THE DEVELOPMENT OF RESISTANCE TO ANTICANCER AGENTS

by

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ABSTRACT

The mechanism of resistance of tumor cells to chemotherapeutic agents is explored using probabilistic methods where it is assumed that resistant cells arise spontaneously with a defined frequency. The resistance process is embedded in a discrete time Markov branching process which models the growth of the tumor and contains three seperate cell types: stem, transitional and end cells. Using the asymptotic properties of such models it is shown that the proportion of each type of cell converge to constants almost surely. It is shown that the parameters relating to stem cell behaviour determine the asymptotic behaviour of the system. It is argued that for biologically likely parameter values, cure of the tumor will occur if, and only if, all stem cells are eliminated.

A model is developed for the acquisition of resistance by stem cells to a single drug. Probability generating functions are derived which describe the behaviour of the process after an arbitrary sequence of drug treatments. The probability of cure, defined as the probability of ultimate extinction of the stem cell compartment, is characterised as the central quantity reflecting the success of therapeutic intervention. Expressions for this function are derived for a number of experimental situations. The effects of variation in the parameter values are examined.

The model is extended to the case where two anticancer drugs are available and formulae for the probability of cure are developed. The problem of therapeutic scheduling is examined and under situations where drugs are of "equal" effectiveness, but may not be given together, it

is shown that the mean number of tumor cells is minimised by sequential alternation of the drugs.

The models are applied to data collected on the L1210 leukemia treated by the drugs Cyclophosphamide and Arabinosylcytosine. In both cases the analysis of the data provide evidence that resistant cells arise spontaneously with a frequency of approximately 10^{-7} per division. When applied to human breast cancer, the model indicates that neo-adjuvant therapy is unlikely to greatly influence the likelihood that the patient will die from the growth of drug-resistant cells.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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1. INTRODUCTION

Resistance is a general term in cancer therapy meaning insensitivity to treatment [1]. This term can be applied to any of the three arms of cancer therapy, surgery, radiotherapy and chemotherapy, but it is usually reserved for the latter two. Here we will be concerned primarily with resistance to chemotherapy which has recently assumed greater importance with the increased use of this modality in clinical cancer therapy. Resistance may be either absolute (no effect of the drug) or partial (reduced effect of the drug). In the discussion which follows we will consider the development of resistance, whether partial or absolute, to chemotherapeutic agents.

Resistance to cancer chemotherapy is known to be multifactorial and there is no reason to believe that all forms have yet been identified. Probably the simplest way in which resistance can arise is that of pharmacologic sanctuary. In this situation tumor cells arise, or are transported to a site which is not accessible to the drug by the usual route of administration. For example, a number of drugs administered intravenously will not gain access to the brain. A second mechanism is the metabolic conversion of the drug to a non-active form. For example, the half life of 5-fluorouracil (5-FU), a drug commonly used in the treatment of gastro-instestinal malignancies, has a measured systemic half-life of six to twenty minutes [2]. Therefore, the tumor exposure time to 5-FU administered by injection is likely to be short and many cells may be expected to escape unaffected. Cells located distantly from the capillary bed are known to experience lower drug levels than those which are closer. Therefore, tumors may display resistance to

chemotherapy because many cells are not exposed to therapeutic doses of the drug. Another mechanism of tumor resistance can result from the phase specific or preferential activity of a drug. Tumor cells, like other dividing cells, move through the various phases of the cell cycle, G_1, S (synthesis), G_2 and M (mitosis) where G_1 and G_2 are intervening periods between the states of chromosomal synthesis and cell division. In some cases drugs act preferentially or exclusively on the cells in particular phases of the cell cycle and thus cells in other phases will appear resistant. Related to this is the relative insensitivity of the state G_0 which is used to designate viable cells not actively in the cell cycle. Cells in this state are non-proliferating and considerably less sensitive to chemotherapeutic agents than actively proliferating cells. Cells in G_0 may later re-enter the cell cycle and continue to proliferate. Therefore tumors with substantial numbers of cells in protected phases will not respond to chemotherapy. This is the main mechanism by which the normal hemopoietic system (which has many cells in G_0) survives the effects of chemotherapy aimed at a tumor.

A further, and important type of resistance is the existence of a subpopulation of cells within the tumor population on which administration of an agent has no or reduced effect when compared to the rest of the tumor cells. This resistance is intrinsic to the cells themselves and persists when such cells are transferred to another host. In-vitro studies of resistant cells have associated the development of resistance with genetic and biochemical differences within these cells when compared to the parent sensitive cells.

One other form of resistance should be mentioned. Certain drugs

show virtually no effect in some types of tumor, whilst they are extremely active in others. Similar variation in response is also seen in different classes of non-tumor cells and it is worth emphasizing the obvious that cells, whether normal or malignant, have varying biochemical properties, and this can be expected to influence their sensitivity to a drug.

It is our objective here to develop a mathematical model for the growth of cell populations where individual cells show the intrinsic differential sensitivity to chemotherapy. It is recognised that we will have to use data from passaged animal tumors even though what we desire to model is the therapy of human malignancy. This phenomenon is of interest since the existence of resistant cells will obviously influence the short term and long term behaviour of tumors treated with chemotherapy. Before proceeding further it is wise to ask whether the mechanism we intend to model is present in human malignancy to any significant extent.

Consider the following common clinical observation. A tumor is treated with an agent (or several agents simultaneously) and appears to shrink. It may even be no longer clinically detectable. Therapy is continued, but it later becomes obvious that the tumor is growing again. Experience indicates that continued therapy with the same agents is fruitless as the tumor is now clinically resistant to these agents. Can any of the previous mechanisms explain this observation?

If there are increases in proportion of cells in G_0, or in the average intermitotic time (the time to go through the cell cycle), then this would imply that the tumor has become resistant since cells will

spend a longer time in resistant phases of the cell cycle. However, it would also imply that the growth rate of the tumor would slow considerably, which does not appear to be the case [3]. Also, if there are fewer cycling cells or the cells have longer cycle times then proportionately fewer cells are in a sensitive state but also fewer cells need be killed to control growth. Although it is not necessarily true that these two effects will move in tandem precisely compensating for one another, they must tend to, to some degree. From this reasoning, and the lack of observation of significantly slower growth rates, it seems reasonable to conclude that this mechanism is not a major cause of tumor regrowth during treatment.

Changes in the host, so that the drug is more rapidly metabolised, also seems an unlikely explanation for tumor regrowth during treatment. Such changes would also imply that the toxic effect frequently seen in normal tissue should decline as the treatment continues, but this does not seem to be the case. Neither the mechanism of pharmacologic sanctuary or total resistance would seem to apply as the tumor responded in the first instance and is regrowing at the original site. Both distance from the capillary bed or the existence of resistant cells provide a plausible self-consistent explanation for the observation of relapse during (initially successful) therapy. Both predict the existence of a subpopulation of resistant cells which upon the application of therapy will be "revealed" and repopulate the tumor. The regrowing tumor can then be expected to be resistant to the drug. Studies of both experimental and human malignancy have shown that resistant tumors contain cells which exhibit structural differences from

the original sensitive cells. Therefore intrinsic cellular resistance provides a logical explanation of this commonly observed phenomenon which is consistent with observation in passaged animal tumors.

Resistance to chemotherapy is thus an important concept whose understanding may better explain the response of tumors to chemotherapy. The variability in response (either survival time or proportion cured) to a fixed treatment protocol of an inbred strain of animals implanted with the same tumor line suggests that the development of resistance involves some random process. In what follows we will thus use stochastic models for tumor growth and the development of resistance.

Earlier work by Goldie and Coldman [4], in which drug resistant mutants were assumed to arise spontaneousely, provided a basic model of this phenomenon. This model provided "quantitative" predictions about the behaviour of tumors which are in broad agreement with experience from experimental and clinical chemotherapy [5]. However, this basic model could not be fit to much experimental data because it assumed: (i) that there was no tumor cell differentiation or loss, (ii) that the drug was only applied once, (iii) that all sensitive cells were killed by the drug and (iv) that resistant cells were absolutely resistant. In Chapter 3 a more general model will be presented in which these assumptions are relaxed. This model will then be fitted to experimental data and the results presented in Chapter 5. For human cancer the age of the tumor is seldom known. In order to use this model of resistance (which is parameterized by time) in human data this parameter must be removed and three methods of accomplishing this, involving differing assumptions, are discussed in Chapter 3.

Experience with both experimental and clinical tumors has shown that for almost all cases there exists a combined chemotherapy (the use of several drugs) which is superior to a single drug in curing disease or increasing survival time. This observation is not surprising since the addition of further anti-cancer agents seems likely, a-priori, to increase the efficacy of any single drug protocol. However, the reason for such an improvement in response is not well understood. These observations may be "explained" by assuming the various drugs in the combination to have differing phase-specific activity so that the combined therapy is more effective than any of the individual agents. However protocols which have attempted to combine agents with differing phase-specific activity generally have not been successful (in improving response), suggesting that other factors may be responsible for the benefits associated with combination chemotherapy. The superiority of combination chemotherapy is "naturally" explained if we assume that the tumor contains subpopulations of cells resistant to particular drugs. The use of combination chemotherapy will thus lead to the preferential selection of those cells which are resistant to all drugs in the protocol, which will usually represent a smaller proportion of the total tumor than that which are resistant to only one of the drugs. In circumstances where the proportion of cells resistant to the combination is smaller than the proportion resistant to any one of the drugs, use of the combination will yield superior results. In order to further model the response of tumors to several drugs it is necessary to consider the joint distribution of multiple types of resistant cells. In Chapter 4 the model developed in Chapter 3 is generalized to two drugs and measures

of the effectiveness of protocols involving two drugs are developed. This leads directly to considerations of maximizing the therapeutic effect of protocols, and results are given indicating the increase in the likelihood of cure obtained in two-drug protocols as compared to single-drug protocols. Examples are developed in Chapter 5 where it is shown that the effects of different protocols depend on the choice of the outcome measure (survival time or proportion cured).

Before continuing, it is worthwhile to emphasize two points. Firstly, in any complex biological system where many, possibly competing, processes are at work, and where any one may produce the same crude end point, it is unrealistic to believe that consideration of one process, no matter how complete, will lead to a comprehensive description of the observed phenomena. However, the consideration of a single process can give important indications of expected behaviour and may provide a framework for the incorporation of other mechanisms. Secondly, mathematical models of processes are seldom, if ever, unique to that process. In particular, the model we will develop can also be used for some of the other resistance mechanisms discussed earlier in this chapter.

1.1 Resistance in Other Biological Systems

Analogous processes were first observed in the study of bacteria exposed to viral infection. In a series of experiments investigating the infection of bacteria by viruses it was found that after chronic exposure to a virus, a subpopulation of the initially sensitive bacterial population, was no longer sensitive to infection by the same virus [6]. In most cases infection by the virus resulted in cell death. Furthermore

although morphologic differences in the cells could sometimes be detected, this was frequently not so, and these resistant bacteria seldom displayed any resistance to infection by other viruses. This observation led to two experimentally indistinguishable hypotheses regarding the origin of resistant subtypes. It was not until 1943 that the pioneering work of Luria and Delbruck [6] permitted the two main competing theories to be compared and experimentally separated. These investigators summarised these two hypotheses as follows:

"1) First hypothesis (mutation): There is a finite probability for any bacterium to mutate during its lifetime from 'sensitive' to 'resistant'. Every offspring of such a mutant will be resistant, unless reverse mutation occurs. The term 'resistant' means here that the bacterium will not be killed (absolute resistance) if exposed to virus, and the possibility of its interaction with virus is left open.

2) Second hypothesis (acquired hereditary immunity): There is a small finite probability for any bacterium to survive an attack by the virus. Survival of an infection confers immunity not only to the individual, but also to its offspring. The probability of survival in the first instance does not run in clones. If we find that a bacterium survives an attack, we cannot from this information infer that close relatives to it, other than descendants, are likely to survive the attack."

Using simple mathematical analysis, Luria and Delbruck showed that for both hypotheses the mean number of resistant cells was proportional to the total number of cells, N, but that the variance of the number of

resistant cells was proportional to N^2 for hypothesis 1 and to N for hypothesis 2. By constructing a suitable experimental method, known as the fluctuation test, they were able to show that their data was incompatible with hypothesis 2 and supportive of hypothesis 1. Assuming hypothesis 1 to be true, they also discussed ways to estimate the mutation rate, which they defined to be the probability that a cell would become resistant.

The work of Luria and Delbruck spurred a great deal of research in both experimental and mathematical analysis of this problem. Lea and Coulson [7], using the probability gerating function and expanding in powers, were the first to derive expressions for the distribution function of the number of resistant cells. This derivation assumed that the growth rates of sensitive and resistant cells were equal and constant, that mutations only occurred from sensitivity to resistance, and that the mutation rate was constant. An error in their derivation was pointed out by Bartlett [8] and a correct solution was given by Armitage [9], who permitted differential growth rates between sensitive and resistant cells, and back mutations from resistance to sensitivity. A theme also explored at this time was the possible effect of a phenomena known as phenotypic delay. This effect related to a possible delay after mutation until the resistance was expressed by the cell, which was modelled by assuming this time to be either fixed, or to depend upon the size of the resistant clone (population of cells from a single parent). These processes were also examined by Kendall [10] who was interested in their application to carcinogenesis.

Crump and Hoel [11] utilised the theory of filtered Poisson

processes, and found analytic results similar to those previously obtained. They also critically examined the properties of estimators for the mutation rate which had been proposed elsewhere in the literature. This approach was more recently extended by Tan [12] to explicitly model mutants at the hypoxanthine-guanine phospheribosil transferase locus in Chinese hamster ovary cells.

Considerable research has been conducted recently in the general theory of branching processes, of which mutational processes are but one special application. Much progress has been made in the asymptotic theory of branching processes and limiting distributions have been derived for cases of fixed transitional rates for both single and multi-type branching processes. A comprehensive survey of results in this area is contained in Athreya and Ney [13]. These results have found wide application in physical problems where large numbers of particles are present (e.g. chemical and nuclear reactions). In this thesis we will be concerned with the distribution of small numbers of resistant cells where asymptotic analysis is not appropriate.

In the following chapters we present and explore the implications of mutation to resistance on the treatment of patients with cancer. Chapter 2 describes a model for tumor growth in order to establish a framework for the development of resistance. Chapter 3 contains a treatment of resistance to a single drug. Chapter 4 establishes a framework for the consideration of more general cases and presents a detailed analysis of the situation when two drugs are available. Chapter 5 presents calculations based on the previously developed theory and discusses some applications of this model both to experimental and human

cancer. The final chapter summarizes the main results and discusses areas for future research.

2. A MODEL FOR TUMOR GROWTH

In this chapter we will discuss a model for tumor growth in discrete time. Results will be presented for the computation of the probability generating function of the tumor growth model and its asymptotic distribution will be derived. We will also discuss how the model parameters can be estimated from experimental observations and indicate how particular aspects of the model can be modelled in continuous time, an idea that is used in subsequent chapters.

Despite (or perhaps because of) the extensive research on models for tumor growth, there does not exist a single commonly accepted model. This is due in part to the fact that two broad, and differing, approaches we will refer to here as "empirical" and "biological" have been taken. In the empirical approach, use is made of serial measurements of tumor size and various mathematical functions are used to fit a model. In the biological approach, assumed processes of cellular division and interaction with the host are synthesized to give a model for the overall tumor growth.

Empirical growth functions have great value in determining useful treatment parameters which cannot be directly observed. For example, knowledge of the growth curve permits the estimation of residual disease after a therapeutic intervention by observing the time at which the disease recurs. However, for human malignancy the requirement of a large number of serial observations has severely limited their usefulness. Further, these mathematical functions may contain parameters which have no obvious biological interpretation.

Alternatively, the biological approach uses processes observed in

severely limited their usefulness. Further, these mathematical functions may contain parameters which have no obvious biological interpretation.

Alternatively, the biological approach uses processes observed in dividing populations of cells and results in models where the effects of single mechanisms can be examined and evaluated independently. However, these models are frequently criticised for failing to take account of all processes, giving results which do not adequately fit data, or yielding models with so many parameters that they could be made to fit almost any data. The latter criticism stems mainly from the fact that many processes, while well-understood in general terms, are not uniquely specified so that any attempt to use them requires the a-posteriori specification of parameter values.

In this discussion we favour the biological approach since we are interested in properties acting at the cellular level. Our aim is to develop a model which will incorporate several known characteristics of human malignant growth. In particular, we require a model which recognises that not all tumors are a homogenous collection of cells with the same proliferative capabilities. Examination of many solid tumors, both experimental and clinical, has shown them to contain cells which are functionally dead, i.e. cells which are incapable of division. Since tumors are believed to grow from microscopic foci, these dead malignant cells represent the descendents of dividing malignant cells. In many populations of dividing cells it is recognised that not all cells are capable of unlimited proliferation. Cells capable of unlimited proliferation are referred to as stem cells and represent a variable fraction (depending upon the tumor type) of the dividing cells in the

tumor. The model we will use here is a slightly modified version of one described by Mackillop et al [14], which is a stem cell model analogous to that used to describe the growth of normal tissue systems such as the hemopoietic system.

This model assumes that cells can be classified into one of three mutually exclusive classes based on their proliferative potential. In common with other work in this area, we will cast this model in a discrete framework in which cells are assumed to divide with a fixed intermitotic interval with division taking place at the beginning of each interval. This biologically unrealistic assumption must be viewed as a first approximation to a complex process in which the intermitotic time can be expected to vary as a function of a large number of factors. Part of this model will be recast in a continuous framework in subsequent chapters, when the behaviour of stem cells alone are considered. The three compartments consist of stem cells, transitional cells and end cells defined as follows:

1. Stem cells denoted (C_1); 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 with probability 1-p-q.
2. Transitional cells ($C_2,..,C_{n+1}$); cells capable of limited proliferation. This class is comprised of disjoint subclasses C_2, ..., C_{n+1} where n is referred to as the clonal expansion number. Transitional cells which are the immediate result of a stem cell division are entered in subclass C_2. Upon division a single C_2 cell gives rise to two

C_3 cells. These processes are repeated for $C_3,\ldots,C_{n+1}$.

3. End cells (C_{n+2}); These are functionally dead cells incapable of further proliferation. Two end cells are formed by the divison of a single C_{n+1} transitional cell.

Dividing cells ($C_1,\ldots,C_{n+1}$) are assumed to divide with a fixed and common interdivision interval. All cells are assumed to behave independently.

For the purpose of this analysis the paramters p, q and n will be considered to be fixed throughout the growth of the tumor, although it is a relatively simple matter to calculate the quantities of interest if these parameters are varied in a systematic way.

The occurrence of metastasis and measurement of experimental tumor systems indicate that substantial numbers of tumor cells are lost from the primary tumor. Cell loss from the primary tumor will be modelled by assuming each cell in compartment C_i to have a fixed probability λ_i ($i=1,\ldots,n+2$) that it will be lost per intermiotic interval, where for the purposes of calculation loss will be assumed to occur at the end of the interval. Losses of cells will be assumed to occur independently and at a fixed rate per intermitotic interval even for the non-dividing cells i.e. C_{n+2}. In this situation loss may be viewed to include lysis of dead cells or migration outside the primary tumor. This model differs from that of Mackillop et al [14] who assumed that $p+q = 1$. This difference will be shown to have important implications when we later consider stem cell resistance. An example where $p+q<1$ had previously been considered by Moolgavkar and Venzon [15] in their model of carcinogenesis.

Some constraints are placed on the choice of p, q and n by the nature of malignant growth. Firstly, from the observation that few, if any, clinically detectable malignancies ever spontaneously become extinct, it seems reasonable to limit n to be less than 30. This is chosen because 2^{30} ($\simeq 10^9$) cells represents the lower limit of detection of primary tumors and since spontaneous complete regression is almost never seen, the likelihood of tumors of this size being composed of totally transitional cells is remote. Similarly, observation of experimental tumors indicates that single cells either have unlimited proliferative potential (stem cells) or can grow to produce clones of no more than 10^6 cells. However, there is in theory no upper limit on n since for any value it is always possible to choose ℓ_i (i=2,...,n+1) to give a model that is consistent with the previous observations.

2.1 Properties of the Growth Model

For the tumor to continue to grow (on the average), the stem cell compartment must grow. Thus the mean number of stem cells produced by a division of a single stem cell must exceed one. From this we have the requirement

$$(1-\ell_1)\ (2p+1-p-q)>1$$

or

$$p-q> \ell_1/(1-\ell_1). \qquad \ldots (2.1)$$

The growth model, although very simple to define, has a complex structure. It is nevertheless a straightforward exercise to write recursive relationships which will give the joint probability generating function of the process.

Let $C_i(t)$ i=1,...,n+2, be random variables representing the number

of cells in compartment C_i at time t where t is measured in units of interdivision times.

Let $\Phi(\underset{\sim}{s};t)$ be the joint probability generating function of the random vector $\underset{\sim}{C}(t)=(C_1(t),\ldots,C_{n+2}(t))$:

$$\Phi(\underset{\sim}{s};t) = E\left[s_1^{C_1(t)} \times \ldots \times s_{n+2}^{C_{n+2}(t)}\right],$$

where $\underset{\sim}{s} = (s_1, \ldots, s_{n+2})$. Let

$$\phi_i(\underset{\sim}{s}) = E\left[s_1^{C_1(1)} \times \ldots \times s_{n+2}^{C_{n+2}(1)} \,|\, \underset{\sim}{C}(0)=\underset{\sim}{e}_i\right]$$

where $\underset{\sim}{e}_i = (0,0,..,1,..,0)$ (the vector with 1 in the i-th position and 0 elsewhere); $\phi_i(\underset{\sim}{s})$ is the probabality generating function after one division of a single cell in state C_i at time 0. Then it can be shown that

$$\begin{aligned}
\phi_1(\underset{\sim}{s}) &= \ell_1+(1-\ell_1)\left[ps_1^2+(1-p-q)s_1s_2+qs_2^2\right],\\
\phi_i(\underset{\sim}{s}) &= \ell_i+(1-\ell_i)s_{i+1}^2 \text{ for } i=2, \ldots, n+1,\\
\phi_{n+2}(\underset{\sim}{s}) &= \ell_{n+2}+(1-\ell_{n+2})s_{n+2}.
\end{aligned}$$

From this we obtain

$$\Phi(\underset{\sim}{s};t+1) = \Phi(\underset{\sim}{\psi}(\underset{\sim}{s});t), \qquad \ldots (2.2)$$

where $\underset{\sim}{\psi}(\underset{\sim}{s})=\left(\phi_1(\underset{\sim}{s}), \ldots, \phi_{n+2}(\underset{\sim}{s})\right)$. Equation (2.2) follows from a well known result [16] for the probability generating function for the sum of a random number of random variables. Let $\underset{\sim}{X}_{ij}=(X_{1ij},\ldots,X_{Jij})$ $(i=1,\ldots,\infty, j=1,\ldots,J)$, $\underset{\sim}{Y}=(Y_1,\ldots,Y_J)$ and $\underset{\sim}{Z}=(Z_1,\ldots,Z_J)$ be non-negative integer valued random vectors with $\underset{\sim}{Z} = \sum_{j=1}^{J} \sum_{i=1}^{Y_j} \underset{\sim}{X}_{ij}$. Assuming $\underset{\sim}{X}_{ij}$ are independent for all i,j, $\underset{\sim}{X}_{ij}$ are identically distributed for all i (for each j), $\underset{\sim}{X}_{ij}$ and Y_j are independent for all i,j, then

$$\phi_{\underset{\sim}{Z}}(\underset{\sim}{s}) = \phi_{\underset{\sim}{Y}}\left(\phi_{\underset{\sim}{X}}(\underset{\sim}{s})\right), \qquad \ldots (2.3)$$

where,

$$\phi_{\underset{\sim}{Z}}(\underset{\sim}{s}) = E\left[s_1^{Z_1} \times \ldots \times s_J^{Z_J} \right],$$

$$\phi_{\underset{\sim}{Y}}(\underset{\sim}{s}) = E\left[s_1^{Y_1} \times \ldots \times s_J^{Y_J} \right],$$

$$\phi_{\underset{\sim}{X}}(\underset{\sim}{s}) = (\phi_{X_1}(\underset{\sim}{s}), \ldots, \phi_{X_J}(\underset{\sim}{s})),$$

and

$$\phi_{X_j}(\underset{\sim}{s}) = E\left[s_1^{X_{11j}} \times \ldots \times s_J^{X_{J1j}} \right].$$

Equation (2.2) follows using (2.3) with $\underset{\sim}{Y}=\underset{\sim}{C}(t)$ and $\underset{\sim}{X}_{1j}=\underset{\sim}{C}(t+1)$ conditional on $\underset{\sim}{C}(t)=\underset{\sim}{e}_j$ (then unconditionally $\underset{\sim}{Z}=\underset{\sim}{C}(t+1)$).

After specification of $\Phi(\underset{\sim}{s};0)$ it is possible to directly calculate $\Phi(\underset{\sim}{s};t)$ by recursive use of (2.2). However, this solution is not very tractable and is of limited use since t is seldom, if ever, known for human malignancy.

Three quantities of interest which are measurable for human cancer, are the growth rate (GR) of the tumor, the proportion of stem cells (P_S) and the proportion of dividing cells (P_D). Consider the following definitions:

$$\begin{aligned} GR(t) &= C(t)/C(t-1), \\ P_S(t) &= C_1(t)/C(t), \\ P_D(t) &= 1-C_{n+2}(t)/C(t) \end{aligned} \qquad \ldots(2.4)$$

and $C(t) = \sum_{i=1}^{n+2} C_i(t)$.

As defined these quantities are random variables which are functions of a possibly unknown parameter t. Consider the limiting quantities:

$$GR = \lim_{t\to\infty} GR(t),$$

$$P_S = \lim_{t\to\infty} P_S(t),$$

$$P_D = \lim_{t\to\infty} P_D(t).$$

We will now show that the limits GR, P_S and P_D exist. In order to do this we will use asymptotic theory developed for multitype branching processes (of which the growth model considered here is one example). Consider the matrix M, where

$$M_{i,j} = E[C_j(1) \mid \underset{\sim}{C}(0)=\underset{\sim}{e}_i] \text{ for } 1\leq i,j\leq n+2.$$

In this case M is given by

$$M = \begin{pmatrix}
(1-\ell_1)(1+p-q) & (1-\ell_1)(1-p+q) & 0 & 0 & \cdots & 0 & 0 \\
0 & 0 & 2(1-\ell_2) & 0 & \cdots & 0 & 0 \\
0 & 0 & 0 & 2(1-\ell_3) & \cdots & 0 & 0 \\
\vdots & \vdots & \vdots & \vdots & \vdots\ \vdots & \vdots & \vdots \\
0 & 0 & 0 & 0 & \cdots & 2(1-\ell_n) & 0 \\
0 & 0 & 0 & 0 & \cdots & 0 & 2(1-\ell_{n+1}) \\
0 & 0 & 0 & 0 & \cdots & 0 & (1-\ell_{n+2})
\end{pmatrix} \qquad \ldots(2.6)$$

Let $M^{(k)}_{i,j}$ denote the (i,j) element of M^k. Two compartments C_i and C_j ($1\leq i,j\leq n+2$) are said to communicate if and only if there exist integers k,m (≥ 0) such that

$$M^{(k)}_{i,j} > 0 \text{ and } M^{(m)}_{j,i} > 0.$$

By convention $M^0 = I$ (the identity matrix) and thus every compartment communicates with itself. Examination of (2.6) shows that the growth model considered here consists of n+2 communicating classes each

consisting of a single cell type. The eigenvalues, λ, of M satisfy the characteristic polynomial $\det|\lambda I-M|=0$, which in this case is

$$(\lambda-A)\lambda^n(\lambda-(1-\ell_{n+2}))=0,$$

where $A = M_{1,1}=(1-\ell_1)(1+p-q)$. The maximal eigenvalue is $\lambda=A>1$ (from (2.1)) which is of multiplicity one. Let $\underset{\sim}{\nu}$ be the left eigenvector of M associated with A, that is,

$$\underset{\sim}{\nu}\, M=A\underset{\sim}{\nu}, \qquad \ldots(2.7)$$

where $\sum_{i=1}^{n+2} \nu_i=1$. Examination of (2.6) reveals that $M_{1,1}>0$, $M_{n+2,n+2}>0$, $M_{i,i+1}>0$ $(i=1,\ldots,n+1)$ and by Theorem 4.1, page 66 in Mode [17], for $\underset{\sim}{C}(0)=\underset{\sim}{e}_1$ we have

$$\frac{\underset{\sim}{C}(t)}{A^t} \to w\underset{\sim}{\nu} \text{ almost surely,} \qquad \ldots(2.8)$$

where w is a non-negative scalar random variable.

It is easily seen that $E[C_j(1) \log C_j(1)|\underset{\sim}{C}(0)=\underset{\sim}{e}_i]<\infty$ for all i,j and we thus have from Theorem 4.1, page 66, in Mode [17] that $E[w|\underset{\sim}{C}(0)=\underset{\sim}{e}_1]>0$. Thus for realizations of the process $\underset{\sim}{C}$ of interest (i.e. those for which $C(t)\to\infty$ as $t\to\infty$) it follows from (2.7) that

$$\frac{C(t)}{A^t} = \frac{\underset{\sim}{C}(t).\underset{\sim}{1}}{A^t} \to w\underset{\sim}{\nu}.\underset{\sim}{1} = w \text{ a.s.},$$

where $\underset{\sim}{1}$ is the vector where each element is 1. Thus,

$$\underset{\sim}{C}(t)/C(t) \to \underset{\sim}{\nu} \text{ a.s.}$$

From this we see directly that P_S and P_D exist and are degenerate. To see that GR exists consider

$$\frac{C(t)}{A^t} - \frac{C(t-1)}{A^{t-1}}.$$

Then we have a.s.

$$0 = \lim_{t\to\infty} \left(\frac{C(t)}{A^t} - \frac{C(t-1)}{A^{t-1}}\right)$$
$$= \frac{1}{A} \lim_{t\to\infty} \frac{C(t-1)}{A^{t-1}} \left(A - \frac{C(t)}{C(t-1)} \right).$$

Thus for realizations of interest we have GR = A a.s.

Notice that the asymptotic growth rate of the tumor is entirely determined by parameters which control the growth of the stem cell compartment, that is A. Furthermore, the random variable w relates to the growth of the stem cell compartment. To see this, we note that if $\underset{\sim}{C}(0)=\underset{\sim}{e}_i$ $(i=2,\ldots,n+2)$, then:

$$C(t) \leq 2^n \text{ for all } t,$$

where n is the clonal expansion number. Thus for A>1,

$$\frac{C(t)}{A^t} \to 0.$$

Therefore for any realization $\underset{\sim}{C}(t)$ with $\underset{\sim}{C}(t')=\underset{\sim}{C}'$,

$$\lim_{t\to\infty} \frac{\underset{\sim}{C}(t)}{A^{t-t'}} = w^* \underset{\sim}{\nu} \quad \text{a.s.},$$

where w* depends only on $C_1(t')$. Because of the independent behaviour of the stem cells, w* is the convolution of w given in (2.8).

In attempting to fit this model to human disease we are faced with situations where only comparatively crude data are available. The fraction of dividing cells can be currently estimated with limited precision [18]. Estimates of stem cell fraction are in the range of 0.001 and above [19]. Therefore when there are at least 10^9 cells, the number of stem cells exceeds 10^6. At the lower limit of the number of stem cells, one or both of the other compartments will be large. The number of cells growing from a single cell (of any type) has a finite mean and variance for a finite time period. Since cells behave inde-

pendently, fluctuations in the proportion of cells in each of the compartments will be small with high probability. In cases where the proportion of stem cells is very high, the proportion of the non-stem cells will be small in comparison to the precision with which it can be measured. From these considerations, we expect the limiting values GR, P_S and P_D will apply to a mature clinical or experimental tumor where we will assume $n \leq 20$. We can then use expressions for GR, P_S and P_D to estimate the parameter values of the tumor. These expressions can be directly calculated by solving (2.7), where M is given by (2.6), and lead to:

$$\nu_2 = \frac{(1-p+q)}{(1+p-q)} \nu_1, \qquad \ldots(2.9.1)$$

$$\nu_{i+1} = \frac{2(1-\ell_i)}{A} \nu_i, \text{ for } i=2,\ldots,n, \qquad \ldots(2.9.2)$$

and

$$\nu_{n+2} = \frac{2(1-\ell_{n+1})}{A-(1-\ell_{n+2})} \nu_{n+1}. \qquad \ldots(2.9.3)$$

The constraint $\sum_{i=1}^{n+2} \nu_i = 1$ and equations (2.9.1-3) yield:

$$\nu_1 = (1+p-q)\left[2+(1-p+q)\left\{\sum_{i=2}^{n}\left(\prod_{j=2}^{i}\frac{2(1-\ell_i)}{A}\right) + \frac{(1-p+q)A}{A-1+\ell_{n+2}}\prod_{j=2}^{n+1}\frac{2(1-\ell_j)}{A}\right\}\right]^{-1}. \qquad \ldots(2.10)$$

Using (2.9.1-3) and (2.10) we will now calculate P_S, P_D and GR for several special cases of the n+2 element vector $\underset{\sim}{\ell}$ of loss rates. We will not indicate the special cases which arise when both $\ell_2=\ldots=\ell_n=\ell$ and $2(1-\ell)=A$.

(I) If $\underset{\sim}{\ell} = (\ell_1, \ell, \ell, \ldots, \ell, \ell_{n+2})$ then

$$GR = (1-\ell_1)\,(1+p-q) = A,$$

$$P_D = \frac{(\ell_1-\ell) + (1-\ell_1)\frac{(1-p+q)}{2}\left(\frac{2(1-\ell)}{A}\right)^n}{(\ell_1-\ell) + (1-\ell_1)\,\frac{1-p+q}{2}\,\left(\frac{2(1-\ell)}{A}\right)^n\,\left[1+\frac{2(1-\ell)-A}{A-1+\ell_{n+2}}\right]}$$

and $$P_S = \frac{(1-\ell) - (1-\ell_1)\,\frac{(1+p-q)}{2}}{(\ell_1-\ell) + (1-\ell_1)\,\left(\frac{1-p+q}{2}\right)\,\left(\frac{2(1-\ell)}{A}\right)^n}\,P_D.$$

(II) If $\underset{\sim}{\ell} = (\ell, \ell, \ldots, \ell, \ell_{n+2})$ then

$$GR = (1-\ell)\,(1+p-q),$$

$$P_D = \frac{A-1+\ell_{n+2}}{2(1-\ell) - (1-\ell_{n+2})}$$

and $$P_S = \left(\frac{1+p-q}{2}\right)^n P_D.$$

(III) If $\underset{\sim}{\ell} = (\ell, \ldots, \ell)$ then

$$GR = (1-\ell)\,(1+p-q),$$

$$P_D = p-q$$

and $$P_S = \left(\frac{1+p-q}{2}\right)^n P_D\,.$$

(IV) If $\underset{\sim}{\ell} = (0, 0, \ldots, 0, \ell)$ then

$$GR = (1+p-q),$$

$$P_D = \left(\frac{p-q+\ell}{1+\ell}\right)$$

and $$P_S = \left(\frac{1+p-q}{2}\right)^n P_D\,.$$

(V) If $\underset{\sim}{\ell} = (\ell, \ell, \ldots, \ell, 0)$ then

$$G = (1-\ell)\,(1+p-q),$$

$$P_D = \frac{(1-\ell)(p-q) - \ell}{1-2\ell}$$

and $$P_S = \left(\frac{1+p-q}{2}\right)^n P_D\,.$$

Example (I) represents the situation where the three types of cells (stem, transitional and end) are lost at three different rates. Example (II) represents the case where all dividing cells are lost at the same rate, and non-dividing cells are lost at a different rate. (III), (IV) and (V) represent special cases where all cells are lost at the same rate, dividing cells are not lost and end cells are not lost respectively. In the absence of specific information for a particular tumor system, example II seems to be a reasonable compromise between complexity of the general case and the likely processes which cause cell loss in a tumor. For example, all dividing cells can be expected to be shed at similar rates into the blood and lymphatic systems because of growth pressure. End cells will be lost at a different rate because they will die at a higher rate (than dividing cells) by their very nature. However, even this model contains five unknown parameters, (p, q, n, ℓ and ℓ_{n+2}) which cannot be uniquely identified from GR, P_D and P_S. To estimate the parameters of this model requires either the a-priori specification of some of the parameters or the collection of data on other tumor characteristics specified by the parameters of this model. Examination of (2.9.1-3) shows that the parameters p and q only appear as the difference $\pm(p-q)$. Therefore even if $\underset{\sim}{\ell}$ and n were known, p and q cannot be inferred (except in the trivial case p-q=1) from experiments measuring GR, P_S and P_D. This problem is not easily resolved since the identification of stem cells, both theoretically and experimentally, is based upon their proliferative potential, and at present it is not easy to separate stem cells from other dividing cells and carry out experiments on them. However, a closer analysis of experiments carried

out to measure P_S shows that further information can be gained.

In order to measure P_S in a human tumor, a biopsy specimen of the tumor is first homogenized and then a sample, N, of cells, are plated out individually onto a medium supplying nutrients and a suitable matrix for growth. After an incubation period the number of cells, r say, which have gone on to form colonies of cells greater than some fixed size, S_M say, are counted. Then the proportion r/N is reported as the fraction of stem cells. If S_M is chosen too small (as determined by n, the clonal expansion number), some of the colonies generated may be the product of transitional cells. If S_M is chosen large enough, the counted colonies will consist entirely of colonies generated by stem cells. However, if S_M is chosen too large many stem cells present will not form colonies of size S_M because the stem cell may initially (or subsequently) divide to form only transitional cells. Thus to design experiments to measure P_S we must know (or have a good idea of) n. However, if S_M is chosen very large then it is possible to obtain an approximate expression for E[r/N] as follows.

Using the same notation for colonies as previously used for tumors, let $\underset{\sim}{C}^i(t)$ be the state vector for the i-th colony at time t and set $C^i(t) = \sum_{j=1}^{n+2} C_j^i(t)$. In this experimental situation we will assume that $\underset{\sim}{\ell}=\underset{\sim}{0}$, that is there is no loss from the colonies.

If S_M is chosen very large then almost all sample paths for which $C^i(t')>S_M$ (for some t') will grow arbitrarily large, that is, these paths will satisfy

$$\lim_{t\to\infty} \frac{C^i(t)}{A^t} > 0. \text{ a.s.}$$

Since the proportion of stem cells approaches ν_1, we have for such paths $\lim_{t\to\infty} P\{C_1^i(t)=k\}=0$ for finite non-zero k. Thus the probability $\kappa=P\{C^i(t')>S_M\}$, is approximately given by $\kappa\simeq 1-\theta$ where the extinction probability $\theta = \lim_{t\to\infty} P\{C_1^i(t)=0\}$. The probability generating function for stem cell growth $\phi(s)$, is given by $\phi_1(s)$ in (2.2) with $\ell_1=0$ and $\underset{\sim}{s}$ = (s,0,...,0), that is

$$\phi(s) = q+(1-p-q)s+ps^2. \qquad ...(2.11)$$

It is a well known result (p. 397 in Karlin and Taylor [20]) that θ is given by the minimum solution of $\theta=\phi(\theta)$. Solving this equation yields $\theta=q/p$ and thus $\kappa\simeq 1-q/p$. The proportion of stem cells will be approximately given by P_S(since the cells are sampled from a mature tumor) and if S_M is very large:

$$E[r/N]\simeq P_S(1-q/p). \qquad ...(2.12)$$

The right hand side of (2.12) depends on the value of p and not just on the difference p-q. Thus by carrying out a series of experiments at values of S_M it is possible to obtain information on the values of p and q.

A further property of this model which is important in the subsequent development is that the stem cell compartment functions autonomously; that is, the size of the stem cell compartment is determined by the history of stem cell divisions and not by any of the other compartments. Assuming that disease is diagnosed at a relatively early stage, then, except in extremely rare cases, elimination of the stem cell compartment is a necessary and sufficient condition for cure of the tumor. This statement is based on the following assumptions:

(i) Diagnosis is made at approximately 10^{10} cells and death will occur at no less than 10^{12} cells;

(ii) The proportionate kill of chemotherapy is the same for all dividing cells (i.e. stem and transitional cells);

(iii) $P_S(t)(\simeq P_S)>10^{-4}$ i.e. at least one in 10^4 cells are stem cells;

(iv) A clonal expansion number (n) in excess of 15 is unlikely;

(v) $(q/p)<0.95$, that is the ratio of stem cell divisions forming only transitional cells compared to those forming only stem cells, is not too large.

By (iii) and (i) there are at least $10^6(10^{-4}\times10^{10})$ stem cells in the tumor. By assumption the stem cells are eliminated and this implies that only sufficient numbers can survive the effects of treatment so that they go spontaneously extinct (yield progeny which are transitional and end cells only). The probability a single stem cell will go spontaneously extinct is (q/p) and because cells behave independently the probability that k would go extinct is $(q/p)^k$. By (v) $(q/p)<0.95$ and thus if $n\geq100$ then $(q/p)^{100}<0.01$. This implies that the probability a stem cell will survive therapy is $<10^{-4}(10^6/10^2)$. Thus by (ii) the expected number of surviving transitional cells is $<10^6(10^{-4}\times10^{10})$. By (iv) each transitional cell can give rise to no more than $2^{15}=3.3\times10^4$ cells. Thus the maximum size the residual tumor can achieve (if all stem cells are eliminated) is $10^6\times3.3\times10^4=3.3\times10^{10}$ which is less than the minimum size which can cause patient death by (i).

As indicated in the previous discussion, the long-term behaviour of the tumor (that is whether it is curable or not) can be assessed by considering whether the stem cell compartment can be eliminated or not.

However, the short-term response of tumors to therapy will naturally be a function of the response of all tumor cells. In attempting to describe tumor behaviour in terms of this model, we will restrict our analysis to considerations of long-term response, based on the behaviour of the stem cell compartment.

By the nature of the growth model presented here, not every sample path passes through the point k (not every path satisfies $C(t)=k$ for some t). In particular if $C_1(t')>k$ for a particular path we cannot conclude that there exists a $t\leq t'$ such that $C_1(t)=k$ for the path. In later chapters we wish to consider t as a continuous parameter, to be able to condition on $C_1(t)$ and require that every path for which $C_1(t')>k$ satisfy $C_1(t)=k$ for some $t\leq t'$. In order to do this we require a model for growth which only changes by increments of +1 or -1. A convenient process which has this property is the linear birth and death process.

In examining long-term response we will utilize a birth and death model for the stem cell compartment. In this model all losses from the stem cell compartment (to transitional cells, cell deaths, etc.) will be termed deaths. Additions of new stem cells by division will be referred to as births. We will assume that for a single cell in a time interval $[t,t+\Delta t)$ divisions resulting in two stem cells occur with probability $b\Delta t+o(\Delta t)$, divisions resulting in one stem cell and one transitional cell with probability $c\Delta t+o(\Delta t)$ and deaths occur with probability rate $d\Delta t+o(\Delta t)$. We make the correspondence between the discrete and continuous models by requiring that b, c and d satisfy the following constraints:

$$\frac{b}{b+c+d} = p(1-\ell_1), \qquad \ldots(2.13.1)$$

$$\frac{c}{b+c+d} = (1-p-q)\ (1-\ell_1), \qquad \ldots(2.13.2)$$

and

$$b-d = \ell n[(1-\ell_1)(1+p-q)]. \qquad \ldots(2.13.3)$$

Conditions (2.13.1) and (2.13.2) result from requiring that the events associated with b, c and d occur in the appropriate limiting frequency with respect to each other. Equation (2.13.3) guarantees that the net mean growth rate will be the same in both formulations. A continuous Markov model is a better, although imperfect, model of cellular division than one in which the inter-mitotic times are constant. A more realistic model of inter-division times would have support on $[x,\infty]$ $x>0$, thus implying a non-zero mode. However the growth process is of secondary interest in this analysis and the mathematically tractable exponential distribution for interdivision times will be used. The relevance of the growth model considered here is that it is biologically plausible and contains parameters which allow the number of stem cells to be varied for a fixed total number of tumor cells. This is important since the porportion of stem cells is suspected to differ greatly between tumor systems.

In the next chapter we will consider the spontaneous evolution of variant stem cells which display resistance to one or more chemotherapeutic agents. Relationships will be developed which relate the curability by chemotherapy of the tumor to the kinetic parameters of the tumor and other parameters reflecting the development of resistance. The growth model developed here will not be explicitly considered in later chapters but is assumed to apply. In what follows we will

concentrate on the stem cell development which, as has been shown, determines the growth and curability of tumor.

3. THE DEVELOPMENT OF RESISTANCE TO A SINGLE CHEMOTHERAPEUTIC AGENT

In Chapter 1 we discussed various mechanisms which lead to resistance to chemotherapy used in the treatment of cancer. In that chapter we discussed how drug resistant cells are known to arise in experimental tumors where they are one of the principal causes of treatment failure. Resistant cells are also thought to be a primary cause of treatment failure in human malignancy although the evidence is not as strong as in the experimental case. We will now consider the development of permanently resistant stem cells within the context of the growth model developed in Chapter 2.

In this chapter we will develop expressions which reflect the development of resistance and the long-term response of tumors treated with a single drug. We will only consider the primary tumor and not the status of any cells contained in distant metastatic deposits. Because of the nature of the tumor growth model presented in Chapter 2 we need only consider the behaviour of stem cells since they alone influence the long-term curability of the tumor. Stem cells will be considered to be in one of two states with respect to a drug: sensitive or resistant. Resistant cells will not be assumed to necessarily be totally resistant, that is, resistant cells may show some response to the drug but this response will be quantitatively less than that exhibited by sensitive cells. The two states are therefore defined with respect to one another and are generally not defined in absolute terms. This definition implicitly involves a notion of the environment of the experiment, which includes the cell line, the drug and the dosage under consideration. A more general description would include a number of states which show

varying sensitivity to the drug. For reasons, which will later become apparent, such a multitype model is difficult to analyze and we will only consider a two state model.

We will assume that in a time interval of length Δt the probability that a single stem cell divides to form two stem cells, is $b\Delta t + o(\Delta t)$, that it divides to form a stem and transitional cell is $c\Delta t + o(\Delta t)$ and that it migrates, dies or forms two transitional cells is $d\Delta t + o(\Delta t)$ (see Chapter 2). These events will be referred to as births, renewals and deaths respectively. The probability of two or more events occuring in a time interval of length Δt will be assumed to be $o(\Delta t)$. In what follows b, c and d will be assumed to be constants for a particular tumor. In common with the theoretical model of Luria and Delbruck [6], we assume that there is a fixed probability α that a birth event in a sensitive cell will result in the addition of a single resistant cell and probability $1-\alpha$ that a sensitive cell is added. Similarly, we assume that there is a probability β that a renewal event to a sensitive cell will result in the replacement of a sensitive stem cell by a resistant stem cell and a probability $1-\beta$ that there is no change in the number of sensitive stem cells. We also assume that a sensitive stem cell may spontaneously mutate from sensitivity to resistance with probability $\gamma\Delta t + o(\Delta t)$ in an interval of length Δt. Resistant stem cells are assumed to have the same parameters b, c and d but all progeny of resistant cells are assumed to remain resistant, that is transitions from the resistant to the sensitive state are assumed not to occur.

In the next section we derive the probability generating function of the process and use it to deduce some quantities which describe the

behaviour of the system. We then give a basic description of the effect of drugs on both normal and malignant cells. These are then integrated into the model for the development of resistance and equations developed for the probability generating function of the distribution of stem cells after an arbitrary sequence of treatments by a single drug. Subsequently we discuss three approaches to developing the probability generating function for the numbers of sensitive and resistant stem cells when the time paramter t is unknown. Finally, we examine the effect of random variation in the resistance parameters on the distribution of resistant and sensitive cells.

