MATHEMATICAL MODEL STUDIES ON THE OPTIMAL SCHEDULING OF THE TREATMENT OF SYSTEMIC MALIGNANT DISEASE BY RADIATION.

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To Mum

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SUMMARY

The work reported in this thesis deals with mathematical model studies on the optimal scheduling of treatment of systemic malignant disease by radiation. To provide the necessary background to the original aspects of the work reviews of several fields are required. Chapter 1 is a general review of normal tissue radiobiology. Chapter 2 is a review of human tumour radiobiology. Chapter 3 focusses on one particular isoeffect model, the linear-quadratic or LQ model, which is employed throughout this thesis to describe the effects of radiation on normal tissues. These basic radiobiological principles are applied to the clinical modalities of total body irradiation (TBI) and biologically targeted radiotherapy (BTR). Chapter 4 reviews the principles of TBI. Chapter 5 is a review of published data on the in-vitro radiosensitivities of human leukaemia/lymphoma and neuroblastoma, two conditions which require a systemic approach to treatment. Chapter 8 is a review of the principles of BTR.

The original work is contained in the appendix to chapter 3, which examines the correspondence between the LQ model and CRE models for continuous radiation exposures with constant and exponentially decaying dose-rates; chapter 6, which examines the question of whether fractionated or low dose-rate TBI is the superior method of treatment; chapter 7, where the optimal scheduling of fractionated TBI is investigated; chapter 9, where the LQ isoeffect model and a dosimetric approach is used for the evaluation of

alternative therapeutic strategies for the treatment of widespread micrometastatic disease by BTR. Finally, in chapter 10 a simple model is used to investigate optimal scheduling of BTR, TBI and marrow rescue.

CONCLUSIONS

1/ Comparison of the LQ model and the CRE model for continuous radiation exposures: for constant dose-rates it is found that, when late-effect parameter values are used in the LQ model, there is a correspondence between the models' predictions. There is no correspondence between models when acute-effect parameter values are used in the LQ model. In the case of exponentially decaying dose-rates the predictions of the CRE and LQ models appear more divergent, although again the use of late rather than acute-effect parameter values in the LQ model gives a closer match to the CRE.

2/ Fractionated TBI is predicted to be preferable to low dose-rate TBI treatment. Although theoretically the methods can be equivalent, low dose-rate treatments would have to be over impractically long treatment times.

3/ In the case of external beam TBI, fractionated low dose-rate treatments do not appear to offer a significant improvement over fractionated high dose-rate treatments. This is because in order to achieve a significant increase in dose or reduction in toxicity impractically long exposure times are required. It is expected that this finding will be true in general for external beam radiotherapy, not just in the case of TBI.

4/ Optimal fractionation schedules for the treatment of leukaemia/lymphoma and neuroblastoma by TBI are predicted to be accelerated and hyperfractionated. It is suggested that a two fraction per day schedule of 10 fractions of 1.3-1.5 Gy is a suitable candidate for clinical evaluation.

5/ It is concluded that knowledge of α/β ratios for tumours and normal tissues is, by itself, insufficient information to enable prediction of optimal schedules.

6/ In the case of BTR, dose-rate effects are predicted to be important for late-responding tissues. Tolerance doses may be greater or less than those for fractionated radiotherapy depending on the effective radionuclide half-life.

7/ When injected activities of targeted radionuclide are restricted by haemopoietic tolerance, curative therapy is unlikely. 131-I appears to be a better radionuclide warhead for therapy of micrometastases than 90-Y.

8/ The use of bone marrow rescue in conjunction with BTR seems to offer curative potential, however reasons are presented why a combined strategy using BTR, TBI and marrow rescue is likely to be preferable.

9/ For optimal scheduling of BTR, TBI and marrow rescue, the main characteristics of BTR which determine curative potential are its specificity and sensitivity. Specificity is defined here as the ratio of initial dose-rate at the tumour cells to that in the dose-limiting tissue. Sensitivity is inversely related to the proportion of

tumour cells which escape targeting. Where biological targeting is highly specific but some tumour cells escape, a phenomenon of "overkill" will largely determine the optimal schedules. It is predicted that these are likely to consist of combinations of BTR and external beam TBI with the TBI component being the greatest in terms of radiation dose to the whole body.

