\mathbf{s};t)$ is given by $\phi(s_1,s_2;t)$ in equation (7) with $\alpha=\alpha_{i,3}$, $\eta=\eta_{i,3}$, $s_1=s_i$ and $s_2=s_3$ for i=1,2. Similarly for a single cell

in R_3 at time 0, the probability-generating function of the number of cells derived from it at time t, $\zeta_3(\mathbf{s};t)$ is given by $y(t)$, [equation (8)] with $s_2=s_3$.

Thus for $t_k \leqslant t < t_{k+1}$ we have

$$\phi(\mathbf{s};t)=\phi(\zeta(\mathbf{s};t-t_k);t_k)\Phi(\mathbf{s};t-t_k) \tag{17}$$

where

$$\zeta(\mathbf{s};u)=(\zeta_1(\mathbf{s};u),\ \zeta_2(\mathbf{s};u),\ \zeta_3(\mathbf{s};u)).$$

Thus by recursive use of equations (16) and (17) we can calculate the probability-generating function at time t in terms of that at time t_1. The probability-generating function at time t_1 is given by (15). These functions are quite complex and it is not possible to calculate the joint distribution of resistant cells. In analogy to equation (14) however, we can calculate the probability that the resistant cells will be eliminated by the therapy P_E, as

$$P_E=\phi(\varepsilon,\ \varepsilon,\ \varepsilon;t_J) \tag{18}$$

where t_J is the time of the last cycle of therapy.

We can similarly calculate the marginal probability that the cells of one particular type are eliminated. For example, the marginal probability that the R_1 cells are eliminated in $\phi(\varepsilon,1,1;t_j)$. Using these formulae [equations (16) and (17)] we can calculate the effect of various treatment protocols on eliminating the various subpopulations of cells.

The probability that the sensitive stem cells are eliminated can be estimated by $\varepsilon^{R_0(t_J)}$ although this is of course approximate. Examples of calculations made using this (Coldman and Goldie, 1983) and other (Day, 1984) models have been presented elsewhere and will not be repeated here.

We may use these calculations to examine the effect of various strategies in applying two treatments so that P_E is maximized. When the treatments are equally effective, i.e. $\alpha_1=\alpha_2$, $\alpha_{1,3}=\alpha_{2,3}$, $\eta_1=\eta_2$, $\eta_{1,3}=\eta_{2,3}$, $\pi_{1,0}=\pi_{2,0}$, $\pi_{1,1}=\pi_{2,2}$, $\pi_{1,2}=\pi_{2,1}$ and $\pi_{1,3}=\pi_{2,3}$ it can be shown (Coldman and Goldie, 1983) that strategies which alternate T_1 and T_2 are optimal in the sense that they minimize $E[N(t)]$. Protocols which maximize P_E cannot in general, be uniquely identified without explicit knowledge of the parameters involved. However, it is possible by examining the effect of various strategies of applying T_1 and T_2, for models with likely values for the various tumor parameters, to construct general rules for the structure of optimum protocols (Day, 1984). Alternatively, one may consider the parameter values to be random variables which follow a distribution. The distributions may be thought of, in this circumstance, as reflecting the level of ignorance as to the

true value of the parameter. In principle it is then possible to examine the effect of various levels of ignorance on the resulting optimum protocol. However, little work has been done using this approach, although the case of single resistance has been examined (Coldman and Goldie, 1986). For a comprehensive discussion of this problem in a quite general setting, see Day (1984).

5. Conclusion. Intrinsic cellular resistance has been recognized, both experimentally and clinically, as a substantial problem in the planning of effective cancer chemotherapy. Using basic probabilistic models for the development of this phenomenon it is possible to examine the effect of various treatment protocols and design strategies which maximize the likelihood that this phenomenon can be overcome. These models are still quite rudimentary and require further development to more accurately model human cancer and its response to treatment. However, if the development to date is reasonably representative, we believe that they will provide greater insight into treatment failure and aid in the logical planning of cancer therapy.

LITERATURE

Birkhead, B. G. and W. M. Gregory. 1984. "Mathematical Model of the Affects of Drug Resistance in Cancer Chemotherapy." *Mathl Biosci.* **72,** 59–70.

Carmen, M. D., J. M. Schornagel, R. S. Rivest, S. Srimatkandada, R. S. Portlock, D. Duffy and J. R. Bertin. 1984. "Resistance to Methotrexate Due to Gene Amplification in a Patient with Acute Leukemia." *J. clin. Oncol.* **2,** 16–20.

Coldman, A. J. and J. H. Goldie. 1983. "A Model for the Resistance of Tumor Cells to Cancer Chemotherapeutic Agents." *Mathl Biosci.* **65,** 291–307.

——— and ———. 1985. "The Effect of Cellular Differentiation on the Development of Permanent Drug Resistance." *Mathl Biosci.* **74,** 177–198.

——— and ———. 1986. "Factors Affecting the Development of Permanent Drug Resistance and its Impact upon Neo-adjuvant Chemotherapy." In *Preoperative (Neo-adjuvant) Chemotherapy*. Berlin: Springer (to appear).

Day, R. 1984. "A Tumor Growth Model with Applications to Treatment Policy and Protocol Choice." Thesis submitted to Harvard School of Public Health, Boston, MA.

John, F. 1982. *Partial Differential Equations,* 4th Edn, pp. 9–14. New York: Springer.

Law, L. W. 1952. "Origin of the Resistance of Leukemic Cells to Folic Acid Antagonists." *Nature* **169,** 628–629.

Ling, V. 1982. "Genetic Basis of Drug Resistance in Mammalian Cells." In *Drug and Hormone Resistance in Neoplasia,* Vol. 1, pp.1–20. Boca Raton, FL: CRC Press.

MacKillop, J., A. Ciampi, J. E. Till and R. N. Buick. 1983. "Stem Cell Model of Human Tumor Growth: Implications for Tumor Cell Clonogenic Assays." *J. natn. Cancer Inst.* **70,** 9–16.

Mode, C. J. 1971. *Multitype Branching Processes—Theory and Applications.* New York: American Elsevier.

Skipper, H. E. 1978. "Cancer Chemotherapy I: Reasons for Success and Failure of Treatment of Murine Leukemias with the Drugs now Employed Treating Human Leukemias." Ann Arbor, MI: University Microfilms Inc.

Till, J. E., A. McCulloch and L. Siminovitch. 1964. "A Stochastic Model of Stem Cell Proliferation Based on the Growth of Spleen Colony-forming Cells." *Proc. natn. Acad. Sci. U.S.A.* **51,** 29–36.

Trent, J. M., R. N. Buick, S. Olson, R. C. Horns and R. T. Schimke. 1984. "Cytologic Evidence for Gene Amplification in Methotrexate Resistant Cells Obtained from a Patient with Ovarian Adenocarcinoma." *J. clin. Oncol.* **2,** 8–15.