3.1 Calculating the Probability Generating Function

Let

$R_0(t)$ = number of sensitive stem cells at time t,

$R_1(t)$ = number of resistant stem cells at time t,

$N(t) = R_0(t)+R_1(t)$

and $P_{i,j}(t) = P\{ R_0(t)=i, R_1(t)=j \}$, for $t>0$.

Table I indicates transitions between states and their associated probabilities. Referring to Table I we may now use the Kolmogorov forward equations [21] to obtain the following family of differential equations for $P_{i,j}(t)$:

$$\frac{dP_{i,j}(t)}{dt} = -[(b+d)j + (b+d+c+\gamma)i]\, P_{i,j}(t) + b(1-\alpha)(i-1)\, P_{i-1,j}(t) + c(1-\beta)\, iP_{i,j}(t) + d(i+1)\, P_{i+1,j}(t) + \alpha bi\, P_{i,j-1}(t) + (\beta c+\gamma)(i+1)\, P_{i+1,j-1}(t) + b(j-1)\, P_{i,j-1}(t) + d(j+1)\, P_{i,j+1}(t) \quad \ldots(3.0)$$

TABLE I

Transitions Occurring in the Stem Cell Compartment in the interval $[t,t+\Delta t)$ which have Probability of Order Δt.

Initial State	Final State	Probability
(i,j)	$(i+1,j)$	$ib(1-\alpha)\Delta t+o(\Delta t)$
(i,j)	(i,j)	$ic(1-\beta)\Delta t$ $+jc\Delta t+o(\Delta t)$
(i,j)	$(i-1,j)$	$id\Delta t+o(\Delta t)$
(i,j)	$(i,j+1)$	$i\alpha b\Delta t+jb\Delta t$ $+o(\Delta t)$
(i,j)	$(i-1,j+1)$	$i(\beta c+\gamma)\Delta t+o(\Delta t)$
(i,j)	$(i,j-1)$	$jd\Delta t+o(\Delta t)$

for $i,j \geq 0$ where $P_{i,j}(t) \equiv 0$ for $i<0$ or $j<0$. Let $\phi(s_0,s_1;t)$ be the probability generating function of $\{R_0(t),R_1(t)\}$, that is

$$\phi(s_0,s_1;t) = \sum_{i=0}^{\infty} \sum_{j=0}^{\infty} P_{i,j}(t)\, s_0^{\,i}\, s_1^{\,j}.$$

In what follows we will specify the initial distribution of cells by the probability generating function at time 0, that is

$$\phi(s_0,s_1;0)=\phi(s_0,s_1).$$

Then using (3.0) we can show (by multiplying by $s_0^i s_1^j$ and summing over i and j and interchanging the order of differentiation and summation) that the probability generating function satisfies,

$$\frac{\partial\phi(s_0,s_1;t)}{\partial t} = [bs_0-d][s_0-1]\frac{\partial\phi(s_0,s_1;t)}{\partial s_0} + [\alpha b s_0+\nu][s_1-s_0]\frac{\partial\phi(s_0,s_1;t)}{\partial s_0} + [bs_1-d][s_1-1]\frac{\partial\phi(s_0,s_1;t)}{\partial s_1} \qquad \ldots(3.1)$$

where $\nu = \beta c + \gamma$.

Using the method of characteristics (see for example John, p. 9 [22]), solution of (3.1) can be reduced to solving the following set of ordinary differential equations:

$$\frac{dt(u)}{du} = 1, \qquad \ldots(3.2.1)$$

$$\frac{d\chi_0(u)}{du} = [\chi_0(u)-1][d-b\chi_0(u)] - [\alpha b\chi_0(u)+\nu][\chi_1(u)-\chi_0(u)], \qquad \ldots(3.2.2)$$

$$\frac{d\chi_1(u)}{du} = [\chi_1(u)-1][d-b\chi_1(u)], \qquad \ldots(3.2.3)$$

where $\chi_0(u)$ and $\chi_1(u)$ are dummy variables.

From (3.2.1) we have,

$$t=u, \qquad \ldots(3.3.1)$$

where, without loss of generality we have set the constant of integration

to zero. Solving (3.2.3) we obtain

$$\chi_1(u) = \frac{d[1-\chi_1(0)] + [b\chi_1(0)-d]\exp(\delta u)}{b[1-\chi_1(0)] + [b\chi_1(0)-d]\exp(\delta u)}, \qquad \ldots(3.3.2)$$

where $\delta=b-d$ and we assume that $b>d$ so that the process is supercritical, that is, it represents a growing tumor.

To solve the differential equation (3.2.2), first notice that $\chi_0(u)=\chi_1(u)$ is a particular solution. Substituting $\chi_0(u)=\chi_1(u)+1/y(u)$ in (3.2.2) yields the following differential equation for y(u):

$$\frac{dy(u)}{du} + y(u)[b+d+\nu-b(2-\alpha)\chi_1(u)] = b(1-\alpha).$$

The solution for y(u) is given by

$$y(u) = \frac{\{\chi_0(0)-\chi_1(0)\}^{-1} + b(1-\alpha)\int_0^u F(x)dx}{F(u)},$$

where $F(x)=\exp\int_0^x g(v)dv$ and $g(v)=b+d+\nu-b(2-\alpha)\chi_1(v)$. Writing $y(u) = [\chi_0(u)-\chi_1(u)]^{-1}$ yields the following expression for $\chi_0(u)$,

$$\chi_0(u) = \chi_1(u) + F(u)\left[\{\chi_0(0)-\chi_1(0)\}^{-1} + b(1-\alpha)\int_0^u F(x)dx\right]^{-1}, \qquad \ldots(3.3.3)$$

where

$$F(x)=\delta^{2-\alpha}\, e^{(\delta+\alpha d+\nu)x}[b[1-\chi_1(0)]+[b\chi_1(0)-d]e^{\delta x}]^{-2+\alpha}. \qquad \ldots(3.4.1)$$

It follows from the method of characteristics that if the substitutions

$$\chi_0(u)=s_0, \quad \chi_1(u)=s_1 \quad \text{and} \quad u=t \qquad \ldots(3.4.2)$$

are made in (3.3.2) and (3.3.3) then the solution of (3.1) is given by

$$\phi(s_0,s_1;t) = \phi(\chi_0(0),\chi_1(0)). \qquad \ldots(3.5)$$

Carrying out these substitutions leads to explicit expressions for $\chi_0(0)$ and $\chi_1(0)$ as functions of s_0, s_1 and t. To emphasize the dependence of

$\chi_0(0)$ and $\chi_1(0)$ on t we will write $w_0(t)=\chi_0(0)$ and $w_1(t)=\chi_1(0)$. Using the substitutions (3.4.2) in (3.3.2) we obtain

$$w_1(t) = \chi_1(0) = \frac{d(1-s_1)+(bs_1-d)\exp(-\delta t)}{b(1-s_1)+(bs_1-d)\exp(-\delta t)} \quad . \qquad \ldots(3.6)$$

Similarly using the substitutions (3.4.2) in (3.3.3) yields, after some algebra,

$$w_0(t) = \chi_0(0) = w_1(t) + \frac{f(t)}{[\delta^{2-\alpha}(s_0-s_1)]^{-1}-b(1-\alpha)\int_0^t f(v)dv} , \qquad \ldots(3.7)$$

where $f(v)=\exp\{-(\delta+\alpha d+\nu)v\}\ [b(1-s_1)+(bs_1-d)e^{-\delta v}]^{-2+\alpha}$.

Notice that (3.6) is the probability generating function for the birth and death process with fixed parameters b and d. As expected, the substitution of $s_0=s_1=s$ in (3.7) yields $w_0(t)= w_1(t)$. Thus the development of the stem cell compartment as a whole is a birth and death process with parameters b and d. Similarly, substituition of $s_1=1$ in (3.7) shows that the sensitive stem cell compartment grows as a birth and death process with parameters $b(1-\alpha)$ and $(d+\nu)$.

For future use we will now calculate some elementary properties of the process $\{R_0(t),R_1(t)\}$. By differentiating (3.1) with respect to s and s_1, setting $s_0=s_1=1$ and interchanging the order of differentiation we obtain the following ordinary differential equations for $m_0(t)=E[R_0(t)]$ and $m_1(t)=[R_1(t)]$ respectively:

$$\frac{dm_0(t)}{dt} = (\delta-\alpha b-\nu)m_0(t),$$

$$\frac{dm_1(t)}{dt} = \delta m_1(t)+(\alpha b+\nu)m_0(t),$$

which yields

$$m_0(t) = m_0 \exp\{(\delta-\alpha b-\nu)t\},$$
$$m_1(t) = [m_1+m_0(1-\exp\{-(\alpha b+\nu)t\})]e^{\delta t}, \qquad \ldots(3.8)$$

where $m_0=m_0(0)$, $m_1=m_1(0)$ are obtained directly from the probability generating function at t=0, $\psi(s_0,s_1)$. From (3.8) we see

$$E[N(t)]=(m_1+m_0)e^{\delta t}.$$

In a similar fashion we can derive ordinary differential equations which the variances and covariance must satisfy. Let $V_0(t)$ and $V_1(t)$ be variances of $R_0(t)$ and $R_1(t)$ respectively and let $V_{01}(t)$ be their covariance. Then

$$\frac{dV_0(t)}{dt} = (b+d-\alpha b+\nu)m_0(t) + 2(\delta-\alpha b-\nu)V_0(t),$$
$$\frac{dV_{01}(t)}{dt} = -\nu\, m_0(t) + (2\delta-\alpha b-\nu)V_{01}(t) + (\alpha b+\nu)V_0(t),$$
$$\frac{dV_1(t)}{dt} = (\alpha b+\nu)m_0(t) + (b+d)m_1(t) + 2(\alpha b+\nu)V_{01}(t) + 2\delta V_1(t).$$

These equations have the following solutions:

$$V_0(t) = [V_0 + A_1(1-\exp\{-(\delta-\alpha b-\nu)t\}] \exp\{2(\delta-\alpha b-\nu)t\},$$
$$V_{01}(t) = [V_{01}+ [V_0 + A_1](1-\exp\{-(\alpha b+\nu)t\}) - A_2(1-\exp(-\delta t))]\exp\{(2\delta-\alpha b-\nu)t\},$$

and

$$V_1(t) = [V_1 + 2[V_{01} + V_0 + A_1 - A_2](1-\exp\{-(\alpha b+\nu)t\}) - [V_0 + A_1](1-\exp\{-2(\alpha b+\nu)t\}) + (b+d)[m_0 + m_1](1-\exp(-\delta t))/\delta - \frac{m_0}{(\delta+\alpha b+\nu)}\left[(b+d-\alpha b-\nu)-2(\alpha b+\nu)\frac{A_2}{m_0}\right](1-\exp\{-(\delta+\alpha b+\nu)t\})]\exp\{2\delta t\},$$

where $A_1= \frac{(b+d-\alpha b+\nu)}{(\delta-\alpha b-\nu)}\, m_0$, $A_2= \frac{[(\alpha b+\nu)(1-\alpha) + (\alpha d+\nu)]}{\delta(\delta-\alpha b-\nu)}\, bm_0$, and

$V_0=V_0(0)$, $V_1=V_1(0)$ and $V_{01}=V_{01}(0)$ are calculated from $\psi(s_0,s_1)$.

Finally we note that the probability that a single stem cell, present at some time $t=t'$, will not have any surviving progeny at $t=\infty$, is given by $\varepsilon=d/b$ (see Karlin and Taylor p.147 [20]). This event will be referred to as spontaneous extinction. Similarly, since the stem cell compartment grows as a birth and death process with parameters $b(1-\alpha)$ and $(d+\nu)$, the probability that a single sensitive stem cell will not any have any surviving sensitive progeny at time $t=\infty$ is $(d+\nu)/b(1-\alpha)$. In order to consider the behaviour of a tumor subject to therapy we must first examine the effects of therapy on the tumor cells and on the normal tissue.

3.2 Effects of Drug Treatment

As mentioned previously the development of resistance to a drug can arise as a mutational process. Evidence for some drugs from experimental tumors shows that resistance can be effectively absolute. An example of this is resistance to Arabinosylcytosine in the L1210 mouse leukemia system [23]. That is, treatment with any dosage of the drug on a cell resistant to it will have no effect. In other cases this is not true, and cells may be identified that show reduced sensitivity when compared to the parent sensitive line. To model the resistance phenomenon we first consider the response of a single cell to chemotherapy.

A large body of experimention, notably by Skipper and his associates [23], has indicated a linear relationship between a single delivered dose and the logarithm of the fraction of cells over a large range of dosages. Repeated courses of chemotherapy to the same population of cells satisfy the same relationship with the same constant of proportionality as long as resistant cells do not emerge. This

relationship has been found to hold for a number of different (non-phase specific) drugs, in several types of tumors and for a range of tumor sizes [24]. From these observations, Skipper and his co-investigators have postulated that tumor cells subject to chemotherapy at dose D have an individual fixed probability, $\pi(D)$ say, of surviving chemotherapy, which may be expressed as $\pi(D)=\exp\{-kD\}$ where k is a constant of proportionality and that the response of each cell is independent of that of the others. For drugs with phase specific effect this relationship also applies providing cells are in the sensitive phase of the cell cycle. We will use this model of chemotherapeutic action in the development that follows.

Consider the binary random variable X, which indicates whether the cell survives (X=1) administration of a single course of the drug or not (X=0). If $\xi(s)$ is the probability generating function of X, then

$$\xi(s)=1-\pi(D)+\pi(D)s \qquad \ldots(3.9.1)$$

for a non-phase specific agent, and

$$\xi(s)=1-p\pi(D)+p\pi(D)s \qquad \ldots(3.9.2)$$

for a phase specific agent where p is the probability that the cell is in the sensitive phase of the cell cycle. This model for drug action was constructed for agents administered over a short period where the drug is rapidly degraded or excreted so that the effect of the drug may be considered as an instantaneous one. In general the dose at some time t, D(t), is defined as

$$D(t) = \int_0^t C(u)du \qquad \ldots(3.10)$$

where the drug is introduced at time t=0 and C(t) is the concentration of

the drug (at the tumor cell under consideration). We assume, without loss of generality, that $C(t)=0$ for $t<0$.

If therapy is phase-specific and is given over an extended period then the likelihood that a cell is in the sensitive phase of the cell cycle, at some time during the therapeutic period, will increase as the duration of therapy is lengthened. Let

$I(t)$ = 1 if the cell is in the sensitive phase at time t,

= 0 otherwise.

Let $C'(t)$ be the effective concentration for the cell at time t, then $C'(t) = C(t)I(t)$ and the effective dose experienced by the cell, $D'(t)$, is

$$D'(t)= \int_0^t C'(u)du \ .$$

Clearly the use of the indicator function $I(t)$ represents an idealization as the transition between phases of the cell cycle will not be instantaneous. However, since the time spent in transition between phases is small compared to the time spent within each phase this approximation seems reasonable.

The form of $C(t)$ is dependent on the method of administration of the drug and will be strongly peaked for a single injection but will be flatter for infusion therapy. A further practical problem to the calculation of effective dose is that some agents tend to block cells from proceeding through the cell cycle however this phenomena will not be modelled here. In the calculation of drug dose it also may be that if $C(t)<k^*$ (say) then the drug has no effect. This may be simply taken into account by considering $C^*(t)$ in the calculation of dose where

$$C^*(t) = C(t) \text{ if } C(t) \geq k^*$$

$$= 0 \quad \text{if } C(t) < k^*$$

It will be noticed that none of the these considerations alter the form of $\xi(s)$ given in (3.9.1-2). They affect the value of the binomial parameter and induce a possible complex time dependency. Assuming that the effect on a cell at time t_i only depends on the dose prior to time t_i, and that the relationships known for instantaneous doses apply, we may calculate the effect of drugs when C(t) varies slowly. To do this we define the instantaneous doses at time t_i as follows:

$$D_i = \int_{t_{i-1}}^{t_i} C(u)du \text{ where } 0=t_0<t_1\ldots<t_J=t.$$

Then

$$D(t) = \sum_{i=1}^{J} D_i.$$

Let $\xi(s;t)$ be the probability generating function for the indicating random variable of cell survival at time t and let $\xi_i(s)$ be the probability generating functions for the indicator random variables of cell survival for the instantaneous doses D_i. Using (3.9.1) we have

$$\xi(s;t) = 1-\pi(D(t)) + \pi(D(t))s$$

and

$$\xi_i(s) = 1-\pi(D_i)+\pi(D_i)s.$$

Then

$$\xi(s;t_j) = \xi_1(\xi_2\ldots\xi_j(s)..) \text{ for } j=1,\ldots,J,$$

if

$$\pi(D(t_j)) = \prod_{i=1}^{j} \pi(D_i) .$$

This condition holds if the logarithm of the probability of cell survival is in proportion to dose as has been found for chemotherapeutic agents

In cases where C(t) varies slowly in time, its effect may be computed using a series of instantaneous effects. In what follows we will assume a single instantaneous effect with the understanding that if this assumption were not appropriate we would consider a series of instantaneous doses as discussed above. This approach will be useful in cases additional different treatments are applied at times t_i (i=1,...,J).

In human malignancy the concentration of drug, C(t), is frequently measured by noting the amount of drug in the serum and not at the tumor. As noted before (Chapter 1) the exposure of a cell to the drug is a function of its distance from the capillary bed and thus may vary between cells. The model of tumor growth we use here does not account for such an effect and incorporation of this feature must be deferred for further research.

3.3 Effects on the Normal Tissue

The effects of treatment regimens are not necessarily specific to the tumor system but can also include the host's normal tissue. To account for these define a random variable:

$T=T\{(D_i,t_i),\ i\varepsilon N\}$ where,

T = 1 if host suffers unacceptable toxicity for any t,

= 0 otherwise,

which reflects the toxicity of the regimen $\{(D_i,t_i),\ i\varepsilon N\}$ where D_i is the dose given at time t_i. Unacceptable toxicity may reflect death when considering animal experimentation and will reflect a (complex) combination of objective and subjective measurements for human disease.

A common objective (althougn not necessarily theoretically optimal)

in experimental and human disease is to select $\{(D_i,t_i),\ i\varepsilon N\}$ so that $P\{T=1\}\leq P_T$ for the whole population where P_T is some constant which depends on the experimenter or clinician. Frequently experimenters use $P_T=0.1$, the so-called LD_{10}.

An assumption commonly made in experimental research is that the likelihood of toxicity depends upon the cumulative dose $D=\sum_i D_i$. We will refer to this as a "cumulative dose toxicity model".

The "model" of toxicity used for chemotherapy in clinical medicine is less explicit. In general regimens are constructed so that the $D_i(i=1,\ldots J)$ and $t_{i+1}-t_i$ $(i=1,.,J-1)$are fixed for a pre-determined series of J cycles of therapy. Here P_T for the complete regimen may be chosen to be quite high since the D_i and t_i may be modified dynamically if toxicity occurs. This will be referred to as the "clinical toxicity model" and will be assumed when considering clinical disease. This approach has its limitations since regimens are constructed using the frequency of acute toxicity with escalating dose and the influence of the timing on the toxicity response surface is not usually examined.

Having examined the effect of chemotherapy on tumor cells and how doses are modified because of toxic side effects we will now discuss how the tumoricidal effects of chemotherapy may be incorporated into the process $\{R_0(t),R_1(t)\}$. We will assume that the dosage schedule has been constructed so that toxicity is at an "acceptable" level.

3.4 Modelling Treatment Effects on the Tumor Cells

In modelling the effects of treatment it is necessary to separate the primary from the secondary malignancies. By primary we refer to a clinically detectable lesion which is subject to treatment. Secondary

disease will refer to any disease present originating from the same initial malignancy as the primary, but which is not clinically detectable. Primary and secondary disease may be located at multiple sites.

Radiation therapy is usually aimed at primary disease but in certain situations it may be used upon secondary disease. The mathematical description of the mechanism of action of radiation is similar to that of chemotherapy. That is, cells behave independently and the survival of each cell can be modelled as a Bernoulli trial. Tumors have been identified which are termed radio-resistant and show a reduced sensitivity to the application of therapy. Insensitivity to radiation is believed to arise as a result of insufficient oxygen because oxygen is known to enhance the cell killing effect of radiation. Tumors with poor vascular supply, or tumor cells within a region of poor vascularisation, will tend to be resistant because of the lower oxygen tension in such regions.

As we are mainly concerned with modelling chemotherapy we will not be greatly concerned with the modelling of radio-resistant cells. We will consider radiotherapy to be a non-selective treatment (i.e. act equally on chemosensitive and chemoresistant cells) and model its effect by considering it to act to increase d, the death rate of cells, over the period of radiation treatment.

Surgery is almost exclusively concerned with the therapy of primary disease. The response of individual cells to surgery may not be as simple as for other modalities. For example, data on the surgery of breast cancer indicates that the variance of the residual number of tumor cells is much greater than would be expected using a binomial model [25].

This "extra-binomial" variation may be modelled by assuming that the number of surviving cells is a binomial variable where the parameter is a random variable. In this case the binomial parameter will be a function of the histology, location and extension of the tumor. This particular model retains independence but offers great versatility. When modelling the effect of surgery we will assume that the binomial parameter has been observed, so that the model of this treatment regimen will be similar to the others. We will use this model when we consider data from breast cancer in Chapter 5.

When a single drug is given alone via injection, we will assume that its effect is instantaneous and independent of other treatments (see Section 3.2). If t_j is the time of the j-th treatment (j=1,...,J), then by (2.3) we have

$$\phi(s_0,s_1;t_j) = \phi(\xi_0(s_0),\xi_1(s_1);t_j^-), \qquad \ldots(3.11.1)$$

where $\xi_0(s_0)$, $\xi_1(s_1)$ are the probability generating functions for the indicator random variables of cell survival for the sensitive and resistant cells respectively and t_j^- represents the time immediately before treatment. At any time t* where $t_j<t^*<t_{j+1}$, the probability generating function for the number of cells is given by

$$\phi(s_0,s_1;t^*) = \phi(w_0(t^*-t_j),w_1(t^*-t_j);t_j), \qquad \ldots(3.11.2)$$

where $w_0(t^*-t_j)$ and $w_1(t^*-t_j)$ are given respectively by (3.7) and (3.6). In particular, the continuity of the functions ϕ and $w_i(t^*-t_j)$ (i=0,1) in t* imply that $\phi(s_0,s_1;t_{j+1}^-)$ is given by the right hand side of (3.11.2) with $t=t_{j+1}$. Notice that these equations also apply to phase specific agents since the $\xi_i(s_i)$ are of the same form. In addition $\phi(s_0,s_1;t_1^-)$ is

given by (3.5) with $t=t_1$.

These relationships may be used recursively to calculate the probability generating function for $\{R_0(t),R_1(t)\}$ after several courses of the same agent. The expected number of resistant and sensitive cells may be recursively calculated using

$$m_0(t_j) = \pi_0(D_j)m_0(t_j^-) \text{ and } m_1(t_j) = \pi_1(D_j)m_1(t_j^-).$$

From (3.8) we also have

$$m_0(t_{j+1}^-) = m_0(t_j)\exp\{(\delta-\alpha b-\nu)\ (t_{j+1}-t_j)\}, \qquad \ldots(3.12.1)$$

$$m_1(t_{j+1}^-) = [m_1(t_j)+m_0(t_j)(1-\exp\{-(\alpha b+\nu)(t_{j+1}-t_j)\})]\exp\{\delta(t_{j+1}-t_j)\}. \qquad \ldots(3.12.2)$$

If chemotherapy is not injected but is given continuously over some finite period, then its effect may be computed as discussed in Section 3.2 . The probability generating functions and expected values may be calculated using (3.11.1-2) and (3.12.1-2) where now the t_j are the times of the approximating instantaneous doses as discussed in Section 3.2. The effects of surgery or radiation on the joint probability generating function can be assessed using the same techniques if it is assumed that the survival of the individual cells are independent Bernoulli trials.

The complex form of (3.7) and (3.5) and the recursive nature of the operation needed to determine $\phi(s_0,s_1;t)$, when treatments have been applied, indicate the need for some simple measure which summarizes the effects of treatment. The expected values $m_0(t)$ and $m_1(t)$ provide one such summary, however we will now develop a more useful summary measure.

3.5 Summarising Treatment Effects

Using the previously described recursive relationships it is possible to calculate the probability generating function

$\phi(s_1,s_2;t)$ for arbitary t. However, the relationships are difficult to invert and in order to obtain the distribution of cell counts at time t. We therefore consider some quantities which will provide a useful summary of the behaviour of the system at time t. The expected values $m_0(t)$, $m_1(t)$ are two useful measures. Another quantity of some interest is $P\{N(t)\equiv R_0(t)+R_1(t)=0\}$ since this is the probability that there are no stem cells at time t. Since the elimination of the stem cells implies that the tumor will eventually become extinct (or not grow sufficiently to kill the patient or animal) this may be thought of as the probability that the tumor can no longer cause the death of the patient. The probability that there are no stem cells at time t is given by $\phi(0,0;t)$ and may be easily calculated from (3.11.1-2). However, $\phi(0,0,;t)$ does not represent the probability that the tumor has been cured by the treatment regimen, for if t_J is the time of the last treatment and $t_1'>t_2'>t_J$ then, for the model under consideration

$$\phi(0,0;t_1')=P\{R_0(t_1')=0,\ R_1(t_1')=0\}>P\{R_0(t_2')=0,\ R_1(t_2')=0\}=\phi(0,0;t_2')$$

with equality if d=0. This motivates consideration of

$$P_{t_J} = E[P\{N(\infty)=0|N(t_J)\}].$$

We will refer to P_{t_J} as the probability of cure, which will of course depend on the regimen being used. Since each cell has a probability $\varepsilon=d/b$ of spontaneous extinction (see the discussion in Section 3.1) and cells behave independently, the probability n cells will go spontaneously extinct is ε^n.

It follows that

$$P_{t_J} = E[\varepsilon^{N(t_J)}] = E[\varepsilon^{R_0(t_J)+R_1(t_J)}] = \phi(\varepsilon,\varepsilon;t_J). \qquad \ldots(3.13)$$

At this point we should note that P_{t_J} will not correspond exactly to the clinical likelihood of cure since it includes the contribution of sample paths destined for extinction, which may nevertheless grow sufficiently to cause patient death. Such paths occur with insignificantly small probability in most practical situations and P_{t_J} will be considered to be equal to the clinical probability of cure.

In some cases, as in the treatment of L1210 leukemia by the drug Ara-C [26], resistance can be effectively absolute for any drug concentration which does not result in animal death. In this case there exists the possiblity that a tumor cannot be cured by the drug no matter what dose is used. If we also assume that at the therapeutic dosage $\pi_0(D)=0$, then it is only necessary to apply a single course of the drug (since subsequent courses will have no effect), and we have the probability of cure, P_{t_1}, is given by

$$P_{t_1} = \phi(\varepsilon,\varepsilon;t_1) = \phi(1,\varepsilon;t_1^-). \qquad \ldots(3.14)$$

This expression may be viewed as an approximation to the probability of cure for cases in which $\pi_1(D)\simeq 1$, $\pi_0(D) \simeq 0$ and the treatments are applied frequently. Using equations (3.5), (3.6) and (3.7) we have

$$P_{t_1} = \phi(G(t_1),\varepsilon), \qquad \ldots(3.15)$$

where $G(t_1)=\varepsilon + \dfrac{(1-\varepsilon)(\delta+\alpha d+\nu)e^{-(\delta+\alpha d+\nu)t_1}}{(\delta+\alpha d+\nu)-\delta\ (1-\alpha)[1-e^{-(\delta+\alpha d+\nu)t_1}]}$

and $\phi(s_0,s_1)=\phi(s_0,s_1;0)$.

If $(\delta+\alpha d+\nu)t_1 >> 1$, then

$$P_{t_1} \simeq \phi(\varepsilon,\varepsilon). \qquad \ldots(3.16)$$

Thus for sufficiently large t_1, P_{t_1} is approximately equal to $\phi(\varepsilon,\varepsilon)$, the

probability, that the tumor will go spontaneously extinct. Equation (3.15) may be used to assess the curability of an experimental tumor where the number of cells implanted has probability generating function $\psi(s_0,s_1)$, the drug parameters are $\pi_0(D)=0$, $\pi_1(D)=1$ and the tumor is treated at time t_1 where t_1 is large. However it also illustrates that the theory developed to this point is of limited use in describing the treatment of large tumors (either clinical or experimental) since it includes spontaneous extinctions (which will largely have occurred in the early history of the neoplasm). This deficiency is especially marked for human disease where the tumor originates with a single sensitive stem cell i.e. $\psi(s_0,s_1)=s_0$ and thus the probability of spontaneous extinction can be large (if ε is large).

Before discussing modifications to exclude spontaneously extinct tumors we will first consider an example which illustrates an application of the theory developed to this point.

Example:

Consider the special case $\pi_1(D)=1$ for all D where the drug considered is not phase specific. Let $\pi_0(D)=\exp\{-kD\}$ as in Section 3.2. Consider a tumor system where $\nu=d=0$ which follows the cumulative dose

Consider the special case $\pi_1(D)=1$ for all D where the drug considered is not phase specific. Let $\pi_0(D)=\exp\{-kD\}$ as in Section 3.2. Consider a tumor system where $\nu=d=0$ which follows the cumulative dose toxicity model. We wish to determine whether it is better to give a single dose of magnitude D at time t_1 or two doses D_1 and D_2 at times t_1 and t_2 where $D_1+D_2=D$ and $t_2>t_1$. A regimen is better if it has a higher probability of cure.

Since $\pi_1(D)=1$ for all D we need not consider resistant cells present at time t_1 as these will be unaffected by either regimen. Thus we will only consider $\phi^*(s_0)=\phi(s_0,0;t_1')$ and assume without loss of generality that there are no resistant cells present at time t_1. If all the drug is given in a single dose at time t_1, then the probability of cure is

$$P_{t_1}=\phi^*(1-\pi_0(D)). \qquad ...(3.17)$$

For the second regimen where two doses are used we must consider the intertreatment development of resistance. Using equations (3.6) and (3.7) we have

$$w_1(u) = \frac{s_1 e^{-bu}}{1-s_1+ s_1 e^{-bu}}, \qquad ...(3.18.1)$$

$$w_0(u) = w_1(u) + \frac{e^{-bu}\,[1-s_1+s_1e^{-bu}]^{-2+\alpha}}{[s_0-s_1]^{-1}-s_1^{-1}[(1-s_1+s_1e^{-bu})^{-1+\alpha}-1]}, \qquad ...(3.18.2)$$

where $u=t_2-t_1$. The probability of cure at time t_2 is

$$P_{t_2} = \phi(0,0;t_2) = \phi(1-\pi_0(D_2),0;t_2^-)$$

since spontaneous death does not occur (d=0). Now by (3.11.2) we have

$$\phi(s_0,s_1;t_2^-) = \phi(w_0(u),w_1(u);t_1),$$

where $w_0(u)$ and $w_1(u)$ are given by (3.18.1-2). Taking the limit as $s_1\to 0$ in (3.18.2) we have

$$P_{t_2} = \phi\Big(\frac{[1-\pi_0(D_2)]e^{-bu}}{1-[1-\pi_0(D_2)](1-\alpha)[1-e^{-bu}]},0;t_1\Big)$$

$$= \phi^*\Big(1-\pi_0(D_1) + \frac{\pi_0(D_1)(1-\pi_0(D_2))e^{-bu}}{1-(1-\pi_0(D_2))(1-\alpha)(1-e^{-bu})}\Big) \qquad ..(3.19)$$

after taking account of cell kill $\pi_0(D_1)$ at time t_1.

Since $\phi^*(s)$ is a probability generating function it is monotonic

non-decreasing on [0,1] and we need only compare the arguments of ϕ^* in equations (3.17) and (3.19). But by assumption $\pi_0(D)=\pi_0(D_1)\pi_0(D_2)$, and thus for $u>0$

$$1-\pi_0(D_1) + \frac{\pi_0(D_1)(1-\pi_0(D_2))e^{-bu}}{1-(1-\pi_0(D_2))(1-\alpha)(1-e^{-bu})}$$

$$= 1-\pi_0(D_1)\pi_0(D_2) - \pi_0(D_1)(1-\pi_0(D_2))\left[1- \frac{e^{-bu}}{1-(1-\pi_0(D_2))\ (1-\alpha)\ (1-e^{-bu})}\right]$$

$$<1-\pi_0(D_1)\pi_0(D_2)=1-\pi_0(D). \qquad \ldots(3.20)$$

Thus giving the total dose at t_1 results in a higher probability of cure than splitting the dose into two parts given at t_1 and $t_2>t_1$. If we set $D_1=0$, $D_2=D$ we also see that giving the total dose later is associated with a lower probability of cure. More generally it is preferable to give a drug in the highest possible dose at the earliest time rather than spread the same dose over a series of smaller doses. This provides a partial justification for the strategy commonly employed in clinical medicine of using the highest possible doses that are tolerable. These observations may also be generalized to cases where $\nu>0, d>0$ and $\pi_1(D)<1$, since the underlying nature of the process is unchanged although the computations become more complex. This completes consideration of this example.

When observing a clinical or experimental tumor the number of resistant stem cells at any point in time is usually unknown. The total number of stem cells can be estimated either by direct experimentation or by applying the appropriate formula for P_S (the proportion of stem cells) developed in Chapter 2 to the observed overall tumor size. In

both these situations we will refer to the number of stem cells as being "observed" even though they may only have been inferred from the observed tumor size. As previously mentioned the theory developed in Section 3.1 describes the growth of the sensitive and resistant stem cells and includes cases where these cells go spontaneously extinct. By the time a tumor has reached a size where it is clinically detectable the likelihood of spontaneous extinction is small. This directly leads to the consideration of $P\{R_1(t)|N(t)\}$. Unfortunately this distribution is not easily obtained because the integral in (3.7) cannot be expressed in terms of standard functions. A further problem in the consideration of human tumors is ignorance of the age, t, of the tumor and it is therefore desirable to construct expressions independent of this parameter. Since these problems are of central importance in the construction of an appropriate distribution for the number of resistant cells we will outline three seperate approaches which provide approximate solutions to this problem and will be of use in various experimental and clinical situations.

3.6 Conditioning on N(t) - Approximation 1

As a first approximation to the problem of conditioning upon N(t) we will examine the process where sample paths that correspond to tumors which go spontaneously extinct (in the absence of treatment) are excluded. The basic idea in this approximation will be to consider the distribution $P\{R_0(t),R_1(t)|N(t)>0\}$ and to approximate it by $P\{R_0(t),R_1(t)|N(\infty)>0\}$ and substitute a plausible value for t derived from consideration of the observed distribution of stem cells. This approach has previously been used elsewhere [27]. In the absence of treatment, we

have for any t_1, t_2 where $t_2 > t_1$ that

$$N(t_2)>0 \rightarrow N(t_1)>0.$$

Thus we may exclude realizations corresponding to tumors which go spontaneously extinct at any time by conditioning on $N(\infty)>0$. This is (approximately) equivalent to including only those realizations corresponding to tumors which, if left untreated, could go on to result in patient or animal death (if we exclude realizations which grow to a sufficient size to cause death but are nevertheless destined for extinction). We will now calculate the probability generating function $\phi'(s_0,s_1;t)$ of the process $\{R_0'(t),R_1'(t)\}$ which consists of all sample paths $\{R_0(t),R_1(t)\}$ for which $N(\infty)>0$. Let

$$\phi'(s_1,s_2;t) = \sum_{\substack{i=0 \\ \{i,j\}\neq\{0,0\}}}^{\infty} \sum_{j=0}^{\infty} P\{ R_0(t)=i, R_1(t)=j \mid N(\infty)>0\} s_0^i s_1^j .$$

To evaluate this probability generating function we first note

$$P\{R_0(t)=i,\ R_1(t)=j \mid N(\infty)>0\}$$
$$= (1-P\{N(\infty)=0\})^{-1}[P\{R_0(t)=i,\ R_1(t)=j\} - P\{R_0(t)=i,\ R_1(t)=j,\ N(\infty)=0\}].$$

Since the cells behave independently, the probability that a single cell (either sensitive or resistant) will go spontaneously extinct is equal to $\varepsilon=d/b$ and it follows that

$$P\{R_0(t)=i,R_1(t)=j,N(\infty)=0\} = P\{R_0(t)=i,R_1(t)=j\}\varepsilon^{i+j}.$$

After a little algebra we obtain

$$\phi'(s_0,s_1;t) =$$
$$(1-P\{N(\infty)=0\})^{-1}(\phi(s_0,s_1;t)-P\{N(t)=0\}- \sum_{\substack{i=0 \\ \{i,j\}\neq\{0,0\}}}^{\infty} \sum_{j=0}^{\infty} P\{R_0(t)=i,R_1(t)=j\}\varepsilon^{i+j}s_0^i s_1^j).$$

Since $P\{N(\infty)=0\}=\psi(\varepsilon,\varepsilon)=\phi(0,0;\infty)$ is the probability that the stem cell compartment will go extinct, the desired probability generating function

may be expressed as

$$\phi'(s_0,s_1;t)=[1-\psi(\varepsilon,\varepsilon)]^{-1}\{\phi(s_0,s_1;t)-\phi(\varepsilon s_0,\varepsilon s_1;t)\}. \qquad \ldots(3.21)$$

We may calculate the first moments $m_0'(t)$ and $m_1'(t)$ of the process by differentiating (3.21) with respect to s_0 and s_1 respectively and evaluating at $s_0=s_1=1$. After some algebra we obtain

$$m_0'(t) = E[R_0'(t)] =$$

$$\frac{1}{1-\psi(\varepsilon,\varepsilon)}\left[m_0\exp(\delta-\alpha b-\nu)t-\left\{\frac{\partial\psi(s,\varepsilon)}{\partial s}\Big|_{s=\varepsilon}\right\}\varepsilon\exp\{-(\delta+\alpha d+\nu)t\}\right], \quad \ldots(3.22.1)$$

and

$$m'(t) = m_1'(t) + m_0'(t)$$

$$= \frac{1}{1-\psi(\varepsilon,\varepsilon)}\left[(m_0+m_1)e^{\delta t}-\left\{\frac{\partial\psi(s,\varepsilon)}{\partial s}\Big|_{s=\varepsilon}+\frac{\partial\psi(\varepsilon,s)}{\partial s}\Big|_{s=\varepsilon}\right\}\varepsilon e^{-\delta t}\right], \quad \ldots(3.22.2)$$

where $m_1'(t)$ is given by the difference of these two expressions.

Equation (3.21) shows that the probability generating function for the conditional (on $N(\infty)>0$) process may be expressed in terms of that of the unconditional process, and thus may be calculated using formulae (3.5) to (3.7). When modelling the effects of treatment we would use (3.21) for the initial growth period and (3.11.2) for growth in the intertreatment intervals. We do not use (3.21) for intertreatment growth, as this would have the effect of assuming that the tumor could not be cured.

If we again consider (as in the previous section) the special case $\pi_0(D)=0$, $\pi_1(D)=1$ then we may calculate P_{t_1}, the probability of cure, for this process with probability generating function given by (3.21). In analogy to (3.14) we have $P_{t_1}=\phi'(1,\varepsilon;t_1^-)$ and thus using (3.21) we obtain

$$P_{t_1}=[1-\psi(\varepsilon,\varepsilon)]^{-1}[\psi(G(t_1),\varepsilon)-\phi(\varepsilon,\varepsilon^2;t_1^-)]$$

where $G(t_1)$ is as specified in (3.15). For the case where $\psi(s_0,s_1)=s_0$,

as is likely in human disease, this simplifies to yield

$$P_{t_1} = (1-\varepsilon)^{-1}\left[\frac{\varepsilon(1-\varepsilon)\,e^{-\delta t_1}}{1+\varepsilon(1-e^{-\delta t_1})} + \frac{g(t_1)(1-\varepsilon)(\delta+\alpha d+\nu)}{\alpha b+\nu+\delta(1-\alpha)g(t_1)} - \frac{g(t_1)h(t_1)}{\varepsilon^{-1}(1-\varepsilon)^{-1}-b(1-\alpha)\int_0^{t_1} g(v)h(v)dv}\right], \qquad \ldots(3.23)$$

where $g(t)=\exp\{-(\delta+\alpha d+\nu)t\}$ and $h(t)=[1+\varepsilon(1-e^{-\delta t})]^{-2+\alpha}$. Examination of (3.23) shows that as expected

$$\lim_{t_1\to\infty} P_{t_1} = 0,$$

that is, cure will occur with vanishingly small probability if treatment is delayed too long. This may be contrasted with (3.16) where, for $d>0$, the probability of cure was always greater than zero since this expression included the likelihood that the tumor did not exist.

As indicated previously, the age of a tumor is only known in certain experimental situations and is of course measured in arbitrary units. For human disease we usually do not know the age of the tumor and thus we do not know the time of the first (or any subsequent) treatment measured on the scale where the tumor originated at time $t=0$. Once one treatment time is specified on this scale then all other treatment times are known. It seems most natural to specify treatment times in terms of t_1, the unknown time of first treatment. A reasonable approach in modelling treatment effects on a specific tumor class is to choose t_1 so that the distribution of stem cells (implicit in (3.21)) at the time of first treatment approximates that observed in the tumor type. If we let $N'(t)$ be the random variable with the distribution of $N(t)$ conditional on $N(\infty)>0$, then we wish to choose $t(=t_1)$ so that the distribution of $N'(t)$ is similar to the observed distribution at diagnosis for the tumor class.

For the special case $\phi(s_0,s_1)=s_0$ the probability generating function for $N'(t)$ is given by (3.21) with $s_0=s_1=s$; that is

$$\phi'(s,s;t)=[1-\varepsilon]^{-1}[\phi(s,s;t)-\phi(\varepsilon s,\varepsilon s;t)] \qquad ...(3.24)$$

Using $\phi(s_0,s_1;t)$ as given by (3.5) with $s_0=s_1=s$, the right hand side of (3.24) may be expanded in powers of s to yield the distribution for $N'(t)$:

$$P\{N'(t)=i\} = \frac{(b^i-d^i)\delta(1-e^{-\delta t})^{i-1}e^{-\delta t}}{(b-de^{-\delta t})^{i+1}} \text{ for } i=1,2,... \qquad ...(3.25)$$

For large i, such that $\varepsilon^i \ll 1$ we have

$$P\{N'(t)=i\} \simeq \frac{1}{(1-\varepsilon e^{-\delta t})^2}\left[1-\frac{(1-\varepsilon)e^{-\delta t}}{(1-\varepsilon e^{-\delta t})}\right]^{i-1}(1-\varepsilon)e^{-\delta t},$$

and if t is also large, so that $\delta t \gg 1$, then to leading order in $e^{-\delta t}$,

$$P\{N'(t)=i\} \simeq (1-(1-\varepsilon)e^{-\delta t})^{i-1}\,(1-\varepsilon)e^{-\delta t}. \qquad ...(3.26)$$

Examination of (3.26) shows that the distribution of $N'(t)$ is approximately geometric and only depends upon b and d through $m'(t)$. This has three implications for the modelling of "large" tumors. Firstly, the approximation to the distribution of $N'(t)$ has only one parameter, its mean value, and thus in attempting to determine an appropriate value of t_1 (in terms of given b and d) one need only employ one summary measure of the distribution. Secondly, whatever summary measure is used (mean, median etc.) this will always result in choosing t_1 to satisfy some relationship in terms of the mean $m'(t_1)$. Thirdly, we may wish to compare the distribution of resistant cells for different tumor models with differing b, c and d but the same α, β and γ. If the different tumor models are required to have the same mean numbers of stem cells, then they will have approximately the same distribution of stem cells. Thus differences in P_{t_1} between such models will not be due

to differences in the distribution of the number of stem cells. The conditional process (with probability generating function given by (3.21)) thus provides a convenient framework for comparing the effects of various parameters (including treatment) on the curability of the tumor for a fixed distribution of stem cells at t_1. However this approach will not be suitable for the modelling of situations where the observed distribution of stem cells at diagnosis is not well approximated by a geometric distribution. We will now examine some elementary properties of this process.

Consider the expected fraction of resistant cells, which is approximately given by $m_1'(t)/m'(t)$. If we assume that $\phi(s_0,s_1)=s_0$ and, using the mean as the summary measure, we choose t_1 so that $N^*=[(1-\varepsilon)^{-1}e^{\delta t_1}]$, ($N^*$ is the mean size of the tumor at diagnosis), then from (3.22.1) and (3.22.2),

$$\frac{m_1'(t_1)}{m'(t_1)} \simeq 1-[(1-\varepsilon)N^*]^{-\left(\frac{\alpha b+\nu}{\delta}\right)}. \qquad \ldots(3.27)$$

From this it may be seen, as expected, that the fraction of resistant cells increases as any of α, β, or γ increase. Increases in c (since $\nu=\beta c+\gamma$) or d also increase the fraction of resistant stem cells although these parameters are also related to P_S, the fraction of stem cells in the tumor (Chapter 2).

We can also examine the effect of the parameters α, β, γ, b, c, and d upon P_{t_1} given by (3.23), where $\pi_0(D)=0$, $\pi_1(D)=1$ and as before t_1 is chosen to be given at a fixed mean stem cell compartment size i.e. $N^*=m'(t_1)$. As expected, increases in α, β or γ decrease the value of this function for fixed N^*. Increases in c are also found to decrease

P_{t_1} but the value of this function is not influenced by changes in d. This function is plotted for various values of the parameters in Figure 1. Although increasing d increases the mean number of resistant cells it does not change the probability that a fixed size stem cell compartment will be curable because of the compensating effect of increases in the spontaneous death rate of resistant cells.

The considerations presented here for the case $\pi_0(D)=0$, $\pi_1(D)=1$ carry over generally to the case $\pi_1(D)=1$, $\pi_0(D)=\pi_0>0$ except, of course, that the magnitude of P_{t_1} will depend upon the effectiveness and timing of subsequent treatments. We will now turn to consideration of a second approximation for conditioning on N(t).

3.7 Conditioning on N(t) - Approximation 2

In most cases of practical interest $\alpha<<1$ and $\nu<<b$ (i.e. transitions to resistance proceed slower than growth) so that, for the majority of sample paths $R_1(t)<<R_0(t)$ and thus $R_0(t)\simeq N(t)$. This suggests that it may be reasonable to approximate the distribution $P\{R_1(t)|N(t)\}$ with the distribution $P\{R_1(t)|R_0(t)\}$. This calculation is complex for general $\phi(s_0,s_1)$ and we will only consider the special case $\phi(s_0,s_1)=s_0$. Thus $\phi(s_0,s_1;t)=w_0(t)$ as given in equation (3.7). Since $\phi(s_0,1;t)$ is the probability generating function of the number of sensitive cells at time t, the coefficient of s_0^i in the expansion of $w_0(t)$ (evaluated at $s_1=1$) in powers of s_0 gives the probability that there will be i sensitive cells at time t.