1.0 NORMAL TISSUE RADIOBIOLOGY

The major object of radiotherapy is to sterilize tumour cells. In general, greater radiation doses produce greater levels of tumour cell sterilization. In principle, any tumour of any size can be sterilized if a high enough dose is given. However, it is not possible to irradiate tumour cells without, simultaneously, irradiating non-tumour normal tissues and it is the capacity of these to withstand radiation insult which provides the limitation on the dose which can be given. Limiting doses are dependent on the types and proportions of normal tissues and organs irradiated. Most radiotherapeutic practice is based on the principle of "treat to tolerance" i.e. give as much dose as the relevant normal tissues can be expected to tolerate. As the probability of tumour cure is dependent on dose, the knowledge of allowable doses to normal organs and how these change as the parameters of the radiotherapeutic technique changes is of vital importance. This chapter consists of a brief description of isoeffect models, a historical overview of how they have developed and an account of the relevant underlying biological processes involved.

1.1 ISOEFFECT MODELS IN CLINICAL RADIOBIOLOGY

An isoeffect model is basically a method of predicting how the parameters of a radiotherapy schedule influence the dose of radiation which brings about a specific biological endpoint. This may be a certain level of functional impairment of an organ or tissue, alternatively it may be

a probability of survival of a whole animal or patient. Isoeffect relationships are arrived at by, in effect, specifying a number of dose schedules which are all judged to be "biologically equivalent". They may exist in the form of graphs or nomograms, as simple or complex mathematical formulae and as the intuition, based on clinical experience, of a radiotherapist. Some data upon which isoeffect relationships are based are derived from clinical practice and usually involve the ranking of radiation effects on some semi-quantitative scale of damage. The work of Turesson and Notter(1988) on the acute and late effects on skin of radiotherapy for breast cancer is a particularly good example of this. Information may also be accumulated through studies of organ function (Depledge et al, 1983), radiographic examination (Mah et al, 1987) and mortality (Fryer et al, 1978) Animal experiments provide an additional source of data which is becoming more important as information about individual organs is required. These in-vivo assays may be on a semi-quantitative scale similar to the clinical case (Fowler et al, 1965; Turesson and Notter, 1979 (a)) but other methods are also available such as LD50 assays (Lehnart and Rybka, 1985; Cardozo et al, 1985; Vegnesa et al, 1985) colony counting assays (Withers and Elkind, 1970; Huczowski and Trott, 1987) and assays of organ function (Travis et al, 1979; Williams and Denekamp, 1983; Stewart et al, 1984 & 1987).

From the earliest days of radiation biology it has been recognized that radiation effects on biological systems

are highly dependent on the pattern of exposure. For example in any one particular tissue or organ using several small radiation doses enables a larger total dose to be given for an equivalent observed biological effect compared to one single big dose. Alternatively if the same total dose is given in small fractions the biological effect will be less. Another important consequence of the pattern of radiation exposure from the point of view of cancer therapy is the dissociation of responses in different tissues or organs, each having their own individual behaviour, when the pattern of radiation exposure changes. The earliest reference to this phenomenon is in the work of Regaud (1922) on the sterilization of the testes of rats by radiation. In order to effect sterilization with a single dose, significant damage was caused to the surrounding skin. When fractionated radiation was used, sterilization could be accomplished without the same degree of skin damage. This experiment demonstrated that the relative sensitivities of two tissues were not invariant but could be changed by manipulating the treatment structure. The dissociation of radiation effects in tissues composed of different cell populations provides the ultimate raison d'etre of isoeffect models i.e. their use in the attempt to increase the therapeutic ratio between damage to tumours and damage to normal organs and tissues. The subject of radiation effects on neoplastic cells is considered in the next chapter. This chapter will deal with the development of mathematical descriptions of isoeffect relationships for

normal tissues.

Radiation therapy can be given in one of two ways :

1/ In the form of multiple high dose-rate exposures e.g. external beam fractionated treatment.

2/ As a single continuous treatment at a low doserate e.g. interstitial implant of radioactive sources

If one had to specify what qualities an "ideal" isoeffect model should have then one important feature would be applicability to both of the above situations. Another would be the ability to express any differences between different biological systems. Yet another would be to account specifically for every variable of importance. At present there is no isoeffect model which embodies this combination of properties; some come closer than others however. The next section deals with the history of isoeffect models in which can be seen the development of successively closer approaches to the ideal.