Performing this expansion yields

$$P\{R_0(t)=i\} = \frac{\lambda^2 e^{-\lambda t}[b(1-\alpha)(1-e^{-\lambda t})]^{i-1}}{[b(1-\alpha)-(d+\nu)e^{-\lambda t}]^{i+1}} \quad \text{for } i=1,2,\ldots, \qquad \ldots(3.28)$$

where $\lambda=\delta-\alpha b-\nu$. Similarly the coefficient of s_0^i in the expansion of $w_0(t)$ (for general s_1) yields

$$\sum_{j=0}^{\infty} P\{(R_0(t)=i,\ R_1(t)=j\}s_1^j = \frac{\delta^{-2+\alpha}\, f(t)\, I(t)^{i-1}}{[\delta^{-2+\alpha} + s_1 I(t)]^{i+1}} \quad i=1,2,\ldots, \qquad \ldots(3.29)$$

where $f(t)$ is given in (3.7) and $I(t)=b(1-\alpha)\int_0^t f(v)dv$. Taking the ratio of (3.29) to (3.28) and setting $s_1=s$ then yields the probability generating function $\zeta_i(s;t)$ of the distribution $P\{R_1(t)|R_0(t)=i\}$ as

$$\zeta_i(s;t) = \frac{\delta^{-2+\alpha}\, f(t) e^{\lambda t}[g(t)]^{i-1}}{\lambda^2\,[h(t)]^{i+1}}, \qquad \ldots(3.30)$$

where $g(t) = I(t)/[b(1-\alpha)(1-e^{-\lambda t})]$ and

$h(t) = [\ \delta^{-2+\alpha} + sI(t)]/[b(1-\alpha)-(d+\nu)e^{-\lambda t}]$.

We may use (3.30) to evaluate $E[R_1(t)|R_0(t)=i]$, by differentiating with respect to s and setting s=1. However, the resulting expressions are rather complex involving the difference of a number of exponential functions. If $\delta>>\alpha b+\nu$ (which implies $\alpha<<1$) then we obtain

$$\begin{aligned} E[R_1(t)|R_0(t)=i] &= i\Big[\frac{(2-\alpha)(\delta-\alpha b-\nu)^2}{\delta(1-\alpha)(2\delta-\alpha b-\nu)}\, e^{(\alpha b+\nu)t}-1\Big] + L_1+L_2 \\ &\simeq i\big[\ e^{(\alpha b+\nu)t}\ -1\big] + L_1 + L_2\ , \qquad \ldots(3.31) \end{aligned}$$

where

$$L_1 = i\Big(1+\frac{d+\nu}{b(1-\alpha)}\Big)\Big(\frac{(2-\alpha)\ (\delta-\alpha b-\nu)^2}{(1-\alpha)\delta(2\delta-\alpha b-\nu)}\Big)e^{-(\delta-2\alpha b-2\nu)t} + O\big(ie^{-(\delta-\alpha b-\nu)t}\big)$$

and

$$L_2 = \frac{b(2-\alpha)\ (\alpha b+\nu)}{\delta(2\delta-\alpha b-\nu)}e^{\delta t} + O(e^{(\alpha b+\nu)t}).$$

For large t ($\delta t>>1$), L_1 is dominated by the first term in (3.31). If $i \gtrsim E(R_0(t)]$ then $ie^{(\alpha b+\nu)t} \gtrsim e^{\delta t}$ and L_2 is dominated by the first term of (3.31). If $i<<E[R_0(t)]$ then L_2 may be comparable or larger than the

first term in (3.31) and the approximation $R_0(t) \simeq N(t)$ may not be a good one. However, this occurs with small probability when $\alpha b+\nu << \delta$. From (3.8), when $m_1(0)=0$, as here, we have

$$E[E\{R_1(t)|R_0(t)\}] = E[R_0(t)](e^{(\alpha b+\nu)t}-1)$$

which shows that the terms L_1 and L_2 in (3.31) have expectation 0 (approximately) with respect to R_0. The first term of (3.31) will in most cases (where $i=<<E[R_0(t)]$) be a reasonable approximation to $E[R_1(t)|R_0(t)]$ for large t except in situations where t is such that $E[R_0(t)]>>i$.

For the special case $\pi_1(D)=1, \pi_0(D)=0$ we may calculate $P_{t_1}(i)$, the probability that a tumor with i sensitive stem cells will be cured by a single course of therapy at time t_1. Using the same argument as previously used in deriving equation (3.14) we have

$$P_{t_1}(i) = \zeta_i(\varepsilon;t_1).$$

Using (3.30) we find

$$\zeta_i(\varepsilon;t_1) = e^{-(\alpha b+\alpha d+2\nu)t_1}\left[\frac{(\delta+\alpha d+\nu)[b(1-\alpha)-(d+\nu)e^{-(\delta-\alpha b-\nu)t_1}}{(\delta-\alpha b-\nu)[b+\nu-d(1-\alpha)e^{-(\delta+\alpha d+\nu)t_1}]}\right]^2$$
$$\cdot \left[\frac{(1-e^{-(\delta+\alpha d+\nu)t_1})(b(1-\alpha)-(d+\nu)e^{-(\delta-\alpha b-\nu)t_1})}{(1-e^{-(\delta-\alpha b-\nu)t_1})(b+\nu-d(1-\alpha)e^{-(\delta+\alpha d+\nu)t_1})}\right]^{i-1}.$$

If $(\delta-\alpha b-\nu)t>>1$ we obtain the approximation

$$P_{t_1}(i)=\zeta_i(\varepsilon;t_1) \simeq e^{-(\alpha b+\alpha d+2\nu)t}\left[\frac{(\delta+\alpha d+\nu)b(1-\alpha)}{(\delta-\alpha b-\nu)(b+\nu)}\right]^2 \left[\frac{b(1-\alpha)}{b+\nu}\right]^{i-1}. \quad ...(3.32)$$

If in addition the individual mutation rates are small so that $(\alpha b+d+2\nu)t<<1$ and $\delta>>\alpha b+\nu$, then

$$e^{-(\alpha b+\alpha d+2\nu)t} \simeq 1, \quad \frac{(\delta+\alpha d+\nu)b(1-\alpha)}{(\delta-\alpha b-\nu)(b+\nu)} \simeq 1, \quad \frac{b(1-\alpha)}{b+\nu} \simeq 1-\alpha-\nu/b$$

and thus

$$\zeta_i(\varepsilon;t_1)=P_{t_1}(i) \simeq [1-\alpha-\nu/b]^{i-1}. \quad ...(3.33)$$

This function is plotted for particular α and ν/b in Figure 1.

The form of this relationship is very simple and makes intuitive sense as follows. In Section 3.6 we found that P_{t_1} did not vary with d (for the conditional process considered there) for fixed mean size N*. We also found that the distribution of $N'(t)$ was approximately geometric and was independendent of d once the mean was fixed. Since P_{t_1} in Section 3.6 is the average probability of cure across the distribution (approximately given by (3.26)) of the number of stem cells and both are approximately independent of d for given mean size, it seems likely that the individual terms representing the curability at a given size are also independent of d. That is exactly what is indicated by equation (3.33) for $N(t) \simeq R_0(t)$. This suggests an approximation to P_{t_1} as given by (3.23) can be obtained by taking the product of the right hand sides of equations (3.26) and (3.33) and summing. Letting $m' = [(1-\varepsilon)e^{-\delta t_1}]^{-1}$ we have

$$P_{t_1} = \sum_{i=1}^{\infty} P\{\text{cure} \mid N'(t_1)=i\} P\{N'(t_1)=i\} \simeq \sum_{i=1}^{\infty} P_{t_1}(i) P\{N'(t_1)=i\}$$

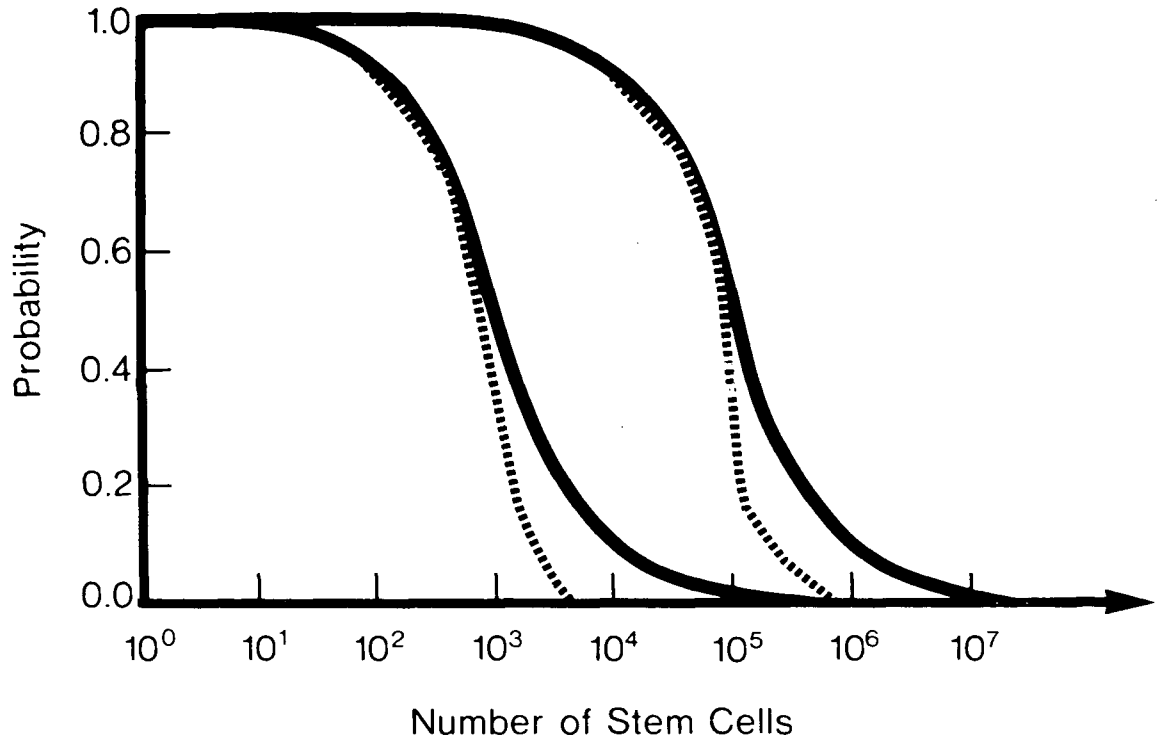
$$\simeq \sum_{i=1}^{\infty} (1-\alpha-\nu/b)^{i-1} m'^{-1} (1-m'^{-1})^{i-1}$$

$$= [1-\alpha-\nu/b+(\alpha+\nu/b)m']^{-1}. \qquad \ldots(3.34)$$

The derivation of (3.34) uses (3.26) where $N'(t)$ was assumed to be large. If m' is large the probability that $N'(t)$ is small will be small and thus (3.34) can be expected to be a reasonable approximation. Numerical evaluation of (3.23) and (3.34) for $\alpha = 10^{-3}, 10^{-4}, \ldots, 10^{-8}$ and $\nu/b = 10\alpha$, α, $10^{-1}\alpha$ shows that the absolute difference between (3.34) and (3.23) is less than 0.01 for $10 \leq N'$, $m' \leq 10^9$. Thus (3.34) provides a reasonable

Figure 1

Probability of Cure for Approximations 1 and 2.



The function P_{t_1} plotted as a function of N* where t_1 is selected to satisfy $N^*=[(1-\varepsilon)e^{-\delta t_1}]^{-1}$, for various values of the mutation rates. The solid curves are for equation (3.23) and dashed curves are for equation (3.33). The two curves to the right have $\alpha=5\times10^{-6}$ and $\nu/b=5\times10^{-6}$, and those to the left have $\alpha=5\times10^{-4}$, $\nu/b=5\times10^{-4}$. These curves do not depend on b (which behaves as a constant for scaling time) and are essentially coincident for all $\varepsilon=d/b$.

approximation to (3.23) and gives a deeper understanding of the nature of P_{t_1}.

Conditioning on $R_0(t)$ appears to be reasonable if t is known, however we are also interested in situations where it is not. The expression for $E[R_1(t)|R_0(t)]$ which is approximated by (3.31), depends upon t and thus its distribution depends upon the choice of t. Here we will propose another more complex method for removing t than that which was presented in Section 3.6 although this will again be approximate. The basis of this approach is to observe that when there are many stem cells present their growth is quite regular. The major contribution to the distribution of the number of stem cells at time t (when grown up from a single cell) results from the variability of growth when small numbers of cells are present. This suggests that it should be reasonable to approximate the growth process by a two phase model in which the growth of sensitive stem cells is first stochastic and later deterministic. A schema illustrating this approach is given in Figure 2. Resistant cells will be assumed to grow stochastically in both phases. In the stochastic phase, which is restricted to the interval $[0,t']$, we use (3.30) and t' is chosen so that the probabilities $P\{R_0(t')>U|R_0(t')\neq 0\}$ and $P\{R_0(t')<L|R_0(t')\neq 0\}$ are both small. L represents a lower limit for which growth is sufficiently regular and $U\leq N$ where N is the observed size of the sensitive stem cell compartment that we are interested in conditioning upon. For example, it is easy to show (using (3.28)) that if the mean of the geometric distribution of $R_0(t)$ is $m_0(t)>>1$ then we can choose U and L where $U/L=10^3$ so that $P\{U>R_0(t')\geq L|R_0(t')\neq 0\}>0.99$. Thus in situations where the number of stem

cells, N (≃ number of sensitive stem cells), at diagnosis satisfies $N>10^6$, we may put $U=10^6$ and $L=10^3$ and choose t' so that $P\{U>R_0(t')\geq L \mid R_0(t')\neq 0\}>0.99$. Thus even at the lower limit L, there will be 1,000 sensitive stem cells and growth after t' can be expected to be approximately regular. After time t', sensitive stem cells will be assumed to grow exponentially with parameter $\delta-\alpha b-\nu$. We will now calculate the probability generating function, $\phi(s;t)$, for the number of resistant cells in the deterministic phase and examine some basic properties of this process.

Consider a model of this process where the sensitive stem cells grow exponentially. In particular we will assume $R_0(t) = A_0 e^{(\delta-\alpha b-\nu)t}$ which is chosen to be the same as their expected growth under a stochastic model; see (3.8). Using a result for filtered Poisson processes [21], the probability generating function $\phi(s;t)$, of the number of resistant cells is given by

$$\phi(s;t)=\exp\{\int_0^t k(u)[\eta(s;t-u)-1]\,du\}, \qquad \ldots(3.35)$$

where $\phi(s;0)=1$, $k(u)= A_0(\alpha b+\nu)e^{(\delta-\alpha b-\nu)u}$ is the rate at which new mutations to resistance occur, and $\eta(s;t)$ is the probability generating function of the birth and death process with parameters b and d. $\eta(s;t)$ is given by $w_1(t)$ (equation (3.6)) with $s_1=s$.

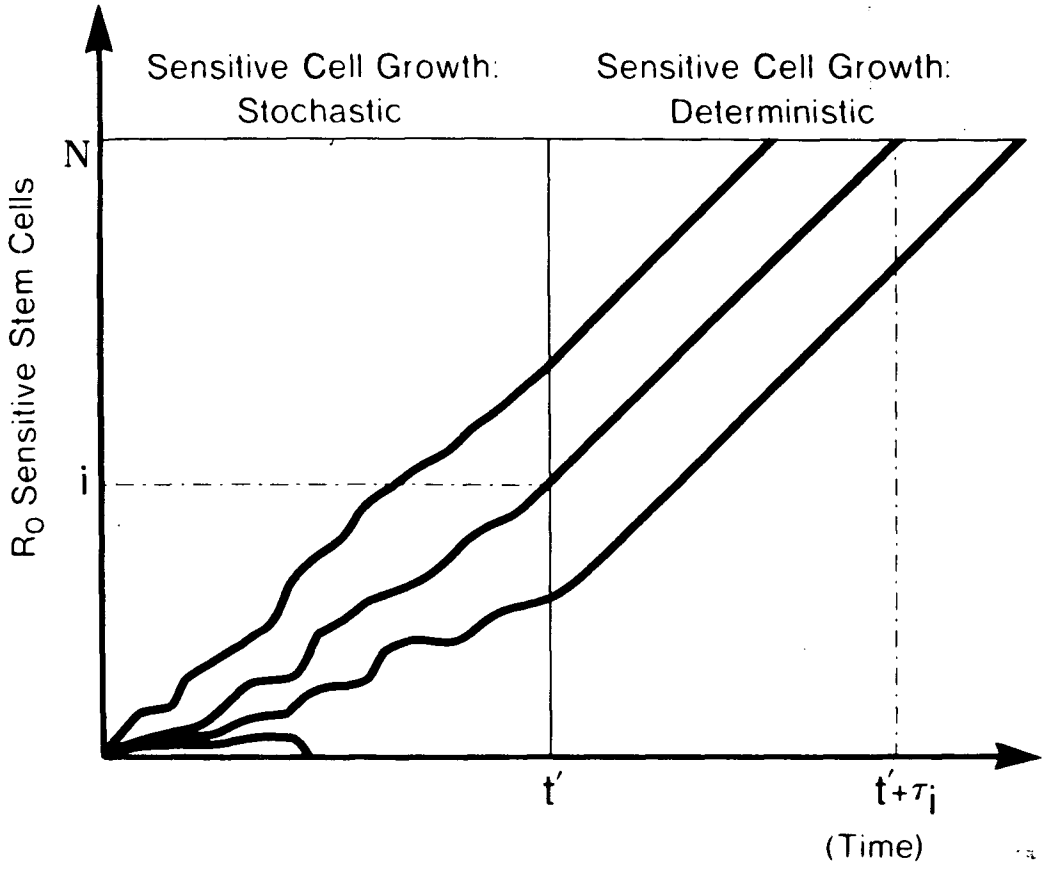
Equation (3.35) cannot be written in terms of standard functions, however the mean may be obtained by differentiating with respect to s and setting s=1; this yields

$$E[R_1(t)] = A_0 e^{(\delta-\alpha b-\nu)t}(e^{(\alpha b+\nu)t}-1) = R_0(t)(e^{(\alpha b+\nu)t}-1). \qquad \ldots(3.36)$$

Further, evaluating (3.35) for $s=\varepsilon$ yields the probability of cure at time t as

Figure 2

Schematic Representation of the Two Phase Growth Process for Sensitive Cell Growth Used in Approximation 2.



$$\phi(\varepsilon;t) = \exp\left\{-\frac{(\alpha b+\nu)(1-\varepsilon)}{(\delta-\alpha b-\nu)}(R_0(t) - A_0)\right\} \simeq (1-\alpha-\nu/b)^{R_0(t)-A_0} \quad \ldots(3.37)$$

for $\delta >> \alpha b+\nu$. We see that this deterministic model yields similar expressions for $E[R_1(t)]$ and P_t(when $\pi_0(D)=0, \pi_1(D)=1$) as for the process conditioned on $R_0(t)$ developed previously in this section; see (3.31) and (3.33).

We will now construct a probability generating function for the distribution of resistant cells for the two phase process which can then be used as an approximation to the probability generating function for a tumor (at diagnosis) of known size but unknown age. Let N be the number of (sensitive) stem cells present when the tumor is observed. Let τ_i be the time required for i sensitive cells present at time t' to grow deterministically to size N (see Figure 2). That is

$$\tau_i = (\delta-\alpha b-\nu)^{-1} \ell n\ (N/i) \quad \text{for } i=1,\ldots,N. \quad \ldots(3.38)$$

Then the probability generating function for the number of new resistant cells (i.e. mutations from sensitivity and their progeny) in the period $[t', t'+\tau_i]$, $\phi_i(s;\tau_i)$, is given by (3.35) with $A_0=i$. The number of resistant cells already present at time t' when there are insensitive cells has probability generating function $\zeta_i(s;t')$ given by (3.30). During the deterministic phase of (sensitive) cell growth these will grow so that the distribution of the number of resistant cells present at $t'+\tau_i$ whose progenitor mutation occurred prior to t' will have probability generating function $\zeta_i(\eta(s;\tau_i);t')$ where $\eta(s;\tau_i)=w_1(\tau_i)$ is given by (3.6). Thus the probability generating function of the number of resistant cells at time $t'+\tau_i$ in a tumor which has i sensitive cells at time t' is

$$\zeta_i(\eta(s;\tau_i);t')\phi_i(s;\tau_i).$$

Since there are i sensitive cells at time t′ with probability $P\{R_0(t')=i\}$ we form the overall probability generating function for the number of resistant cells at size N, $\Phi(s;N)$, as

$$\Phi(s;N) = K \sum_{i=L}^{U} \zeta_i(\eta(s;\tau_i);t')\phi_i(s;\tau_i)P\{R_0(t')=i\}, \qquad \ldots(3.39)$$

where K is chosen so that $\Phi(1;N)=1$. In what follows we shall set U=N and L=1 to simplify the evaluation of (3.39). Immediately we have $K=[1-P\{R_0(t')=0\}-P\{R_0(t')>N\}]^{-1}$.

A number of improvements can be suggested to increase the accuracy of the approximation (for example include sample paths for which $R_0(t')>N$ or adjust $P\{R_0(t')=i\}$ for spontaneous extinctions in the interval $[t',t'+\tau_i]$) however these will not be discussed here as they complicate an already difficult computation. We will now calculate approximate expressions for the mean number of cells $E[R_1(N)]$ and the probability of cure P_N for the random variable $R_1(N)$ which has probability generating function given by (3.39). The mean is then given by

$$E[R_1(N)] = \left.\frac{\partial\Phi(s;N)}{\partial s}\right|_{s=1}$$

$$= K \sum_{i=1}^{N} P\{R_0(t')=i\} \left[\left.\frac{\partial\zeta_i(s;t')}{\partial s}\right|_{s=1} \left.\frac{\partial\eta(s;\tau_i)}{\partial s}\right|_{s=1} + \left.\frac{\partial\phi_i(s;\tau_i)}{\partial s}\right|_{s=1}\right].$$

We will now evaluate the above function and in order to do this we will assume that $\delta>>\alpha b+\nu$ and that t′ is large. Then

$$P\{R_0(t')=0\} \simeq P\{R_0(\infty)=0\} = \frac{d+\nu}{b(1-\alpha)} \simeq \varepsilon+\alpha\varepsilon+\nu/b$$

since the sensitive cells grow as a birth and death process with paramters $b(1-\alpha)$ and $(d+\nu)$ in the first phase. If $N>>E[R_0(t')]$ then

$$K\simeq(1-P\{R_0(t')=0\})^{-1} \simeq (1-\varepsilon-\alpha\varepsilon-\nu/b)^{-1}.$$

Also we have,

$$\left.\frac{\partial \zeta_i(s;t')}{\partial s}\right|_{s=1} \simeq i(e^{(\alpha b+\nu)t'}-1) \text{ from (3.31)},$$

$$\left.\frac{\partial \eta(s;\tau_i)}{\partial s}\right|_{s=1} = (N/i)^{(1-(\alpha b+\nu)/\delta)^{-1}} \simeq N/i \text{ from (3.6)},$$

and

$$\left.\frac{\partial \phi_i(s;\tau_i)}{\partial s}\right|_{s=1} = ie^{\delta t_i}[\, e^{(\alpha b+\nu)\tau_i}-1\,] \text{ from (3.36)}.$$

Thus we have

$$E[R_1(N)] \simeq K \sum_{i=1}^{N} P\{R_0(t')=i\}[N(e^{(\alpha b+\nu)t'}-1)+N(e^{(\alpha b+\nu)\tau_i}-1)].$$

In most cases $R_1(t)<<R_0(t)$ and thus $(e^{(\alpha b+\nu)t}-1)<<1$ and we will approximate $e^{(\alpha b+\nu)t}-1 \simeq (\alpha b+\nu)t$ for $t=t'$ and $t=\tau_i$. Then

$$E[R_1(N)] \simeq K \sum_{i=1}^{N} P\{R_0(t')=i\}\; N(\alpha b+\nu)\{t'+(\delta-\alpha b-\nu)^{-1}\ln(N/i)\},$$

$$= \frac{N(\alpha b+\nu)}{(\delta-\alpha b-\nu)}\; [\ln N + K \sum_{i=1}^{N} [(\delta-\alpha b-\nu)t'-\ln(i)]P\{R_0(t')=i\}].$$

In this process we require that $R_0(t')>0$ and we have

$$m_0^*(t')=E[R_0(t')|R_0(t')>0] \simeq (1-\varepsilon-\alpha\varepsilon-\nu/b)^{-1}e^{(\delta-\alpha b-\nu)t'},$$

and thus

$$(\delta-\alpha b-\nu)t' \simeq \ln[(1-\varepsilon-\alpha\varepsilon-\nu/b)m_0^*(t')].$$

Using the above expression we obtain

$$E[R_1(N)] \simeq \frac{(\alpha+\nu/b)\; N}{(1-\varepsilon-\alpha-\nu/b)}\; [\ln\{N(1-\varepsilon-\alpha\varepsilon-\nu/b)\} + D],$$

where $D= \ln(E[R_0(t')|R_0(t')>0]) - E[\{\ln R_0(t')|R_0(t')>0\}]$. By analogy with the discussion presented in Section 3.6, the distribution of $R_0(t')$ conditional on $R_0(t')>0$ is geometric, and thus D does not depend on the parameters b, d, α and ν after the mean is fixed. If $E[R_0(t')] = 10^6$, then direct calculation yields D=1.15. Thus for $N=10^7$ and

$\varepsilon+\alpha\varepsilon+\nu/b<0.99$, then $\ell n[N(1-\varepsilon-\alpha\varepsilon-\nu/b)]\geq 12>10D$ and we may approximate $E[R_1(N)]$ by

$$E[R_1(N)] = \frac{(\alpha+\nu/b)N}{(1-\varepsilon)} \ell n\ (N(1-\varepsilon)). \qquad \ldots(3.40)$$

This relationship will hold (approximately) for large $N(\geq 10^7)$ where $N>>E[R_0(t')|R_0(t')>0]$ as required by the original assumptions of the development presented here. We will now calculate $P_N=\Phi(\varepsilon;N)$, the probability the tumor is curable at size N for the special case $\pi_0(D)=0$, $\pi_1(D)=1$. Using (3.37) and (3.33) we have

$$P_N=\Phi(\varepsilon;N)\simeq(1-\varepsilon-\alpha\varepsilon-\nu/b)^{-1} \sum_{i=1}^{N} [1-\alpha-\nu/b]^{i-1}[1-\alpha-\nu/b]^{N-i}\ P\{R_0(t')=i\}$$
$$\simeq[1-\alpha-\nu/b]^{N-1}. \qquad \ldots(3.41)$$

The use of this approach is limited for the modelling of experimental and human cancer because of the complex nature of the resulting probability generating function: equation (3.39). However, in contrast with the previous approximation (Section 3.7) it does permit calculation of the probability generating function conditional on a single value of $R_0(\simeq N)$ rather than for a fixed distribution of N. We notice that (3.41) is of the same form as (3.33). This is to be expected since the right hand side of (3.33) is independent of t and thus the curability when t is unknown will be the same.

It is interesting to note that if we use the deterministic model of sensitive cell growth presented here for the whole period [0,t], we can choose A_0 and t (see (3.35)) so that the mean number of resistant cells and probability of cure is approximately the same as that for the process with probability generating function $\Phi(s;N)$ given by (3.40) and (3.41) respectively. Specifically this is achieved by setting $A_0=(1-\varepsilon)^{-1}$ and

$t=\delta^{-1}\ell n\ (N(1-\varepsilon))$. We will use this approximation of (deterministic) sensitive stem cell growth when we consider drug resistance further in Chapter 4. We will now consider our third approximation to the distribution of resistant cells at diagnosis. This method is similar to the first method, in that N(t) has a distribution at diagnosis, but permits some flexibility in selecting this distribution.

3.8 Conditioning on N(t) - Approximation 3

The final approximation to be discussed here will consider not only the growth of tumors but also the rate at which they are initiated. The basic approach will be to 'integrate out' the time parameter present in the previous discussions and develop formulae by summing across a distribution for N(t). Again we will only consider the special case $\psi(s_0,s_1)=s_0$. Consider the following idealization of the detection of a tumor. An individual is selected at random and is found to be of age t. The individual is examined and a tumor is diagnosed with a probability which depends on the number, n, of stem cells present. Notice that t now represents the age of the individual and not the age of the tumor (as previously). We wish to calculate $P_{r|n}(t)$, the probability that there are r resistant cells in a tumor containing n stem cells detected in an individual of age t and will show that for values of t of interest it may be well approximated by $P_{r|n}(\infty)\equiv P_{r|n}$.

We will assume that a tumor is created by the transformation of a single normal cell and that the number of transformations (in an individual) is a Poisson random variable, I(t), with mean $\mu(t)$ ($\mu(t)\equiv 0$, $t<0$). At time t, conditional on I(t)=i, define i-dimensional random vectors $\underset{\sim}{U}(t)$, $\underset{\sim}{R}(t)$, and $\underset{\sim}{N}(t)$ with elements as follows:

$U_j(t)$ = time of initiation of the j-th tumor, $1 \leq j \leq i$,

$R_j(t)$ = number of resistant cells in j-th tumor, $1 \leq j \leq i$,

and

$N_j(t)$ = number of cells in j-th tumor, $1 \leq j \leq i$.

Notice that $t - U_j(t)$ is the (random) age of the j-th tumor. For each t the tumors are labelled randomly (the $U_j(t)$ are not ordered).

Conditional upon $I(t)=i$ we have,

$$P\{\underset{\sim}{N}(t)=\underset{\sim}{n} \mid I(t)=i\} = \int_0^t \cdots \int_0^t P\{\underset{\sim}{N}(t)=\underset{\sim}{n} \mid \underset{\sim}{U}(t)=\underset{\sim}{u}, I(t)=i\}\, dF_{\underset{\sim}{U}(t)\mid I(t)}(\underset{\sim}{u}).$$

Assuming that each cell grows independently,

$$P\{\underset{\sim}{N}(t)=\underset{\sim}{n} \mid \underset{\sim}{U}(t)=\underset{\sim}{u}, I(t)=i\} = \prod_{j=1}^{i} P\{N(t-u_j)=n_j\},$$

where $P\{N(t-u_j)=n_j\}$ is as defined in Section 3.1. Conditional on $I(t)=i$ we have

$$dF_{\underset{\sim}{U}(t)\mid I(t)}(\underset{\sim}{u}) = \prod_{j=1}^{i} \frac{1}{\mu(t)} X_{[0,t]}(u_j)\, d\mu(u_j),$$

where

$$X_{[0,t]}(u) = 1 \text{ if } u \in [0,t],$$

$$= 0 \text{ otherwise.}$$

Combining the above equations we obtain,

$$P\{\underset{\sim}{N}(t)=\underset{\sim}{n} \mid I(t)=i\} = \prod_{j=1}^{i} \int_0^t P\{N(t-u_j)=n_j\} \frac{1}{\mu(t)}\, d\mu(u_j).$$

Similarly we have

$$P\{\underset{\sim}{R}(t)=\underset{\sim}{r}, \underset{\sim}{N}(t)=\underset{\sim}{n} \mid I(t)=i\}$$

$$= \prod_{j=1}^{i} \int_0^t P_{n_j-r_j, r_j}(t-u_j) \frac{1}{\mu(t)}\, d\mu(u_j),$$

where $P_{x,y}(t)$ is as defined in Section 3.1. If we assume that detection of the tumor depends only upon the size (number of stem cells) of the

largest tumor in the individual, $N_m(t)$, then we will calculate the conditional distribution of the number of resistant cells present, $R_m(t)$. Then conditional on $I(t)=i$,

$$P\{R_m(t)=r|N_m(t)=n,I(t)=i\} = \frac{P\{R_m(t)=r,N_m(t)=n|I(t)=i\}}{P\{N_m(t)=n|I(t)=i\}}$$

$$= \frac{\int_0^t P_{n-r,r}(t-u)\, d\mu(u)}{\int_0^t P\{N(t-u)=n\}\, d\mu(u)} \qquad \text{for } i>0,$$

$$= X_{[0,0]}(r) \qquad \text{for } i=0.$$

The above result follows from a simple consideration of the order statistics for independent identically distributed random variables. In most cases arising in human disease, tumors arise quite infrequently so that the likelihood of an individual having more than one cancer is small.

The form of $\mu(t)$ will naturally depend upon the animal and tumor under consideration. We will assume here that $\mu(t)=\lambda t$. This form is assumed since it leads to tractable results and is not an unreasonable approximation for tumors which do not posess a strong age dependent initiation rate. This form is also of interest since it provides a contrast with the two previous approaches where the initiation time was implicitly assumed to be fixed. Substituting for $\mu(t)$ and letting $v=t-u$ we have,

$$P_{r|n}(t) \equiv P\{R_m(t)=r|N_m(t)=n,I(t)>0\} = \frac{\int_0^t P_{n-r,r}(v)dv}{\int_0^t P\{N(v)=n\}dv}. \qquad \ldots(3.42)$$

We now wish to remove the time parameter t in order to obtain expressions for the distribution of the number of resistant cells conditional on the observed number of stem cells, $P_{r|n}$. For any finite

non-zero n we have,

$$\lim_{t\to\infty} \int_t^\infty P\{N(v)=n\}dv = 0$$

and thus, if the age of the subject is great ($t>>0$), we may put

$$\int_0^t P\{N(v)=n\}dv \simeq \int_0^\infty P\{N(v)=n\}dv.$$

A similar argument yields

$$\int_0^t P_{n-r,r}(v)dv \simeq \int_0^\infty P_{n-r,r}(v)dv.$$

Thus if the age of the animal is much greater than the likely time a tumor has taken to grow to size n a reasonable approximation to $P_{r|n}$ is provided by

$$P_{r|n} = \frac{\int_0^\infty P_{n-r,r}(v)\ dv}{\int_0^\infty P\{N(v)=n\}dv} \text{ for } r\leq n, \qquad \ldots(3.43)$$

and $P_{r|n} \equiv 0$ for $r>n$.

Unfortunately (3.43) may not be simply evaluated because $P_{n-r,r}(v)$ is complicated. However, as we will now show, if the number of stem cells follows a particular distribution at the time of diagnosis for a tumor class, it is possible to obtain the probability generating function for the number of resistant cells (at diagnosis). We will now specify the form of the distribution for the number of stem cells at diagnosis and derive expressions for the resulting probability generating function of the number of resistant cells.

When modelling clinical disease there is no unique size, n, at which a tumor is detected but rather a distribution of such sizes. If we let g(n) be the probability that a tumor will be detected at size n (assuming no dependence on t, the age of the patient);

$$g(j) = P\{N=j \,|\, \text{Tumor is diagnosed}\}.$$

The probability that a tumor will have r resistant cells at detection, P_r, (where the dependence on g is suppressed) is given by

$$P_r = \sum_{n=1}^{\infty} g(n)\, P_{r|n}$$

where $g(0) = 0$ implies that a tumor will not be diagnosed if it has no stem cells. We may pass g(n) through the integral sign in (3.43) to obtain

$$P_r = \int_0^{\infty} \sum_{n=1}^{\infty} g(n) \left\{ \frac{P_{n-r,r}(t)}{\int_0^{\infty} P\{N(v)=n\}dv} \right\} dt. \qquad \ldots(3.44)$$

But $P\{N(v)=n\}$ is given by equation (3.28) with $\alpha=\nu=0$ and $t=v$, since this is then the probability distribution for a birth and death process with parameters b and d. Integration gives

$$\int_0^{\infty} P\{N(v)=n\}dv = (bn)^{-1} \text{ for } n>0,$$

and thus

$$P_r = \int_0^{\infty} \sum_{n=1}^{\infty} bng(n) P_{n-r,r}(t)\, dt.$$

For general g(n), P_r is difficult to evaluate because $P_{n-r,r}(t)$ is not easily evaluated. However, consider the special case (which is of the same form as one previously considered by Day [34]),

$$g(n) = \sum_{j=1}^{J} a_j q_j^n, \qquad \ldots(3.45)$$

where $\sum_{j=1}^{J} a_j q_j^n \geq 0$ for all n, $q_j<1$, $\sum_{j=1}^{J} a_j=0$ and $\sum_{j=1}^{J} a_j q_j (1-q_j)^{-1} = \sum_{n=1}^{\infty} g(n)=1$.

If N is a random variable on the non-negative integers, where $P\{N=n\}=g(n)$ (of the form (3.45)), then it is easily shown that

$$E[N] = \sum_{j=1}^{J} a_j q_j (1-q_j)^{-2}$$

and

$$E[N^2] = \sum_{j=1}^{J} a_j q_j (1-q_j)^{-3}.$$

In particular if J=2 then

$$E[N] = \frac{(1-q_1 q_2)}{(1-q_1)(1-q_2)}$$

and

$$\text{var}(N) = \frac{q_1}{(1-q_1)^2} + \frac{q_2}{(1-q_2)^2}.$$

If E[N]>>0 and J=2 then it is straightforward to show that

$$\text{C.V.}[N] > 2^{-1/2},$$

where C.V. is the coefficient of variation. Some examples of the distribution g(n) (of the form (3.45) for J=2) are given in Table II.

Unfortunately distributions g(n) of the form (3.45) do not constitute a sufficiently rich set to accurately model an abitrary distribution at diagnosis. The major limitation arises because these distributions cannot give enough weight (99% or more probability) to a range of tumor stem cell sizes (Nmin,Nmax) where $\frac{\text{Nmax}}{\text{Nmin}} < 10^2$. This corresponds to a relative difference of 5 fold in the linear dimensions of a spherical tumor. However, for clinical neoplasms, data is fairly coarse and we may use g(n) of this form to approximate the diagnostic distribution. Let $\Theta(s)$ be the probability generating function for the distribution P_r and let R_1 be the number of resistant cells, i.e. a random variable where $P\{R_1=r\}=P_r$. It may be shown from the definition of the probability generating function that

$$\Theta(s) = \sum_{r=0}^{\infty} P_r s^r = \sum_{j=1}^{J} a_j q_j b \int_0^{\infty} \frac{\partial \phi(s_0, s_0 s; t)}{\partial s_0} \Big|_{s_0=q_j} dt, \qquad \ldots(3.46)$$

where $\phi(s_0,s_0 s;t)$ is given by equation (3.5). We will only consider the

TABLE II

The Probablity of Diagnosis Distribution

$g(n) = a_1 q_1^n + a_2 q_2^n = P\{N=n\}$, where $E[N]=10^{10}$.

$1-q_1$	$1-q_2$	Range of N $[N_{min}, N_{max}]$	$\sum_{n=N_{min}}^{N_{max}-1} g(n)$	$\sum_{n=0}^{N_{max}-1} g(n)$	S.D.(N)
		$[1, 5\times10^8)$	0.012	0.012	
		$[5\times10^8, 1\times10^9)$	0.028	0.039	
		$[1\times10^9, 5\times10^9)$	0.316	0.355	9.1×10^9
1.111×10^{-10}	1.000×10^{-9}	$[5\times10^9, 1\times10^{10})$	0.274	0.630	
		$[1\times10^{10}, 5\times10^{10})$	0.366	0.996	
		$[5\times10^{10}, 1\times10^{11})$	0.004	1.000	
		$[1\times10^{11}, \infty)$	0.000	1.000	
		$[1, 5\times10^8)$	0.005	0.005	
		$[5\times10^8, 1\times10^9)$	0.013	0.018	
		$[1\times10^9, 5\times10^9)$	0.251	0.269	
1.667×10^{-10}	2.500×10^{-10}	$[5\times10^9, 1\times10^{10})$	0.328	0.598	7.2×10^9
		$[1\times10^{10}, 5\times10^{10})$	0.402	0.999	
		$[5\times10^{10}, 1\times10^{11})$	0.001	1.000	
		$[1\times10^{11}, \infty)$	0.000	1.000	
		$[1, 5\times10^8)$	0.005	0.005	
		$[5\times10^8, 1\times10^9)$	0.013	0.018	
		$[1\times10^9, 5\times10^9)$	0.247	0.264	
1.961×10^{-10}	2.041×10^{-10}	$[5\times10^9, 1\times10^{10})$	0.330	0.594	7.1×10^9
		$[1\times10^{10}, 5\times10^{10})$	0.405	0.999	
		$[5\times10^{10}, 1\times10^{11})$	0.001	1.000	
		$[1\times10^{11}, \infty)$	0.000	1.000	
		$[1, 5\times10^8)$	0.005	0.005	
		$[5\times10^8, 1\times10^9)$	0.013	0.018	
		$[1\times10^9, 5\times10^9)$	0.247	0.264	
1.996×10^{-10}	2.004×10^{-10}	$[5\times10^9, 1\times10^{10})$	0.330	0.594	7.1×10^9
		$[1\times10^{10}, 5\times10^{10})$	0.406	1.000	
		$[5\times10^{10}, 1\times10^{11})$	0.000	1.000	
		$[1\times10^{11}, \infty)$	0.000	1.000	

case $\phi(s_0,s_1) = s_0$ and we have by integrating (3.7) with $s_1=s_0s$,

$$\int_0^\infty \frac{\partial\phi(s_0,s_0s;t)}{\partial s_0}\Big|_{s_0=q_j} dt = G_j(s)+H_j(s), \qquad ...(3.47)$$

where $G_j(s) = \frac{s}{b(1-q_js)}$, $H_j(s) = \frac{\int_0^\infty U_j(s,t)V_j(s,t)\,dt}{1-b(1-\alpha)\int_0^\infty V_j(s,t)\,dt}$,

$$U_j(s,t) = \frac{[1-\varepsilon e^{-\delta t} + q_js(1-\alpha)(1-e^{-\delta t})]}{q_j[1-\varepsilon e^{-\delta t} - q_js(1-e^{-\delta t})]},$$

and

$$V_j(s,t) = q_j(1-s)(1-\varepsilon)^{2-\alpha}[1-\varepsilon e^{-\delta t}-q_js(1-e^{-\delta t})]^{-2+\alpha}\, e^{-(\delta+\alpha d+\nu)t}.$$

The term $G_i(s)$ in (3.47) is obtained by direct integration of the derivative (with respect to s) of the first term in (3.7). The second term, $H_j(s)$, is most simply obtained by interchanging the order of differentiation and integration of the second term in (3.7).

We may calculate the probability of cure, P_g (where g indicates dependence on the distribution g) for the special case $\pi_0(D)=0, \pi_1(D)=1$ by evaluating (3.46) for $s=\varepsilon$. This function must be evaluated by numerical methods. $E[R_1]$ and $E[R_1^2]$ may be calculated by differentiating (3.46) with respect to s and evaluating at s=1. Carrying out this operation and interchanging the order of differentiation and integration yields

$$E[R_1] = \frac{d\theta(s)}{ds}\Big|_{s=1} = \sum_{j=1}^{J} a_jq_jb\left[\frac{1}{b(1-q_j)^2} + I_{1j}\right],$$

$$E[R_1^2] = \frac{d^2\theta(s)}{ds^2}\Big|_{s=1} + \frac{d\theta(s)}{ds}\Big|_{s=1}$$

$$= \sum_{j=1}^{J} a_jq_jb\left[\frac{1+q_j}{b(1-q_j)^3} + I_{1j} + I_{2j} + 2b(1-\alpha)I_{1j}I_{3j}\right],$$

where

$$I_{1j} = \int_0^\infty U_j(1,t)\, \frac{\partial V_j(s,t)}{\partial s}\Big|_{s=1} dt,$$

$$I_{2j} = \int_0^\infty \{2\frac{\partial U_j(s,t)}{\partial s}\Big|_{s=1}\frac{\partial V_j(s,t)}{\partial s}\Big|_{s=1} + U_j(1,t)\frac{\partial^2 V_j(s,t)}{\partial s^2}\Big|_{s=1}\}dt,$$

$$I_{3j} = \int_0^\infty \frac{\partial V_j(s,t)}{\partial s}\Big|_{s=1} dt.$$

For general d and ν these integrals must be evaluated numerically. When $q_j \simeq 1$, (which is the usual case), close attention must be paid to the accuracy with which these integrals are evaluated. This is necessary since most of the integrals have large absolute value, however they do not have the same sign, and the differences (in numeric value) are comparatively small. Therefore it is of some practical interest to determine whether special cases exist which lead to simple forms for (3.46). Inspection of (3.47) shows that the special case d=0 (no stem cell death) and ν=0 (mutations occur only at division) permits considerable simplification yielding:

$$\theta(s) = \sum_{j=1}^{J} a_j q_j \left[\frac{s}{(1-q_j s)} + \frac{s(1-s)}{(1-q_j s)^{2-\alpha} - (1-s)(1-q_j s)}\right].$$

Then we have the probability, $\theta(0)$, that the tumor is curable when $\pi_0(D)=0$, $\pi_1(D)=1$, is

$$\theta(0) = \sum_{j=1}^{J} a_j q_j (1-(1-\alpha)q_j)^{-1} = \sum_{n=1}^{\infty} g(n)\,(1-\alpha)^{n-1}. \qquad \ldots(3.48)$$

The mean and second moment are given by

$$E[R_1] = \theta'(1) = \sum_{j=1}^{J} \frac{a_j q_j}{(1-q_j)^2}\left[1-(1-q_j)^{\alpha}\right]$$

and $$E[R_1^2] = \sum_{j=1}^{J} \frac{a_j q_j}{(1-q_j)^3}\{(1+q_j-2(1-q_j)^{\alpha})(1-(1-q_j)^{\alpha}) + 2\alpha q_j (1-q_j)^{\alpha}\}.$$

Table III gives computed values of the probability of cure P and the

mean and variance of the number of resistant cells for several choices of α, ν and ε where J=2, q_1=0.99, q_2=0.9. Table III also contains the analogous quantities which would be obtained using the deterministic model previously presented (see discussion following (3.41) in Section 3.7) when the constant $A_0=(1-\varepsilon)^{-1}$ as suggested there. The probability of cure (written at P_g to emphasize its dependence on the distribution at diagnosis), mean and variance are calculated using the deterministic model for each tumor size n and are then averaged over g(n) so that these quantities may be compared using the same underlying distribution of tumor size.

We see from Table III that the deterministic model has greater variance than the comparable "full" model; this result probably arises from the condition $R_1 \leq N$ which is not satisfied by the deterministic model and the different distribution of initiation times implicit in each model. On the other hand examination of this table shows that at least for the examples considered, the coefficient of variation is quite similar for both models.

We will now discuss the relative merits of the three approximations presented in this chapter.

3.9 Comparing the Three Approximations

The main strength of the first approximation is that the resulting probability generating function of the number of resistant cells is a simple function of the probability generating function of the underlying process. It provides a reasonable framework for the comparison of treatment effects because of the approximate stability of the underlying geometric distribution of the number of stem cells. However, because the

TABLE III

The Probability of Cure P_g, Expected Number and Standard Deviation of the Number of Resistant Cells.

J=2 q_1=0.99 q_2=0.9

ε	α	ν/b	P_g		$E(R_1)$		$S.D.(R_1)$	
0.0	0.01	0.0	0.46	0.46	4.9	5.4	14.9	20.9
0.5	0.01	0.0	0.46	0.46	9.3	9.1	25.1	29.6
0.9	0.01	0.0	0.47	0.46	29.9	25.9	55.6	66.2
0.0	0.02	0.0	0.28	0.28	9.6	10.4	21.6	29.4
0.5	0.02	0.0	0.29	0.28	17.7	17.4	35.6	41.5
0.9	0.02	0.0	0.31	0.28	50.8	45.2	72.7	90.6
0.0	0.01	0.01	0.28	0.28	10.6	10.4	25.7	29.4
0.5	0.01	0.01	0.29	0.28	18.6	17.4	37.9	41.5
0.9	0.01	0.01	0.31	0.28	51.2	45.2	73.2	90.6

The left hand column represents calculations based on the probability generating function given by (3.46) and right hand column is that based on the deterministic model given by (3.35) averaged over g(n); see (3.45). P_g is the probability of cure for the distribution at diagnosis g(n).

underlying distribution is approximately fixed it does not provide a suitable framework for estimating the distribution of resistant cells when the true distribution of stem cells is not geometric. This method is the simplest of the three and for this reason it is probably the most useful for estimating the effects of different treatment regimens (differing timing and dosages) when the distribution of the number of stem cells at diagnosis is unspecified.