1.2 HISTORICAL DEVELOPMENT OF ISOEFFECT MODELS

The first isoeffect rule for fractionated radiotherapy, the "cube root law" was purely empirical and stated that the total dose for a given biological effect should be proportional to the cube root of the number of fractions. i.e.

$$D = k N^{1/3} \qquad (1.1)$$

This meant that if it were desired to change a fractionation schedule of total dose D_1 given in N_1 fractions to a "biologically equivalent" schedule consisting of N_2 fractions, the new total dose was given by

$$D_2 = D_1 \left(\frac{N_2}{N_1}\right)^{1/3} \qquad (1.2)$$

Strandqvist in 1944 published a curve of isoeffective total dose versus duration of treatment based on many years' experience in the treatment of carcinoma of the skin and lip. By finding the curve which most effectively divided patients into cures and failures he found a relationship.

$$D_2 = D_1 \left(\frac{T_2}{T_1}\right)^{0.22} \qquad (1.3)$$

When expressed on a log-log plot this relationship was represented by a straight line. This method of expressing total dose for a tissue isoeffect became known as a Strandqvist plot. Alternatively total dose may be plotted against fraction number in a Fowler-Stern plot (Fowler and Stern, 1963).

The next development in the history of isoeffect models was due to Ellis and co-workers in the late 1960's (Ellis, 1969; Winston et al, 1969). Ellis reasoned from consideration of isoeffect curves for skin tolerance, erythema and squamous carcinoma cure that the effects of fraction size and overall time could be separated. This led to the concept of nominal standard dose, NSD. NSD and its derivatives proved to be very influential in the historical development of radiotherapy. It is instructive to examine the assumptions underlying this approach.

Briefly, Ellis's reasoning went as follows : the main factor in the healing of skin epithelium is the condition of the underlying connective tissue. By and large, connective tissue is similar throughout the body including the vicinity of tumours. Both cutaneous tolerance and erythema have the same slope on a Strandqvist plot, i.e. 0.33, suggesting that the same mechanisms are responsible for the expression of and recovery from radiation damage. As squamous carcinoma is not subject to homeostatic control of growth the radiation effect depends only on fractionation and not on temporal factors. The slope of the isoeffect curve for cure of squamous carcinoma is 0.22. Therefore the effects of time can be represented by the difference between the slopes, i.e. 0.11. As the radiotherapeutic schedules represented by the curves used 5 fractions per week, the regression coefficient with respect to number of fractions was modified to become 0.24. This was summarised in the equation

$$NSD = D\ N^{-0.24}\ T^{-0.11} \qquad (1.4)$$

the parameter NSD representing connective tissue tolerance. For schedules which did not result in tolerance, the concept of partial tolerance (PT) was introduced.

$$PT = NSD\left(\frac{n}{N}\right) \qquad (1.5)$$

where N was the number of fractions of a specified dose and frequency which produced tolerance and n was the actual number of fractions in the schedule. Regimes consisting of a series of sub-tolerance schedules could be represented by a PT value which was the sum of the partial

tolerances of the schedules.

$$PT = PT_1 + PT_2 + \cdots \qquad (1.6)$$

In retrospect Ellis's reasoning was flawed. He was not justified in assuming that fractionation had the same effect on normal connective tissue and tumour. Another conceivable interpretation would be that the slope of connective tissue tolerance was entirely due to fractionation with effectively no time dependence whilst that for tumour cure was composed of a small fractionation effect and also a factor due to time. The alternative interpretation is what would be expected for a normal tissue having a slow turnover time and a proliferating tumour cell population. The problem was further complicated by the fact that epithelial tissue (Potten, 1986) and vascular endothelium (Withers et al, 1980), which are both important in the context of tolerance, represent distinct cell populations. Whilst separation of the fractionation and time components may have led to negligible clinical consequences for epithelial tissue this was not so for the critical cells of the vascular endothelium. Needless to say, this was not evident at the time.