The second approximation provides the probability generating function when the number of stem cells is fixed and addresses the problem of conditional distribution of resistant cells most directly. However, it is approximate and its calculation is quite complex. This hybrid stochastic-deterministic model of sensitive cell growth may be approximated (to give the same mean number of resistant cells and probability of cure) by a purely deterministic model. In this case the deterministic growth curve for the number of sensitive stem cells is approximately the same as the mean value function of the number of stem cells found for the first approximation. This suggests one can reasonably approximate the distribution of resistant cells using a deterministic model of sensitive stem cell growth, and that the deterministic growth function should be the expected growth function under a stochastic model where extinction has been eliminated. We will use the purely deterministic model of sensitive stem cell growth in Chapter 4.

The third approximation presents the most realistic model for the distribution of resistant cells in spontaneously occurring human or animal tumors since it implicitly incorporates the spontaneous incidence

rate of the tumor. However the calculation of the probability generating function represents a considerable problem for cases other than the one considered, where $\mu(t)=\lambda t$; even in this case the probability generating function of the process is complex when cell loss is present. Table III shows that the third approximation and (a modified form of) the second approximation do not yield the same distribution of resistant cells for the same distribution of stem cell burden. The main contributor to this difference is, of course, the assumption (in the third approximation) that new tumors are being initiated uniformly in time. In most experiments cells are implanted and thus the third approximation will not be suitable. Human tumors appear to be initiated throughout life and thus to accurately model resistance in such tumors it is necessary to consider the appropriate distribution of initiation times.

In conclusion, each approximation has its strengths and weaknesses and the choice of one of these will depend upon the experimental or clinical situation to be modelled and on the ultimate object of the modelling. In Chapter 4 we will use a deterministic model of sensitive cell growth in order to facilitate further development of this theory. Before completing our description of single drug resistance we will consider the possible effect of variation in the paramters α, β and γ.

3.10 Variation in the Resistance Parameters α, β and γ

Up to this point we have assumed that α, β and γ are fixed. In passaged animal tumor systems this assumption appears reasonable and has been assumed in all analyses of these systems. These tumor systems also possess little variation in a number of other physical properties. This is not unexpected since the process by which these tumors are chosen for

study tends to select those which maintain their characteristics after serial passaging. Spontaneous tumors, whether animal or human, do not undergo such a selection process and exhibit a greater variability in a number of physical characteristics than do passaged tumors. For example, experimental tumors display quite regular growth rates especially when many cells are present. In contrast, human tumors of almost every type display considerable variation in growth rates. Possible variation in α, β and γ can be thought of as occurring in two distinct ways. Firstly, these parameters may be considered to "evolve" (either deterministically or stochastically) as a tumor grows. One special case of this would be the possible effects of treatment on these parameters. Radiation and many drugs used in cancer therapy are known to be mutagenic and the values of α, β and γ may be expected to increase subsequent to treatment. Secondly, the parameters α, β and γ may vary between tumors within the same class with each class having some distinct distribution of α, β and γ.

Modelling the effect of mutagenicity of treatment is relatively straight-forward if we assume that the effect of treatment brings about a deterministic change in the value of the mutation rates for all the tumor cells. Since the probability generating function for the appearance of new mutations to resistance is independent for disjoint time intervals, we may use recursive relationships such as (3.11.1-2) to determine the probability generating function after treatment. If the effect of treatment is to induce a random change in the mutation rates of all the cells in a tumor (for a finite or an infinite time period) then this is extremely complex to model. Here we will only examine the effects of

variations in the mutation rates between tumors of a given class which are constant in time. As we know little regarding the relative magnitude of α, β and γ (most experiments measure the quantity $\alpha+\nu/b$) it is not necessary to consider their joint distribution. If we let $\phi(s;t,a)$ be the probability generating function of the distribution of the number of resistant cells (computed using the second of the three approximations previously presented) now viewed as conditional on $a=\alpha+\nu/b$, we have that the unconditional probability generating function, $\Psi(s;t)$, is given by

$$\Psi(s;t) = \int \phi(s;t,a)dF(a), \qquad \ldots(3.49)$$

where $F(a)$ is the cumulative distribution function for a.

Little is known about the distribution $F(a)$, since almost all experiments have assumed a to be fixed. We will therefore choose a convenient distribution which has support on a subset of $[0,1]$. An obvious choice for the distribution of a is to use the conjugate of $P\{R(t)|N(t),a\}$; however, this probability distribution function has not been determined. We propose to use the beta distribution which has support $[0,1]$ and is conjugate to the Bernoulli distribution. We have already shown that the probability of cure at size N for fixed a where $\pi_0(D)=0$, $\pi_1(D)=1$, is $P_N(a)\simeq(1-a)^{N-1}$; see equation (3.41). Then the cure probability, P_N, for the class of tumors is given by

$$P_N \simeq \int_0^1 (1-a)^{N-1}\beta(a;u,v)da,$$

where $\{u,v\}$ are the parameters of the beta distribution, and we assume that a and N are independent. It follows that

$$P_N = \frac{\Gamma(u+v)\Gamma(v+N-1)}{\Gamma(v)\Gamma(u+v+N-1)} = \prod_{x=0}^{N-2} \left(\frac{v+x}{u+v+x}\right), \qquad \ldots(3.50)$$

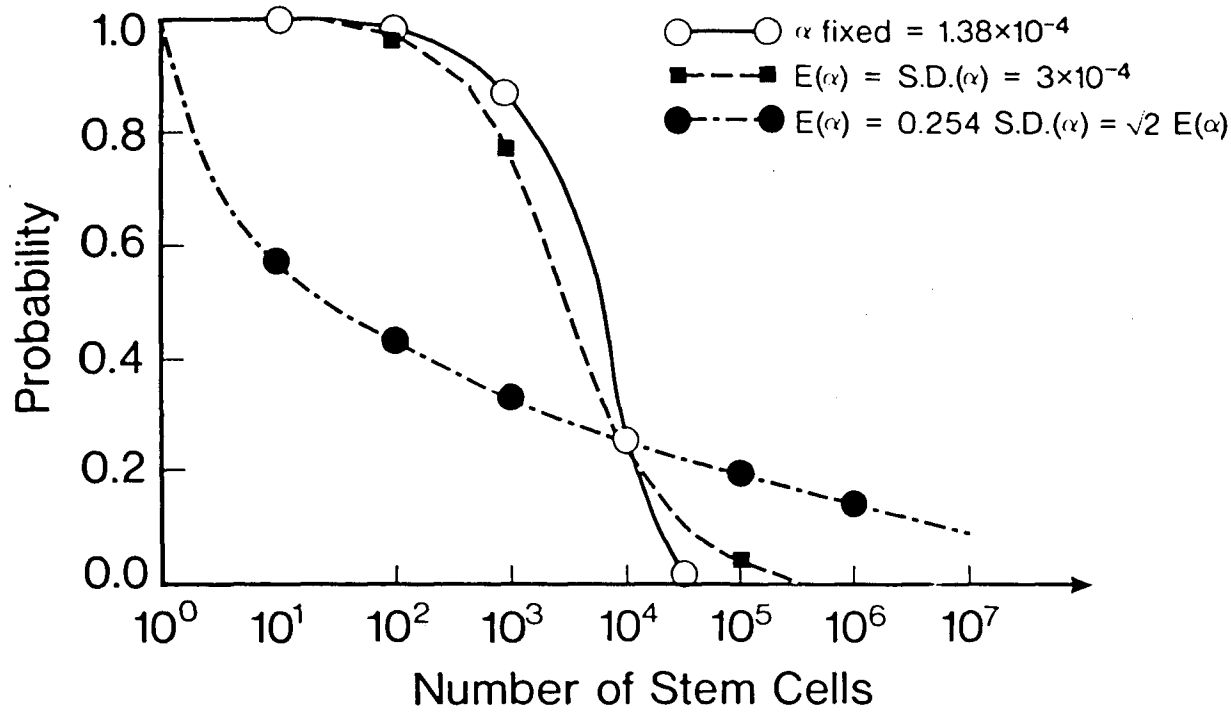
where Γ is the gamma function.

It is a simple matter to evaluate (3.50). In order to estimate the significance of variations in a on this P_N, it is necessary to fix a frame of reference. We choose here to assume that for some specified reference size there is a constant cure rate. Then we explore the effect of different choices of u and v at sizes other than the reference point. Examples are presented in Figure 3, where it may be seen that the values of u and v can effect the shape of the curve considerably.

Figure 3 shows that variation in a will affect the probability of no resistant cells and thus affect the likelihood that the tumor will be curable as a function of size. This observation seems important since not only does this formula relate to the probability of cure in clinical disease but it also relates to current methods used to estimate (assumed fixed) mutation rates in animal tumors. Experimental estimation of mutation rates is frequently based on destructive testing where it is assumed that $\pi_0(D)=0$, $\pi_1(D)=1$. The percentage of surviving animals is measured for various tumor burdens, and the mutation rate is estimated using an equation like (3.41). Thus the fitting of this type of data to equation (3.49) allows one to estimate the variability present in the mutation rates. However, other factors which affect curability may also cause similar departures from the form (3.41) and thus it is not possible to uniquely identify variability in mutation rates as the only cause. The curves in Figure 3 are, of course, strongly dependent upon the assumption of the beta-distribution. If the distribution of a is not adequately approximated by a beta distribution the curves of P_N may be quite different. The effect of variation of a in the mean number of resistant cells is easily calculated. From (3.40) the corresponding

Figure 3

Probability of Cure when Variation in the Mutation Rate is Present.



Plots of the probability of cure, P_N, as a function of the number of stem cells where the mutation rates are assumed to follow a distribution (3.50). The parameters were chosen so that the mean and standard deviation of a were as given. Each curve has been constructed to pass through the point $N=10^4$, $P_N=0.25$.

number of resistant cells is given by

$$\frac{N}{(1-\varepsilon)} \ln(N(1-\varepsilon)) \int_0^1 a\beta(a;u,v)da = \frac{u}{(u+v)} \frac{N}{(1-\varepsilon)} \ln (N(1-\varepsilon)).$$

This concludes our treatment of single drug resistance. In the next chapter we will consider the problem of resistance to two drugs.

4. RESISTANCE TO TWO OR MORE CHEMOTHERAPEUTIC AGENTS

The previous chapter considered the development of resistance to a single drug by tumor stem cells. In the chemotherapy of many human malignancies several active drugs are available. Where possible these drugs may be combined to form regimens which are more effective than either of their individual constituents. Here we will consider the development of resistance to two drugs.

The possible combinations (individual drugs and their dosages) are limited because of their effects on the host normal tissue systems. The construction of combined regimens depends on a variety of considerations, which include consideration of the activity of potential drugs on each component of the normal system of the host, pharmacokinetics of the drugs and other factors which relate to the "acceptability" of the resulting regimen. The final regimen may also include radiation or surgery. The construction of regimens (especially in the light of the restricted and imperfect information available) requires consideration of factors which we do not propose to model here. Therefore, we will consider that the drugs, their dosages and the timings of administration are fixed. We will consider a general framework for the development of resistance in stem cells and will provide a detailed examination of the case of two drugs.

Consider the case where there are n different antitumor agents available , T_1, ..., T_n. An individual tumor cell may then be characterized as being in one of 2^n mutually exclusive states with respect to these agents, according to which therapies it is resistant to and which not. As before a cell will be defined as resistant if the probability of

cell death after administration of chemotherapy is lower than in the parent (sensitive) line.

Let $R_{ij\ldots m}(t)$ be the number of stem cells at time t which are resistant to the set of drugs $\{T_i, T_j, \ldots, T_m\}$ and not resistant to any in the set $\{T_1, \ldots, T_n\}$ $\{T_i, T_j, \ldots, T_m\}$ and refer to such cells as being in the state $R_{ij\ldots m}$. Those stem cells sensitive to all drugs will be identified as members of R_ϕ, (ϕ is the empty set), which will be written R_0. The possible states for the individual tumor cells will be written as R_{Q_i}, where Q_i, i=0, 1,..., 2^n-1 ($Q_0=\phi$) are the 2^n distinct subsets of $\{1,2,\ldots,n\}$.

We will assume that when a stem cell in R_{Q_i} divides to form two new stem cells, one of them will be in R_{Q_i} and the other will be in R_{Q_j} with probability α_{Q_i,Q_j} where $\sum_{j=0}^{2^n-1} \alpha_{Q_i,Q_j} = 1$. As in the single drug case these probabilities will depend on the tumor type, the drug concentration, and the length of time the drug is administered. Similarly, we will define β_{Q_i,Q_j} as the probability that a stem cell transits from R_{Q_i} to R_{Q_j} when the cell divides forming a stem cell and a transitional cell. Also let $\gamma_{Q_i,Q_j}\Delta t + o(\Delta t)$ be the probability that a stem cell mutates from R_{Q_i} to R_{Q_j} in the interval [t, t+Δt). Transitions from the sensitive state R_0 to the resistant state R_{Q_j} will have as parameters α_{ϕ,Q_j}, β_{ϕ,Q_j} and γ_{ϕ,Q_j} for the three different types of transition. We will write these rates α_{Q_j}, β_{Q_j} and γ_{Q_j} respectively.

To simplify notation we will omit braces in the rate parameters and

use 0 to represent the empty set. For example $\alpha_{\{1\},\{12\}}$ will be written $\alpha_{1,12}$, $\beta_{\{1\}}$ as β_1, $\gamma_{\{1\},\phi}$ as $\gamma_{1,0}$, etc. We will now concentrate attention on the special case n=2, that is, two drugs. This case is both tractable and informative. As before we will assume that the probability of two transitions between states occurring in a time interval of length Δt is of the order $o(\Delta t)$. As in Chapter 3 we will assume that the acquisition of resistance is permanent. This implies $\alpha_{1,0}=\beta_{1,0}=\gamma_{1,0}=0$, $\alpha_{2,0}=\beta_{2,0}=\gamma_{2,0}=0$, $\alpha_{12,0}=\beta_{12,0}=\gamma_{12,0}=0$, $\alpha_{1,2}=\beta_{1,2}=\gamma_{1,2}=\alpha_{2,1}=\beta_{2,1}=\gamma_{2,1}=0$ and $\alpha_{12,1}=\beta_{12,1}=\gamma_{12,1}=\alpha_{12,2}=\beta_{12,2}=\gamma_{12,2}=0$. As in Chapter 3 we will only "keep track" of stem cells and the development of transitional and end cells (irrespective of their resistance status) will not be considered explicitly. Similarly we will assume that the growth parameters of all cells are the same. This assumption appears reasonable for some drugs and tumor types but others display differential growth rates for the sensitive and resistant cells. We will now discuss the calculation of the probability generating function for the process.

4.1 Probability Generating Function for Double Resistance

Define $P_{i,j,k,\ell}(t) = P\{R_0(t)=i,\ R_1(t) = j,\ R_2(t) = k,\ R_{12}(t) = \ell\}$ and

$$N(t) = R_0(t)+R_1(t)+R_2(t)+R_{12}(t).$$

Table IV indicates the permitted transitions with their associated probabilities. We continue by writing down the Kolmogorov forward equations [21] for the process which yields the following family of differential equations:

$$\frac{\partial P_{i,j,k,\ell}(t)}{\partial t}$$

TABLE IV

Transitions Occurring in the Stem Cell Compartment in the interval $[t,t+\Delta t)$ which have Probability of Order Δt.

Initial State	Final State	Probability
(i,j,k,ℓ)	$(i+1,j,k,\ell)$	$ib(1-\alpha_2-\alpha_2-\alpha_{12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	(i,j,k,ℓ)	$ic(1-\beta_1-\beta_2-\beta_{12})\Delta t$ $+jc(1-\beta_{1,12})\Delta t+\ell c\Delta t$ $+kc(1-\beta_{2,12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i-1,j,k,\ell)$	$id\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j+1,k,\ell)$	$ib\alpha_1\Delta t$ $+jb(1-\alpha_{1,12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i-1,j+1,k,\ell)$	$i(\beta_1 c+\gamma_1)\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j,k+1,\ell)$	$ib\alpha_2\Delta t$ $+kb(1-\alpha_{2,12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i-1,j,k+1,\ell)$	$i(\beta_2 c+\gamma_2)\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j,k,\ell+1)$	$ib\alpha_{12}\Delta t+jb\alpha_{1,12}\Delta t$ $+kb\alpha_{2,12}\Delta t+\ell b\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i-1,j,k,\ell+1)$	$i(\beta_{12}c+\gamma_{12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j-1,k,\ell)$	$jd\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j-1,k,\ell+1)$	$j(\beta_{1,12}c+\gamma_{1,12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j,k-1,\ell)$	$kd\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j,k-1,\ell+1)$	$k(\beta_{2,12}c+\gamma_{2,12})\Delta t+o(\Delta t)$
(i,j,k,ℓ)	$(i,j,k,\ell-1)$	$\ell d\Delta t+o(\Delta t)$

$$= -[(b+d+c)(i+j+k+\ell) + \gamma_{1,12}j + \gamma_{2,12}k + (\gamma_1+\gamma_2+\gamma_{12})i]P_{i,j,k,\ell}(t)$$
$$+ b(1-\alpha_1-\alpha_2-\alpha_{12})(i-1)P_{i-1,j,k,\ell}(t) + \alpha_1 biP_{i,j-1,k,\ell}(t)$$
$$+ \alpha_2 biP_{i,j,k-1,\ell}(t) + d(i+1)P_{i+1,j,k,\ell}(t)$$
$$+ c(1-\beta_1-\beta_2-\beta_{12})iP_{i,j,k,\ell}(t) + (\beta_1 c+ \gamma_1)(i+1)P_{i+1,j-1,k,\ell}(t)$$
$$+ (\beta_2 c+\gamma_2)(i+1)P_{i+1,j,k-1,\ell}(t) + b\alpha_{12}iP_{i,j,k,\ell-1}(t)$$
$$+ (\beta_{12}c+\gamma_{12})(i+1)P_{i+1,j,k,\ell-1}(t) + b(1-\alpha_{1,12})(j-1)P_{i,j-1,\ k,\ell}(t)$$
$$+ b\alpha_{1,12}jP_{i,j,k,\ell-1}(t) + d(j+1)P_{i,j+1,k,\ell}(t)$$
$$+ c(1-\beta_{1,12})jP_{i,j,k,\ell}(t) + (c\beta_{1,12}+\gamma_{1,12})(j+1)P_{i,j+1,k,\ell-1}(t)$$
$$+ b(1-\alpha_{2,12})(k-1)P_{i,j,k-1,\ell}(t) + b\alpha_{2,12}kP_{i,j,k,\ell-1}(t)$$
$$+ d(k+1)P_{i,j,k+1,\ell}(t) + c(1-\beta_{2,12})kP_{i,j,k,\ell}(t)$$
$$+ (c\beta_{2,12}+\gamma_{2,12})(k+1)P_{i,j,k,\ell-1}(t) + b(\ell-1)P_{i,j,k,\ell-1}(t)$$
$$+ d(\ell+1)P_{i,j,k,\ell+1}(t) + c\ell P_{i,j,k,\ell}(t) \qquad \ldots(4.1)$$

for all of $i,j,k,\ell \geq 0$ and where $P_{i,j,k,\ell}(t) \equiv 0$ for any of i,j,k or $\ell<0$. We will assume that $P_{i,j,k,\ell}(0)$ is known.

Let $\phi(\underset{\sim}{s};t)$ be the probability generating function for the process, that is

$$\phi(\underset{\sim}{s};t) = \phi(s_0,s_1,s_2,s_3;t)$$
$$= \sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\sum_{k=0}^{\infty}\sum_{\ell=0}^{\infty} P_{i,j,k,\ell}(t)\, s_0^i\, s_1^j\, s_2^k\, s_3^\ell.$$

Then multiplying (4.1) by $s_0^i\, s_1^j\, s_2^k\, s_3^\ell$ and summing i,j,k,ℓ over 0 to ∞ yields

$$\frac{\partial\phi(\underset{\sim}{s};t)}{\partial t} = \sum_{i=0}^{3}(bs_i-d)(s_i-1)\frac{\partial\phi(\underset{\sim}{s};t)}{\partial s_i}$$
$$+ \sum_{i=1}^{2}\{(\alpha_{i,12}bs_i+\nu_{i,12})(s_3-s_i)\frac{\partial\phi(\underset{\sim}{s};t)}{\partial s_i}$$
$$+ (\alpha_i bs_0+\nu_i)(s_i-s_0)\frac{\partial\phi(\underset{\sim}{s};t)}{\partial s_0}\} + (\alpha_{12}bs_0+\nu_{12})(s_3-s_0)\frac{\partial\phi(\underset{\sim}{s};t)}{\partial s_0}$$
$$\ldots(4.2)$$

where $\nu_i=c\beta_i+\gamma_i$, $\nu_{i,12}=c\beta_{i,12}+\gamma_{i,12}$ for i=1,2 and $\nu_{12}=c\beta_{12}+\gamma_{12}$.

We may use the method of characteristics [22] to reduce the solution of (4.2) to the solution of the following set of five ordinary differential equations:

$$\frac{dt(u)}{du} = 1,$$

$$\frac{d\chi_i(u)}{du} = (1-\chi_i(u))(b\chi_i(u)-d) + (\chi_i(u)-\chi_3(u))(b\alpha_{i,12}\chi_i(u)+\nu_{i,12}),$$

for i=1,2,

$$\frac{d\chi_3(u)}{du} = (1-\chi_3(u))(b\chi_3(u)-d),$$

$$\frac{d\chi_0(u)}{du} = (1-\chi_0(u))(b\chi_0(u)-d) + \sum_{i=1}^{2}(\alpha_i b\chi_0(u)+\nu_i)(\chi_0(u)-\chi_i(u)) + (\alpha_{12}b\chi_0(u)+\nu_{12})(\chi_0(u)-\chi_3(u)),$$

where u, $\chi_1(u)$, $\chi_2(u)$, $\chi_3(u)$ and $\chi_4(u)$ are dummy variables. Unfortunately, although the first four equations are straightforward to solve, the final equation (involving $\chi_0(u)$) is complicated and a closed form solution is not apparent. However, we have already shown (in the case of single resistance) that if t and $R_0(t)$ are known, then the distribution of the number of resistant cells can be reasonably well approximated (Section 3.7) by using a continuous deterministic function for the growth of the sensitive cells. From this point on in this chapter we will assume that sensitive stem cells grow deterministically and to emphasize this we will set $R_0(t)=B(t)$; the compartments R_1, R_2 and R_{12} will grow as before. A less general form of this model has previously been considered by Coldman et al [27]. Let

$$P^*_{i,j,k}(t) = P\{R_1(t)=i,R_2(t)=j,R_{12}(t)=k \mid R_1(0)=0,R_2(0)=0,R_{12}(0)=0\}$$

and

$$\Phi(\underset{\sim}{s};t) = \Phi(s_0,s_1,s_2,s_3;t) = \sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\sum_{k=0}^{\infty} P^*_{i,j,k}(t)\, s_1^i\, s_2^j\, s_3^k$$

be the joint probability generating function for the number of resistant (R_1, R_2 and R_{12}) cells derived from sensitive stem cells (R_0) after time t=0, excluding cells in R_1, R_2 or R_{12} present at time t=0, that is $P^*_{000}(0)=1$ and thus $\Phi(\underset{\sim}{s};0)=1$. This generating function is dependant on the function B(t), but this dependence will not be explicitly indicated.

The assumption of deterministic sensitive cell growth alters the form of the transition probabilities given in Table IV and thus in (4.1). The effect is to delete the state R_0 and set to zero all probabilities which applied to changes in the numbers of cells in R_0 alone (i.e. those without changes in the numbers of cells in either R_1, R_2 or R_{12} as well). The probabilities for transition involving the number of cells in R_0 and the numbers in either R_1, R_2 or R_{12} are unchanged except that i is replaced by B(t). Transitions between other states are as before. We may then derive the following partial differential equation for $\Phi(\underset{\sim}{s};t)$ in the same way as (4.2) was obtained:

$$\frac{\partial\Phi(\underset{\sim}{s};t)}{\partial t} = \sum_{i=1}^{3} [bs_i-d]\,[s_i-1]\,\frac{\partial\Phi(\underset{\sim}{s};t)}{\partial s_i}$$

$$+ \sum_{i=1}^{2}\left\{(\alpha_{i,12}bs_i+\nu_{i,12})(s_3-s_i)\,\frac{\partial\Phi(\underset{\sim}{s};t)}{\partial s_i} + (\alpha_i b+\nu_i)(s_i-1)\,B(t)\Phi(\underset{\sim}{s};t)\right\}$$

$$+ (\alpha_{12}b+\nu_{12})\,(s_3-1)\,B(t)\Phi(\underset{\sim}{s};t). \qquad \ldots(4.3)$$

The result can also be obtained by setting $s_0=1$, $\phi(\underset{\sim}{s};t)=\Phi(\underset{\sim}{s};t)$ and $\frac{\partial\phi(s;t)}{\partial s_0} = B(t)\Phi(\underset{\sim}{s};t)$ in (4.2).

Using the method of characteristics [22] the solution of (4.3) is obtained by solving the following series of differential equations:

$$\frac{dt(u)}{du} = 1, \qquad \ldots(4.4.1)$$

$$\frac{d\chi_i(u)}{du} = (1-\chi_i(u))\,(b\chi_i(u)-d) + (\chi_i(u)-\chi_3(u))\,(b\alpha_{i,12}\chi_i(u)+\nu_{i,12}),\ i=1,2, \qquad \ldots(4.4.2)$$

$$\frac{d\chi_3(u)}{du} = (1-\chi_3(u))\,(b\chi_3(u)-d), \qquad \ldots(4.4.3)$$

$$\Phi(\underset{\sim}{\chi}(u);u)^{-1}\,\frac{d\Phi(\underset{\sim}{\chi}(u);u)}{du} = \Big\{\sum_{i=1}^{2}(\alpha_i b+\nu_i)\,(\chi_i(u)-1) + (\alpha_{12}b+\nu_{12})\,(\chi_3(u)-1)\Big\}B(u). \qquad \ldots(4.4.4)$$

Now we note that the equation (4.4.3) for $\chi_3(u)$ is simply solved as before (see equation (3.2.3)):

$$\chi_3(u) = \frac{d[1-\chi_3(0)] + [b\chi_3(0)-d]e^{\delta u}}{b[1-\chi_3(0)] + [b\chi_3(0)-d]e^{\delta u}}. \qquad \ldots(4.5)$$

Noting that $\chi_i(u) = \chi_3(u)$ is a particular solution for (4.4.2) we have (in analogy to the solution of (3.2.2))

$$\chi_i(u) = \chi_3(u) + \frac{F_i(u)}{[\chi_i(0)-\chi_3(0)]^{-1} + b(1-\alpha_{i,12})\int_0^u F_i(x)dx}, \text{ for } i=1,2 \qquad \ldots(4.6)$$

where

$$F_i(x) = \delta^{2-\alpha_{i,12}}\exp\{(\delta+\alpha_{i,12}d+\nu_{i,12})x\} \times[b[1-\chi_3(0)] + [b\chi_3(0)-d]e^{\delta x}]^{-2+\alpha_{i,12}}.$$

Equation (4.4.4) may then be solved directly by substituting (4.6) for $\chi_i(u)(i=1,2)$ and (4.5) for $\chi_3(u)$ and integrating the left and right hand sides directly. The required solution $\Phi(\underset{\sim}{s};t)$ is then obtained by setting $u=t$ and $\chi_i(u)=s_i$ (for $i=1,2,3$) and inverting (4.5) and (4.6) so that $\chi_i(0)$ $(i=1,2,3)$ are expressed in terms of $\underset{\sim}{s}$ and t. These values are then substituted into the expression obtained by integration of the right hand side of (4.4.4). Carrying out this substitution we obtain, after some

simplification, the following expression for $\Phi(\underset{\sim}{s};t)$:

$$\ln \Phi(\underset{\sim}{s};t) = I_0 B_0 + B_0 \sum_{i=1}^{2} (\alpha_i b+\nu_i) I_i(s_i) , \qquad \ldots(4.7)$$

where

$$I_0 = \{\alpha_{12} b+\nu_{12} + \sum_{i=1}^{2}(\alpha_i b+\nu_i)\}\ \delta(s_3-1) \int_0^t \frac{B'(t-v)\ dv}{b(1-s_3) + (bs_3-d)e^{-\delta v}} ,$$

$$I_i(s) = \int_0^t \frac{B'(t-u)\ g_i(u)\ du}{[\delta^{2-\alpha_{i,12}}(s-s_3)]^{-1} - b(1-\alpha_{i,12}) \int_0^u g_i(v)\ dv} ,$$

with

$$g_i(v) = e^{-(\delta+\alpha_{i,12}d+\nu_{i,12})v} [b(1-s_3) + (bs_3-d)e^{-\delta v}]^{-2+\alpha_{i,12}},$$

$B'(u) = B(u)/B_0$ and $B_0 = B(0)$. Equation (4.7) generalizes a previous result found by Coldman et al [27].

The function $\Phi(\underset{\sim}{s};t)$, given by (4.7), is the probability generating function for the number of singly and doubly resistant cells derived from the growth of the sensitive cells over the interval [0,t] conditional on $R_1(0)=R_2(0)=R_{12}(0)=0$. Our objective is to derive $\Psi(\underset{\sim}{s};t)$ the unconditional probability generating function for an arbitrary distribution of sensitive and resistant cells at t=0. We will now examine the development of resistant cells from singly resistant cells present at t=0. This is quite straightforward since the development of double resistance in cells already resistant to one agent is analogous to the development of single resistance in sensitive cells considered in Chapter 3.

Let $\phi_i(\underset{\sim}{s};t)$, i=1,2, be the probability generating functions of the number of resistant cells derived from (progeny of) a single cell in R_i at time t=0, i.e. conditional on $R_0(t)=0$, $R_i(0)=1$, $R_{3-i}(0)=0$ and

$R_{12}(0)=0$. Then

$$\phi_i(\underset{\sim}{s};t) = w_0(t), \quad i=1,2, \qquad \ldots(4.8)$$

where $w_0(t)$ is given by (3.7) with $s_0=s_i$, $s_1=s_3$, $\alpha=\alpha_{i,12}$ and $\nu=\nu_{i,12}$. Similarly, let $\phi_3(\underset{\sim}{s};t)$ be the probability generating function of the number of resistant cells derived from (progeny of) a single cell in R_{12} at time t=0, i.e. conditional on $R_0(t)=0$, $R_1(0)=0$, $R_2(0)$ and $R_{12}(0)=1$. Then

$$\phi_3(\underset{\sim}{s};t) = w_1(t), \qquad \ldots(4.9)$$

where $w_1(t)$ is given by (3.6) with $s_1=s_3$.

For future use it is convenient to include a term in the unconditional generating function reflecting the number of sensitive cells at time t. To do this we will multiply the generating function by $s_0^{[B(t)]}$, which may be viewed as the approximate generating function for the number of sensitive cells. Using the general result (2.3), $\Psi(\underset{\sim}{s};t)$, the unconditional probability generating function of $\{B(t),R_1(t),R_2(t),R_{12}(t)\}$, is given by

$$\Psi(\underset{\sim}{s};t) = \phi(1,\phi_1(\underset{\sim}{s};t),\phi_2(\underset{\sim}{s};t),\phi_3(\underset{\sim}{s};t))\Phi(\underset{\sim}{s};t)s_0^{[B(t)]} \qquad \ldots(4.10)$$

where $\phi(1,s_1,s_2,s_3)= \Psi(1,s_1,s_2,s_3;0)$ is the probability generating function for the distribution of $\{R_1(0), R_2(0), R_{12}(0)\}$.

For future reference we will now calculate $m_1(t)=E[R_1(t)]$, $m_2(t)=E[R_2(t)]$ and $m_{12}(t)=E[R_{12}(t)]$. Differentiating (4.10) with respect to s_i, (i=1,2,3) and setting $\underset{\sim}{s} = (1,1,1,1)$ yields the following relationships:

$$m_i(t) = e^{(\delta-a_{i,12})t}\left(m_i(0) + a_i\int_0^t e^{-(\delta-a_{i,12})u}B(u)du\right), \quad i=1,2, \quad \ldots(4.11.1)$$

where $a_i=\alpha_i b+\nu_i$, $a_{i,12}=b\alpha_{i,12}+\nu_{i,12}$ and

$$m_{12}(t) = e^{\delta t}\{m_{12}(0) + \sum_{i=1}^{2} m_i(0)\,[1-e^{-a_{i,12}t}] + \sum_{i=1}^{2} a_i[\int_0^t B(u)e^{-\delta u}\,du - e^{-a_{i,12}t}\int_0^t B(u)e^{-(\delta-a_{i,12})u}\,du] + a_{12}\int_0^t B(u)e^{-\delta u}du\}, \quad \ldots(4.11.2)$$

where $a_{12}=\alpha_{12}b+\nu_{12}$.

The special case $B(u)=B_0\exp(ku)$, $(k\neq\delta-a_{i,12})$ is of particular interest since it is the mean growth function for a birth and death process with fixed rates. In this case the expected values are

$$m_i(t) = \exp\{(\delta-a_{i,12})t\}\,(m_i(0) + \frac{a_i B_0[e^{(k-\delta+a_{i,12})t}-1]}{[k-\delta+a_{i,12}]}), \quad \ldots(4.12.1)$$

and

$$m_{12}(t) = e^{\delta t}\{m_{12}(0) + \sum_{i=1}^{2} m_i(0)[1-e^{-a_{i,12}t}] + B_0\sum_{i=1}^{2}\frac{a_i a_{i,12}}{[k-\delta+a_{i,12}]}[\frac{e^{(k-\delta)t}-1}{(k-\delta)} - (\frac{1-e^{-a_{i,12}t}}{a_{i,12}})] + \frac{B_0 a_{12}}{[k-\delta]}[e^{(k-\delta)t}-1]\}. \quad \ldots(4.12.2)$$

The choice $k=\delta-a_1-a_2-a_{12}$ yields the same expected numbers of singly resistant cells as in the fully stochastic case, i.e. that with joint probability generating function satisfying (4.2). This may be shown by differentiating (4.2) with respect to s_i $(i=0,1,2,3)$ setting $\underset{\sim}{s}=\underset{\sim}{1}$ and obtaining differential equations for $m_0(t)$, $m_1(t)$, $m_2(t)$ and $m_{12}(t)$. In particular the ordinary differential equation for $m_0(t) = E[R_0(t)]$ obtained from (4.2) is

$$\frac{dm_0(t)}{dt} = (\delta-a_1-a_2-a_{12})m_0(t),$$

which has solution $m_0(t) = m_0 \exp\{(\delta - a_1 - a_2 - a_{12})\}t$. Repeating the procedure for $m_1(t)$, $m_2(t)$ and $m_{12}(t)$ shows that (4.12.1-2) are solutions to the appropriate differential equations when $k = \delta - a_1 - a_2 - a_{12}$. In the following analysis of the two drug case we will assume that $B(u) = B_0 e^{ku}$ where $k = \delta - a_1 - a_2 - a_{12}$.

As discussed in Chapter 3 we will be interested in situations of growth from a single sensitive stem cell where the tumor size (stem cells) N is observed, but t is unknown. We will then use the approximation suggested in Section 3.7, equations (3.38) and (3.40), and assume that the overall growth of the stem cell compartment is given by $B_0 e^{\delta t}$ where $B_0 = (1-\varepsilon)^{-1}$. Thus we will set

$$t = \delta^{-1} \ell n\ (N(1-\varepsilon)), \qquad \ldots(4.13)$$

where the term $(1-\varepsilon)$ arises from excluding tumor growth paths in which the stem cell compartment goes spontaneously extinct. This factor is retained since, although the stem cell compartment cannot go extinct (because the sensitive cells are growing deterministically), it yields a better approximation to the fully stochastic model. Now if we observe R_0^* sensitive cells at some time t (which may not be known on the scale where t=0 is the origin time of the progenitor stem cell) then we would use t given by (4.13) in (4.10) and set the last factor on the right hand side of (4.10) to be,

$$s_0^{[B(t)]} = s_0^{R_0^*} \qquad \ldots(4.14)$$

In most cases of practical interest N is observed and R_0^* is unknown. In such cases we will set $R_0^* = [N - m_1(t) - m_2(t) - m_{12}(t)]$ $(\simeq N)$ where t is given by (4.14). We will now consider the modelling of treatment effects in the two drug case.

4.2 Modelling Treatment Effects

Radiotherapy and surgery will be modelled in the same way (with cell survival as Bernoulli random variables) as presented in Section 3.4 and the effect of each will be the same for all resistant subtypes. To model the effects of chemotherapy upon stem cells we will assume that the drugs obey the same laws of kill as outlined in Section 3.2 [26] and define the following quantities for $Q\varepsilon\{\{0\},\{1\},\{2\},\{12\}\}$:

$\pi_{i,Q}(D)$ = P{a cell in R_Q will survive administration of a single course of the drug T_i at dose D} for i=1,2.

We will generally omit the dependence of $\pi_{i,Q}(D)$ on D where it is understood to relate to some fixed but possibly unspecified dose.

We define the variable $X_{i,Q}$ as follows:

$X_{i,Q}$ = 1 if a cell in R_Q survives administration of T_i,

= 0 otherwise.

Then $\xi_{i,Q}(s)$, the probability generating function for $X_{i,Q}$, is given by

$$\xi_{i,Q}(s) = 1-\pi_{i,Q} + \pi_{i,Q}\, s.$$

For simplicity, as before, we will write $\pi_{i,\{1\}}$ as $\pi_{i,1}$ etc. Now if treatment T_i is given at time t_1 then

$$\Psi(\underset{\sim}{s};t_1) = \Psi(\xi_i(\underset{\sim}{s});t_1^-), \qquad \ldots(4.15)$$

where

$$\xi_i(\underset{\sim}{s}) = (\xi_{i,0}(s_0),\ \xi_{i,1}(s_1),\ \xi_{i,2}(s_2),\ \xi_{i,12}(s_3)). \qquad \ldots(4.16)$$

This result follows from (2.3) and is similar to result (3.11.1) for the single drug case.

Equation (4.15) deserves some comment since $\Psi(\underset{\sim}{s};t_1)$ contains one part in which the number of sensitive cells is deterministic and another in which it is random. This arose because we assumed $R_0(t)=B(t)$ in order

to derive the probability generating function for the number of resistance cells derived from sensitive cells. We have also written a probability generating function for the number of sensitive cells at time t_1, (4.14), and used it to derive the probability generating function of the number of sensitive cells after treatment, (4.15). We have done this to obtain a better approximation to the behaviour of the fully stochastic model. In intertreatment intervals we may consider stem cell growth to be stochastic, but to calculate the distribution of resistant cells which arise from sensitive cells (in that interval) we use the deterministic growth model for $R_0(t)$. We know from Section 3.1 that in the case of single resistance the stem cell compartment grows (stochastically) as a birth and death process with parameters $b(1-\alpha)$ and $\nu+d$. Since the ultimate destination of cells leaving the sensitive compartment is irrelevant to the growth of this compartment we deduce that, in the fully stochastic model for resistance to two agents, the sensitive cell compartment will grow as a birth and death process with parameters $b(1-\alpha_1-\alpha_2-\alpha_{12})$ and $(d+\nu_1+\nu_2+\nu_{12})$. If we let $\phi_0(\underset{\sim}{s};t)$ be the probability generating function of the number of sensitive stem cells in this fully stochastic model

$$\phi_0(\underset{\sim}{s};t) = w_1(t), \qquad \ldots(4.17)$$

where $w_1(t)$ is given by (3.6) with $s_1=s_0$, b replaced by $b(1-\alpha_1-\alpha_2-\alpha_{12})$ and d by $(d+\nu_1+\nu_2+\nu_{12})$.

We may use the stochastic model for the growth of the sensitive cell compartment to "update" the probability generating function for newly resistant stem cells as follows. In deriving the probability generating function (4.7) we assumed that B_0 was a constant. If instead we consider

B_0 to be a random variable with distribution not dependent on t, then $\Phi(\underset{\sim}{s};t)$ can be viewed as being conditional on B_0. If we emphasize this by writing $\Phi_{B_0}(\underset{\sim}{s};t)$, then we see from (4.7) that

$$\Phi_{B_0}(\underset{\sim}{s};t)=[\Phi_1(\underset{\sim}{s};t)]^{B_0}.$$

Furthermore if B_0 has a distribution with support on the non-negative integers with probability generating function $\Theta(s)$ say, then the unconditional probability generating function of the number of cells is given by $\Theta(\Phi_1(\underset{\sim}{s};t))$.

In particular this will be useful here since after treatment the number of stem cells is random. Using (2.3), we may write an expression for the probability generating function in an intertreatment interval as

$$\Psi(\underset{\sim}{s};t_j+v)=\Psi\left(\Phi_1(\underset{\sim}{s};v)\phi_0(\underset{\sim}{s};v),\phi_1(\underset{\sim}{s};v),\phi_2(\underset{\sim}{s};v),\phi_3(\underset{\sim}{s};v);t_j\right), \quad \ldots(4.18)$$

where $t_j<t_j+v\leq t_{j+1}$, $t_j(j=1,\ldots,J)$ are treatment times, $\Phi_1(\underset{\sim}{s};v)$ is given by (4.7) with $B_0=1$, $\phi_i(\underset{\sim}{s};v)$ $(i=1,2)$ is given by (4.8), $\phi_3(\underset{\sim}{s};v)$ is given by (4.9), and $\phi_0(\underset{\sim}{s};v)$ is given by (4.17).

We may therefore use equations (4.15) and (4.18) to calculate recursively the resulting probability generating function for the growth process corresponding to various treatment sequences by setting $v=t_{j+1}-t_j$ for the interval $[t_j,t_{j+1})$ where the initial probability generating function at time t_1^- is given by (4.10).

Notice that we may use (4.18) recursively at times where treatment is not given in order to improve the approximation to the fully stochastic model. In general we would not do this prior to t_1 as this would then induce (a non-degenerate) distribution for $R_0(t_1)$ with all the attendant problems this produces (as extensively discussed in Chapter 3).

In the situation to be considered later (Chapter 5) we will only use (4.18) at times of treatment, that is $v=t_{j+1}-t_j$. We will use (4.10) for the interval $[0,t_1)$, chose t_1 as given in (4.13) and use (4.14) with $R_0^*=[N-m_1(t_1^-)-m_2(t_1^-)-m_{12}(t_1^-)]$ (the integer part) where $m_i(t_1^-)$ (i=1,2), $m_{12}(t_1^-)$ are calculated from (4.11.1-2) and N is the "observed" stem cell compartment size.

The incorporation of a stochastic element to the growth of the sensitive stem cells is somewhat 'artificial' however it does improve the approximation of the model to the fully stochastic one. It also allows a reasonable determination of $P\{N(t)=0\}$ which would otherwise be identically zero if $R_0(t)$ were left purely deterministic. The model can be expected to be a reasonable reflection of reality since when there are large numbers of sensitive stem cells, growth can be expected to be quite regular and thus well approximated by the deterministic assumption. When the number of sensitive cells is small, the likelihood that new resistant cells will arise (from R_0) is small and thus the assumption of deterministic growth should not cause a great distortion to the distribution of the number of resistant cells. As in Chapter 3 we will now consider some special cases which illustrate the behaviour of the model.

In many cases two drugs may not be given together because of their overlapping toxicity on normal tissue. Consider the special case where the drugs act independently and can be given together, with $N(0)=R_0(0)=1$ (the tumor originates from a single sensitive stem cell), $\pi_{1,12}=\pi_{2,12}=1$, and $\pi_{1,0}=\pi_{2,0}=\pi_{1,2}=\pi_{2,1}=0$ ($\pi_{1,1}$ and $\pi_{2,2}$ are arbitrary) i.e. when the two drugs are given together all stem cells are killed except those in R_{12}.

When $N(0)= R_0(0)=1$ we will set $B_0=(1-\varepsilon)^{-1}$ as described in the discussion leading to (4.13). If both drugs are given at t_1 then the probability the tumor will be cured, P_{t_1}, is given by $\Psi(1,1,1,\varepsilon;t_1^-)$, (see (3.14)), where $\Psi(\underset{\sim}{s};t)$ is given by (4.10). This reduces to

$$P_{t_1} = \Phi(1,1,1,\varepsilon;t_1^-),$$

since $\phi(1,s_1,s_2,s_3) = 1$. To simplify notation we will write P_t for P_{t_1}.

Examining the terms in (4.7) we have for $s_3 = \varepsilon$ that

$$\int_0^u g_i(v)dv = \frac{\delta^{-2+\alpha_{i,12}}}{(\delta+a_{i,12}\ d+\nu_{i,12})}[1-e^{-(\delta+\alpha_{i,12}d+\nu_{i,12})u}].$$

For $i=1,2$ and $s_3=\varepsilon$ we also have

$$I_i(1) = \frac{\delta(\delta+\alpha_{i,12}d+\nu_{i,12})}{b}\int_0^t \frac{B'(t-u)\ du}{(\alpha_{i,12}b+\nu_{i,12})e^{(\delta+\alpha_{i,12}\ d+\nu_{i,12})u} + \delta(1-\alpha_{i,12})}.$$

After some simplification we obtain

$$\ln P_t = -\sum_{i=1}^{2} \frac{a_i a_{i,12}\delta}{b} \int_0^t \frac{(e^{(\delta+a^*_{i,12})u}-1)\ B(t-u)\ du}{a_{i,12}e^{(\delta+a^*_{i,12})u} + \delta(1-\alpha_{i,12})} - \frac{a_{12}\delta}{b}\int_0^t B(t-u)\ du, \qquad \ldots(4.19)$$

where a_i, $a_{i,12}(i=1,2)$, a_{12} are as given in (4.11.1-2) and $a^*_{i,12}=a_{i,12}-\alpha_{i,12}\delta$.

Using $B(t) = B_0 \exp\{(\delta-a_1-a_2-a_{12})t\}$, the formula for P_t may be numerically evaluated. As we are primarily interested in treatment applied at some fixed size but unknown time, we will restrict attention to the calculation of P_t where $t = \delta^{-1}\ln[N(1-\varepsilon)]$ and in this case we will designate the probability of cure as P_N. P_N is plotted as a function of N for various mutation rates in Figure 4. In most cases of

interest we will have $\delta >> a_1+a_2+a_{12}$ and $\delta >> a_{i,12}$ for i=1,2. Figure 4 shows that for some sample values of a_1, a_2, a_{12}, $a_{i,12}$ (i=1,2) the shape of the resulting curves of P_N against N are similar to those obtained for the analogous case in single resistance (Figure 1). This suggests that in analogy to (3.37) and (3.41) it may be possible to approximate P_N by a function of the form $(1-a^*)^{N-1}$, or $\exp\{-a^*(N-1)\}$, (which are numerically similar for $a^* << 1$) where a^* is a function of a_1, a_2, a_{12}, $a_{1,12}$ and $a_{2,12}$. We will thus attempt to approximate (4.19) for fixed N; to do this we will first bound P_N.