The NSD formula gave rise to the derivatives of cumulative radiation effect, CRE, and time, dose and fractionation factors, TDF. As the fractionated versions of these models are based on the original Ellis equation, and thereby the same biological reasoning, they suffer from the same flaws. The CRE model for fractionated radiotherapy (Kirk et al, 1971) used the regression

factors of Ellis for normal connective tissue to establish a scale of radiation damage. Kirk et al reasoned that Ellis's NSD parameter defined a particular value of a more general function which, as it took on different values, corresponded to different levels of sub-tolerance radiation damage

$$CRE_F = D\, N^{-0.24}\, T^{-0.11} \qquad (1.7)$$

The CRE value at tolerance was equal to the NSD parameter. In the CRE approach, instead of having an analogue of partial tolerance, schedules which did not result in tolerance were represented by values of the CRE function. The overall effect of a series of sub-tolerance schedules was found by applying equivalence conditions at the junctions between schedules. In a series of papers the CRE concept was extended to describe continuous radiation therapy with long-lived (Kirk et al, 1972) and short-lived radionuclides (Kirk et al, 1973), volume effects (Kirk et al , 1975(a)) and the consequences of gaps in treatment (Kirk et al, 1975(b)).

Although the same name was used for both the fractionated and continuous isoeffect models, the clinical databases and mathematical interpretations were totally different. In the continuous case the clinically derived isoeffective dose curves of Paterson and Green (referred to by Kirk et al, 1972) for radium treatments were used. Kirk et al reasoned that the CRE for continuous radiation at a constant dose-rate could be expressed as

$$CRE_c = k_q\, D\, T^{-0.29} = k_q \wedge T^{0.71} \qquad (1.8)$$

where D was the total dose given in time T

r was the dose-rate (= D/T)

q was a factor accounting for differences in the relative biological effectiveness (RBE) of different radiations and k was a normalising constant which was required to ensure that the same value of CRE described the same level of biological effect for both continuous and fractionated radiation. The value of k was estimated to be 0.53 $(day)^{0.18}$ from comparisons of fractionated and continuous treatments which were said to achieve tolerance.

The extension of the CRE to continuous radiation had a dual purpose, firstly to compare continuous radiation treatment at differing dose-rates and secondly to relate fractionated treatments at high dose-rate to continuous treatment at low dose-rate. In the first of these applications the continuous CRE formula was successful, not surprisingly as it was simply the expression in mathematical terms of a clinically observed isoeffect relationship uncomplicated by attempts to separate the effects of time and dose-rate, in contrast to the case of the fractionated CRE. The application of the CRE formulae to comparisons between fractionated and continuous radiation was less successful (Liversage, 1980). It is tempting to suggest that the whole idea of relating the fractionated to the continuous CRE was flawed due to the different biological databases used. An alternative method of extending the NSD concept to sub-tolerance radiotherapy schedules was provided by the time, dose and fractionation factor (TDF) approach (Orton and Ellis, 1973). This procedure enables the effects of sub-tolerance schedules

to be added and had practical advantages over the NSD and PT approach. TDF was extended to continuous radiation with the aid of Patersons data on isoeffective doses for different radium dose-rates (Orton, 1974). The oontinuous versions of the CRE and TDF are thus, to all intents and purposes, identical. The inadequacies of the Ellis equation and its derivatives have been exposed by accumulating clinical evidence and animal studies (Arcangeli et al, 1974; Bates and Peters, 1975; Singh, 1978; Berry et al, 1974; Turesson and Notter, 1979(b); Overgaard et al, 1987). The biological basis of the failure of NSD is well established. It is a direct consequence of the dissociation of radiation responses in different tissues and organs. The general concept of normal connective tissue tolerance appears to be no longer a useful one and tissues and organs require to be studied on an individual basis. There have been a variety of attempts to modify the basic N/T exponent equation of Ellis for use with specific tissues like lung (Wara et al, 1973) and brain (Pezner and Archambeau, 1981). This may be done by changing the exponents for individual tissues. For example, increasing the exponent of N and decreasing that for T will describe a tissue with a greater fraction size dependence and a lesser dependence on time. An alternative method of modifying the Ellis equation has been used by Turesson and Notter (1979). This approach involves the use of fraction size dependent correction factors evaluated through experiments on delayed radiation effects in pigs' skin. Even with these

attempts to extend the usefulness of the Ellis equation it is difficult to avoid the conclusion that the stage of its clinical relevance is over. It is perhaps unfortunate that the fractionated and continuous isoeffect formulae are described by the same name and thus tend to be condemned together. There is no evidence which casts doubt on the continuous versions of the CRE and TDF and these empirical relationships appear to be as valid now as they were when proposed.