To simplify further presentation we note that the scale of measurement of t is unimportant in the calculation of P_N. Thus we will choose a scale for which b=1 and assume that the other rates are all relative to this time scale. This will be emphasized by writing $\varepsilon(=d/b)$ rather than d. Thus using $B(t) = (1-\varepsilon)^{-1}\exp\{(1-\varepsilon-a_1-a_2-a_{12})t\}$ and $a=a_1+a_2+a_{12}$ in (4.19) we obtain

$$\ell n P_N =$$

$$-\sum_{i=1}^{2} a_i a_{i,12} N[N(1-\varepsilon)]^{-a(1-\varepsilon)^{-1}} \int_0^t \frac{e^{(a+a^*_{i,12})u}\{1-e^{-(1-\varepsilon+a^*_{i,12})u}\}}{(1-\varepsilon)^{-1}a_{i,12}e^{(1-\varepsilon+a^*_{i,12})u} + 1-\alpha_{i,12}} du$$

$$- \frac{a_{12}}{(1-\varepsilon-a)}\{[N(1-\varepsilon)]^{1-a(1-\varepsilon)^{-1}} - 1\}, \qquad \ldots(4.20)$$

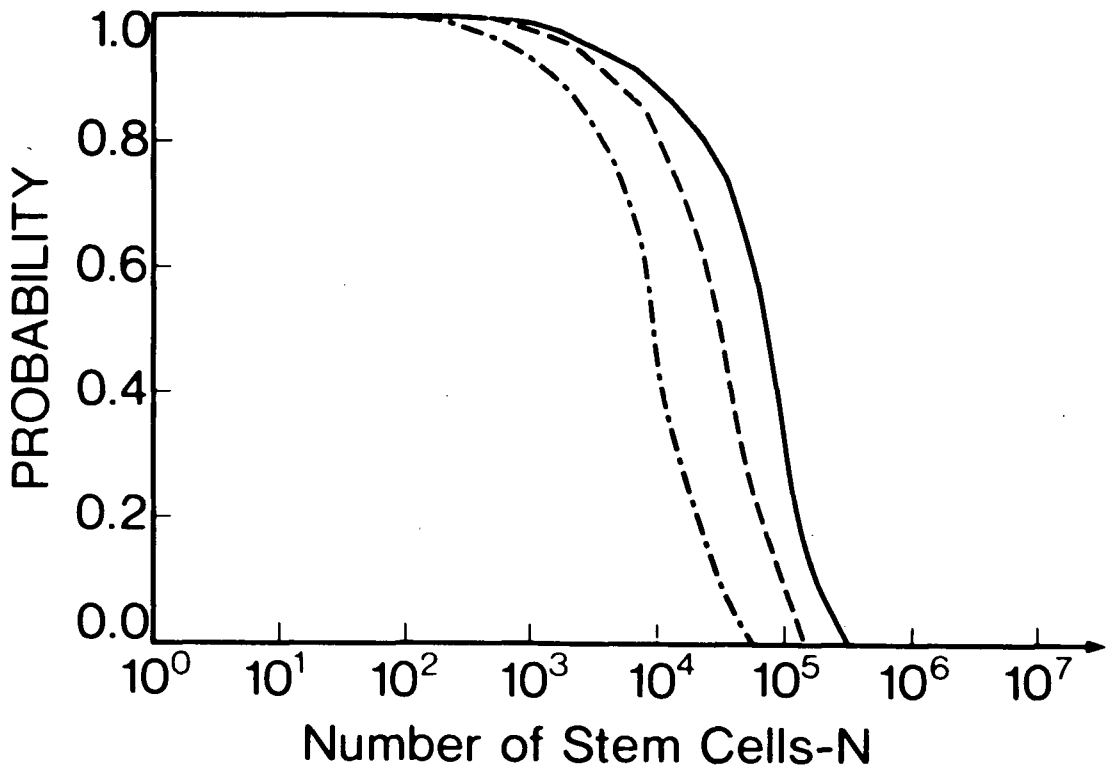
where $t=(1-\varepsilon)^{-1}\ell n\,[N(1-\varepsilon)]$. We will now bound $\ell n P_N$.

For i=1,2 let $h_i(u)$ be the integrand on the right hand side of (4.20). Then if

$$U_i(u) = \frac{\{1-e^{-2(1-\varepsilon-a)u}\}}{(1-\varepsilon)^{-1}a_{i,12}\ e^{(1-\varepsilon-a)u} + (1-\alpha_{i,12})e^{-(1-\varepsilon-a)u}},$$

Figure 4

Probability of Cure when Loss is Present.



Probability of cure P_N as a function of stem cell burden at diagnosis where T_1 and T_2 are given simultaneously at that time, $\pi_{i,0}=0$, $\pi_{i,i}$ arbitrary, $\pi_{i,12}=1$ for $i=1,2$ $\pi_{1,2}=\pi_{2,1}=0$, $\alpha_i+\nu_i/b=\alpha_{i,12}+\nu_{i,12}/b=10^{-3}$ for $i=1,2$ and $b=1$. For $x_i=\alpha_i+\nu_i/b<10^{-2}$ and $x_{i,12}=\alpha_{i,12}+\nu_{i,12}/b<10^{-2}$, P_N depends (up to the fourth decimal place) on x_i and $x_{i,12}$ only and not on the individual α_i,ν_i etc. that sum to x_i. P_N as given in (4.19) with $t=\delta^{-1}\ln[N(1-\varepsilon)]$ is plotted for three values of ε:

(i) ———— $\varepsilon=0$

(ii) - - - - - $\varepsilon=0.5$

(iii) — - — - $\varepsilon=0.9$

$$L_i(u) = \frac{\{1-e^{-(1-\varepsilon-a)u}\}}{(1-\varepsilon)^{-1}a_{i,12}\ e^{(1-\varepsilon-a)u} + 1-\alpha_{i,12}},$$

we have

$$U_i(u) - h_i(u) \geq 0, \text{ if } u \geq 0 \text{ and } 1-\varepsilon-2a > a^*_{i,12},$$

$$L_i(u) - h_i(u) \leq 0, \text{ if } u \geq 0,$$

and thus

$$U_i(u) \geq h_i(u) \geq L_i(u) \text{ for } u \geq 0 \text{ and } 1-\varepsilon-2a > a^*_{i,12}. \qquad \ldots(4.21)$$

By integrating the bounding functions over [0,t] we obtain

$$\int_0^t U_i(u)du = \frac{1}{\lambda}\left\{(k_0^{-1}+k_1^{-1})\left(\frac{k_0}{k_1}\right)^{1/2}\left[\tan^{-1}\left(\frac{k_1}{k_0}\right)^{1/2} - \tan^{-1}\left[\left(\frac{k_1}{k_0}\right)^{1/2} e^{-\lambda t}\right]\right] + \frac{1}{k_1}\left[e^{-\lambda t}-1\right]\right\}$$

and

$$\int_0^t L_i(u)du = \frac{1}{\lambda}\left\{\left(\frac{k_1+k_0}{k_1^2}\right)\ln\left[\frac{k_0+k_1}{k_0+k_1 e^{-\lambda t}}\right] + \frac{1}{k_1}\left[e^{-\lambda t}-1\right]\right\},$$

where $\lambda = 1-\varepsilon-a$, $k_0 = a_{i,12}(1-\varepsilon)^{-1}$, and $k_1 = 1-\alpha_{i,12}$. Now $t = (1-\varepsilon)^{-1}\ln(N(1-\varepsilon))$ and thus

$$e^{\lambda t} = [N(1-\varepsilon)]^{1-a(1-\varepsilon)^{-1}}.$$

For large N, $e^{-\lambda t} \simeq 0$. If also $(1-\varepsilon) >> a_{1,12}+a_{2,12}$ then k_0 is small and $k_1 \simeq 1$. If in addition $k_0^{-1/2}e^{-\lambda t}$ is small then,

$$\int_0^t U_i(u)du \simeq (1-\varepsilon-a)^{-1}\left\{\frac{\pi}{2}\left[a_{i,12}(1-\varepsilon)^{-1}\right]^{-1/2} - 1\right\}$$

and

$$\int_0^t L_i(u)du \simeq (1-\varepsilon-a)^{-1}\{\ln[(1-\varepsilon)/a_{i,12}]-1\}.$$

To this order of approximation, we have

$$(1-\varepsilon-a)^{-1}\left\{\frac{\pi}{2}\left[a_{i,12}(1-\varepsilon)^{-1}\right]^{-1/2} - 1\right\} > \int_0^t h_i(u)du > (1-\varepsilon-a)^{-1}\{\ln[(1-\varepsilon)/a_{i,12}]-1\}. \qquad \ldots(4.22)$$

In Chapter 3 we found that P_N (N fixed, single resistance) did not

depend upon ε. However $\int_0^t h_i(u)du$ does depend upon ε since if $\varepsilon=0$, $a<<1$, we have

$$\int_0^t U_i(u)du \simeq \frac{\pi}{2}\left[a_{i,12}\right]^{-1/2} - 1,$$

whereas if $1-\varepsilon\leq\left[a_{i,12}\right]^{1/2}$, we have

$$\int_0^t L_i(u) \simeq \left[a_{i,12}\right]^{-1/2}\left[-\frac{1}{2}\ln\left(a_{i,12}\right)-1\right].$$

Since $a_{i,12}<<1$, the lower bound can exceed the upper bound (for different ε). For example consider the inequalities for the two cases $\varepsilon=1-10^{-3}$ and $\varepsilon=0$ where $a_{i,12}=10^{-6}$, $a=10^{-5}$. Equation (4.22) implies $\int_0^t h_i(u)du$, and therefore P_N, varies with ε (in contrast to what was found for single resistance). Numeric evaluation of (4.20) for $10^{-9}\leq\alpha\leq10^{-1}$, $10\leq N\leq10^9$ reveals that the lower bound $\int_0^t L_i(u)du$ is close to $\int_0^t h_i(u)du$, and can be used in approximating P_N for most cases arising in practice. Using the right hand side of (4.22) and approximating $N^{1-a(1-\varepsilon)^{-1}}$ by N, $(1-\varepsilon)^{-a(1-\varepsilon)^{-1}}$ by 1, and $1-\varepsilon-a$ by $1-\varepsilon$ and using (4.20) in the resulting expression for $\ln P_N$ in (4.20) we obtain

$$\ln P_N \simeq -(1-\varepsilon)^{-1}N\{a_{12}(1-\varepsilon)+\sum_{i=1}^{2} a_i a_{i,12}[\ln((1-\varepsilon)/a_{i,12})-1]\}. \quad \ldots(4.23)$$

This approximation is of the form expected i.e. $P_N = \exp\{-a^*N\}$, where

$$a^* = a_{12} + (1-\varepsilon)^{-1}\sum_{i=1}^{2} a_i a_{i,12}\,[\ln((1-\varepsilon)/a_{i,12})-1]. \quad \ldots(4.24)$$

A similar approximation has been derived previously in a less general setting [27]. Note that a^* depends upon ε (i.e. d). If ε is large, say 0.9, the effect on P_N can be considerable. A similar effect on P_N would be seen for cases in which $\pi_1, \pi_2, \pi_{1,2}, \pi_{2,1}$ are not necessarily 0 although the curability of the tumor will then depend upon the total treatment

protocol.

The structure exhibited in equation (4.23) has implications for the general analysis of these processes. Resistance to some drugs appears to arise from a single discrete change in the genetic material. In such cases resistance may be almost absolute. In other cases resistance may arise incrementally, such as in processes involving gene amplification [28]. In these circumstances the acquisition of each gene copy may be viewed as a separate stage. Therefore the distribution of the numbers of cells possessing a specified level of resistance (i.e. some minimum number of gene copies) will be that of a multistage process and not that of a single stage process. This clearly represents a diffficult problem when attempting to analyze experiments designed to estimate mutation rates to drug resistance. Indeed in a multistage process there is no single parameter to estimate but rather a variable number depending on the number of stages involved. The number of stages would also be needed to be estimated (if not known) from such experiments and given the extremely variable nature of the basic process, it seems that estimation of parameters will be quite difficult. Furthermore, even when the number of stages is known, it is not possible (in general) to write down expressions for the distribution functions for the multistage process. This problem is in need of much more detailed exploration.

We will now consider the problem of planning treatment and how this model may be used in this context.

4.3 Optimal Scheduling

In attempting to find an optimal treatment plan it is necessary to consider two factors: a criteria which quantitatively measures the value

of a treatment plan, and the set of treatment regimens which are to be considered. Ideally the criterion would include measurement of both the therapeutic and toxic effects of a treatment plan on the subject. Unfortunately, the side effects of various treatment regimens are often difficult to describe in a quantitative form. We shall assume that each regimen to be considered has acceptable side effects and that the "value" of the therapy may be measured by its (tumor specific) therapeutic effects. A natural criterion for the value of any regimen is the probability of cure, since cure is the usual object of therapy. In this case P_N, the probability of cure for a tumor first treated at size N, will be defined as the limit $t\to\infty$ if $P\{N(t)=0|N(t_1)=N\}$. When all the tumor and drug parameters are known it is possible to examine the effect of various dosages and schedules of administration on the probability of cure for the tumor using equations (4.10), (4.15) and (4.18). In cases where cure is unlikely another "natural criterion" is the expected number of cells at some time after the completion of therapy $E[N(t)]$, $t>t_J$ where t_J is the time of the last treatment in the regimen. This quantity may be simply evaluated using (4.12.1-2) in conjunction with equations generalizing (3.12.1-2). For a given set of therapeutic regimens it seems desirable that the optimal regimen be the same for either criterion (P_N or $E[N(t)]$). Unfortunately this is not always the case, although in many cases of interest the optimal strategies are the same (as will be discussed later).

One way to restrict the set of possible protocols is to consider those of some fixed length, that is, those where there are a fixed number of times at which treatments are applied (protocols of fixed length).

Notice that it is always possible to "improve" a protocol, that is, increase P_N or decrease $E[N(t)]$, by adding further treatment applications to the end of the regimen. By this reasoning any protocol of length J-1 (number of cycles of therapy) will be no better than at least two protocols of length J (i.e. those which add a single cycle of either T_1 or T_2 to the protocol of length J-1). The length of the regimen will therefore depend on a decision about the value of any further increase in the probability of cure versus the "costs" (both human and financial) associated with extra cycles of treatment. Protocols of fixed length are of some interest since they correspond to the structure of many clinical protocols.

Another way to restrict the set of possible treatment regimens is to consider only those which satisfy some constraint placed on the measure of the therapeutic effect. That is, we can restrict attention to protocols for which $P_N \geq \Delta$ $(0<\Delta<1)$ or $E[N(t)]<k$ $(k>0$, where care must be taken in the selection of t used in this case). Sets of protocols (when not empty) satisfying such a condition are of some interest when it is desired to reduce the duration and quantity of therapy without unduly influencing therapeutic results. The optimal regimen will then be one where the number of treatments J is minimal, among regimens satisfying the condition imposed. Notice, that once J is determined then the optimal protocol of length J (determined from the set of protocols of length J) will be an optimal protocol by this criterion. Thus the optimal protocol of length J is of quite general interest. Examination of the efficacy of the optimal protocol of length J, for a range of values of J, is thus useful for determining both the length and "content"

of the protocol of clinical interest.

In principle the specification of the criterion for efficacy and the set of permissable protocols permit identification of the optimum regimen for a given set of tumor parameters, although this will usually be rather a lengthy exercise. Many tumor parameters are not under control either in the laboratory or in the clinic and thus it is not necessary to analyze the effect of changing these parameters on the optimal regimen (for a particular type of tumor). One parameter which is under control in the experimental setting is the size of the tumor at first treatment. In the clinic individual patients, with tumors of the same type, present with differing tumor burdens. It is thus of some interest to know whether regimens which are optimal for one size (at first treatment) are optimal at other sizes. In general the optimal regimen depends upon the size of the tumor when the first treatment is applied. Thus when identifying the optimal treatment plan for a particular situation, care must be taken to verify that the plan is optimal at all sizes likely to be encountered.

A practical problem arises in the therapy of clinical disease when few of the relevant tumor parameters are known with any accuracy. Clearly ignorance of the parameters makes it difficult to evaluate optimal strategies. However, it is possible to derive optimal rules in the particular case, where two drugs are of equal effectiveness. Since this case is of some practical interest, we will now examine it in some detail.

4.4 Optimum Scheduling for Two Equivalent Agents

One special case which is of some practical interest is the

situation where two drugs (or combinations) are available which are of approximately equal efficacy. This appears to arise in the treatment of Hodgkin's Disease where two combinations, MOPP (Nitrogen Mustard, Oncovin, Procarbazine and Prednisone) and ABVD (Adriamycin, Bleomycin, Vinblastine and Dacarbazine) produce similar cure rates and remission rates when delivered over the same time interval [29]. These observations suggest that the development of resistance to each combination proceeds at the same rate and that cell kills of each combination are similar. The available evidence also suggests that each combination is equally successful in producing remissions and cures in tumors which have previously failed with the other therapy. This implies that each combination's effect is approximately the same in cells resistant to the other. As a first approximation we may consider the two drug combinations as having equal values for the model parameters. In this situation we will refer to the two combination as being equivalent, and by that we will mean that each drug has identical values for all parameters.

In what follows we will model two agents as two individual drugs. When an agent consists of a combination of drugs this model must be considered a first approximation since resistance to multiple agents is more complex than that to a single agent (see discussion in Section 4.2). Explicitly two agents will be said to be equivalent if $\pi_{1,0}=\pi_{2,0}$, $\pi_{1,1}=\pi_{2,2}$, $\pi_{1,12}=\pi_{2,12}$, $\pi_{1,2}=\pi_{2,1}$, $\alpha_1=\alpha_2$, $\nu_1=\nu_2$, $\nu_{1,12}=\nu_{2,12}$ and the intertreatment times $t_{j+1}-t_j$, $j=1,\ldots,J-1$ are constant. In this case, if

$$\Psi(s_0,s_1,s_2,s_3;0) = \Psi(s_0,s_2,s_1,s_3;0), \qquad \ldots(4.25)$$

that is

$$P\{R_1(0)=i,R_2(0)=j|R_0(0),R_{12}(0)\}=P\{R_1(0)=j,R_2(0)=i|R_0(0),R_{12}(0)\}$$

then for $t<t_1$ (the time of first treatment), we have

$$\Psi(s_0,s_1,s_2,s_3;t) = \Psi(s_0,s_2,s_1,s_3;t). \qquad \ldots(4.26)$$

Here we will assume (4.25) holds, which is reasonable since otherwise we would expect the response of the tumor to therapy by T_1 (alone) to be different from the response to T_2 (alone) and thus the agents would not appear equivalent.

This definition of equivalent agents has been used previously in the consideration of the effects of cancer therapy [30,31]. Intertreatment times are usually selected to be the minimum times necessary for the recovery of normal tissues between cycles of treatment. By assuming that intertreatment times are the same for each treatment we indicate that the minimum recovery time for each treatment is the same. The term "equivalent" is motivated by the observation that if either of the drugs is used alone then the distribution of the total number of cells will be the same for each drug. Note that from the general definition of the resistant states, we have $\pi_{i,0}<\pi_{i,i}\leq\pi_{i,12}$ and $\pi_{i,3-i}<\pi_{i,i}$ for $i=1,2$. The tumor parameters b, c and d are fixed and will not be explicitly specified.

As noted before (Section 3.3), chemotherapy is given in repeating cycles for clinical disease in which the doses and drugs used are fixed in advance [32]. The intervening time between repeat applications is determined by the recovery time of the patients' normal tissues. This recovery time is selected to be the minimum time for the necessary recovery. Protocols which administer the cycles at greater than the minimum interval will be less effective than those giving the same drugs

at the same dose in the same sequence as frequently as permissable, since longer intertreatment times allow more time for regrowth.

We will now consider the construction of optimal rules for sequencing the administration of two equivalent agents. In this section we will consider the construction of the optimal treatment regimen within the set of protocols of fixed length J (number of times a treatment is applied). We will refer to the treatment plan as a strategy, which represents the sequence in which treatments are administered (the times of administration being already specified).

First we will fix J, the number of times of administration of treatments in the regimen. A therapeutic strategy, S, will be represented by a vector which consists of a sequence of J 1's or 2's with each number referring to the subscript of the treatment given (either T_1 or T_2), and the sequence indicating the order in which they are given. There will be 2^J such strategies and we will write $S(\nu)$ when we wish to refer to a particular strategy in the set. A solution to the fixed length problem, which of course will depend on J, will be referred to as an optimal strategy for each criteria of treatment efficacy. At least one optimal strategy exists because the number of strategies of fixed length J is finite. When a tumor is treated with strategy $S(\nu)$ we will write the probability of cure as $P_N(S(\nu))$ and the expected number of cells as $E[N_{S(\nu)}(t)]$.

Having defined the set of strategies to be considered it remains to specify the criterion for the efficacy of the therapy. As before, two natural candidates are P_N and $E[N(t)]$. From (4.25) and general considerations of the behaviour of the process, at least two distinct

strategies have the same value of P_N because the drugs are equivalent and each strategy has a "mirror image" (i.e. 1's and 2's interchanged). Similar considerations apply to E[N(t)] and to any symmetric functional (with respect to $R_1(t)$ and $R_2(t)$) of the distribution of $\{R_0(t),R_1(t),R_2(t),R_{12}(t)\}$. We wish to show that there exist optimal strategies which are independent of the drug and tumor parameters for any pair of equivalent drugs. Such optimal strategies do exist for the criterion E[N(t)] as we will show subsequently. Unfortunately these strategies are not necessarily optimal for P_N, as will be shown by producing a counterexample (see Chapter 5).

We may formally link minimizing E[N(t)] and maximizing P_N under particular circumstances as follows. For two strategies S(i) and S(j), if

$$P\{N_{S(i)}(t)\geq k\}\geq P\{N_{S(j)}(t)\geq k\} \text{ for all } k, \qquad \ldots(4.27)$$

then it follows immediately that

$$E[N_{S(i)}(t)] \geq E[N_{S(j)}(t)] \text{ and } P_N(S(i)) \leq P_N(S(j)).$$

Thus the intuitive idea of minimising E[N(t)] will also be formally equivalent to maximising $P_N(S)$ in situations where the rather strong condition (4.27) of stochastic ordering applies. However, it is doubtful that this condition could ever be verified in practice.

The particular situation of equivalent drugs permits the consideration of criteria of efficacy other than P_N and E[N(t)]. A second quantity which can be minimized may be motivated by the consideration of the resistant subcompartments of the tumor. Initially we observe that cells in R_0 "see only" one drug, since each drug has the same effect on R_0 cells. Thus the only component of the

strategy which affects the distribution of the number of cells in R_0 at time $t>t_J$, is the length J of the strategy. Similarly the effect of the strategy on cells already in R_{12} at t_1^- depends only on the length J and not on the order in which the drugs are given. Therefore the "value of" strategies result from their differential effect on the cells in R_1 and R_2.

For any strategy to be of some value it must be capable of causing a net overall decline in the mean number of singly resistant cells. This, of course, does not follow from any formal constraints placed on this model but from a consideration of what this would imply about the regrowth of the resistant cells. It is possible for treatments to eliminate singly resistant compartments (when treatment is given) with a non-negligible probability, even though the net mean growth of these cells may be positive because of a very large regrowth between treatments. There is no evidence to suggest that this occurs in clinical disease although there are probably many cases where small cell kills are 'balanced' by regrowth between treatments. An interesting case of this has been identified by Skipper in his analysis of the response of a mouse mammary tumor to treatment by the CAF (Cyclophosphamide, Adriamycin and 5-Fluorouracil) regimen [33].

The objective of the therapeutic strategy is to cause a net decline (to extinction) of the singly resistant cells in such a way as to minimize the number of transitions to double resistance. Clearly, the cases of greatest importance are those where the growth of the cells already in R_{12} cannot be made subcritical and it is necessary to plan the strategy so that the likelihood of transitions from the singly

resistant to the doubly resistant state is minimized. Even in cases where the chemotherapy can make the growth of doubly resistant cells subcritical (over the treatment plan) one would wish to minimize the number of new doubly resistant cells since they are by definition the most difficult to treat. We will now develop an expression for the mean number of transitions from single to double resistance during the treatment period and subsquently derive the form of strategies which minimize this quantity.

Consider the number of cells in R_1 at any time $t\varepsilon(t_j, t_{j+1})$ which are derived from cells in R_1 at time t_j. Conditional on $R_1(t_j)$ the expected number of such cells at time t, $g_1(t_j, t)$, is given by the first term on the right hand side of (4.12.1) with $m_i(0)=R_1(t_j)$:

$$g_1(t_j,t) = R_1(t_j) \exp\{(\delta-a_{1,12})(t-t_j)\}.$$

Conditional on $R_1(t_j)$, the expected number of transitions to double resistance in the interval (t_j,t) by these cells, $\mu_{R_1}(t_j,t)$, is given by

$$\mu_{R_1}(t_j,t) = a_{1,12}\int_{t_j}^{t} g_1(t_j,u)du.$$

The simplest way to obtain the above relationship is to consider cells in R_1 as being sensitive (to T_2) and in R_{12} as being resistant (to T_2) and use the differential equations leading to (3.8) as follows. Set $m_0(t)=g_1(t_j,t)$, $\alpha b+\nu=a_{1,12}$ and $m_1(t)=\mu_{R_1}(t_j,t)$. Then solve the differential equation for $\mu_{R_1}(t_j,t)$ noting that the term $\delta\mu_{R_1}(t_j,t)\equiv 0$ because here we are counting the number of transitions (which have no intrinsic growth). Letting $\mu_1(t_j,t)=E[\mu_{R_1}(t_j,t)]$, it follows that

$$\mu_1(t_j,t) = a_{1,12}\int_{t_j}^{t} m_1(t_j)e^{(\delta-a_{1,12})(u-t_j)}\,du. \qquad \ldots(4.28)$$

Similarly we may define $\mu_2(t_j,t)$ for transitions from R_2 to R_{12}. The expected number of transitions to double resistance in $[t_j,t_{j+1})$ from singly resistant cells at t_j is thus $\mu_1(t_j,t_{j+1})+\mu_2(t_j,t_{j+1})$. Thus the mean number of those events which occur in some $[t_j, t_{j+1})$ for $j=0,\ldots,J$ ($t_0=0$) is given by

$$M' = \sum_{j=0}^{J} \sum_{i=1}^{2} \mu_i(t_j,t_{j+1}), \qquad \ldots(4.29)$$

where for simplicity we set $t_{J+1}=t_J+(t_J-t_{J-1})$. We seek strategies which minimize (4.29). In seeking to minimize (4.29) we may minimize any function of the form $KM'+C$ where K (>0) and C are not dependent upon the strategy. In particular we may replace each term $\mu_i(t_j,t_{j+1})$ of the form (4.28) by

$$a_{i,12}\int_{t_j}^{t_{j+1}^-} \{m_i(t_j) + \frac{a_i m_0(t_j)[e^{(k-\delta+a_{i,12})(u-t_j)}-1]}{[k-\delta+a_{i,12}]}\} e^{(\delta-a_{i,12})(u-t_j)} du$$

$$= a_{i,12}\int_{t_j}^{t_{j+1}^-} m_i(u)du$$

from (4.12.1). The added terms do not depend upon the strategy and thus minimizing M' is equivalent to minimizing M^*, where

$$M^* = \sum_{j=1}^{J} \int_{t_j}^{t_{j+1}^-} [m_1(u)+m_2(u)]du. \qquad \ldots(4.29)$$

Minimization of this quantity has previously been considered for the special case $c=d=0$, $\pi_{i,i}=\pi_{i,12}=1$ [31]. We will now proceed to characterize the strategies which minimize (4.29) and then show that these strategies also minimize $E[N(t)]$, $t\geq t_J$.

In order to do this we now define some new quantities. Let $E_{i,j}(t)$ be the expected number of cells resistant to T_i alone at time t, which derive from (have grown from, including the effects of treatment) "new

mutations" (transitions from R_0 to R_i) in the interval $[t_{j-1}, t_j)$ for $j=1,\ldots,J+1$. Define $E_{i,j}(t)=0$ if $t\leq t_{j-1}$. Singly resistant cells present at t=0 will be included in the interval $[t_0,t_1)=[0,t_1)$. Then for i=1,2 and $u\leq t_{J+1}$

$$m_i(u)=\sum_{k=1}^{J+1} E_{i,k}(u),$$

and we may then write (4.29) as

$$M^*=\sum_{j=1}^{J}\int_{t_j}^{\bar{t}_{j+1}}\sum_{k=1}^{j+1}[E_{1,k}(u)+E_{2,k}(u)]du,$$

which gives

$$M^*=\sum_{j=1}^{J}\sum_{k=1}^{j}\int_{t_j}^{\bar{t}_{j+1}}[E_{1,k}(u)+E_{2,k}(u)]du$$

$$+\sum_{j=1}^{J}\int_{t_j}^{\bar{t}_{j+1}}[E_{1,j+1}(u)+E_{2,j+1}(u)]du. \qquad \ldots(4.30)$$

The second term on the right hand side of (4.30) represents "new mutations" arising from cells in R_0 between cycles of treatment which have not been exposed to either drug. Conditional on the treatment times and the number of treatment cycles, the distribution of cells in R_0 is the same for all treatment strategies (because the two drugs are equivalent) for abitrary t, and thus the second term is the same for all such strategies. Thus minimizing M* is equivalent to minimizing the first term on the right hand side of (4.30).

The growth of $E_{i,k}(u)$ over $[t_j,t_{j+1}]$ for all j, k where $j\geq k$ is exponential with parameter $(\delta-a_{i,12})$ (see first term in (4.12.1)). Thus

$$\int_{t_j}^{\bar{t}_{j+1}} E_{i,k}(u)du = E_{i,k}(t_j)(\delta-a_{i,12})^{-1}\{\exp[(\delta-a_{i,12})(t_{j+1}-t_j)]-1\}.$$

Using (4.30) we have

$$M^* = (\delta - a_{1,12})^{-1} \exp\{(\delta - a_{1,12})(t_2 - t_1) - 1\} M + K$$

(since $a_{1,12} = a_{2,12}$ and $t_{j+1} - t_j$ is constant for $j=1,\ldots,J$) where K is a constant given by the second term of (4.30) and

$$M = \sum_{j=1}^{J} \sum_{k=1}^{j} \sum_{i=1}^{2} E_{i,k}(t_j). \qquad \ldots(4.31)$$

Thus minimizing M* is equivalent to minimizing M.

We will now proceed to develop our notation in order to explicitly minimize M and thus minimize M'. Define

$$C_k(S(\nu)) = \sum_{i=1}^{2} \sum_{j=k}^{J} E_{i,k}(t_j),$$

where the $E_{ik}(t)$ are calculated for the treatment strategy $S(\nu)$; then

$$M = \sum_{k=1}^{J} C_k(S(\nu)) .$$

Define $\delta_j(\nu) = 1$ if the j-th treatment in $S(\nu)$ is T_1,

$= 0$ otherwise, $\qquad \ldots(4.32)$

and let

$$X_k(S(\nu),\ell) = \left| \sum_{j=k}^{\ell} \delta_j(\nu) - [1-\delta_j(\nu)] \right|, \text{ for } 1 \leq k \leq \ell \leq J,$$

the modulus of the number of times T_1 is given minus the number of times T_2 is given between the k-th and ℓ-th times of treatment. For $k=1,\ldots,J$, let

$$K_k\{S(\nu)\} = \{i : X_k(S(\nu),i) = \max_{k \leq \ell \leq J} X_k(S(\nu),\ell)\}, \qquad \ldots(4.33)$$

the indices of treatment times where the modulus of the difference in the number of T_1's and T_2's is maximized commencing at k. Let

$$B_k = \{S(\nu) : \max_{k \leq \ell \leq J} X_k(S(\nu),\ell) = 1\} \text{ for } k=1,\ldots,J, \qquad \ldots(4.34)$$

the set of strategies where, commencing at the k-th time of treatment,

the maximum modulus of the difference in the number of times T_1 and T_2 are given equals 1. Let

$$g = \exp\{(\delta - a_{1,12})\ (t_{j+1} - t_j)\} \text{ for } j=1, \ldots, J.$$

As before,

$$\pi_{i,Q} = P\{\text{a cell in } R_Q \text{ will survive one cycle of } T_i\} \text{ for } i = 1,2$$

To simplify notation, let $\pi_0 = \pi_{1,1}(=\pi_{2,2})$ and $\pi_1 = \pi_{1,2}(=\pi_{2,1})$ where $\pi_1 < \pi_0$ from the general definition of resistance. By equivalence $E_{1,j}(\bar{t_j}) = E_{2,j}(\bar{t_j})$ and we will let $E_j = E_{i,j}(\bar{t_j})$. Define

$$n_j(k) = \sum_{i=j}^{k} \delta_i(\nu),$$

(see 4.32), the number of times T_1 is given between the j-th and k-th cycles of therapy where reference to the strategy, indexed by ν, is supressed for simplicity.

Using this notation it is then straightforward to show

$$C_j(S(\nu)) = E_j \sum_{k=j}^{J} g^{k-j} \left[\pi_0^{n_j(k)}\, \pi_1^{k-j+1-n_j(k)} + \pi_0^{k-j+1-n_j(k)}\, \pi_1^{n_j(k)}\right]. \quad \ldots(4.35)$$

We see from (4.35) that, as expected, mirror image strategies (i.e. 1 and 2's interchanged) have the same value of $C_j(S(\nu))$ since E_j does not depend on $S(\nu)$.

Having developed the required notation we will now show that M given by (4.31) and thus M′ given by (4.29) is minimized by the alternating strategies $\{1,2,1,2,\ldots\}, \{2,1,2,1,\ldots\}$ amongst those of fixed length J.

THEOREM 1

Among all strategies of fixed length J, $M = \sum_{j=1}^{J} C_j(S(\nu))$ is minimized only by the two strategies which alternate therapy at each cycle.

Proof:

The proof will be achieved by characterizing the strategies $S(\nu)$ which minimize $C_j(S(\nu))$ for abitrary j. We will then show that the alternating strategies minimize $C_j(S(\nu))$ for all j. The proof will consist of three parts:

(i) Choose arbitrary j. For any $S(\nu)$ not in B_j (see (4.34)), there exists $S(\nu^*) \varepsilon B_j$ such that $C_j(S(\nu^*)) < C_j(S(\nu))$.

(ii) If $S(\nu)$, $S(\nu^*) \varepsilon B_j$ then $C_j(S(\nu)) = C_j(S(\nu^*))$.

(iii) If $S(1) = (1,2,1,2,\ldots)$ and $S(2) = (2,1,2,1,\ldots)$ then $S(1)$ and $S(2)$ minimize $\sum_{j=1}^{J} C_j(S(\nu))$ among all strategies of length J.

(i) Choose arbitrary j. If $S(\nu)$ is not in B_j choose one $k \varepsilon K_j(S(\nu))$, as defined in (4.33). Consider first the case $k<J$ and $\delta_k(\nu)=1$, that is the k-th cycle is T_1. Let σ_k be the operator which interchanges the k-th and k+1-st elements of a strategy. Now $k \varepsilon K_j(S(\nu))$ and $k<J$ implies that $S_{k+1}(\nu)=2$. Consider the strategy $\sigma_k S(\nu)$. Using (4.35) we have

$$C_j(S(\nu)) - C_j(\sigma_k S(\nu))$$
$$= E_j g^{k-j} [\pi_0^{n_j(k)-1} \pi_1^{k-j+1-n_j(k)} (\pi_0-\pi_1) + \pi_0^{k-j+1-n_j(k)} \pi_1^{n_j(k)-1} (\pi_1-\pi_0)]$$

where $n_j(k)$ is calculated for $S(\nu)$. Thus we may write

$$C_j(S(\nu)) - C_j(\sigma_k S(\nu))$$
$$= E_j g^{k-j} [\pi_0^{n_j(k)-1} \pi_1^{k-j+1-n_j(k)} - \pi_0^{k-j+1-n_j(k)} \pi_1^{n_j(k)-1}]$$

where $E_j g^{k-j}$ does not depend on $S(\nu)$.

Now since $S(\nu)$ is not in B_j and $\delta_k(\nu)=1$, we have $n_j(k) > (k-j+2)/2$, Since $\pi_0>\pi_1$ we have $\pi_0^{n_j(k)-1} \pi_1^{k-j+1-n_j(k)} > \pi_0^{k-j+1-n_j(k)} \pi_1^{n_j(k)-1}$

and thus

$$C_j(S(\nu)) - C_j(\sigma_k S(\nu)) > 0.$$

For $k<J$ and $\delta_k(\nu)=0$ then we can also show the above result using similar considerations. For $\{J\}=K_j(S(\nu))$ consider the strategy $S(\nu')$ where the J-th treatment is replaced by the other treatment and obtain a similar inequality for $C_j(S(\nu))-C_j(S(\nu'))$.

We may now apply the same considerations to the new strategy which we have created (either $\sigma_k S(\nu)$ or $S(\nu')$) and obtain a sequence of distinct strategies, $\{S(\nu)\}$ say, which have strictly decreasing $C_j(S(\nu))$. Now the number of possible strategies is finite (for finite J), and this process of producing new strategies must terminate since each strategy is distinct. Since there is at least one ν such that $S(\nu) \in B_j$ (and the process of improving strategies is valid for all ν such that $S(\nu)$ is not in B_j) we conclude that the sequence of strategies terminates with the last member being contained in B_j. This proves the desired result.

(ii) For all j, $1 \leq j \leq J$, B_j contains $2^{j+[(J-j)/2]}$ elements and therefore consider the non-trivial case $S(\nu) \neq S(\nu^*)$. Using (4.35) we have

$$C_j(S(\nu)) - C_j(S(\nu^*)) =$$

$$E_j \sum_{k=j}^{J} g^{k-j} \left[\pi_0^{n_j(k)} \pi_1^{k-j+1-n_j(k)} + \pi_0^{k-j+1-n_j(k)} \pi_1^{n_j(k)} - \pi_0^{n_j^*(k)} \pi_1^{k-j+1-n_j^*(k)} - \pi_0^{k-j+1-n_j^*(k)} \pi_1^{n_j^*(k)}\right]. \quad \ldots(4.36)$$

where $n_j^*(k)$ is calculated for strategy $S(\nu^*)$. Since $S(\nu)$, $S(\nu^*) \in B_j$ we have

$$n_j(k) = \frac{k-j+1}{2} = n_j^*(k) \text{ for } k-j+1 \text{ even},$$

and

$$n_j(k) = n_j^*(k) \text{ or } n_j(k) = k-j+1-n_j^*(k) \text{ for } k-j+1 \text{ odd}.$$

Thus each term in the sum (4.36) is zero, and therefore $C_j(S(\nu)) = C_j(S(\nu^*))$, proving the required result.

(iii) Now $S(1), S(2) \in B_j$ for all $1 \leq j \leq J$. Furthermore only $S(1)$ and $S(2)$ have this property. But $S(1)$ and $S(2)$ minimize $C_j(S(\nu))$ for all j and thus only $S(1)$ and $S(2)$ minimize

$$M = \sum_{j=1}^{J} C_j(S(\nu)).$$

The proof is complete.

The proof of a special case of this theorem ($c=d=0$, $\pi_{i,i}=\pi_{i,12}=1$) has been presented previously [31]. We will now show that the alternating strategies minimize $E[N(t)]$ for $t>t_J$.

Theorem 2

Among the strategies of fixed length J, $S(1)$ and $S(2)$ minimize $E[N(t)]$ for arbitrary $t>t_J$.

Proof:

We will evaluate $E[N(t)]$ at time $t_{J+1}=t_J+(t_J-t_{J-1})$ (as before) without loss of generality. Consider the development of doubly resistant cells in the interval $[t_j, t_{j+1})$ for $j=0,\ldots J$ from cells which were not doubly resistant at time t_j. Each such cell must have grown from one of three types of progenitor at time t_j, i.e. either a R_0, a R_1 or a R_2 cell. The treatment sequence does not affect the distribution of $R_0(t)$ (only the length does because of equivalent treatments), so the number of doubly resistant cells at t_{j+1}^- derived from R_0 cells at t_j does not depend on the treatment sequence. Thus the differential effect of various strategies on the number of doubly resistant cells at time t, $t_j<t<t_{j+1}$, results from its differential effect on singly resistant cells

present at the treatment times $t_1,\ldots t_j$. We will now calculate the expected number of doubly resistant cells which have arisen from singly resistant cells present at treatment times.

Let $R_{12}(t,t',t'')$ $(t''>t',\ t\geq t')$ be the number of doubly resistant cells present at time t whose progenitor (first doubly resistant cell) originated as a mutation from a singly resistant cell (either R_1 or R_2) in $[t',\ t'')$. Using (4.12.2) we can write

$$E[R_{12}(\bar{t}_{k+1},\ t_k,\ t_{k+1})|R_1(t_k),\ R_2(t_k)] = [R_1(t_k) + R_2(t_k)]\ h, \quad \text{for } k=1,\ldots,J, \qquad \ldots(4.37)$$

where $h=e^{\delta(t_{k+1}-t_k)}(1-e^{-a_{1,12}(t_{k+1}-t_k)})$. Let $\pi_2=\pi_{1,12}$ $(=\pi_{2,12})$ then

$$E[R_{12}(t_j)] = \pi_2\ E[R_{12}(\bar{t}_j)] \text{ for } j=1,\ldots J.$$

If we let $g^*=e^{\delta(t_{k+1}-t_k)}$ then we have by (4.12.2)

$$E[R_{12}(t_{J+1},\ t_k,\ t_{k+1})|R_1(t_k),\ R_2(t_k)] = [R_1(t_k)+R_2(t_k)]\ h\ (\pi_2 g^*)^{J-k}, \text{ for } k=1,\ldots J. \qquad \ldots(4.38)$$

From the same considerations used in deducing (4.35) we have

$$E[R_1(t_k) + R_2(t_k)] = \sum_{j=1}^{k} E_j g^{k-j} \{\pi_0^{n_j(k)}\ \pi_1^{k-j+1-n_j(k)} + \pi_0^{k-j+1-n_j(k)}\ \pi_1^{n_j(k)}\}, \qquad \ldots(4.39)$$

where E_j and $n_j(k)$ are the same as in the proof of Theorem 1. Thus using (4.37), (4.38) and (4.39) we have

$$E[R_{12}(t_{J+1},t_1,t_{J+1})] = \sum_{k=1}^{J} h\ (\pi_2 g^*)^{J-k} \sum_{j=1}^{k} E_j\ g^{k-j} \{\pi_0^{n_j(k)}\ \pi_1^{k-j+1-n_j(k)} + \pi_0^{k-j+1-n_j(k)}\ \pi_1^{n_j(k)}\}. \qquad \ldots(4.40)$$

Now $E[R_{12}(t_{J+1})] = E[R_{12}(t_{J+1},t_1,t_{J+1})] + E[R_{12}(t_{J+1},t_0,t_1)]$, where $E[R_{12}(t_{J+1},t_0,t_1)]$ does not depend on the strategy $S(\nu)$. Also from (4.39)

we have

$$E[R_1(t_{J+1}) + R_2(t_{J+1})]$$
$$= \sum_{j=1}^{J+1} E_j\, g^{J-j+1} \{\pi_0^{n_j(J)}\, \pi_1^{J-j+1-n_j(J)} + \pi_0^{J-j+1-n_j(J)}\, \pi_1^{n_j(J)}\}, \quad \ldots(4.41)$$

where $n_j(k)=0$ if $j>k$. Using (4.40) and (4.41) we obtain

$$E[N(t_{J+1})] = E[R_0(t_{J+1})] + E[R_1(t_{J+1})] + E[R_2(t_{J+1})] + E[R_{12}(t_{J+1})]$$
$$= K + \sum_{j=1}^{J+1} E_j g^{J-j+1} \{\pi_0^{n_j(J)}\, \pi_1^{J-j+1-n_j(J)} + \pi_0^{J-j+1-n_j(J)}\, \pi_1^{n_j(J)}\}$$
$$+ \sum_{k=1}^{J} h\,(\pi_2 g^*)^{J-k} \sum_{j=1}^{k} E_j\, g^{k-j} \{\pi_0^{n_j(k)}\, \pi_1^{k-j+1-n_j(k)} + \pi_0^{k-j+1-n_j(k)}\, \pi_1^{n_j(k)}\}$$
$$\ldots(4.42)$$

where K does not depend on the strategy $S(\nu)$.

The terms within the summations in (4.42) have both been seen to be minimized by strategies belonging to B_j; it follows that the summations are uniquely minimized by S(1) and S(2). Thus S(1) and S(2) minimize $E[N(t)]$ for $t>t_J$. This completes the proof of the theorem.

We have found that there is one "pattern" of strategies which is optimal (in terms of minimizing E[N(t)]) for any treatment parameters providing the two drugs are equivalent. This property is extremely convenient since in any situation where treatment must be stopped early (i.e. patient toxicity or refusal), the truncated regimen is then optimal for the number of treatments given. Similarly if it is decided to increase the treatment regimen we may still construct the optimal plan of the required length by adding cycles of the drugs to the pre-existing regimen.

As previously indicated, however, the probability of cure P_N, is not

necessarily maximized by those strategies which minimize E[N(t)] for $t>t_J$ (or minimize M') when treatments are equivalent. An example of this is given in Chapter 5 and the accompanying discussion suggests that this phenomena will only occur in the particular set of circumstances when regrowth between treatments is large and the composite process of treatment and regrowth (for singly resistant cells) is not strongly subcritical. This situation is unlikely to be encountered in human disease since growth over periods of one month (which is greater than most intertreatment intervals) is modest for the majority of human tumors. However, such conditions may be encountered in several experimental cancers where doubling times in the order of twelve hours are not uncommon.

The two theorems, with the preceding discussion, indicate that in cases of human cancer where two equivalent agents are available, which may not be used concurrently, the best way to use these two will be in an alternating strategy. This result is of interest both because of its generality (it does not depend on the particular parameter values) and because it is not current clinical practice.

In clinical medicine protocols are developed whereby active agents are combined, as much as possible, into regimens which are then repeated a fixed number of cycles. Where two such regimens are available the common practice is to use one continuously until there is evidence of relapse when the other regimen is employed. Conversely, although alternating strategies represent a departure from clinical practice, they are compatible with the clinical concept of combination chemotherapy. Combination chemotherapy uses drugs given at constant times during a

cycle and this cycle is repeated a fixed number of times. In each regimen the drugs are frequently not given simultaneously but on different days. An alternating regimen can be viewed as combination chemotherapy with repeated cycles of the regimen T_1T_2 (or T_2T_1) over a longer intertreatment interval.

4.5 Discussion

The identification of optimal strategies (i.e. those which maximize P_N) represents a considerable problem in computation when the parameters are known. For example, when J=12 there are 2^{12} possible strategies. Thus it is desirable to seek heuristics to reduce the set of strategies which must be considered. For a strategy to be effective the treatments must be able to make the net growth of $R_0(t)$, $R_1(t)$ and $R_2(t)$ subcritical (over the treatment period); otherwise no cure is possible. In particular the cells present at time t_1 in R_0, R_1 and R_2 must be eliminated with a "large" probability. Following this reasoning we infer that the expected number of these cells should be small at completion of the treatment regimen. That is, "reasonable" strategies would be expected to satisfy,

$$E[R_i(t_1)]\ [\pi_{1,i}^{n_1(J)}\ \pi_{2,i}^{J-n_1(J)}] < k, \qquad \ldots(4.43)$$

for i=0,1,2 where k is chosen as a function of d (i.e. it will be larger if the death rate is larger; a possible choice is $k=0.5(1-\varepsilon)^{-1}$). In certain cases the set of inequalities (4.43) may provide useful lower and upper bounds on $n_1(J)$ (i.e. not 0 and J), thus eliminating some strategies from consideration. These inequalities may also indicate that J is too small so that the search for an optimal rule of length J may not be of great use.

The search for optimal strategies, using P_N as the criterion, has been examined in considerable detail by Day [34], who considered 16 strategies (chosen to "span" the set of possible strategies) for the case J=12, and calculated their effect on the probability of cure for 256 different combinations of drug and tumor parameters. He showed that it is possible to identify certain patterns in the best (of the 16) treatment strategies as the degree of asymmetry in the parameters of the two drugs increases. In a particular clinical problem strategies "close" to the best of the 16 determined by Day could be examined. The details of such a search remain to be worked out and we will return to this problem in Chapter 5.

It should be remarked that the assumption of a fixed number of treatments may not be a reasonable model for the clinical situation when the two drugs have different recovery times before further therapy is possible. In such cases it may be more reasonable to fix the total treatment interval $[t_1,t']$ where J will be chosen so that $t_J<t'$. If the tumor parameters are known then it is straightforward, although computationally demanding, to calculate the optimum strategy. In order to treat the problem of optimizing strategies comprehensively, we need a precise statement of the relationship between dose and toxicity for each of the drugs. If this were specified then it would be possible to construct optimum dosages as well as optimum schedules. However little theoretical work has been undertaken in this area and at present it is not possible to include considerations of toxicity in modelling the effects of treatment. This concludes the consideration of optimizing treatment strategies. We will now consider variation in mutation rates

on the development of double resistance.

4.6 Variation in the Mutation Rates

In the previous chapter dealing with resistance to a single agent we examined the effect of variation in the rate $a=\alpha+\nu/b$ (Section 3.10). Here (in analogy to the case of single resistance) we will consider variations in mutation rates where the rates for an individual tumor are fixed but follow a distribution for tumors of that type. In particular we will consider variations in the vector of parameters $\underset{\sim}{A}^*$, where

$$\underset{\sim}{A}^* = (A_1, A_2, A_3, A_4, A_5)$$
$$= (\alpha_1 b+\nu_1, \alpha_2 b+\nu_2, \alpha_{1,12} b+\nu_{1,12}, \alpha_{2,12} b+\nu_{2,12}, \alpha_{12} b+\nu_{12}).$$

We will assume $\alpha_{12} b+\nu_{12} = 0$ since we are primarily interested in examining the effect of variation in rates on the two step development of double resistance; the one step process having been essentially covered in Section 3.10. Thus we will consider $\underset{\sim}{A}$, the first four elements of $\underset{\sim}{A}^*$, at this point although we will consider $\underset{\sim}{A}^*$ later in a different context. Also, because the distribution function of $\{R_0(t), R_1(t), R_2(t), R_{12}(t)\}$ cannot be obtained in explicit form, we will (as in Section 3.10) consider the effect of variations in $\underset{\sim}{A}$ on the probability of cure. The scale of measurement of t is, of course, arbitrary. In order to simplify presentation we will assume, without any loss of generality, that t is measured on a scale for which b=1.

The probability of cure depends on the treatment strategy for arbitrary $\pi_{i,Q}$. In analogy with the case for single resistance we will only consider the special case $\pi_{1,0}=\pi_{2,0}=\pi_{1,2}=\pi_{2,1}=0$, $\pi_{1,12}=\pi_{2,12}=1$ ($\pi_{1,1}$ and $\pi_{2,2}$ are arbitrary) and assume that both drugs are given together. Thus all cells, except the doubly resistant ones, are eliminated by the

first application of the combination of the two drugs. In this case the probability of cure depends only on the first time of administration of the combination since subsequent application has no effect on the remaining doubly resistant stem cells. Even in this case the probability of cure is a complicated function (involving integrals) and thus we will use the approximation given by (4.23). In what follows we will assume that $\underset{\sim}{A}$ is random and will indicate the dependence of P_N on $\underset{\sim}{A}$ by writing writing $P_N(\underset{\sim}{a})$. We wish to select a distribution for $\underset{\sim}{A}$ which leads to an expression for $E[P_N(\underset{\sim}{A})]$ which is reasonably simple to calculate. We assume that there exists a density function for the random variable $\underset{\sim}{A}$, $f(\underset{\sim}{a})$ say. Unfortunately, little information is available as to the form of $f(\underset{\sim}{a})$ since no experiments have been undertaken to attempt to identify it.

Given our ignorance on the form of $f(\underset{\sim}{a})$ it seems reasonable to require that $f(\underset{\sim}{a})$ have structure which accords with our physical understanding about the nature of the processes involved. We have, generally,

$$f(\underset{\sim}{a}) = g(a_3,a_4|a_1,a_2)\, h(a_1,a_2),$$

where $g(a_3,a_4|a_1,a_2)$ is the density of (A_3,A_4) conditional on $(A_1=a_1,A_2=a_2)$ and $h(a_1,a_2)$ is the marginal density of (A_1,A_2). We postulate here that (A_3,A_4) are conditionally (on (A_1,A_2)) independent:

$$g(a_3,a_4|a_1,a_2) = g_1(a_3|a_1,a_2)\, g_2(a_4|a_1,a_2),$$

where $g_1(a_3|a_1,a_2)$ and $g_2(a_4|a_1,a_2)$ are the marginal densities of A_3 and A_4 respectively, conditional upon $(A_1=a_1,A_2=a_2)$. Also we postulate that

$$g_1(a_3|a_1,a_2)=g_1(a_3|a_2), \qquad g_2(a_4|a_1,a_2)=g_2(a_4|a_1),$$

that is, the development of resistance to T_j in cells resistant to T_i

is dependent only on the realized parameter for the acquisition of resistance to T_j in sensitive cells. Combining the above postulates we have,

$$f(\underset{\sim}{a}) = g_1(a_3|a_2)\, g_2(a_4|a_1)\, h(a_1,a_2). \qquad \ldots(4.44)$$

This implies that if A_1 and A_2 are marginally independent then (A_2,A_3) and (A_1,A_4) are independent. The structure for $f(\underset{\sim}{a})$ expressed in (4.44) seems a reasonable simplification to impose since it implies that the pairs (A_2,A_3) and (A_1,A_4) are independent if, and only if, A_1 and A_2 are independent. Also the distribution of rates to double resistance depends only on the analogous rates to single resistance. In common with the single resistance case (section 3.10) we will use a beta distribution to model variation in the mutation rates as detailed below.

Reference to (4.44) shows that there are three seperate densities whose form must be specified. We would like to model $h(a_1,a_2)$ by a bivariate beta distribtuion. The "natural" bivariate beta distribution (which is obtained by conditioning on sums of gamma random variables) has a negative correlation for all parameter values. Since instability in the stem cell genome is likely to lead to higher mutation rates of all kinds, mutation rates to drug resistance are more likely to be positively, than negatively correlated. Rather than attempt to construct a positively correlated bivariate distribution with beta marginals, we will consider two particular forms for $h(a_1,a_2)$ as follows:

(i) independence: $h(a_1,a_2) = h_1(a_1)h_2(a_2)$ where $h_1(a_1),h_2(a_2)$ are both univariate beta-distributions. $\ldots(4.45)$

(ii) dependence: $A_2=A_1$ with probability 1 where A_1 has a beta distribution. $\ldots(4.46)$

To motivate the choice of the densities $g_1(a_3|a_2)$ and $g_2(a_4|a_1)$, it is helpful to consider some underlying structure for their expected values. A convenient form is the linear model, that is

$$E[A_{i+2}|A_j=a_j] = \mu_{i+2}+k_j(a_j-\mu_j) \qquad i=1,2,\ j=3-i, \qquad \ldots(4.47)$$

where $\mu_j = E[A_j]$ and thus $E[A_{i+2}]=\mu_{i+2}$. Since we must have $0\leq E[A_{i+2}|A_j=a_j]\leq 1$, we require that

$$\max\left(\frac{-\mu_{i+2}}{1-\mu_j},\ \frac{\mu_{i+2}-1}{\mu_j}\right) \leq k_j \leq \min\left(\frac{1-\mu_{i+2}}{1-\mu_j},\ \frac{\mu_{i+2}}{\mu_j}\right).$$

We will consider two different forms for the distributions $g_1(\cdot)$ and $g_2(\cdot)$ which exhibit this linear structure as follows.

(i) <u>First Form</u>

$$A_{i+2} = \mu_{i+2}+k_j(A_j-\mu_j) \text{ with probability 1.}$$

This may be viewed as the limit of a beta distribution (for the conditional distribution given $A_j=a_j$) with parameters (u,v) where $u\to\infty$, $v\to\infty$ in such a way that

$$\frac{u}{u+v} = \mu_{i+2}+k_j(a_j-\mu_j),\ i=1,2,\ j=3-i.$$

Letting

$$P_N = E[P_N(\underset{\sim}{A})] = \int P_N(\underset{\sim}{a})\ f(\underset{\sim}{a})d\underset{\sim}{a}\ ,$$

then from (4.24) we have

$$P_N \simeq \int_0^1\int_0^1 \exp\Big\{-(1-\varepsilon)^{-1}N \sum_{i=1}^{2} a_i\left(\mu_{i+2}+k_j(a_j-\mu_j)\right) \ln\left[(1-\varepsilon)/e(\mu_{i+2}+k_j(a_j-\mu_j))\right]\Big\}\ h(a_1,a_2)da_1da_2, \qquad \ldots(4.48)$$

where j=3-i. As previously mentioned, two forms for $h(a_1,a_2)$ will be used: (4.45) and (4.46). In calculating (4.48) we will be concerned mainly with cases where the standard deviations of A_1 and A_2 are small, since it is clear (by analogy with the case of single resistance, Section 3.10) that when the standard deviation is large, P_N will vary slowly

with N. By examining the cases where S.D.(A_i) is small, we will be able to examine the effects upon curability of variability in the mutation rates which lie close to the level of detectability even in experimental systems. Figures 5 and 6 plot equation (4.48) as a function of N for $d=0, \mu_3=\mu_4=\mu_1=\mu_2=10^{-3}$, S.D.($A_1$)=S.D.($A_2$)=$10^{-3}$ and where $k_1=k_2=0$ and $k_1=k_2=1$ respectively. As may be seen the most marked effect of variability in the rates is to produce a pronounced tail in P_N (for increasing N) which is not evident when the rates are fixed.

(ii) Second Form

Here we will assume that A_{i+2} (i=1,2) have beta distributions (for the conditional distributions given $A_j = a_j$) where

$$E[A_{i+2}|A_j=a_j] = \mu_{i+2}+k_j(a_j-\mu_j) \text{ for } i=1,2,\ j=3-i.$$

This does not uniquely specify the beta distribution (which has two parameters) and thus we will also require that the coefficients of variation are the same, that is,

$$C.V.[A_{i+2}|A_j] = C.V.\ [A_j],\ \text{for } i=1,2,\ j=3-i. \qquad \ldots(4.49)$$

We assume that the conditional coefficient of variation is constant since variation in mutation rates are likely to be proportional to their absolute magnitude. The integrals to be calculated to evaluate P_N for the second form are more complex than the first form and involve the numerical calculation of one more nested integral. Examples are presented in Figures 7 and 8. Examination of these figures shows a similar tail for P_N to that seen previously where A_{i+2}(i=1,2) was a degenerate function of A_j (j=3-i). For the most pronounced case, where $k_1=k_2=1$, a considerable change is produced from the case where the rates are fixed (Figure 8).

In summary we can conclude that even modest variations (S.D.($\underset{\sim}{A}$)≃μ($\underset{\sim}{A}$)) in the mutation rates can lead to substantial changes in the function P_N for the special case $\pi_{i,0}=0$, $\pi_{i,j}=0$ $(i\neq j)$ and $\pi_{i,12}=1$ $(i=1,2)$. Clearly these effects will apply to other situations where the π's are arbitrary, however the effects are then more difficult to calculate because they depend on the full treatment protocol. An example (with further discussion) of a case where $\pi_{i,0}\neq 0$ and $\pi_{i,j}\neq 0$ $(i,j=1,2)$ is given in Chapter 5. However if we assume that we may use the example presented as a model for the (more complex) situations encountered in real tumor systems, we may make some tentative observations. If a particular tumor type has a small, but significant, cure rate when treated at an advanced stage (large bulk of tumor), then the predicted curability at lesser tumor burdens (of the same type) will be a function of the amount of variablity in the mutation rates. For example a five-fold reduction in size would imply a large increase in curablity and the size of this increment will decrease as the degree of variablity in the rates increases. This observation has implications for the therapy of human disease where the curablity of a regimen is observed and little is known of the mutation rates.

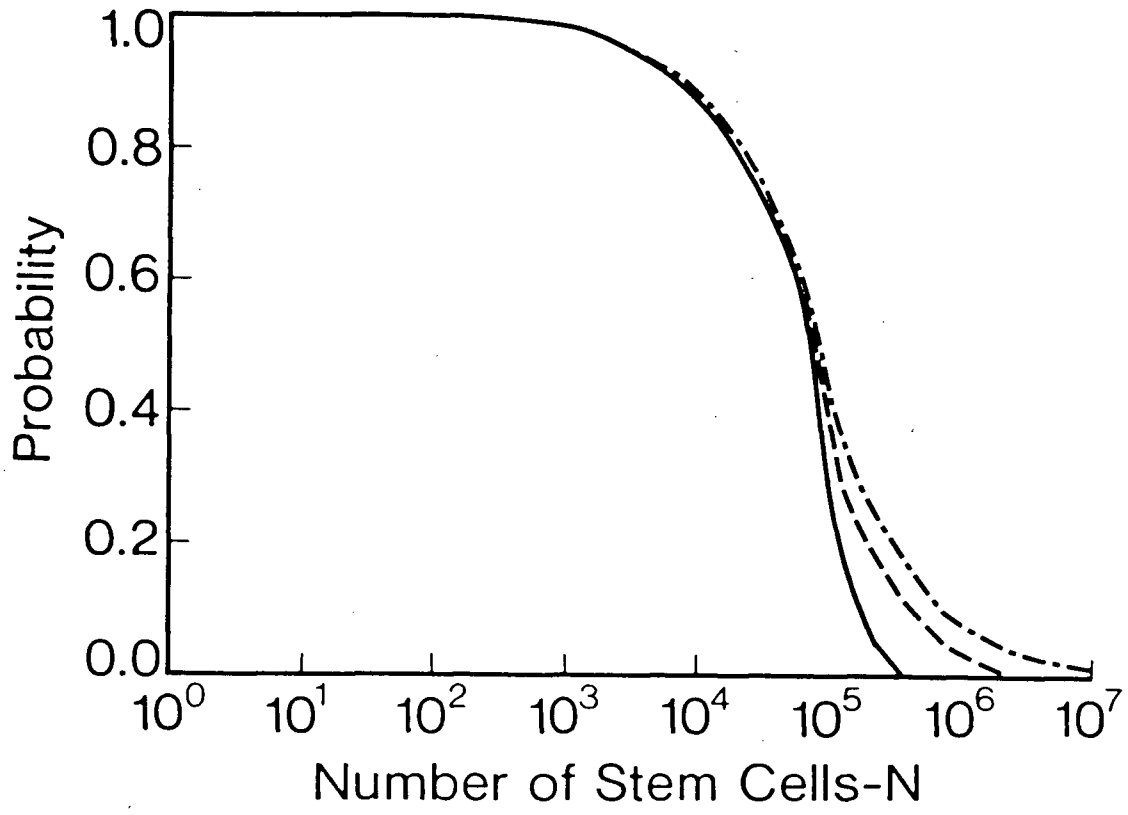
The effect of variation in $\underset{\sim}{A}$ on the mean number of doubly resistant cells is more easily evaluated. Using (4.12.2) and approximating all exponentials (except $e^{\delta t}$) by the first three terms in their expansion yields

$$m_{12}(t)\simeq e^{\delta t}\{m_{12}(0)+\sum_{i=1}^{2} m_i(0)t[a_{i,12}-\frac{t}{2}a_{i,12}^2]+B_0t^2/2\sum_{i=1}^{2} a_i a_{i,12}\}.$$

Taking the expected value of this expression (with respect to $\underset{\sim}{A}$) and

Figure 5

Probability of Cure when Variation is Present - 1.



Probability of cure P_N plotted as a function of stem cell burden at diagnosis where T_1 and T_2 are given simultaneously at time of diagnosis, $\pi_{i,0}=0$, $\pi_{i,i}$ arbitrary, $\pi_{i,12}=1.0$ for $i=1,2$, $\pi_{1,2}=\pi_{2,1}=0$, $b=1$ and $d=0$. The function is plotted for three separate cases where $k_1=k_2=0$ in (4.48):

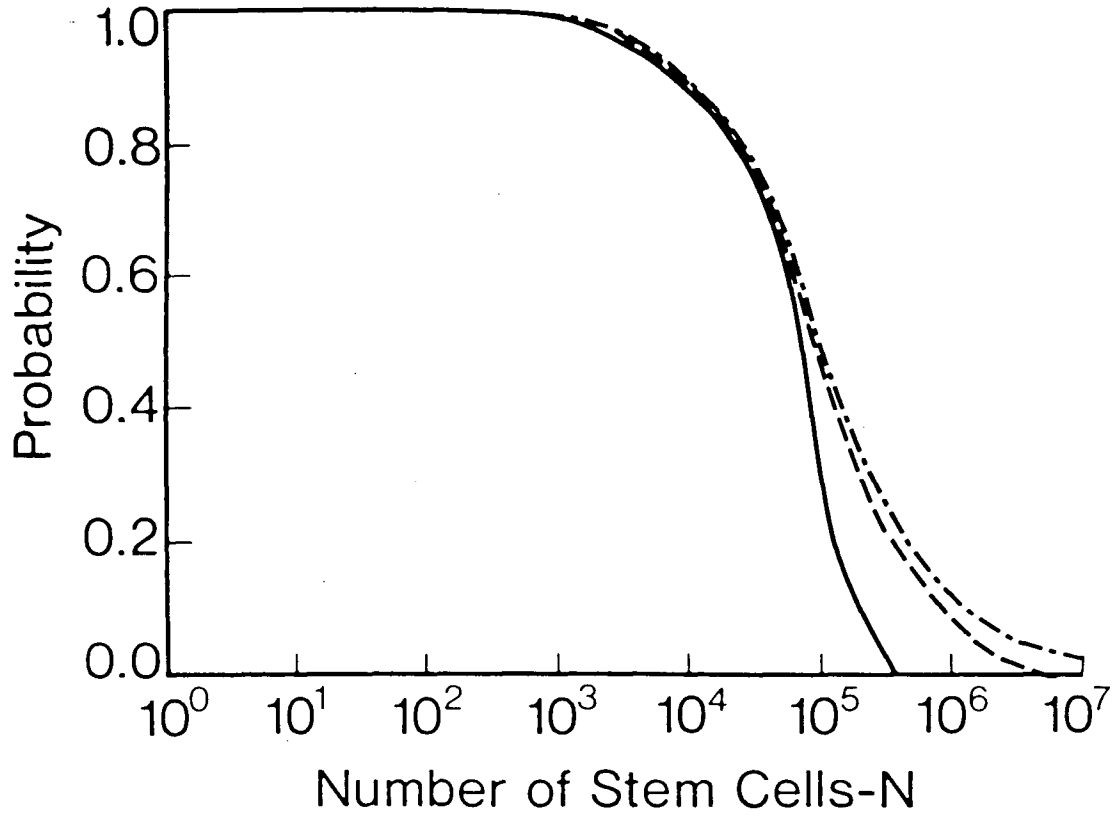
(i) ———— $A_1=A_2=A_3=A_4=10^{-3}$, mutation rates fixed.

(ii) - - - - - $A_3=A_4=10^{-3}$, A_1 and A_2 independent with β-distribution with $E[A_i]=$ S.D.$[A_i]=10^{-3}$ for $i=1,2$.

(iii) — — — — $A_3=A_4=10^{-3}$, $A_1=A_2$ with probability 1, where A_1 has a β-distribution with $E[A_1]=$S.D.$[A_1]=10^{-3}$.

Figure 6

Probability of Cure when Variation is Present - 2.



Probability of cure P_N plotted as a function of stem cell burden at diagnosis where T_1 and T_2 are given simultaneously at time of diagnosis, $\pi_{i,0}=0$, $\pi_{i,i}$ arbitrary, $\pi_{i,12}=1.0$ for i=1,2, $\pi_{1,2}=\pi_{2,1}=0$, b=1 and d=0. The function is plotted for three separate cases where $k_1=k_2=1$ in (4.48):

(i) ———— $A_1=A_2=A_3=A_4=10^{-3}$, mutation rates fixed.

(ii) - - - - - $A_{i+2}=A_{3-i}$ with probability 1 for i=1,2, A_1 and A_2 independent and follow a β-distribution with $E[A_i]=S.D.[A_i]=10^{-3}$ for i=1,2.

(iii) — — — — $A_{i+2}=A_{3-i}$ and $A_1=A_2$ with probability 1 for i=1,2, where A_1 has a β distribution with $E[A_1]=S.D.[A_1]=10^{-3}$

assuming the structure expressed by (4.44), (4.47) and (4.49) we have

$$E[a_{i,12} - \frac{t}{2} a_{i,12}^2] = \mu_{i+2} - \frac{t}{2}[\mu_{i+2}^2 + k_j^2 \text{ var } (A_j)] \left[\frac{\text{var } (A_j)}{\mu_j^2} + 1\right]$$

and

$$E[\sum_{i=1}^{2} a_i \, a_{i,12}] = \sum_{i=1}^{2} [\mu_i \mu_{i+2} + k_j(\text{cov } (A_1, A_2)].$$

From this we have

$$E[R_{12}(t)] \simeq e^{\delta t}\{m_{12}(0) + t \sum_{i=1}^{2} m_i(0) \left[\mu_{i+2} - \frac{t}{2} \left(\mu_{i+2}^2 + k_j^2 \text{var } (A_j)\right)\left[\frac{\text{var}(A_j)}{\mu_j^2} + 1\right]\right] + B_0 t^2/2 \sum_{i=1}^{2} [\mu_i \mu_{i+2} + k_j \text{ cov } (A_1, A_2)]\} \quad \text{where } j=3-i.$$

For clinical disease, where we assume $m_1(0) = m_2(0) = m_{12}(0) = 0$, the net effect of variation in mutation rates on $E[R_{12}(t)]$ will depend on k_1, k_2 and $\text{cov}(A_1, A_2)$. Thus even when A_1 and A_2 have a small correlation, if their variance is large the mean number of doubly resistant cells may be quite different from when the rates are fixed.

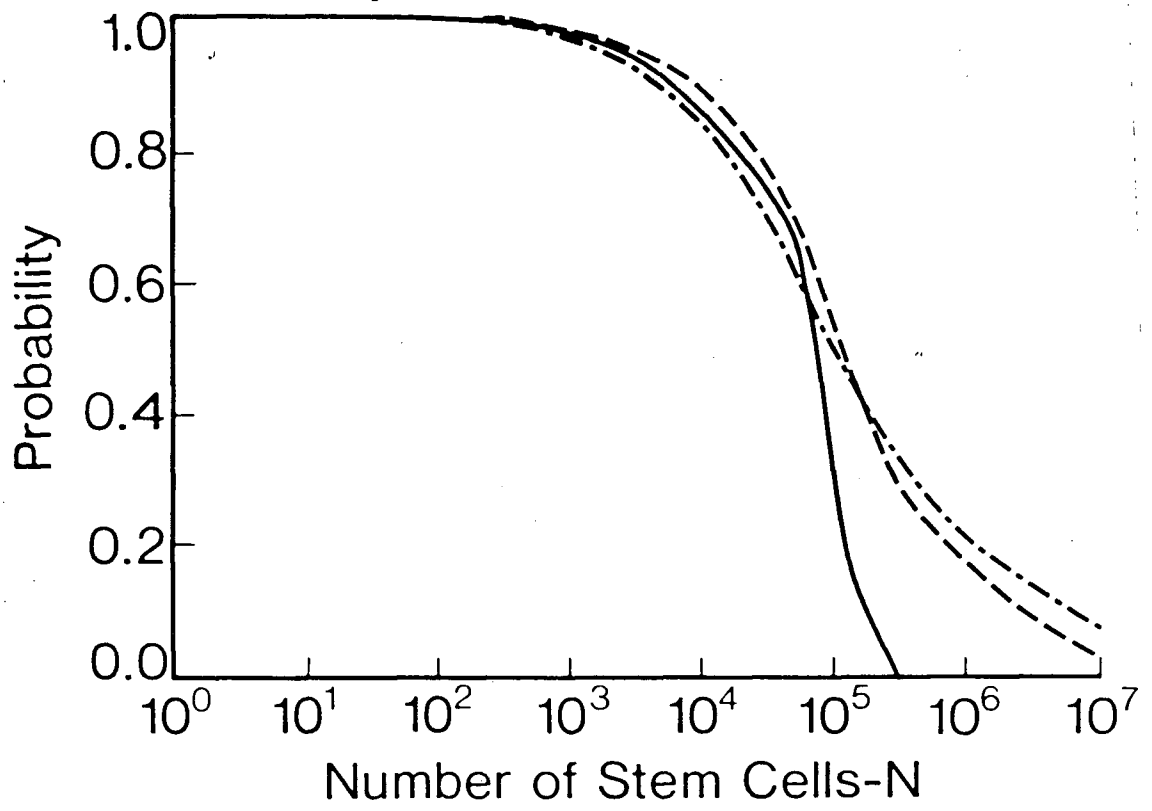
It is natural to consider whether it is possible to generalize the notion of equivalent agents (where each component of $\underset{\sim}{A}^*$ is fixed, as in Section 4.3) to include the situation where $\underset{\sim}{A}^*$ $(A_5 \neq 0)$ has a nondegenerate distribution. Assuming $\underset{\sim}{A}^*$ to have a density function $f_{A_1,A_2,A_3,A_4,A_5}(\underset{\sim}{x})$ say, then a natural definition of equivalent agents is

$$f_{A_1,A_2,A_3,A_4,A_5}(\underset{\sim}{x}) = f_{A_2,A_1,A_4,A_3,A_5}(\underset{\sim}{x}) , \qquad \ldots(4.50)$$

where as before $\pi_{1,0}=\pi_{2,0}$, $\pi_{1,1}=\pi_{2,2}$, $\pi_{1,2}=\pi_{2,1}$, $\pi_{1,12}=\pi_{2,12}$ and $(t_{j+1}-t_j)$ are fixed for $j=1,\ldots,J-1$. We may extend Theorem 2 to this situation. However, Theorem 1 may not be simply extended (in general) since the rationale behind its construction (minimizing transitions from

Figure 7

Probablity of Cure when Variation is Present -3.



Probability of cure P_N plotted as a function of stem cell burden at diagnosis where T_1 and T_2 are given simultaneously at time of diagnosis, $\pi_{i,0}=0$, $\pi_{i,i}$ arbitrary, $\pi_{i,12}=1.0$ for i=1,2, $\pi_{1,2}=\pi_{2,1}=0$, b=1 and d=0. The function is plotted for three separate cases where $k_1=k_2=0$ in (4.48):

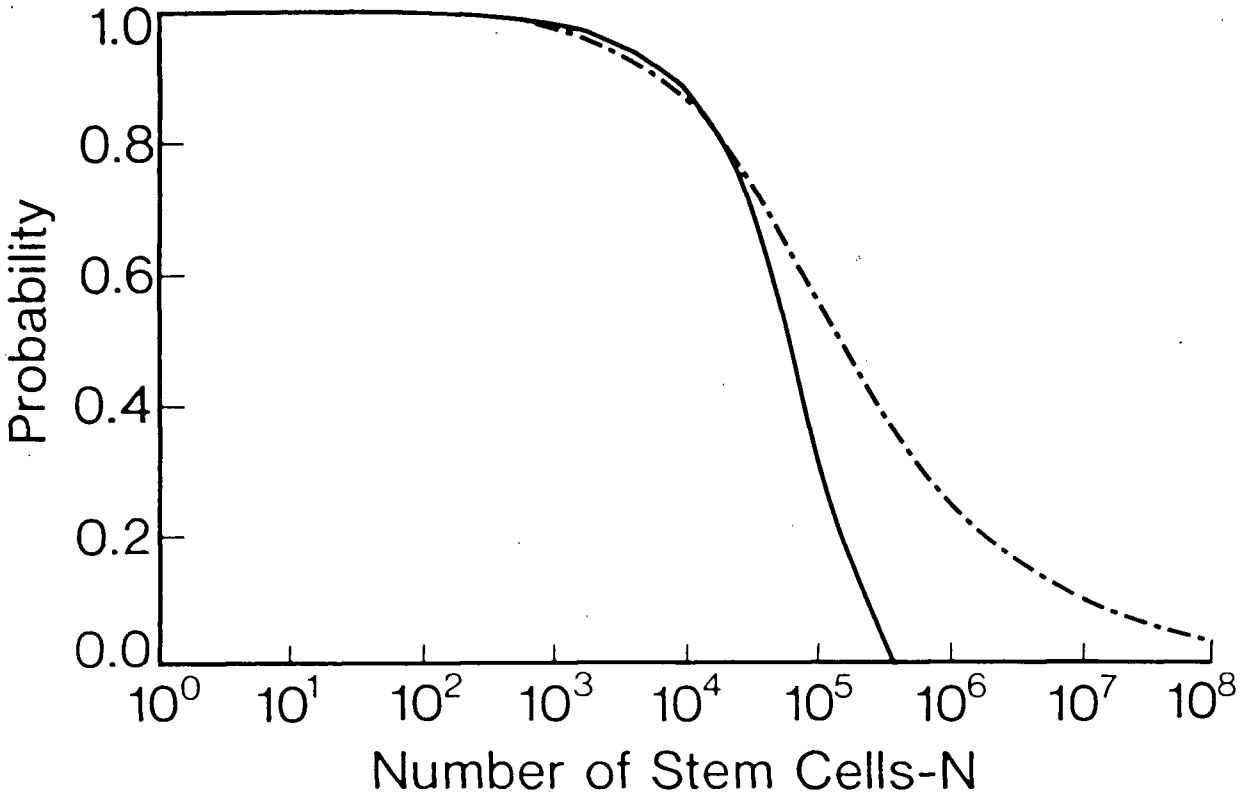
(i) ———— $A_1=A_2=A_3=A_4=10^{-3}$, mutation rates fixed.

(ii) - - - - - A_3 and A_4 independent and follow a beta distribution where $E[A_{i+2}]=S.D.[A_{i+2}]=10^{-3}$ for i=1,2, A_1 and A_2 independent with beta distribution and $E[A_i]=S.D.[A_i]=10^{-3}$, for i=1,2. (A_3,A_4) are independent of (A_1,A_2).

(iii) — — — A_3 and A_4 independent and follow a beta distribution where $E[A_{i+2}]=S.D.[A_{i+2}]=10^{-3}$ for i=1,2. $A_1=A_2$ with probability 1 where A_1 has a beta distribution with $E[A_1]=S.D.[A_1]=10^{-3}$. (A_3,A_4) are independent of (A_1,A_2).

Figure 8

Probability of Cure when Variation is Present - 4.



Probability of cure P_N plotted as a function of stem cell burden at diagnosis where T_1 and T_2 are given simultaneously at time of diagnosis, $\pi_{i,0}=0$, $\pi_{i,i}$ arbitrary, $\pi_{i,12}=1.0$ for $i=1,2$, $\pi_{1,2}=\pi_{2,1}=0$, $b=1$ and $d=0$. The function is plotted for two cases where $k_1=k_2=1$ in (4.48):

(i) ———— $A_1=A_2=A_3=A_4=10^{-3}$, mutation rates fixed.

(ii) - - - - - A_3 and A_4 follow a β-distribution where $E[A_{i+2}|A_{3-i}=a_{3-i}]=a_{3-i}$, $S.D.[A_{i+2}|A_{3-i}=a_{3-i}]=a_{3-i}$ for $i=1,2$. $A_1=A_2$ with probability 1 where A_1 has a β-distribution with $E[A_1]=S.D.[A_1]=10^{-3}$.

R_1 and R_2 to R_{12}) implicitly assumed that $A_3 = A_4$ with probability 1.

Corollary 1 (to Theorem 2)

For strategies of fixed length J, if

$$f_{A_1,A_2,A_3,A_4,A_5}(\underset{\sim}{x}) = f_{A_2,A_1,A_4,A_3,A_5}(\underset{\sim}{x}),$$

then the alternating strategies S(1) and S(2) minimize $E[N(t)]$ for arbitrary $t>t_J$.

Proof:

Without loss of generality assume that t is measured on a scale where b=1. To proceed we will first condition on $\underset{\sim}{A}^*=\underset{\sim}{a}^*$. Firstly we note

that $E_{i,j}(\bar{t}_j)$ (i=1,2, j=1,...,J) depends upon $\underset{\sim}{a}^*$ and thus in general $E_{i,j}=E_{i,j}(\bar{t}_j)\neq E_{2,j}(\bar{t}_j)=E_{2,j}$ for j=1,...,J. Similarly the terms g and h (used in Theorem 2) depend on $\underset{\sim}{a}^*$ and are not in general the same for R_1 and R_2. Thus define

$$g_i=\exp\{(\delta-a_{i+2})(t_{k+1}-t_k)\}$$

and

$$h_i=e^{\delta(t_{k+1}-t_k)}\left[1-e^{-a_{i+2}(t_{k+1}-t_k)}\right] \text{ for } k=1,\ldots,J,\ i=1,2.$$

Examining (4.42) we see that the constant K depends on $\underset{\sim}{a}^*$ but is not dependent on the treatment strategy. The two summations in (4.42) only depend on $\underset{\sim}{a}^*$ through $E_{i,j}$, g_i and h_i. Carrying out the appropriate substitutions of $E_{i,j}$, g_i and h_i for E_j, g and h in (4.42) we have

$$E[N(t_{J+1})|\underset{\sim}{A}^*=\underset{\sim}{a}^*] = K(\underset{\sim}{a}^*)$$
$$+\sum_{j=1}^{J+1}\{E_{1,j}\,g_1^{J-j+1}\,\pi_0^{n_j(J)}\,\pi_1^{J-j+1-n_j(J)} + E_{2,j}g_2^{J-j+1}\,\pi_0^{J-j+1-n_j(J)}\,\pi_1^{n_j(J)}\}$$
$$+\sum_{k=1}^{J}(\pi_2 g^*)^{J-k}\sum_{j=1}^{k}\{E_{1,j}h_1g_1^{k-j}\,\pi_0^{n_j(k)}\,\pi_1^{k-j+1-n_j(k)} + E_{2,j}h_2g_2^{k-j}\,\pi_0^{k-j+1-n(k)}\,\pi_1^{n_j(k)}\}$$

...(4.51)

where g* is as used in (4.38). Conditional on $\underset{\sim}{A}^*=\underset{\sim}{a}^*$ we may use (4.12.1) to calculate $E_{i,j}$. In order to do this we use $k=\delta-a_1-a_2-a_5$ and notice that $m_i(0)=0$ (by the definition of $E_{i,j}$), which gives

$$E_{i,j} = \frac{a_i\, B(t_{j-1})\, e^{\delta\Delta t}\left[\, e^{-a_{2+i}\Delta t} - e^{-(a_1+a_2+a_5)\Delta t}\right]}{a_1 + a_2 + a_5 - a_{2+i}}$$

where $j=1,..,J$, $\Delta t=t_2-t_1$ and $i=1,2$ for all $\underset{\sim}{a}^*$ such that $a_1+a_2+a_5-a_{2+i}\neq 0$. We will assume that $a_1+a_2+a_5-a_{2+i}\neq 0$ with probability 1 and thus from the definition of equivalence (4.50),

$$E[E_{1,j}g_1^{\,J-j+1}] = E[E_{2,j}g_2^{\,J-j+1}] \qquad \ldots(4.52.1)$$

and

$$E[E_{1,j}h_1g_1^{\,k-j}] = E[E_{2,j}h_2g_2^{\,k-j}]. \qquad \ldots(4.52.2)$$

From (4.51) and (4.52.1-2) we have $E[N(t_{J+1})] = E\left[E[N(t_{J+1})\,|\,\underset{\sim}{A}]\right]$ is of the same form as (4.42) and thus the corollary is proved.

Notice that the proofs of Theorems 1 and 2 and Corollary 1 do not require the assumption of deterministic growth of sensitive stem cells since (conditional on $\underset{\sim}{a}^*$) the E_{ij} have the same values under the fully stochastic model.

This concludes our consideration of variation in mutation rates. We will now briefly consider extensions of the proposed model.

4.7 Extensions

Generalizing this model to n drugs is possible in principle, however the complexity of the process increases rapidly as a function of n. For n drugs there are 2^n resistant states and $3n2^{n-1}$ parameters. Thus explicit solution of the full problem will likely be of little practical

value in human disease for n>2 because of the large number of paramaters which must be specified.

Even in the case where n=2, the large number of parameters require that we simplify the problem and carry out sensitivity analyses to assess the effects of assuming different choices of these parameters [34]. Under the strong assumption of equivalent agents (using the natural extension of its definition to multiple agents) the specification of 3n parameters would be required. A fruitful approach therefore seems to examine multidrug therapies and determine whether it is possible to consider them as two drugs. In multidrug regimens (n>2) for clinical cancer it is frequently possible to identify one of the drugs as being much more effective than the others. We may thus attempt to model the regimen by considering it to be composed of two drugs (the most effective and the others) and try to approximate the effect of the regimen using the case n=2. We would argue that this approach is reasonable, especially in light of the possibility that resistance to any one of the drugs may arise in a series of stages anyway.

This approach is not of great use in the construction of protocols where it is desired to choose drugs and the dosages that are to be used. However, the major obstacle to using these models in the planning of protocols is a comprehensive description of the nature of toxicity associated with drug combinations and how this depends on the individual dosages used. This is an extremely important problem which has not been extensively explored. Moreover, since most drugs overlap in toxicity on only two or three normal tissue systems (i.e. hemopoietic, gastro-intestinal, etc.), it may be possible to summarize the toxic effects of

drugs using a vector with as little as two or three elements (one for each system).

This completes the consideration of multitype drug resistance. In the next chapter we will present some applications of the theory developed in Chapter 3 and 4 to experimental and clinical cancer.

5. APPLICATIONS OF THE THEORY

In the previous chapters we have presented theory for the development of resistance to one or two drugs as a result of spontaneous transitions from the sensitive state. As remarked in Chapter 1, this is one of many mechanisms which can lead to clinical resistance and thus the model presented here can only be considered to be tentative for the response of clinical disease to chemotherapy. Nevertheless, it is possible to examine observations on clinical and experimental cancer in the context of this model and assess their "fit". The model presented is clearly not comprehensive, since it ignores many processes, but it is intended to be of general applicability to a large variety of experimental and clinical tumor systems. However, even within the context of the process of resistance considered, further generalization may still be required in order to accurately model the process in clinical and experimental cancer. For example, we have assumed that the rates α and ν do not vary with time. If these rates vary continuously in time, we may approximate the resulting process by partitioning the growth and treatment periods into a number of intervals and assuming that the rates are fixed within each interval. The resulting overall probability generating function may then be constructed using the recursive relationships presented in Chapters 3 and 4. The interdivision time of cells has been assumed to be exponentially distributed with a common parameter. This is not an accurate reflection of reality where very small divisions times (in relation to the mean) may not occur. Although we may vary the values of b, c and d throughout the growth period (in the same way as for α and ν), we cannot relax the distributional assumption

included in this model. Furthermore we have assumed that the growth parameters for sensitive and resistant cells are the same.

Keeping these limitations in mind, we now propose to examine the application of the model presented in three different cases. Firstly, using the theory presented in Chapter 4, we will present calculations of the effect of various treatment strategies on curability. Secondly, we will examine experimental data collected on the treatment of a mouse leukemia with two chemotherapeutic agents. Thirdly, we will examine the concept of neo-adjuvant chemotherapy in the light of this model.

5.1 The Effect of Treatment Strategies on Curability

A computer program was written which incorporates the relationships presented in (4.11), (4.17) and (4.18). Numerical integration is performed using Simpson's rule. The integrals are generally well-behaved and may be evaluated to 8 figure accuracy by partitioning the interval of integration into no more than 100 subintervals. Input consists of parameters which define the behaviour of the tumor and of the drugs and are described in more detail below.

The basic treatment parameters are $\pi_{i,Q}$, the probability of a cell in compartment R_Q surviving administration of treatment i, and T(i), the recovery time after treatment i, i.e. the minimum time before any further treatment may be "safely" administered. Five treatments are considered as follows: i=1,2 correspond to specific chemotherapeutic agents T_1 and T_2, i=3 corresponds to the two agents (T_3) being given together $\pi_{3,Q} = \pi_{1,Q}\pi_{2,Q}$, i=4 represents a non-chemotherapeutic treatment (T_4) which affects all stem cells equally, that is $\pi_{4,Q}=k$ $(0\leqslant k\leqslant 1)$ for all Q, and i=5 represents a null treatment (T_5) where $\pi_{5,Q}=1.0$ for all Q. It is assumed

that no treatment may be administered within the (minimum) recovery time for the preceding treatment. T_5 is included so that other treatments may be applied at arbitrary times after the minimum recovery time. In the examples which follow treatments will be applied at the minimum recovery times. The following parameters are also input:

N = number of stem cells at diagnosis,

DT = the doubling time of the tumor,

ε = d/b the relative rate of cell death,

c* = c/b the relative rate of cell renewal,

α_{Q_i,Q_j}, β_{Q_i,Q_j} = transition parameters for resistance to the drug T_i (i=1,2) where $Q_i,Q_j \in \{0, 1, 2, 12\}$,

$\gamma^*_{Q_i,Q_j}=\gamma_{Q_i,Q_j}/b$ = relative rates for spontaneous development of resistance $Q_i,Q_j \in \{0, 1, 2, 12\}$,

J = number of times treatments are administered.

There is no implicit time scale used but each parameter reflecting times (DT and T(i)) must be entered using the same scale i.e. days, hours etc. In all cases the tumor is assumed to have grown from a single stem cell.

The output from the program includes $E[R_0(t)]$, $E[R_1(t)]$, $E[R_2(t)]$, $E[R_{12}(t)]$ evaluated at t_j^-, and t_j, for j=1,...,J. The following probabilities are also calculated: $P_0(t_j)=\phi(\varepsilon,1,1,1;t_j)$, $P_1(t_j)=\phi(1,\varepsilon,1,1;t_j)$, $P_2(t_j)=\phi(1,1,\varepsilon,1;t_j)$, $P_{12}(t_j)=\phi(1,1,1,\varepsilon;t_j)$ and $P(t_j)=\phi(\varepsilon,\varepsilon,\varepsilon,\varepsilon;t_j)$, (the probability of cure after the j-th treatment), for j=1,...,J. The first four of these quantities correspond to the

marginal probabilities that cells in R_0, R_1, R_2 and R_{12} respectively at time t_j will go spontaneously extinct at some later time. $P(t_j)$ is the probability of cure. Notice that $P_0(t_j)$ is the probability that the sensitive cells at time t_j will go spontaneously extinct (all cells derived from these cells go extinct) and not the probability that there will be no sensitive stem cells at time $t=\infty$. This observation also applies (for the appropriate states) to $P_1(t_j)$, $P_2(t_j)$ and $P_{12}(t_j)$.

We will present an example with parameters chosen to be in the range of those seen in passaged experimental tumors. The parameter values are indicated in Table V. The parameters ε and c^* were chosen to be zero, implying that all cells are stem cells which seems to be approximately true for a number of experimental tumors. The doubling times (DT) and intertreatment times (T(i)) were chosen to be 5 and 3 days respectively. This doubling time represents the upper limit for most experimental tumors and the lower limit of those measured for human disease. However, as noted previously, the unit of measurement is irrelevant to these computations and it is only the ratio (5/3) of the quantities which is important. As noted in Chapter 4, when $\alpha_{Q_i,Q_j}+\nu_{Q_i,Q_j}/b$ is fixed, the various values of α_{Q_i,Q_j} and ν_{Q_i,Q_j} have little real effect on the probability of cure. Thus for simplicity we have chosen $\nu_{Q_i,Q_j}=0$ and $\gamma^*_{Q_i,Q_j}=\beta_{Q_i,Q_j}=0$. For simplicity we have assumed that $\alpha_{12}=\nu_{12}=0$, that is direct transitions from sensitivity to double resistance do not occur. The therapeutic parameters have been chosen so that resistant cells are absolutely resistant to the particular drug. The number of times therapy is administered, J, has been set to 8. Parameters were chosen so that

the drugs satisfy the definition of equivalence given in Section 4.4. In all simulations which follow the intertreatment interval has been assumed to be the minimum permitted by the recovery time of the previous treatment (in this case 3 days).

Tables VI, VII and VIII show the effect of three treatment strategies on curability: S(1)=(1,1,1,1,1,1,1,1), S(2)=(1,1,1,1,2,2,2,2) and S(3)=(1,2,1,2,1,2,1,2). That is, S(1), represents eight cycles of T_1 given at 3 day intervals with the first cycle being given when the tumor consists of 10^7 stem cells etc. Since the treatments are equivalent, each strategy has its mirror image which has the same probability of cure. Figures 9, 10 and 11 plot the expected number of cells for the treatment strategies S(1), S(2) and S(3).

Tables VI, VII, and VIII show, for this example, that among the three strategies of length J=8 which give a single drug per treatment, the probability of cure is maximized by the alternating strategy S(3). As can be seen by referring to Tables VI-VIII, all three strategies control (eliminate with high probability) the sensitive cells but the strategies have differential effect in controlling the various resistant compartments. S(2) and S(3) successfully control both the singly resistant compartments but have a differential effect on cells in R_{12}. Furthermore since neither T_1 or T_2 have any effect on cells in R_{12} further treatment (after t_8 with either T_1 or T_2) cannot increase the probability of cure to a value which exceeds $P_{12}(t_8)$. The question arises as to whether S(3) is best, in the sense that it maximizes $P(t_8)$ over all strategies with J=8 which use either T_1 or T_2 at the minimum permissable treatment times? Since the treatments are equivalent there

are at most $2^7=128$ strategies with distinct probabilities of cure (since each strategy has a mirror image). We will now consider general arguments to reduce the set of strategies which must be considered, in order to determine the optimal one.

We have assumed, in this example, that $\pi_{1,12}=\pi_{2,12}=1$ and we have for any strategy that $P(t)\leq P_{12}(t')$ where $t\geq t'$. Examining Table VIII we see that the alternating strategies of length J=8 have $P_{12}(t_8)=0.569$. The strategies considered in Table VI and Table VII are not optimal since after 3 consecutive applications of the same drug, $P_{12}(t_3)=0.487<0.568$. When $\pi_{1,12}=\pi_{2,12}$ the value of $P_{12}(t_j)$ does not depend on which drug is given at time t_j (either T_1 or T_2) but only depends on preceding applications of therapy. Thus a strategy whose first three cycles are (1,1,2) have the same value for $P_{12}(t_3)$ as that of a strategy commencing with (1,1,1). Thus $P_{12}(t_3)=0.487$ if T_1 (or T_2) is given as the first two cycles of the strategy. From this we conclude that the optimum strategy must begin with the alternation of T_1 and T_2. Examination of Tables VI and VII also show that strategies which include four cycles of T_1 and four cycles of T_2 are sufficient to eliminate the singly resistant stem cells with probability ≥ 0.999. If there are only three cycles of T_2 (T_1) then the likelihood that the R_1 (R_2) cells will be eliminated is significantly reduced; for example compare $P_1(t_7)$ and $P_1(t_8)$ in Tables VII or VIII. Thus we need only consider $\binom{6}{3}=20$ strategies to determine the best of length J=8, i.e. those that begin with (1,2) and have four cycles of T_1. Simulations of these 20 strategies indicates that there is little to choose between strategies which commence with either (1,2,1,2) or (1,2,2,1) and have four cycles of

T_1 in the total treatment strategy. Although these considerations only apply to the model with the particular values of the parameters specified, arguments similar to these may usually be applied to reduce the number of strategies which must be considered to determine the optimal one.

Figures 9-11 present plots of the mean number of cells (for each of the resistant subcompartments and overall) for the tumor model with parameters given in Table V for the three strategies S(1), S(2) and S(3) respectively. Judged by $E[N(t_8)]$, S(1) is clearly inferior to S(2) and S(3), however there is little difference between the latter two ($E[N(t_8)]$=131.6 and 122.5 respectively). The relatively small difference in $E[N(t_8)]$ for the two strategies can be contrasted with the large difference in predicted curability between S(2) and S(3) (Tables VII and VIII). This indicates that the effects of strategies on curability may not be reflected by similar proportionate changes in $E[N(t_8)]$ and this has clinical implications as follows.

In the analysis of clinical and experimental chemotherapy two measures of efficacy are in common use: cure rate (probability of cure) and survival time or time to relapse. Time to relapse (or survival time) depends on the growth rate of the neoplasm and the post-treatment tumor burden. If the effects of treatment are similar on the two proliferative compartments of the tumor (i.e. stem cells and transitional cells) and produce a large net reduction in the number of tumor cells, then the tumor will regrow at the rate determined in Chapter 2 and the time taken for it to reach some predetermined size will depend on the post-treatment stem cell burden. Thus in an experiment where recurrence times are

measured in genetically identical animals, the times will be a function of the post-treatment stem cell burden. It is common to view these two measures of treatment efficacy (cure rate and relapse time) as measuring the same underlying efficacy of the treatment protocol. Indeed we have argued in Chapter 4 that this is likely to be so, that is, $P(S(\nu))$ is maximized and $E[N_{S(\nu)}(t)]$ is minimized by the same strategy. However, even when these two criteria do induce a similar ordering on the set of strategies, this does not imply that differences between strategies will be quantitatively similar using either measure of efficacy. In the previous example we saw that the $P(t_8)$ for S(3) was 0.569 and for S(2) was 0.275 whereas the corresponding values of $E[N(t_8)]$ were 122.5 and 131.6 respectively. In an experiment carried out on a tumor where this model was appropriate and the parameter values were as given in Table V the large difference in cure probabilities would be readily apparent. However, consider the same example except that $\alpha_1=5\times10^{-4}$ $(=\alpha_2=\alpha_{1,12}=\alpha_{2,12})$. In this case the probability of cure is negligible for all strategies which use T_1 and/or T_2 only, since for $\pi_{1,12}=\pi_{2,12}=1$ we have for $t\geq t_1$, $P(t)<P_{12}(t_1)<10^{-10}$. If we apply S(2) and S(3) we find that $E[N(t_8)]$ = 13,064 and 12,158 respectively. The effective extension of the time to relapse is

$$\frac{\ell n\ [13,064/12,158]}{\ell n\ [2]} \times \text{Doubling time} = 0.10 \times 5 \text{ days}$$

$$= 0.5 \text{ day}$$

When this is compared against an estimated 86 days from time of first treatment to relapse (at 10^8 cells) we see that improvements of the order of 0.5 day will be very difficult to detect. Thus even in cases where Theorem 2 applies (i.e. treatments are equivalent), increases in

disease-free survival may be difficult to distinguish experimentally.

We may continue this example and consider the survival time under various strategies. If, for simplicity, we assume that death occurs at 10^{10} cells, then we still have a mean difference of 0.5 day (between S(2) and S(3)) and a mean survival time of approximately 119 days. We may contrast this with the protocol where T_1 is given until relapse (at 10^8 cells) when the treatment is switched to T_2 which is continued until death (at 10^{10} cells). In this case an animal has an approximate mean survival time (from first treatment) of 120 days. Thus the approximate difference in mean survival time between the last strategy and S(3) (the best strategy) is 1 day. Given possible uncontrolled variations in experimental conditions, variations in the number of resistant cells and the intrinsic precision of measurement, it will be extremely difficult to detect differences of this order in real systems. From consideration of this example we see that the value of strategies as reflected by their ability to produce cures (in cases where this is possible) may not be equally reflected in mean disease-free intervals or survival times when cure is unlikely.

The strategies considered up to this point have all assumed that T_1 and T_2 may not be given simultaneously. We will now consider cases where they can be given together. In each of the following two cases the parameters values are as given in Table V except as indicated. Table IX and Figure 12 contain details of the effect of the strategy S(4)=(3,3,3,3,3,3,3,3) where $\pi_{3,Q}=\pi_{1,Q}\pi_{2,Q}$ and T(3)=T(1)=T(2). Table X and Figure 13 contain the same information for the strategy S(4) where $\pi_{3,0}= 10^{-2}$, $\pi_{3,1}=\pi_{3,2}=10^{-1}$, $\pi_{3,12}=1$ and T(3)=T(1)=T(2). The parameter

values chosen for treatment 3 in the calculations presented in Table IX correspond to a case where T_1 and T_2 have no overlapping toxicity and thus may be given in full dose together. The parameter values chosen for treatment 3 in Table X correspond to a case where toxicity overlaps on one or more normal tissues and in order to give them together the drug dose of each is halved.

As expected, when there is no overlapping toxicity, S(4) has the highest probability of cure (of all strategies considered); this indicates that where possible active drugs should be combined (Table IX). Comparison of Tables VIII and X indicates that when the individual drug dosages are reduced (in order to combine them) the resulting strategy can be better than cyclic administration of the two agents singly. Notice that in this case we have assumed that the net-kill per cycle of the combination is the same to sensitive cells as that of either of the drugs given alone in full dose. If this were not true then such regimens might not be superior to one or more strategies involving cyclic administration of each drug at full dose.

As discussed in Chapter 4 minimizing E[N(t)] is not necessarily equivalent to maximizing the probability of cure, P(t). We will now present an example where P(t) is not maximised by alternation of two equivalent drugs (where of couse E[N(t)] is minimized). Table XI contains the parameter values and Table XII the results of three strategies $S'(1)=(1,2,1,2)$, $S'(2)=(1,2,2,1)$ and $S'(3)=(1,2,2,2)$ for this example. It can be seen that the alternating strategy is clearly inferior and that $S'(3)$ is a superior strategy. Calculation shows, as expected, that the alternating strategy $S'(1)$ minimizes the expected

tumor size at time t_4. Calculation also shows that $S'(3)$ is the best of the sixteen strategies of length J=4, i.e. that which maximizes $P(t_4)$. Examination of Table XII shows that extending the length of the strategies may improve the curability of the regimens since $P_{12}(t_4) > P(t_4)$ for each of the three strategies. However, notice that $P_{12}(t_4)$ for $S'(1)$ is much less than $P(t_4)$ for either $S'(2)$ or $S'(3)$, and thus all alternating regimens (of length $J \geq 4$) will have a lower probability of cure P(t) than at least two other strategies (those that begin with either $S'(2)$ or $S'(3)$). Examination of Table XII shows that the reason the alternating strategy does not maximise $P(t_4)$ is because the R_2 cells are eliminated, with probability 0.912, by the first course of T_1. Because of the fast regrowth of the cells several courses of T_2 must be given to eliminate cells in R_1. This combination of circumstances seems unlikely to occur in the treatment of human cancer, but could arise in the therapy of experimental neoplasms.

In Chapter 4 we examined the effects of variability in mutation rates on the probability of cure, for the special case where both drugs were given together and eliminated all but the R_{12} cells. We will now examine the effects on more general treatment strategies. In this example we will use the parameter values as given in Table V except that the mutation rates follow a distribution. We will assume that $\underset{\sim}{A}$ $(A_5=0)$ satisfies $A_1=A_2=A_3=A_4$ with probability 1 (see Section 4.6) where A_i follows a beta distribution with $E(A_i)=S.D.(A_i)$. This corresponds to a particular example of the dependent case (4.46) for $g_1(\cdot)$ and $g_2(\cdot)$ of the second form (4.49). This single specialized example is considered because of the complexity of the calculations involved. Even in this

situation where $\underset{\sim}{A}$ is essentially a scalar random variable, it is necessary to approximate its true distribution. In order to provide comparability with the previous example (Table V) we will assume that $\nu_i=\nu_{i,12}=0$ for i=1,2, $\nu_{12}=0$ and $E[A_i]=5\times10^{-5}$ for i=1,...,4. The effect of variation in $\underset{\sim}{A}$ is difficult to compute exactly because of the recursive nature of the relationships involved (see (4.11), (4.17) and (4.18)) where $E[\phi(\underset{\sim}{s};t,\underset{\sim}{a})]$ is not of closed form. We will therefore approximate the beta distribution by a set of 10 discrete mass points of weight 0.1 placed at the 5,15,...,95 percentiles of the beta distribution. The points are given in Table XIII. Tables XIV-XVI give the results of applying the strategies S(1), S(2) and S(3) to the tumor system. A similar calculation using 20 mass points (at the 2.5,...,97.5 percentiles) yielded results which were the same (to four decimel places) as those presented and thus the discrete approximation to the beta distribution can be expected to be reasonable for the puposes of this calculation.

As is to be expected, the probability of no doubly resistant cells at the commencement of therapy, $P_{12}(t_1^-)$, is different from the situation when the mutation rates were fixed. However, the difference is quite small. We find that, as when mutation rates were fixed, the alternating regimen S(3) is superior to either S(1) or S(2); in fact it maximizes $P(t_8)$ among the strategies which only give one treatment per treatment time. However, there are differences in the effects of the strategies on the two different tumor systems. Comparison of Tables VI-VIII and Tables XIV-XVI, shows that the probability of extinction of the sensitive stem cells is virtually the same in the two series of computations.

Similarly, differences in the probability of extinction of the singly resistant cells are small and of the type expected (see Figure 3). That is, when the mutation rates are variable, $P_1(t)$ and $P_2(t)$ increase earlier in the treatment regimen but require approximately the same number of treatments to approach unity. Comparison of Tables VII and XV shows that changes in $P_{12}(t)$ occur more slowly during the treatment period when variation is present. This behaviour is to be expected as may be seen from the following observation. From (4.24) we may approximate the probability of no doubly resistant cells prior to treatment, $P_{12}(t_1^-)$, in the form exp (-a*N). Thus the effect on $P_{12}(t_1^-)$ of any variation in a* will have the same effect as an analogous variation in N when a* is fixed. Thus we may consider $P_{12}(t_1^-)$ as the weighted sum of points of the function P_N given in Figure 4. As time increases (and the tumor grows) each point will experience a different rate of change of P_N. In the example considered, the fixed mutation rate case experiences a high (in absolute value) rate of change of P_N and P_N will decline comparatively quickly. For the variable mutation rate $P_{12}(t_1^-)$ may be considered to decline as a mixture of variable rates of change in P_N (some large and some small in absolute value), and thus $P_{12}(t_1^-)$ will decline more slowly than when the mutation rates are fixed. This argument also indicates that the probability of cure will not always change more slowly (during the treatment period) when variability in mutation rates is present than when it is not. When the mutation rates are fixed and the rate of change in P_N is small then $P_{12}(t_1^-)$ may decline at a faster rate when mutation rates have considerable variability.

This behaviour may be of some practical interest. Consider a class

TABLE V

Parameter Values for Simulations Presented in Tables VI-X.

Parameter	Value
N	10^7
DT	5 days
ε	0
c*	0
α ($=\alpha_1 = \alpha_2 = \alpha_{1,12} = \alpha_{2,12}$)	5×10^{-5}
ν ($=\nu_1 = \nu_2 = \nu_{1,12} = \nu_{2,12}$)	0
α_{12}	0
ν_{12}	0
$\pi_{1,0}$ ($=\pi_{2,0} = \pi_{1,2} = \pi_{2,1}$)	10^{-2}
$\pi_{1,1}$ ($=\pi_{2,2} = \pi_{1,12} = \pi_{2,12}$)	1
T(1)(=T(2))	3 days
J	8

TABLE VI

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for Strategy S(1)=(1,1,1,1,1,1,1,1).

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.641	0
t_1	T_1	0	0	0	0.641	0
t_2	T_1	0	0	0.500	0.573	0
t_3	T_1	0	0	0.984	0.487	0
t_4	T_1	0.707	0	1.000	0.386	0
t_5	T_1	0.995	0	1.000	0.277	0
t_6	T_1	1.000	0	1.000	0.172	0
t_7	T_1	1.000	0	1.000	0.087	0
t_8	T_1	1.000	0	1.000	0.033	0

TABLE VII

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for Strategy S(2)=(1,1,1,1,2,2,2,2).

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.641	0
t_1	T_1	0	0	0	0.641	0
t_2	T_1	0	0	0.500	0.573	0
t_3	T_1	0	0	0.984	0.487	0
t_4	T_1	0.707	0	1.000	0.386	0
t_5	T_2	0.995	0	1.000	0.277	0
t_6	T_2	1.000	0.059	1.000	0.275	0.022
t_7	T_2	1.000	0.934	1.000	0.275	0.263
t_8	T_2	1.000	0.999	1.000	0.275	0.275

TABLE VIII

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for Strategy S(3)=(1,2,1,2,1,2,1,2).

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.641	0
t_1	T_1	0	0	0	0.641	0
t_2	T_2	0	0	0	0.573	0
t_3	T_1	0	0	0.369	0.571	0
t_4	T_2	0.707	0.254	0.368	0.569	0.044
t_5	T_1	0.995	0.254	0.968	0.569	0.155
t_6	T_2	1.000	0.955	0.968	0.569	0.537
t_7	T_1	1.000	0.955	0.999	0.569	0.550
t_8	T_2	1.000	0.999	0.999	0.569	0.568

TABLE IX

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for Strategy S(4)=(3,3,3,3,3,3,3,3) and $\pi_{3,0}=10^{-4}$, $\pi_{3,1}=\pi_{3,2}=10^{-2}$, $\pi_{3,12}=1$.

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.641	0
t_1	T_3	0	0	0	0.641	0
t_2	T_3	0.859	0.515	0.515	0.640	0.163
t_3	T_3	1.000	0.985	0.985	0.640	0.627
t_4	T_3	1.000	1.000	1.000	0.640	0.639
t_5	T_3	1.000	1.000	1.000	0.640	0.640
t_6	T_3	1.000	1.000	1.000	0.640	0.640
t_7	T_3	1.000	1.000	1.000	0.640	0.640
t_8	T_3	1.000	1.000	1.000	0.640	0.640

TABLE X

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for Strategy S(4)=(3,3,3,3,3,3,3,3) and $\pi_{3,0}=10^{-2}$, $\pi_{3,1}=\pi_{3,2}=10^{-1}$, $\pi_{3,12}=1$.

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.641	0
t_1	T_3	0	0	0	0.641	0
t_2	T_3	0	0	0	0.627	0
t_3	T_3	0	0	0	0.624	0
t_4	T_3	0.707	0.266	0.266	0.624	0.036
t_5	T_3	0.995	0.781	0.781	0.624	0.406
t_6	T_3	1.000	0.956	0.956	0.624	0.584
t_7	T_3	1.000	0.992	0.992	0.624	0.618
t_8	T_3	1.000	0.999	0.999	0.624	0.623

TABLE XI

Parameter Values for Simulations Presented in Table XII.

Parameter	Value
N	10^7
DT	0.3 days
ε	0
c*	0
$\alpha_1 (=\alpha_2=\alpha_{1,12}=\alpha_{2,12})$	10^{-5}
$\nu_1 (=\nu_2=\nu_{1,12}=\nu_{2,12})$	0
α_{12}	0
ν_{12}	0
$\pi_{1,0}$ $(=\pi_{2,0})$	10^{-5}
$\pi_{1,2}$ $(=\pi_{2,1})$	10^{-4}
$\pi_{1,1}$ $(=\pi_{2,2}=\pi_{1,12}=\pi_{2,12})$	1
T(1)(=T(2))	3 days
J	4

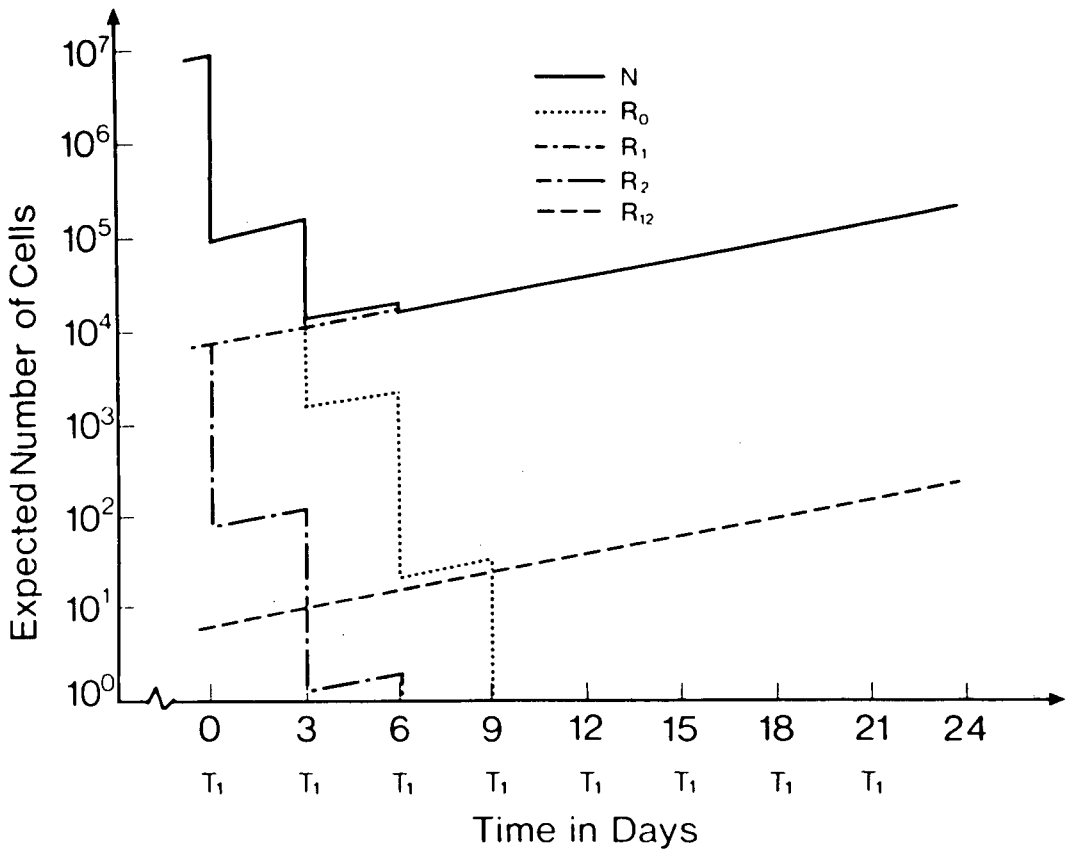
TABLE XII

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table XI for the Strategies S′(1)=(1,2,1,2), S′(2)=(1,2,2,1) and S′(3)=(1,2,2,2).

Strategy	Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	$P(t)$
	t_1^-		0	0	0	0.979	0.0
S′(1)	t_1	T_1	0	0	0.912	0.979	0.0
	t_2	T_2	0.363	2.7×10^{-11}	0.329	0.009	7.5×10^{-13}
	t_3	T_1	0.990	2.7×10^{-11}	0.712	0.005	4.3×10^{-12}
	t_4	T_2	1.000	2.7×10^{-11}	0.712	1.9×10^{-11}	4.3×10^{-12}
S′(2)	t_1	T_1	0	0	0.912	0.979	0.0
	t_2	T_2	0.363	2.7×10^{-11}	0.329	0.009	7.5×10^{-13}
	t_3	T_2	0.990	0.012	0.326	0.005	1.0×10^{-4}
	t_4	T_1	1.000	0.012	0.326	0.002	1.1×10^{-4}
S′(3)	t_1	T_1	0	0	0.912	0.979	0.0
	t_2	T_2	0.363	2.7×10^{-11}	0.329	0.009	7.5×10^{-13}
	t_3	T_2	0.990	0.012	0.326	0.005	1.0×10^{-4}
	t_4	T_2	1.000	0.512	0.326	0.002	0.001

Figure 9

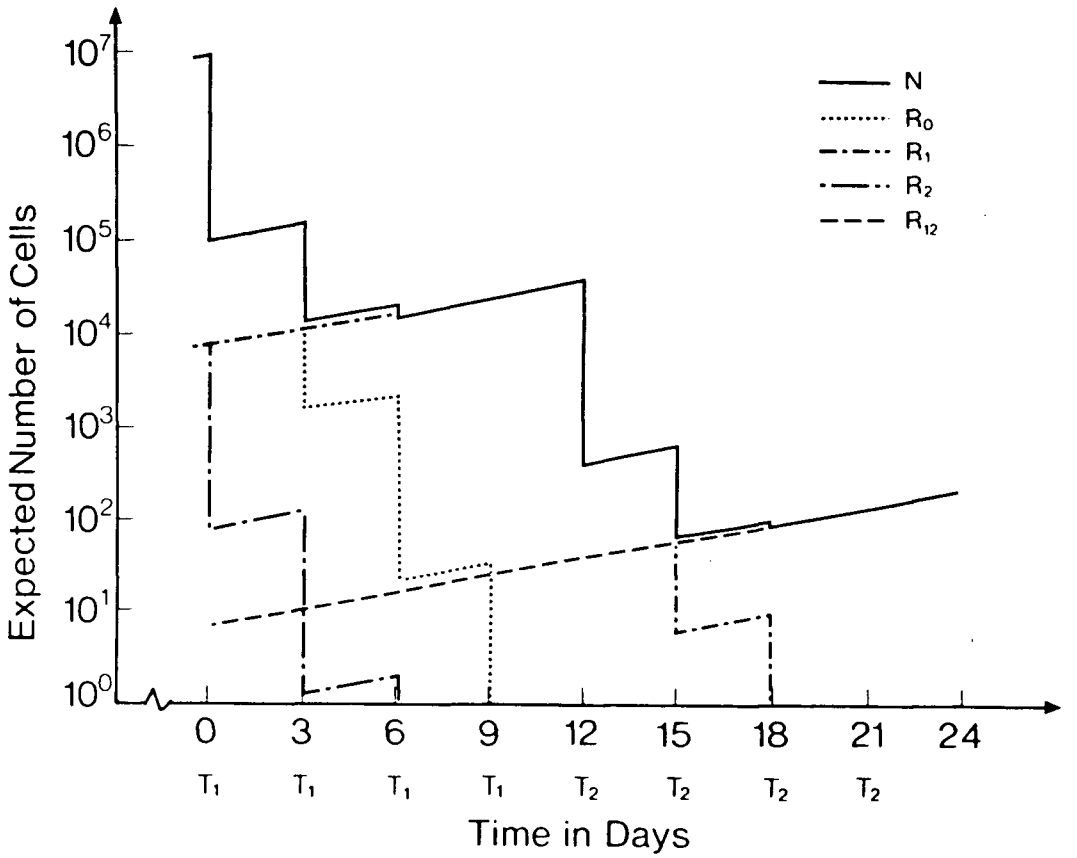
Expected Numbers of Cells for Treatment Strategy S(1).



Plot of expected number of stem cells in each of the resistant compartments for the tumor with parameters given in Table V treated with $S(1)=\{1,1,1,1,1,1,1,1\}$.

Figure 10

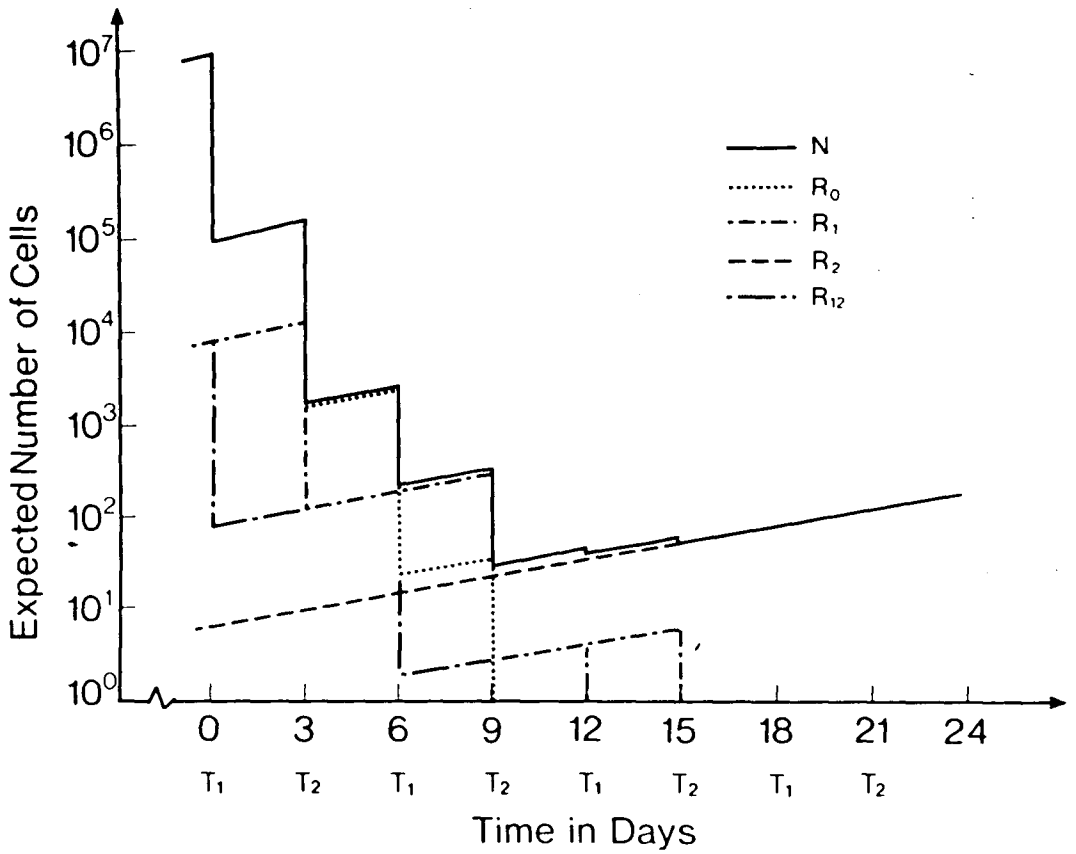
Expected Numbers of Cells for Treatment Strategy S(2).



Plot of expected number of stem cells in each of the resistant compartments for the tumor with parameters given in Table V treated with $S(2)=\{1,1,1,1,2,2,2,2\}$.

Figure 11

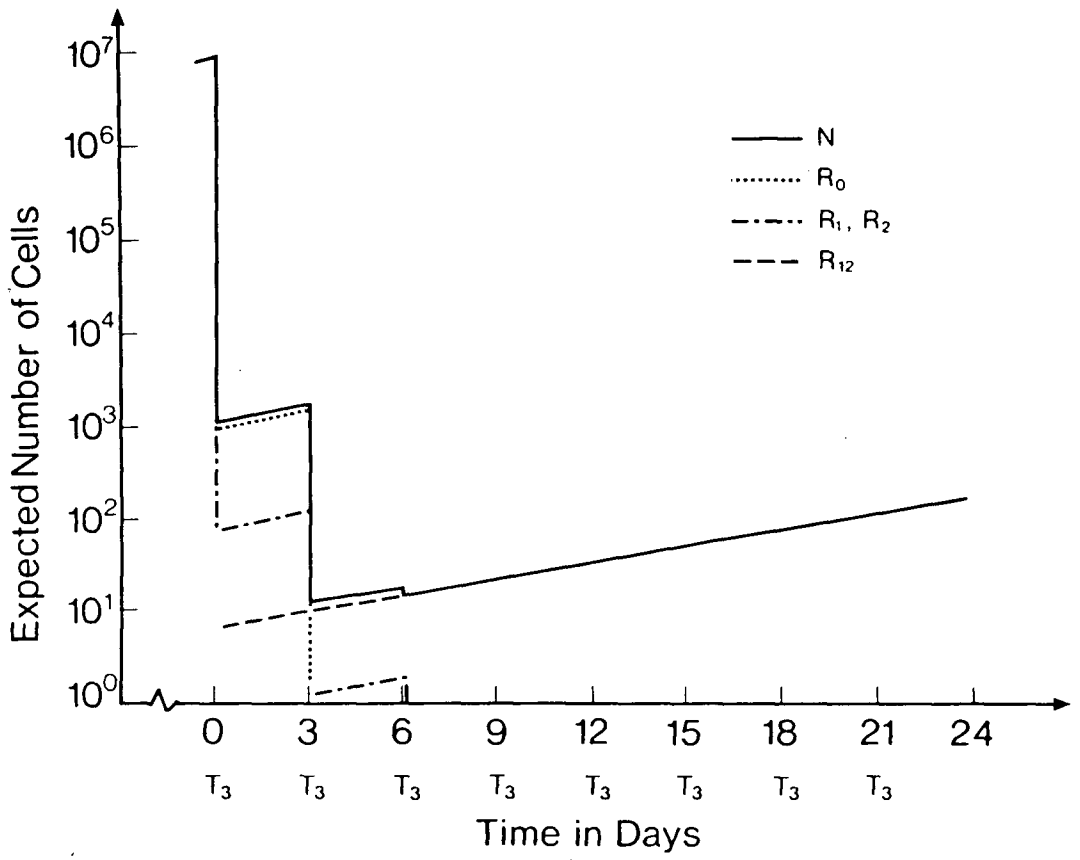
Expected Number of Cells for Treatment Strategy S(3).



Plot of expected number of stem cells in each of the resistant compartments for the tumor with parameters given in Table V treated with $S(3)=\{1,2,1,2,1,2,1,2\}$.

Figure 12

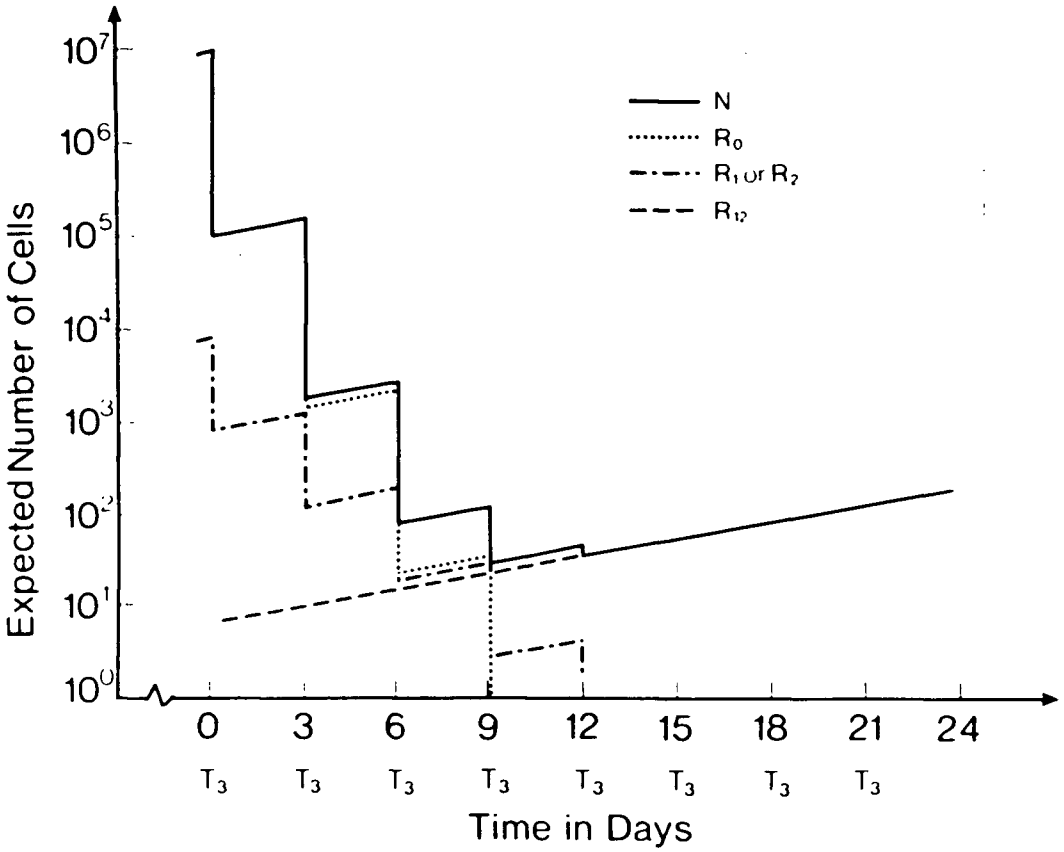
Expected Number of Cells for Treatment Strategy S(4) - 1.



Plot of expected number of stem cells in each of the resistant compartments for the tumor with parameters given in Table V treated with $S(4)=\{3,3,3,3,3,3,3,3\}$ where $\pi_{3,Q}=\pi_{1,Q}\pi_{2,Q}$.

Figure 13

Expected Number of Cells for Treatment Strategy S(4) - 2.



Plot of expected number of stem cells in each of the resistant compartments for the tumor with parameters given in Table V treated with $S(4)=\{3,3,3,3,3,3,3,3\}$ where $\pi_{3,0}=10^{-2}$, $\pi_{3,1}=\pi_{3,2}=10^{-1}$ and $\pi_{3,12}=1$

TABLE XIII

Mass Points for the Approximation to the Beta Distribution with $E[A_i]=5\times10^{-5}$, S.D. $[A_i]=5\times10^{-5}$.

The parameters of the beta distribution $\beta(a;u,v)$ are $u=1-10^{-4}$, $v=(1-10^{-4})(2\times10^{-4}-1)$.

Point	Percentile	Mass
2.6×10^{-6}	0.05	0.10
8.1×10^{-6}	0.15	0.10
1.4×10^{-5}	0.25	0.10
2.2×10^{-5}	0.35	0.10
3.0×10^{-5}	0.45	0.10
4.0×10^{-5}	0.55	0.10
5.3×10^{-5}	0.65	0.10
6.9×10^{-5}	0.75	0.10
9.5×10^{-5}	0.85	0.10
1.5×10^{-4}	0.95	0.10

TABLE XIV

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for the Strategy S(1)=(1,1,1,1,1,1,1,1) where the Mutation Rates are Equal with Probability 1 and have the Distribution Given in Table XIII.

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.676	0
t_1	T_1	0	0	0.032	0.676	0
t_2	T_1	0	0	0.591	0.639	0
t_3	T_1	0	0	0.985	0.597	0
t_4	T_1	0.707	0	1.000	0.551	0
t_5	T_1	0.995	0	1.0	0.501	0
t_6	T_1	1.000	0	1.0	0.449	0
t_7	T_1	1.000	0.	1.0	0.398	0
t_8	T_1	1.000	0	1.0	0.349	0

TABLE XV

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for the Strategy S(2)=(1,1,1,1,2,2,2,2) where the Mutation Rates are Equal with Probability 1 and have the Distribution Given in Table XIII.

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.676	0
t_1	T_1	0	0	0.032	0.676	0
t_2	T_1	0	0	0.591	0.639	0
t_3	T_1	0	0	0.985	0.597	0
t_4	T_1	0.707	0	1.000	0.551	0
t_5	T_2	0.995	0.002	1.000	0.501	0.002
t_6	T_2	1.000	0.259	1.000	0.500	0.232
t_7	T_2	1.000	0.938	1.000	0.500	0.491
t_8	T_2	1.000	0.999	1.000	0.500	0.500

TABLE XVI

Probability of Extinction of Cells at Times of Treatment for Parameter Values given in Table V for the Strategy S(3)=(1,2,1,2,1,2,1,2) where the Mutation Rates are Equal with Probability 1 and have the Distribution Given in Table XIII.

Time t	Treatment	$P_0(t)$	$P_1(t)$	$P_2(t)$	$P_{12}(t)$	P(t)
t_1^-		0	0	0	0.676	0
t_1	T_1	0	0	0.032	0.676	0
t_2	T_2	0	0.019	0.028	0.639	0
t_3	T_1	0	0.019	0.500	0.638	0
t_4	T_2	0.707	0.421	0.500	0.638	0.197
t_5	T_1	0.995	0.421	0.969	0.638	0.366
t_6	T_2	1.000	0.957	0.969	0.638	0.619
t_7	T_1	1.000	0.957	0.999	0.638	0.626
t_8	T_2	1.000	0.999	0.999	0.638	0.637

of tumors treated with two agents, T_1 and T_2, where the doubly resistant cells are absolutely resistant and mutation rates are fixed. If q_R is the probability that an individual tumor will contain no doubly resistant cells at the time of first treatment, then the effectiveness (as judged by the probability of cure) of various treatment strategies result from their ability (or lack of it) to decrease the likelihood that double resistance will develop in the remaining proportion $(1-q_R)$ of tumors. Consider the same situation, where again a proportion q_R of tumors contain no doubly resistant cells, where now the mutation rates are nondegenerate random variables. In this case the tumors with existing doubly resistant cells at diagnosis tend to contain a greater proportion of tumors with higher mutation rates and vice-versa. As before the effect of the treatment strategies, which use only T_1 and T_2, is on those tumors where doubly resistant cells have not emerged prior to the commencement of treatment. Since these tumours will tend to have lower mutation rates the rate of development of double resistance will be "slower". This will result in the differences in curability between various strategies being diminished (compared to the situation where rates are fixed). For example, in the most extreme case, the mutation rates among the tumors without existing resistant cells at the time of first treatment may be all identically zero. In this extreme case all strategies of fixed length which give T_1 the same number of times will be of equal effectiveness. Thus as the variability in mutation rates increases the differences in the probability of cure for strategies of the same length will decline, possibly to the point where they become experimentally indistinguisable. Similar arguements apply to the effects

of variation in the size of the tumor, N, at first treatment, which will cause a similar reduction in the relative benefits of various strategies when compared to the case where N is fixed. In summary, random variation in parameters may act to decrease the differences in effectiveness among strategies and thus it is necessary to consider such variation in the modelling of real systems.

This completes our examination of response when two drugs are available. We will now examine some experimental data to determine the appropriateness of the model presented here.

5.2 Fitting the Model to Experimental Data

We will examine experimental data collected by H. Skipper, F. Schabel and co-workers on the treatment of L1210 (mouse) leukemia by two drugs: Cyclophosphamide (Cyc) and Arabinosylcytosine (Ara-C)[26]. This tumor and these drugs were chosen because of the extensive data collected on them by a single group of investigators in the same laboratory using the same breed of mouse. These drugs are also representative of two of the major types of drugs used in cancer chemotherapy, the alkylating agents (Cyc) and the antimetabolites (Ara-C). The data to be used in the examination of response to Cyclophosphamide alone is given in Table XVII; all data is for single doses given up to the LD_{10} which occurs at about 300 mg/kg.

This information has been compiled from a number of clinical trials carried out by the investigators for intraperitoneally (IP) and intravenously (IV) implanted L1210 leukemia. The data is collected from experiments in which a fixed number (usually in the range of 100-1000) of cells are implanted in an animal. The growth of the tumor is known to be

regular (for innoculums in this range) and the size at any later time can be accurately estimated given the size of the original innoculum [3]. Autopsies of animals indicate that 45 day survivors (after the completion of any treatment) are free of any measurable L1210 leukemia [3]. The data presented in Table XVII gives the number of 45 day survivors.

The L1210 leukemia has been extensively studied and many of its physical properties are well known. Observation of the tumor (using thymidine labelling) suggests that the median intermitotic time of the tumor is close to the median doubling time [26]. This implies that most cells are actively dividing and consequently that the end cell compartment is small, and that cell loss is small. Limiting dilution assays (where a liquid suspension of cells are successively diluted and then injected into animals) suggests that a single cell is sufficient to cause animal death (from the leukemia) [26]. This implies that almost all the cells are stem cells. We will assume that all cells are stem cells and thus we have a model in which c=d=0 (and there are no transitional cells). Data on cells from this tumor which have been selected for Cyclophosphamide resistance suggests that such resistance is effectively absolute (resistant cells survive administration of the drug with probability 1), that is $\pi_1(D)=1.0$ for all achievable doses D (see Section 3.1). Data on the mode of therapeutic action of Cyclophosphamide shows that it has general activity on all phases of the cell cycle (see Section 3.2). If the cells behave independently we see that the probability a tumor of size S sensitive stem cells will be cured by administration of the drug at dose D is $[1-\pi_0(D)]^S$, where $\pi_0(D)$ is the probability that a single sensitive cell will survive administration of

the drug.

The form of $\pi_0(D)$ may be estimated from observation on growth delay curves (time taken to reach some fixed size after treatments of varying dosages carried out at a common initial size). These observations indicate (assuming cells behave independently) that

$$\pi_0(D)=\exp\{-kD\}, \qquad \ldots(5.1)$$

for a range of doses up to the LD_{10} (Section 3.2). There is some indication that (5.1) may not be accurate for doses approaching the LD_{10}, where the therapeutic effect may be less than predicted by (5.1) [26]. This observation may be explained in at least two ways. Firstly, it may be that the form of (5.1) should be modified at high doses because some mechanism (possibly drug transport into the cell) becomes saturated so that the effect of increasingly large doses is limited. Secondly, we note that estimates of $\pi_0(D)$ are based on observations of the whole tumor and not just on sensitive cells. Since large therapeutic effects can only be measured in large tumors, it is possible that resistant cells have emerged in these large tumors and contribute to the regrowth of the tumor. Thus a deviation from (5.1) would be expected in large tumors where estimates of $\pi_0(D)$ are based on the response of the total tumor. Since it is known that resistant cells are present in large tumors, we will assume that the second explanation is the true one.

Let t_1 be the time of treatment (only one cycle is given) and N the number of stem cells (all the tumor in this case). Then since c=0, d=0 we have

$$P_N \equiv P\{\text{cure} \mid N(t_1^-)=N\} = P\{R_0(t_1)=0, R_1(t_1)=0 \mid N(t_1^-)=N\}.$$

Since $\pi_1(D)=1.0$ we have

$$P_N = P\{R_0(t_1)=0, R_1(t_1^-)=0 \mid N(t_1^-)=N\},$$
$$= P\{R_0(t_1)=0 \mid R_0(t_1^-)=N\}\ P\{R_1(t_1^-)=0 \mid N(t_1^-)=N\}. \qquad \ldots(5.2)$$

Assuming that the effect of therapy in each cell is independent, the first term of (5.2) is given by

$$P\{R_0(t_1)=0 \mid R_0(t_1^-)=N\} = [1-\pi_0(D)]^N,$$

since the probability a single stem cell will survive therapy is $\pi_0(D)$. Using the approximation suggested in Section 3.7, that is replacing $N(t_1^-)$ by $R_0(t_1^-)$ in the second term in (5.2), we have

$$P\{R_1(t_1^-)=0 \mid N(t_1^-)=N\} \simeq P\{R_1(t_1^-)=0 \mid R_0(t_1^-)=N\} = (1-\alpha-\nu/b)^{N-1}$$

from (3.33). Since we cannot distinguish α and ν from one another (without further information) we set $a=\alpha+\nu/b$ and obtain

$$P_N \simeq [1-a]^{N-1}[1-\pi_0(D)]^N.$$

From (5.1) we have

$$P_N \simeq [1-a]^{N-1}[1-\exp(-kD)]^N.$$

Equation (3.33) was derived under the assumption that the tumor grew from a single sensitive stem cell. In the situation under consideration a number (100-1000) of cells are implanted and this formula must be viewed as approximate. We will set

$$P_N \simeq [1-a]^N[1-\exp(-kD)]^N \qquad \ldots(5.3)$$

since $N=N(t_1^-)$ is large in all cases.

The approximate log-likelihood, L(a,k), for the data is given by

$$L(a,k) = \sum_{i=1}^{I}\sum_{j=1}^{J} n_{ij}[f_{ij}\ell nP_{ij}+(1-f_{ij})\ell n(1-P_{ij})] \qquad \ldots(5.4)$$

where N_i = size of tumor at treatment $i=1,\ldots,I$,

D_j = dosage of treatment applied $j=1,\ldots,J$,

n_{ij} = number of animals tested at size N_i and dosage D_j,

s_{ij} = number of animals cured among the n_{ij} treated,

$f_{ij} = s_{ij}/n_{ij}$, the observed proportion of animals cured,

and P_{ij} = probability of cure at size N_i and dose D_j, where,

$$P_{ij} = [1-a]^{N_i}\,[1-\exp\{-kD_j\}]^{N_i}.$$

We may then differentiate (5.4) to obtain equations in a and k, which the maximum likelihood estimators a* and k* must satisfy:

$$\frac{\partial L}{\partial a} = \sum_{i=1}^{I}\sum_{j=1}^{J} n_{ij}\left[\frac{f_{ij}}{P_{ij}} - \frac{(1-f_{ij})}{(1-P_{ij})}\right]\frac{\partial P}{\partial a}_{ij}$$

$$= -\sum_{i=1}^{I}\sum_{j=1}^{J} n_{ij}\left[\frac{f_{ij}}{P_{ij}} - \frac{(1-f_{ij})}{(1-P_{ij})}\right]\frac{N_i P_{ij}}{(1-a)} = 0,$$

$$\frac{\partial L}{\partial k} = \sum_{i=1}^{I}\sum_{j=1}^{J} n_{ij}\left[\frac{f_{ij}}{P_{ij}} - \frac{(1-f_{ij})}{(1-P_{ij})}\right]\frac{\partial P}{\partial k}_{ij}$$

$$= \sum_{i=1}^{I}\sum_{j=1}^{J} n_{ij}\left[\frac{f_{ij}}{P_{ij}} - \frac{(1-f_{ij})}{(1-P_{ij})}\right]\frac{N_i D_j \exp\{-kD_j\} P_{ij}}{[1-\exp\{-kD_j\}]} = 0.$$

The data for IP innoculation in Table XVII were first modelled using the previous equations with a≡0 which yielded a maximum likelihood estimate k*=0.0678 and a corresponding log-likelihood of L(0,k*) =-1262.94. The full model was then fit and the maximum likelihood estimates were $a^*=1.04\times10^{-7}$, k*=0.0780 with L(a*,k*)=-530.20. Using the asymptotic χ^2 distribution for twice the difference in the log-likelihoods a test of H_0:a=0 versus H_1:a≠0 has χ^2_1=1465.48 providing strong evidence that a≠0. The predicted values of P_{ij} using the maximum likelihood estimates a* and k* are given in Table XVIII. The fit of the model to this data is not good, as judged by a log-likelihood goodness-of-fit statistic of χ^2_{15}= 453.26. Nevertheless the data analysis provides evidence for the development of drug resistant mutants. Coupled with observational evidence that drug (Cyclophosphamide) resistant cells may

be selected from this tumor, we conclude that this analysis is compatible with the idea that these drug resistant cells arise via spontaneous mutations although the goodness-of-fit indicates that this model is not a complete description of the data.

Calculations based on growth delay curves indicate that the therapeutic effect (probability of sensitive cell survival) of cyclophosphamide is greater for IV implanted tumors than for IP implanted tumors [26]. Repeating the preceeding analysis for the data on IV implanted tumors in Table XVII, we find that when $a\equiv 0$ that the maximum likelihood estimate is $k^*=0.0648$ with a log-likelihood of $L(0,k^*)=-478.01$. Fitting the full model we find $a^*=1.06\times10^{-7}$, $k^*=0.0802$ and $L(a^*,k^*)=-175.65$. A test of $H_0:a=0$ has associated $\chi^2_1=604.72$ providing strong evidence that $a\neq 0$. Again the fit of the model is not good as assessed by the log- likelihood goodness-of-fit of $\chi^2_{13}= 141.99$. The predicted values of P_{ij} for the full model are presented in Table XVIII.

The analysis presented thus provides some evidence that the therapeutic effect on sensitive cells, k, is increased in the IV innoculated tumors but the estimated values of a are almost identical. By combining the data sets we may test whether the parameters a and k vary with route of implantation. Let a_i, k_i (i=1 for IP and i=2 for IV) be the parameters for the two groups. We will first fit the model $a_1,a_2,k_1=k_2=k$. Proceeding as before we obtain the maximum likelihood estimates $a_1^*=1.06\times10^{-7}$, $a_2^*=1.01\times10^{-7}$, $k^*=0.0784$ with associated log-likelihood $L(a_1^*,a_2^*,k^*,k^*)=-706.82$. Using the log-likelihoods calculated from the separate models presented previously we have an

asymptotic test for $H_0:k_1=k_2$ versus $H_1:k_1\neq k_2$. This yields $\chi_1^2 = 1.94$ and thus we may conclude that there is no evidence (from this analysis) that the parameter k is affected by the route of implantation of the tumor. A-priori we would postulate that the mutation rate, a, should be the same for both IP and IV innoculated tumors since it has been assumed to be a property of the tumor cells. This hypothesis may be tested by fitting the model $a_1=a_2=a$ and $k_1=k_2=k$. Fitting this model we obtain the maximum likelihood estimates $a^*=1.048\times10^{-7}$ and $k^*=0.0784$ with $L(a^*,a^*,k^*,k^*)=-706.89$. Comparing this with the previous model we have a test for the hypothesis $H_0:a_1=a_2$ versus $H_1:a_1\neq a_2$ with associated $\chi_1^2=0.14$. On the basis of this analysis (and data), we conclude that the mutation rate does not vary with the route of implantation.

The analysis presented so far has assumed that the mutation rates are fixed. In Section 3.10 we presented theory which modelled the mutation rates as random variables with beta distributions. We may use that development to determine whether this data provides evidence for variability in the mutation rates (of a type which may be approximated by the beta distribution) and estimate the parameters of the distribution. A technical problem arises because the probability of no resistant cells is given by (3.50), which requires computing the product of 8×10^7 terms (the largest size in the data), that is

$$P\{R_1(t_1^-)=0 \mid R_0(t_1^-)=N\} \simeq \prod_{x=0}^{N-2} \left(\frac{v+x}{u+v+x}\right).$$

where (u,v) are the parameters of the beta distribution. In the preceeding analysis, when rates were fixed we found $a\simeq10^{-7}$. We would therefore expect that the mean of this beta distribution, u/(u+v), would

be small and thus that u<<v. If this is indeed the case, then we may approximate the product as follows:

$$\ell n\left\{\prod_{x=0}^{N-2}\left(\frac{v+x}{u+v+x}\right)\right\} = -\sum_{x=0}^{N-2}\ell n\ (1+\frac{u}{v+x})$$
$$\simeq -u\sum_{x=0}^{N-2}(v+x)^{-1}$$
$$\simeq -u\int_{v}^{v+N-2}\frac{dw}{w} = -u\ \ell n\left[\frac{v+N-2}{v}\right].$$

Using this approximation to (3.50),then from (5.3) we have

$$P_N \simeq \left[\frac{v+N-2}{v}\right]^{-u}\ [1-\exp(-kD)]^N. \qquad \ldots(5.5)$$

Fitting this model to the IP data using the log-likelihood function (5.4) with

$$P_{ij} = \left[\frac{v+N_i-2}{v}\right]^{-u}\ [1-\exp(-kD_j)]^{N_i},$$

yielded the maximum likelihood estimates u*=0.301, v*=0.578x10^5 and k*=0.0857 with associated log-likelihood L(u*,v*,k*)=-347.42. The fixed mutation rate model is a special case ($u\to\infty$, $v\to\infty$ such that $u/(u+v)\to a$) of the variable mutation rate model and we may construct a test assessing whether the fit of the model is improved by permitting variability. This yields χ^2_1=365.56 which provides evidence that the fit of the model is considerably improved by permitting variability. Despite this improvement there still remains considerable residual variation as judged by the log-likelihood goodness-of-fit statistic of χ^2_{14}= 87.70.

Repeating this analysis for the data on IV implanted tumors yields u*=0.633, v*=4.912x10^5, k*=0.0846 with a log-likelihood L(u*,v*,k*) = -117.41. As in the IP case we find that permitting the rates to vary (with a beta distribution) improves the fit of the model with an associated χ^2_1 =116.48. However, as before we find that this model does

not adequately fit the data as judged by the log-likelihood goodness-of-fit statistic of $\chi^2_{12} = 25.49$.

If we let i (=1 for IP and =2 for IV) index the route of implantation, we may analyse the combined data set and test whether $k_1=k_2=k$. Fitting this model yields $L(u_1^*,v_1^*,u_2^*,v_2^*,k^*)=-464.95$ and thus a test of $H_0:k_1=k_2$ has $\chi^2_1=0.24$ providing no evidence for a difference in the therapeutic parameters. Fitting the model $u_1=u_2=u$, $v_1=v_2=v$, $k_1=k_2=k$ we obtain $L(u^*,v^*,k^*)=-470.24$ and a test of $H_0:u_1=u_2,v_1=v_2$ (assuming $k_1=k_2$) is given by $\chi^2_2=10.58$ thus providing some evidence that the distributions of a may not be the same for IV and IP implanted tumors. The estimated values of the cure rates for the IP and IV implanted tumors using the maximum likelihood estimators u_i^*, v_i^* and k_i^* (i=1,2) are given in Table XVIII.

Interpreting these results is not straightforward since if variability in mutation rates exists we would not expect it to vary with route of implantation. The evidence that variability exists must remain hypothetical and we can only say that the analysis of the data presented here is compatible with this idea. This subject is worthy of future (experimental) study although this will not be easy.

Data on survival of animals having L1210 tumors treated with Ara-C is given in Table XIX. Ara-C is especially active against cells in the S-phase of the cell cycle and thus its effect is limited by the proportion of cells in this phase during treatment [26]. This drug is best administered in doses far below the LD_{10} since large doses have no greater tumoricidal effect. After much experimentation with this drug, Skipper and his associates have found that doses of 15mg/kg may be

repeated every 3 hours up to 8 times without resulting in undue toxicity [26]. Observations on the growth delay of tumors treated with between 1 and 8 cycles of Ara-C (every 3 hours) suggest that the log of the fraction of surviving cells is linearly proportional to the number of cycles given. This would imply that the effect of each cycle of Ara-C is the same (assuming independence) and that the cells sufficiently redistribute themselves about the cell cycle so that, approximately, a constant proportion of cells are in the S phase at each application of the drug. Further cycles of therapy beyond 8 (every 3 hours) produce considerable toxicity, however, if therapy is not given for three days the animal's normal tissues recover sufficiently for therapy to be applied again. A regimen of 8 cycles of Ara-C given every 3 hours will be referred to as a course [26].

Up to four courses may be given, with intervening three day recovery periods, without undue toxicity. Data from experiments using between one and four courses, for various initial tumor burdens, are given in Table XIX. We propose to model this data using the model presented in Section 3.7. In what follows $j=1,\ldots,4$ will index the number of courses of Ara-C given. As earlier the log-likelihood, L, is given by (5.4).

In this case, however, P_{ij} is of a more complex form. Here we will use the approximation developed in Section 3.7 where we assumed that $R_0(t_1) \simeq N(t_1)$ where t_1 is the time of the first cycle of the first course. Since the death rate for this tumor is assumed to be zero, the tumor is cured if, and only if, $\{R_0(t_J)=0, R_1(t_J)=0\}$ where t_J is the time of the last cycle of therapy. For simplicity we will assume that $\nu=0$ and estimate α only, that is we will assume that transitions to resistance

occur only at cell division. Observation on Ara-C resistant cells suggest that this resistance is effectively absolute, i.e. $\pi_1(D)=1$ for the doses of Ara-C used.

If $\phi(s_0,s_1;t)$ is the probability generating function for the distribution of sensitive and resistant cells in the tumor, then the probability of cure is $\phi(0,0;t_J)$; see (3.13). Since $\pi_1(D)=1$, from (3.11.1) we have

$$\phi(s_0,0;t_j) = \phi(\xi_0(s_0),0;t_j^-), \qquad \text{for } j=1,\ldots,J,$$

where from (3.9.1),

$$\xi_0(s_0)=1-\pi_0(D)+\pi_0(D)s_0.$$

From (3.11.2) we have,

$$\phi(s_0,0;t_{j+1}^-) = \phi(w_0(t_{j+1}-t_j),0;t_j) \qquad \text{for } j=1,\ldots,J-1,$$

where $w_1(t)=0$ for $s_1=0$ and

$$w_0(t) = \frac{s_0 e^{-bt}}{[1-s_0(1-\alpha)\ (1-e^{-bt})]}$$

since $s_1=0$ and $\nu=0$ (see (3.6) and (3.7)). Using (3.30) we may write

$$\phi(s_0,s_1;t_1^-) = s_0^{R_0}\ \zeta_{R_0}(s_1;t_1^-),$$

where R_0 is the "observed" number of sensitive stem cells at time t_1^- and we will set $R_0=N$, the total tumor size at first treatment. From (3.33) we have

$$\phi(s_0,0;t_1^-)=s_0^N\ (1-\alpha)^{N-1}.$$

Using the above equations we may estimate the probability of cure for various values of the parameters α and $\pi_0(D)$. Notice that the drug is only given at a single dose level (15 mg/kg) so it is not necessary to specify the form of $\pi_0(D)$. The complex form of P_{ij} makes it infeasible

to set up equations for the maximum likelihood estimates of α and $\pi_0(15)=\pi_0$. Thus a direct approach was taken by selecting "likely" values of the parameters and iterating in directions so that the log-likelihood increases. Initially this method was used on a version of this model in which $\alpha\equiv 0$. In this case it proved difficult to compute the log-likelihood since for all choices of π_0 either $P_{ij}\simeq 1$ or $P_{ij}\simeq 0$ for some i,j. When the log-likelihood was calculated at least one term in the sum overflowed yielding the following inequality for the log-likelihood: $L(0,\pi_0^*)<-10^{38}$. However, joint estimation of α and π_0 did produce easily computed likelihoods for a range of these two parameters. Maximum likelihood estimates were obtained for a number of starting values ($10^{-9}\leq\alpha\leq 10^{-5}$, $0.1\leq\pi_0\leq 0.3$) and in all cases (in which the log-likelihood did not overflow) each sequence converged to the same estimates. The maximum likelihood estimates were given by $\alpha^*=1.791\times 10^{-7}$, $\pi_0^*=0.186$ with $L(\alpha^*,\pi_0^*)=-209.41$. The log-likelihood goodness-of-fit of $\chi^2_{10}=22.38$ indicates that variation exists which is not explained by the model. The predicted estimates of P_{ij} using this model are given in Table XIX. There is thus considerable evidence that spontaneously resistant cells do arise with a frequency of the order of 10^{-7}.

Fitting a model incorporating variable mutation rates poses a considerable technical problem since the recursive nature of the relationships involved do not permit an approximation of the type used in (5.5). We will approximate the effect of variation in α (following a beta distribution) using a discrete distribution similar to that used in Section 5.1. The number of mass points used in the approximation was was varied (5,10 and 20) and each lead to essentially the same result (to

six significant figures in the likelihood). Using the same notation as in the analysis of Cyclophosphamide, the maximum likelihood estimates were $u^*=3.298$, $v^*=1.574\times10^8$ (where u and v are the parameters of the beta distribution which generate the percentiles used in the discrete approximation) and $\pi_0^*=0.186$ with $L(u^*,v^*,\pi_0^*)=-209.15$. The asymptotic χ^2 distribution of the difference in log-likelihoods yielded a test for the presence of variability in α of $\chi_1^2=0.52$ providing no evidence for variation in α (following an approximate beta distribution).

The analysis of the data on two quite different drugs (one phase specific and one not) appear to provide evidence compatible with the hypothesis that drug resistant cells do arise as a result of random mutations. In one case (Cyc) there was evidence that the mutation rate may be random, whereas the analysis of the data for Ara-C provided no evidence for this. We cannot conclude that the mutation rate has been demonstrated to be random for resistance to Cyc in the L1210 leukemia because there still exists considerable unexplained variation in the data. The existence of random variation in mutation rates for spontaneous tumors cannot be determined from the analysis of passaged animal tumors because each spontaneous tumor is unique whereas each animal implanted with a passaged tumor (L1210) should be considered to have a sample of a single tumor. By testing for random variation in mutation rates in a single type of experimental tumor we are testing whether these rates spontaneously evolve during the serial passaging of the tumor. In summary the presence of variation in mutation rates can properly be determined only by analyzing data from a series of de-novo spontaneous tumors. Since variation can influence the value of various

TABLE XVII

Response of Intraperitoneally (IP) and Intravenously (IV) Innoculated L1210 Leukemia to Single Doses of Cyclophosphamide.*

Dose mg/kg	Size at treatment	IP		IV	
		# of Animals Treated	# of Survivors	# of animals Treated	# of Survivors
300	$8x10^7$	94	7	80	4
	$8x10^6$	148	60	30	10
	$8x10^5$	39	30	20	14
250	$8x10^7$	-	-	66	1
	$8x10^6$	-	-	30	3
	$8x10^5$	-	-	30	17
230	$8x10^6$	50	7	-	-
	$8x10^5$	40	10	-	-
	$8x10^4$	50	41	-	-
200	$8x10^7$	109	3	60	0
	$8x10^6$	160	11	40	3
	$8x10^5$	60	11	10	0
	$8x10^4$	10	8	-	-
	$8x10^3$	10	10	-	-
150	$8x10^7$	30	0	245	0
	$8x10^6$	19	0	60	0
	$8x10^5$	20	1	50	3
100	$8x10^7$	10	0	130	0
	$8x10^6$	20	0	30	0
	$8x10^5$	144	0	20	0

* Abstracted from reference [26].

TABLE XVIII

Observed (Obs) and Predicted values for the Probability of Cure for IP and IV Innoculated L1210 Leukemia Treated with Cyclophosphamide Using the Maximum Likelihood Parameter Estimates for Fixed Rates (Pred1) and for Variable Rates (Pred2).

Dose mg/kg	Size at treatment	t IP			IV		
		Obs	Pred1	Pred2	Obs	Pred1	Pred2
300	$8x10^7$	0.074	0.000	0.109	0.050	0.000	0.040
	$8x10^6$	0.405	0.435	0.223	0.333	0.428	0.165
	$8x10^5$	0.769	0.920	0.446	0.700	0.919	0.544
250	$8x10^7$	-	-	-	0.015	0.000	0.038
	$8x10^6$	-	-	-	0.100	0.422	0.164
	$8x10^5$	-	-	-	0.567	0.917	0.544
230	$8x10^6$	0.140	0.393	0.218	-	-	-
	$8x10^5$	0.250	0.911	0.445	-	-	-
	$8x10^4$	0.820	0.992	0.778	-	-	-
200	$8x10^7$	0.028	0.000	0.008	0.000	0.000	0.001
	$8x10^6$	0.069	0.145	0.173	0.075	0.180	0.115
	$8x10^5$	0.183	0.824	0.435	0.000	0.843	0.524
	$8x10^4$	0.800	0.981	0.776	-	-	-
	$8x10^3$	1.000	0.998	0.964	-	-	-
150	$8x10^7$	0.000	0.000	0.000	0.000	0.000	0.000
	$8x10^6$	0.000	0.000	0.000	0.000	0.000	0.000
	$8x10^5$	0.050	0.003	0.066	0.060	0.008	0.045
100	$8x10^7$	0.000	0.000	0.000	0.000	0.000	0.000
	$8x10^6$	0.000	0.000	0.000	0.000	0.000	0.000
	$8x10^5$	0.000	0.000	0.000	0.000	0.000	0.000

TABLE XIX

Observed and Predicted Rates of Cure for Intravenously Innoculated L1210 Leukemia Treated with Repetitive Courses of Ara-C.*

Dose mg/kg	Schedule	Size at treatment	# of Animals Treated	# of Survivors	Observed Cure Rate	Predicted Cure Rate
15	q 3hr (x8) 1 course	$8x10^6$	10	0	0.000	0.000
		$8x10^5$	60	2	0.033	0.021
		$8x10^4$	20	11	0.550	0.681
	q 3hr (x8) 2 courses	$8x10^7$	20	0	0.000	0.000
		$8x10^6$	40	3	0.075	0.223
		$8x10^5$	19	11	0.579	0.860
	q 3 hr (x8) 3 courses	$8x10^6$	9	3	0.333	0.224
		$8x10^5$	30	25	0.833	0.861
	q 3 hr (x8) 4 courses	$8x10^7$	59	0	0.000	0.000
		$8x10^6$	80	25	0.313	0.224
		$8x10^5$	215	187	0.870	0.861
		$8x10^4$	30	30	1.000	0.985

*Data abstracted from reference [26].

therapeutic interventions this subject is worthy of further study.

5.3 Neo-Adjuvant Chemotherapy

Adjuvant is a term applied to chemotherapy which is used in addition to other forms of therapy (i.e. radiotherapy or surgery) [32]. Adjuvant chemotherapy is commonly used in a large number of solid human tumors and has proven successful in increasing the curability of several of these tumors (i.e. breast cancer) [35]. The use of adjuvant chemotherapy has been particularly successful (and initially somewhat controversial) when used in individuals with no observable disease (perhaps after surgery), but who are believed to have microscopic disease present (based on the experience of individuals with similar disease). In all these cases chemotherapy is given subsequent to "curative" therapy (usually surgery).

For some types of tumor, individuals may present with advanced disease where surgery, although desirable, is not possible. In particular tumors (initially head and neck cancer) a new concept has been proposed, that of neo-adjuvant (or pre-operative) chemotherapy [36]. In this approach chemotherapy is given first in order to shrink the primary tumor so that surgery is possible. After surgery the patient then receives the appropriate therapy. Like any good idea it has been applied to a variety of cases where it is more or less appropriate. In particular neo-adjuvant chemotherapy has been advocated, and is currently being tested, in situations where surgery is already possible without the neo-adjuvant chemotherapy. In breast cancer, which is one such case, neo-adjuvant chemotherapy amounts to starting the programme of adjuvant therapy at a time prior to surgery rather than after [37].

In this section we will consider the case of human breast cancer in some detail. Many advantages are espoused for the neo-adjuvant application of therapy, however, we will be concerned with only one of them here; that neo-adjuvant therapy reduces the likelihood of treatment failure from drug resistance. At this point we will provide an overview of the approach to be taken. We will assume that prior to diagnosis the distribution of tumor cells is given by the approximation of Section 3.6. We will set up an ad-hoc model for the effect of surgery on the distribution of tumor cells and fit it to observations from human breast cancer. We will not assume that we have a particular drug (with given α, ν, etc.), but require that the drug used is "reasonably" effective (against the sensitive cells) and examine the curability for various values of the mutation rates. Using the models developed for the effect of surgery and chemotherapy (Chapter 3) we will then examine the effect on curability of an extra neo-adjuvant cycle of therapy.

We know, from the example considered in Section 3.5, that for a given treatment strategy the earlier the treatment is begun the greater will be the probability of cure. The critical question is the magnitude of the increase in the probability of cure produced by an extra neo-adjuvant cycle of therapy. We will use breast cancer as an example although this approach can, in principle, be extended to other tumor types. The adjuvant therapy will be assumed to be a single drug which is given in a fixed number of cycles. This application of the theory differs from those considered previously since we are now considering the effect of two modalities of therapy (chemotherapy and surgery) rather than chemotherapy alone.

As mentioned before (Chapter 3), the effect of surgery (in terms of tumor reduction) is complex and depends on many factors. One of the principal difficulties is that metastasis of the tumor to other sites may not be apparent at diagnosis. For example in breast cancer, the finding of lymph node involvement is strongly indicative of tumor dissemination to other sites. This is the idea implicit in the clinical and pathological staging systems for tumors although other prognostic factors not included in these systems have been identified. In breast cancer two prognostic factors which have been identified are commonly used in the planning of clinical trials: menopausal status (pre-menopausal or post-menopausal) and lymph node status (0 positive nodes, 1-3 positive nodes, 4+ positive nodes) [25]. We will consider separately the six groups defined by menopausal status and nodal status for women with breast cancer.

In order to estimate the effects of surgery within each of these groups it is necessary to analyse data on recurrence times of individuals with breast cancer treated by surgery. Ideally such data would include individual measurement of recurrence times and growth rates for women treated surgically (using a standard procedure) for breast cancer. Unfortunately such data does not appear to be available since individual growth rates are seldom reported. Here we propose to use the results of an analysis by Skipper [38] of data of Valagussa et al consisting of women treated surgically for breast cancer [25]. In that analysis the following assumptions were made:

(i) All premenopausal disease grows at a fixed rate,

(ii) All postmenopausal disease grows at a fixed rate,

(iii) Recurrence occurs when the tumor burden at a single site exceeds 10^9 cells,

(iv) Individuals not recurring within 10 years after surgery are cured,

(v) All cells are stem cells.

The first four assumptions are certainly not precisely true but are not unreasonable approximations. The fifth assumption is not explicitly stated by the author but is implicit in the development of the estimates of residual tumor burden. In this development we would have preferred not to make this assumption however the raw data was not available for analysis. For consistency, we have thus assumed that c=d=0 in what follows although this is not required by the subsequent development. The estimates of residual tumor burden (stem cell burden) subsequent to surgery are given in Table XX. We will also require the following further assumptions to continue with this analysis:

(vi) The removal of cells by surgery is a random process and does not distinguish between cell types i.e. drug sensitive and drug resistant,

(vii) The failure of drug therapy is solely due to the presence of drug resistant cells arising by the process described in Chapter 3,

(viii) The two modalities (chemotherapy and surgery) do not interact with one another i.e. the effect of each modality for an individual is independent of the time at which it is given,

(ix) The number of tumor cells at the site of the first recurrence (if it occurs) is much greater than the number of tumor cells at

any other sites in the same individual,

(x) The resistant cells survive chemotherapy with probability 1,

(xi) The sensitive cell kill of the chemotherapy is sufficiently large and the therapy is applied sufficiently frequently so that the net growth of the sensitive cells during the treatment period is strongly sub-critical,

(xii) The distribution of the number of tumor cells after surgery is not related to the pre-surgery tumor burden.

It seems appropriate, at this point, to indicate the reasons for these further assumptions. Assumption (vi) seems reasonable and considerably simplifies the later development. Assumption (vii) relates to the intended objective of this section which is to examine the effect of neo-adjuvant chemotherapy in preventing the development of drug resistance. Assumption (viii) permits analysis of the effect of timing and is a reasonable simplification of the behaviour of these two very different modalities. Assumption (ix) implies that we may approximate the total tumor burden of the individual by the number of tumor cells at the site of recurrence. We may then approximate the post-surgical probability generating function for the total number of cells by the probability generating function for the number of cells at the site of recurrence. Also from (i) and (ii) we may make the preceeding approximation at all times after the time of surgery. Assumptions (x) and (xi) are are simplifying assumptions which imply that the probability of cure for the chemotherapy is approximately equal to the probability of cure at the first cycle of therapy. Thus the probability of cure is only weakly dependent on the details of the way in which the chemotherapy is

applied (after the first cycle). Assumption (xii) is clearly incorrect, however we are forced to make this assumption because of a lack of detailed information on the pre-surgical tumor burden.

We will approximate the post-surgical distribution of tumor cells seperately for each of the six prognostic groups (menopausal x nodal combinations). Approximation (xii) may not be as bad as it first seems since the relative difference in initial tumor burden before surgery is likely to be much smaller than the relative difference in tumor burden after surgery. This assertion is based on the assumption that the majority of the tumor burden prior to therapy is located in the breast lesion which is (almost totally) excised in all cases, thus leaving the more variable metastatic burden in place. We are now in a position to determine analytic expressions which summarise the effects of applying the chemotherapy early (neo-adjuvant).

The estimates of tumor burden after surgery (in the absence of neo-adjuvant therapy) derived by H. Skipper are given in Table XX for the six prognostic groups. Clearly the variation in residual tumor burden is quite large. We can now proceed to fit a distribution to the data given in Table XX, however, the possible mathematical form of the distribution which can be used in subsequent analysis is limited. The reason is that we do not have the distribution function for the number of sensitive and resistant cells in explicit form. The natural model for the response (removal or not) of a single cell to surgery is a Bernoulli variable, where the parameter, θ, is a function of the individual (tumor) and the surgical technique. The parameter is unknown and cannot be estimated reliably since we only have one observation per individual. We will

assume that the parameters $\{\theta\}$ only take a finite number of values and then fit this model to the observed data. This model is quite ad-hoc, but our aim here is to estimate the post-surgical distribution of sensitive and resistant cells and thus we only need to calculate the effect of surgery and not construct a valid model of the mechanism of action. We will set

$$\theta_i = 10^{-i} \qquad i=1,\ldots,11,$$

which spans the likely range of $\{\theta\}$. Let $j(=1,2)$ and $k(=1,2,3)$ index the prognostic groups and define

$$C_{ijk}=P\{\theta=\theta_i \mid \text{individual is in prognostic group } j,k\}.$$

We will assume that the distribution of the number of stem cells prior to surgery has probability generating function, $\zeta(s;t)$, given by (3.24). The probability generating function of the number of cells after surgery given at time t_* in prognostic group j,k , $\zeta_{jk}(s;t_*)$, is given by

$$\zeta_{jk}(s;t_*)= \sum_{i=1}^{11} C_{ijk}\, \zeta(\xi_i(s);t_*) \text{ for } j=1,2,\ k=1,2,3,$$

where $\xi_i(s)=1-\theta_i+\theta_i s$ for $i=1,\ldots,11$.

Let $N_{jk}(t_*)$ be the post-surgical number of stem cells for individuals in prognostic group j,k. Then $P\{N_{jk}(t_*)=n\}$ is given by the coefficient of s^n in the above expression. Expanding and identifying the coefficient of s^n, we have

$$P\{N_{jk}(t_*)=n\} = \sum_{i=1}^{11} C_{ijk} \frac{\theta_i e^{-bt_*}[\theta_i(1-e^{-bt_*})]^{n-1}}{[\theta_i+(1-\theta_i)e^{-bt_*}]^{n+1}} \quad \text{for } n>0,$$

and

$$P\{N_{jk}(t_*)=0\} = \sum_{i=1}^{11} C_{ijk} \frac{(1-\theta_i)e^{-bt_*}}{[\theta_i+(1-\theta_i)e^{-bt_*}]}.$$

$$P\{N_{jk}(t_*)=0\} = \sum_{i=1}^{11} C_{ijk} \frac{(1-\theta_i)e^{-bt_*}}{[\theta_i+(1-\theta_i)e^{-bt_*}]} .$$

Assuming that the mean number of cells prior to surgery is 10^{10} (approximately a 2 cm. diameter spherical tumor), we set $e^{bt_*}=10^{10}$. Then values of C_{ijk} may be chosen and the post-surgical tumor burden examined and compared with the "observed" values (Table XX). The values selected for C_{ijk} are given in Table XXI and the predicted post-surgical distribution is given in Table XXII. These values are not unique and their "fit" is not perfect as may be seen by comparing Tables XXI and XXII. These values were selected by an informal procedure of trial and error until the fitted values were within ±1% of the observed values. Given that the observed distribution of post-surgical tumor burden has considerable random error (since it was estimated from data on 716 cases) the fitted model seems adequate.

Let $\phi(s_0,s_1;t)$ be the probability generating function for the number of sensitive and resistant cells in the tumor at time t (see Section 3.1). For the neo-adjuvant approach, chemotherapy is applied first, at time t_1 say. Then from (3.9.1) we have:

$$\phi(s_0,s_1;t_1) = \phi(\xi_0(s_0),s_1;t_1^-),$$

where $\xi_0(s)=1-\pi_0+\pi_0 s_0$. If surgery is applied at time t_2, we have

$$\phi(s_0,s_1;t_2^-) = \phi(w_0(t_2-t_1),w_1(t_2-t_1);t_1),$$

where $w_0(t)$ is given by (3.7), $w_1(t)$ by (3.6) with c=d=0. The effect of surgery on the probability generating function of the number of cells for a tumor in the prognostic group j, k is then given by

$$\phi(s_0,s_1;t_2) = \sum_{i=1}^{11} C_{ijk}\, \phi(\xi_i(s_0),\xi_i(s_1);t_2^-),$$

for j=1,2, k=1,2,3.

To analyse the effect of neo-adjuvant therapy we must consider the parameters which are related to the chemotherapy. We will assume that $\nu=0$ and calculate the curability for a number of values of α. From assumptions (x) and (xi), if the protocol is sufficiently long (i.e. J large), the curability of the regimen will not depend very strongly on the parameter π_0 (the probability of sensitive cell survival for a cycle of chemotherapy). In his analysis of this data, Skipper found that the doubling time was 56 days for premenopausal disease and 69 days for postmenopausal disease. We will model conventional adjuvant chemotherapy as consisting of six cycles of chemotherapy where the first cycle is given 28 days after surgery and then given in cycles with 21 day interval. Calculations based on this model show that the curability is approximately the same for all cases where $\pi_0 \leq 0.1$, $J \geq 4$ (the number of treatment times) and the interval between cycles of chemotherapy is less then thirty days. Neo-adjuvant therapy will be modelled by assuming that an single extra cycle of therapy is given two days before surgery and then followed by the same post-surgical adjuvant therapy as above. In both cases the date of surgery is the same, that is, the inclusion of the neo-adjuvant cycle does not affect the timing of other therapy. Tables XXIII-XXIV give the estimated curability of the tumor as a function of the mutation rate to resistance, α, for the conventional adjuvant protocol and the increase in the probability of cure associated with a neo-adjuvant cycle of therapy added to the same protocol for each of the prognostic groups.

The most obvious result which may be seen from examination of Tables XXIII-XXIV is that in no case does the calculated increase in curability

exceed 0.01. Thus the likelihood of any measurable affect of neo-adjuvant therapy of the type described here for the development of resistance to a single drug for breast cancer is negligible. The modelling procedure is not ideal, as has already been described, however it would seem that inaccuracies in the modelling of surgery or the effects of chemotherapy are unlikely to cause an order of magnitude change in the advantage of neo-adjuvant therapy. Secondly, it can be seen that the curability of the tumor (in any of the prognostic groups) varies quite slowly with the mutation rate. Large improvements in the cure rates obtained with adjuvant chemotherapy will thus require significant reductions in the overall mutation rate. For example, an improvement in curability of 0.10 in premenopausal negative node group requires a chemotherapy with a mutation rate of 10^{-4}. A further improvement in curablity of 0.10 would require a chemotherapy with a mutation rate of 10^{-7}. The principle reason that neo-adjuvant therapy is predicted to have little effect (on the development of resistance) in this tumor is the highly variable post-surgical tumor burden. If the post-surgical tumor burden lies in a narrow range then the relationship between curability and mutation rate will be quite different from that displayed in Tables XXIII-XXIV. In this situation curability will rapidly change (as a function of the mutation rate) in the region where the inverse of the mutation rate is approximately equal to the mean residual tumor burden. In such situations an extra neo-adjuvant cycle of may have considerable impact in preventing the development of resistance.

In conclusion, if neo-adjuvant chemotherapy is to have any

measurable effect in this tumor, its primary effect must be on other mechanisms of treatment failure and not on the development of spontaneous resistance. This completes the consideration of applications of this model.

TABLE XX

Distribution of Post-Surgical Tumor Burden for 716 Cases of Breast Cancer as a Function of Nodal Status and Menopausal Classification.*

	Premenopausal			Postmenopausal		
	Number of Positive Nodes			Number of Positive Nodes		
Tumor Burden	0	1-3	4+	0	1-3	4+
[0]	0.69	0.31	0.12	0.74	0.35	0.15
$[10^0, 10^1)$	0.07	0.22	0.11	0.05	0.10	0.08
$[10^1, 10^2)$	0.00	0.07	0.03	0.01	0.03	0.04
$[10^2, 10^3)$	0.04	0.02	0.03	0.02	0.00	0.03
$[10^3, 10^4)$	0.03	0.04	0.09	0.02	0.08	0.04
$[10^4, 10^5)$	0.01	0.07	0.14	0.02	0.06	0.05
$[10^5, 10^6)$	0.04	0.07	0.13	0.03	0.08	0.08
$[10^6, 10^7)$	0.08	0.09	0.07	0.04	0.09	0.20
$[10^7, 10^8)$	0.03	0.11	0.18	0.03	0.11	0.20
$[10^8, \infty)$	0.00	0.01	0.11	0.04	0.08	0.14
TOTAL	1.00	1.00	1.00	1.00	1.00	1.00

* Abstracted from reference [38].

TABLE XXI

Table of Values of C_{ijk}, the Probability of Bernoulli parameter θ_i, for the Six Prognostic Categories.

Bernoulli Parameter	Premenopausal			Postmenopausal		
	Number of Positive Nodes			Number of Positive Nodes		
θ_i	0	1-3	4+	0	1-3	4+
10^{-11}	0.64	0.00	0.00	0.75	0.34	0.04
10^{-10}	0.12	0.44	0.16	0.03	0.00	0.17
10^{-9}	0.00	0.07	0.09	0.00	0.13	0.02
10^{-8}	0.00	0.08	0.02	0.01	0.01	0.05
10^{-7}	0.05	0.00	0.01	0.02	0.00	0.01
10^{-6}	0.04	0.00	0.09	0.02	0.11	0.05
10^{-5}	0.00	0.04	0.15	0.02	0.04	0.05
10^{-4}	0.03	0.07	0.15	0.03	0.09	0.05
10^{-3}	0.11	0.07	0.02	0.04	0.08	0.22
10^{-2}	0.01	0.08	0.23	0.03	0.12	0.22
10^{-1}	0.00	0.14	0.09	0.05	0.08	0.13
TOTAL	1.00	1.00	1.00	1.00	1.00	1.00

TABLE XXII

Predicted Distribution of Residual Tumor Burden after Surgery using the values of θ_i and C_{ijk} in Table XXI.

	Premenopausal			Postmenopausal		
	Number of Positive Nodes			Number of Positive Nodes		
Tumor Burden	0	1-3	4+	0	1-3	4+
[0]	0.688	0.310	0.121	0.736	0.344	0.154
$[10^0, 10^1)$	0.072	0.208	0.114	0.048	0.106	0.079
$[10^1, 10^2)$	0.009	0.068	0.029	0.012	0.030	0.041
$[10^2, 10^3)$	0.041	0.019	0.028	0.017	0.020*	0.024
$[10^3, 10^4)$	0.032	0.041	0.088	0.018	0.085	0.042
$[10^4, 10^5)$	0.012	0.068	0.140	0.023	0.057	0.051
$[10^5, 10^6)$	0.039	0.072	0.130	0.034	0.081	0.077
$[10^6, 10^7)$	0.083	0.088	0.070	0.037	0.088	0.194
$[10^7, 10^8)$	0.022	0.107	0.174	0.032	0.107	0.203
$[10^8, \infty)$	0.001	0.019	0.106	0.044	0.082	0.134
TOTAL	1.000	1.000	1.000	1.000	1.000	1.000

* Observed and predicted tumor burden distribution differ by more than 0.01.

TABLE XXIII

Predicted Curability of Breast Cancer for Premenopausal Disease as a Function of α and the Increase in Curability Associated with an Extra (Neo-adjuvant) Cycle.

Mutation Rate α	Probability of cure for adjuvant Chemotherapy Number of Nodes			Increase in Probability of Cure with Neo-adjuvant Therapy Number of Nodes		
	0	1-3	4+	0	1-3	4+
10^{-9}	0.994	0.968	0.897	4×10^{-4}t	22×10^{-4}	70×10^{-4}
10^{-8}	0.960	0.872	0.736	16×10^{-4}	43×10^{-4}	47×10^{-4}
10^{-7}	0.891	0.773	0.616	15×10^{-4}	22×10^{-4}	29×10^{-4}
10^{-6}	0.851	0.694	0.475	4×10^{-4}	13×10^{-4}	26×10^{-4}
10^{-5}	0.828	0.631	0.347	4×10^{-4}	8×10^{-4}	15×10^{-4}
10^{-4}	0.791	0.594	0.279	5×10^{-4}	4×10^{-4}	5×10^{-4}
10^{-3}	0.763	0.545	0.246	2×10^{-4}	7×10^{-4}	4×10^{-4}
10^{-2}	0.742	0.447	0.189	3×10^{-4}	14×10^{-4}	7×10^{-4}
10^{-1}	0.693	0.319	0.125	3×10^{-4}	6×10^{-4}	3×10^{-4}
Observed when no chemotherapy	0.692	0.309	0.120			

TABLE XXIV

Predicted Curability of Breast Cancer for Postmenopausal Disease as a Function of α and the Increase in Curability Associated with an Extra (Neo-adjuvant) Cycle.

Mutation Rate α	Probability of cure for adjuvant Chemotherapy Number of Nodes			Increase in Probability of Cure with Neo-adjuvant Therapy Number of Nodes		
	0	1-3	4+	0	1-3	4+
10^{-9}	0.965	0.924	0.872	19×10^{-4}	40×10^{-4}	68×10^{-4}
10^{-8}	0.923	0.812	0.660	12×10^{-4}	33×10^{-4}	65×10^{-4}
10^{-7}	0.882	0.708	0.470	8×10^{-4}	19×10^{-4}	30×10^{-4}
10^{-6}	0.848	0.625	0.379	4×10^{-4}	11×10^{-4}	10×10^{-4}
10^{-5}	0.824	0.551	0.325	2×10^{-4}	9×10^{-4}	6×10^{-4}
10^{-4}	0.805	0.490	0.287	2×10^{-4}	4×10^{-4}	3×10^{-4}
10^{-3}	0.790	0.462	0.252	1×10^{-4}	3×10^{-4}	3×10^{-4}
10^{-2}	0.773	0.402	0.207	2×10^{-4}	6×10^{-4}	4×10^{-4}
10^{-1}	0.740	0.347	0.158	2×10^{-4}	2×10^{-4}	2×10^{-4}
Observed when no chemotherapy	0.736	0.353	0.154			

6. CONCLUSION

In the previous chapters we developed a model for the resistance of tumor cells to chemotherapeutic agents. This model is predicated on the assumption that tumor cells spontaneously acquire resistance to drugs as these cells grow. This model uses a growth model (developed in Chapter 2) which assumes that tumors, in analogy to normal tissues, are composed of three types of cells: stem cells, transitional cells and end cells. The growth of these cells is described by a discrete-time Markov model with constant transition probabilities for each cell. Using known results the asymptotic distribution of the number of cells at time t was derived. For unbounded realisations of tumor growth, it was shown that the asymptotic distribution depends only on the number of stem cells at time t_0. For all unbounded realisations, having the same growth parameters, the proportion of each type of cell converges almost surely to a fixed limit. It was argued that, for most parameter values which are likely to arise in practice, this asymptotic distribution would approximate the true distribution for tumors of clinical dimensions. In this case, the number of cells of each type can be estimated from a knowledge of the parameter values and the observed size of the tumor. In particular the number of stem cells can be estimated and curability of the tumor reduces to consideration of the stem cells alone.

The preceding model of tumor growth must be regarded as approximate since it takes no account of local and systemic conditions which influence growth. Furthermore, the assumption that cells grow independently must be considered a first approximation since interactions between cells have been demonstrated in a number of systems.

Further work is needed to develop models describing the growth of tumors which preserve the discrete nature of the process and incorporate the random nature of individual cellular events. It is unlikely that such models will strongly influence the distribution of resistant cells unless there is some, presently unrecognised, relationship between parameters governing growth and those governing the development of resistance.

In Chapter 3 a model was constructed for the development of stem cell resistance to a single drug. It was assumed that stem cells behave independently and grow as a birth and death process with fixed parameters. The probability generating function of the number of sensitive and resistant stem cells was derived for a tumor of known parameters that began with a single cell. It was shown that the mean proportion of resistant cells increases in time. Recursive relationships were developed for the calculation of the probability generating function of the process after an arbitrary sequence of treatments. The formulation assumes that all cells behave independently and that their interdivision times are exponentially distributed with the same parameters. To model situations where the growth rate of resistant and sensitive cells are different, it could be necessary to use a model in which this is permitted: such a model has been described by Day [34]. Models which permit cells to have interdivision times which are not exponentially distributed are of interest. However these models will generally not have the simple Markov structure of the one used here and their development will be more complicated.

Using the model developed in Chapter 3 it was shown that the best strategy for maximizing the probability of cure for a given total dosage,

D, of a drug over a period $[t_1,\infty)$ is to give the whole dose at time t_1. Therapies which best approximate this strategy (in real systems) have previously been recommended, as a result of empirical research, on the basis that they maximise $P\{R_0(\infty)=0\}$. In particular the knowledge that such a dosage schedule also maximizes $P\{R_1(\infty)=0\}$ mandates its use (or the clinically feasible regimen which best "approximates" it). This may be of particular importance since a number of different regimens may have similar values for $P\{R_0(\infty)=0\}$ but divergent values for $P\{R_1(\infty)=0\}$ whereas the reverse is not true (since $R_0(\infty)>0$ implies $R_1(\infty)>0$).

A central problem arising in the analysis of spontaneous tumors is the specification of the age of the tumor when first seen. Coupled to this is the fact that certain realisations of the growth model have zero stem cells at $t=\infty$ and should not be included in the consideration of large tumors. Three possible approaches were developed to address these problems:

1) Delete sample paths where $N(t)\to 0$ and choose t' so that the distribution $\{N(t')|N(\infty)>0\}$ approximates that observed,

2) Approximate the distribution $\{R_1(t)|N(t)\}$ by the distribution $\{R_1(t)|R_0(t)\}$ over the early period of growth of the tumor, assume that the subsequent growth of R_0 cells is deterministic and derive the resulting distribution of $R_1(t^*)$ for some observed $R_0(t^*)$,

3) Assume that tumors are initiated uniformly in time and then calculate the resulting distribution of resistant cells for a tumor distribution at diagnosis of a particular prescribed form.

Each of these approaches represent solutions to different problems

and as such are generally not directly comparable to one another. Each case is of use for a particular situation. In terms of the model developed, the first two solutions can be generalized by redefining the concept of the size at diagnosis. One approach is to define the critical tumor burden to have a distribution across individuals (with that tumor) and assume that diagnosis will occur when the size of the tumor first exceeds the criticial size in that individual. This would require the consideration of first passage times and would be quite complex. The third approach can be generalised in several directions. The resulting distribution of resistant cells can be examined for a variety of mean incidence functions, $\mu(t)$, which do not have the simple form (i.e. constant) assumed in Chapter 3. Possible forms of this function are available from the mathematical modelling of carcinogenesis [15]. In such cases the modelling of resistance is unlikely to yield simple expressions for the probability generating function and numerical evaluation will be necessary. The use of incidence functions of this type will permit the examination of the distribution of resistant cells as a function of the age of the subject. In advance it does not seem likely that a strong relationship will exist, however, it is worthy of exploration. The major conclusion from the analysis of the three approaches is that the quantitative description of resistance depends upon the description of the system under consideration and that attention must be paid to the particular experimental situation. However, qualitatively the systems behave similarly and one or other of the approaches presented is likely to be of use in most situations.

In the last section of Chapter 3 we introduce the concept of

intrinsic variability in the mutation. There is little direct evidence for such variability however given the experimental complexity involved in testing for such variation we analysed its effect assuming that the appropriate parameters to follow a beta distribution. It was shown that variability in the mutation rates affect the form of the probability of cure and thus it may be possible to identify this phenomenon in experimental systems. This phenomena was examined in Chapter 5 for the experimental data on the L1210 leukemia treated with Cyclophosphamide and Arabinosylcytosine. Initially a model was fit where all cells were considered sensitive and the logarithm of the probability of cell survival, after treatment, was proportional to the dose used. This model did not fit the data well for either drug. Generalising this model to permit the existence of resistant cells considerably improved the fit to the data for each drug. Allowing the mutation rates to vary improved the fit of the model for the data on treatment with Cyclophosphamide, but not for Arabinosylcytosine. In both cases there still remained unexplained variation. These considerations apply only to a single well behaved tumor system treated with two drugs. It is quite possible that spontaneous tumors may have more variable mutation rates. In particular, we have analysed data on a single tumor, the L1210 leukemia, and we cannot generalise results from a particular leukemia to all leukemias (in the same animal). To determine whether variation in mutation rates exists is necessary to compare estimates of the mutation rates for a variety of experimental tumors of the same type.

In addition, the data used in the preceding analysis did not include the cause of death (whether due to resistant or sensitive cells). The

analysis of similar data with cause of death information would allow more accurate determination of mutation and pharmacokinetic parameters. Such an analysis may also be useful in determining the source of the residual variation unexplained by the present model. Further analysis of such data is desirable since the concepts developed from such experiments are used in the construction of protocols for the treatment of human cancer.

In Chapter 4 a model was developed for resistance to two drugs. Expressions were developed which enabled the joint probability generating function of the number of stem cells to be calculated for an arbitrary treatment regimen. Although not explicitly detailed, the effects on the probability of cure of the timing and dosage of a single drug are seen to carry over to this situation. However, the optimum use of two drugs remains an unresolved problem. The major problem is that there is no common scale of measurement for the effects of drugs on normal tissue. There is a need for models of toxicity since the construction of protocols critically depends on them (both in theory and practice). However, given that such a dosage and timing schedule have been described then it is possible to examine how the ordering of treatments may effect the probability of cure. In particular, it was shown that if the treatments are "equivalent" (i.e. each has the same effect on sensitive cells and cells resistant to it and are given at the same times) then the expected number of stem cells is minimized by giving these drugs in an alternating fashion. It was also argued that, in most cases of practical interest, the probability of cure will also be maximized by such strategies. Although equivalence may not usually arise in practice, its examination leads to the conclusion that treatments must be interspersed

to maximize the probability of cure. For non-equivalent drugs the pattern of interspersement will depend on a number of parameters which reflect the effectiveness of the drugs in each of the stem cell sub-compartments. This problem has been extensively studied by Day [34] who has examined the relationship between the tumor and drug parameters and the pattern of application of each drug in the "optimal" strategies. In cases where the parameters are known the optimal strategy may be determined. In cases where some parameters are not accurately known it seems reasonable to give these parameters a distribution reflecting the precision with which they are known. Optimal strategies may then be determined for this system. Such a calculation was presented for equivalent agents (using the generalized definition) in Chapter 5 where the mutation rates follow a distribution. It was shown (in Section 4.6) that the optimal strategy (for $E[N(t)]$) is the same as for the fixed mutation rate case (that is, the drugs should be alternated). However, in other cases the optimal strategy may depend on the amount of variability (or lack of precision). This problem is worthy of further exploration.

The generalization of this model to more than two drugs represents a considerable technical problem. This situation is probably best approached using a model similar to that developed by Day [34]. As in the example of two drugs, an unresolved question is the way in which drugs may be combined. This requires a knowledge of their joint effect on toxicity.

Chapter 5 presented applications of the theory developed in preceding chapters to experimental and clinical tumors. In addition to

those situations already discussed, the model was applied to the neo-adjuvant chemotherapy of breast cancer. Using an ad-hoc model for the effect of surgery on the distribution of stem cells, we assessed the influence of an extra neo-adjuvant cycle of chemotherapy on the probability of cure. Chemotherapy was assumed to consist of a single drug with unspecified pharmacokinetic and mutation parameters. Generally it is found that the application of the extra neo-adjuvant cycle had little effect on the probability that the tumor is cured. This lack of improvement results mainly from the high variability in the post-surgical tumor burden as estimated by Skipper [38]. In situations where the variation in burden is much smaller, the effect of neo-adjuvant therapy can be expected to be greater. However, it should be emphasized that this conclusion only applies to the development of spontaneous drug resistant cells and if other mechanisms of tumor sensitivity are influenced by this early cycle of therapy, then the resultant effect may be considerably larger. Of more general interest, this analysis illustrates the sensitivity of this model to variation in the overall stem cell burden. This is not surprising, at least in retrospect, but it does illustrate that the quantitative effect of therapeutic strategies determined for animal models may not translate simply to human disease where the variation in tumor burden at treatment is much greater. Further work in modelling human disease is desirable, since an understanding of the parameters which influence the clinical therapy of cancer is the ultimate objective of such research. The greatest obstacle to such research is the relative paucity of quantitative information available for human disease. At present a most fruitful approach would

seem to be to model clinical systems where the parameters have considerable variation which may be taken to reflect the heterogeneity or imprecision in their specification.

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INDEX OF NOTATION

Index of first appearance of notation. Greek symbols are listed seperately.

page