Computer based isoeffect models have been described by Cohen (Cohen and Creditor, 1983(a); Cohen and Creditor, 1983(b); Cohen, 1987). These are based on the multitarget two component (TC) or linear-quadratic cell survival curves (see next chapter) and estimates of parameters such as cell cycle time and the maximum number of compensatory cell doublings allowed. Cohen has derived parameter values by computer-aided statistical analyses of clinical data and has produced isoeffective dose curves for different organs with various fractionation patterns. Cohen's work represents the most developed form of isoeffect model for normal tissues. However, it suffers from the disadvantages of being not easily accessible and inconvenient to apply in a clinical setting. The years since 1982 have seen the rapidly increasing application of the linear quadratic or LQ equation which is now the normal tissue isoeffect formalism most widespread in use. The LQ model will be employed throughout this thesis and is described in detail in chapter 3.

1.3 BIOLOGY OF RADIATION DAMAGE TO NORMAL TISSUES

Although individual normal tissues probably have individual radiobiological properties, it has been found useful to classify them into two general types, acute responders and late responders (Thames et al, 1982; Fowler, 1984). Barendsen (1982) has suggested that three distinct classes may usefully be identified, however here only two groups will be considered :

1/ Acute or early responders where the time required for the expression of radiation damage can be measured in days or weeks

2/ Late responders in which the time to expression of radiation damage is a matter of months or even years.

Haemopoietic tissues and intestinal, skin and testicular epithelium are all acute responders. In general these cell lines have a dependence on fraction size, and by implication a capacity to repair radiation damage, which is less than that of late responding tissues. However, there are also variations within the acute responding category, as indeed there are within the late responding category, e.g. the fraction size dependence of skin epithelium appears to be greater than that of the haemopoietic system. Conventionally changes occurring two to three months or longer after the end of radiation therapy are considered as "late effects". However, there is no general "late effect" syndrome. It has been suggested (e.g. Duncan and Nias, 1977; Hopewell et al, 1986) that the vascular system may be the main target, damage to which would result in late radiation effects. In

contrast, the following three arguments have been cited against the origin of late effects being due to vascular damage and instead being directly attributable to depletion of parenchymal cells (Withers et al, 1980) :

1/ The doses which may be given safely to different organs (i.e. tolerance doses) are different.

2/ Different radiations vary in their RBE values from one organ to another.

3/ The times required for the expression of functional impairment are different.

Kidney, spinal cord, lung and vascular endothelium are all late responding organs.

The time required for the manifestation of radiation damage is dependent on the kinetics of tissue renewal and the organizational structure of the tissue. Acute responders, such as intestinal epithelium and haemopoietic tissues, have what has been described as a "hierarchical" or H-type organization (Michalowski, 1981), meaning a relatively small compartment of clonogenic but functionally incompetent stem cells feeding into a maturing compartment with limited proliferative ability and also a limited functional capacity, finally feeding a fully functional compartment with no ability for division and from which cells are lost through "wear and tear" or programmed ageing. The functional lifetime of a cell in an acute responding tissue is dependent on type but may typically be from days to weeks. The transit time from stem cell to functional cell is typically a few days. Radiation damage is also carried through the different

compartments. Initially stem cells are sterilized but the damage is not seen till functional cells are lost in the normal way without being replaced. The behaviour of acute responding tissues following radiation insult is characterized by a progressive diminution of functional cell number followed, after a time lag, by compensatory accelerated proliferation. This would mean, from the perspective of an isoeffect model, that the time during which radiation exposure takes place is irrelevant when it is less than the delay period and of great importance when it is longer. It therefore implies that the sort of time function used in the Ellis equation cannot be correct, as this has most effect for short times. However taking account of the time factor in acute tissue isoeffect models is not a simple process (Whèldon and Amin, 1988) and remains an unsolved problem to the present day.

Late reponding organs, such as kidney, liver and nervous tissue, do not have the same kinetic structure as the acutely reacting tissues, but instead appear to consist of a population of stable, functionally competent cells. Replacement of functional cells lost through "wear and tear" processes may be achieved by division and differentiation of stem cells or by division of functionally competent cells. In normal circumstances cells are not noticeably involved in proliferation and the existence of a separate compartment of clonogenic stem cells is not established. Isoeffect curves of total dose versus dose per fraction are steeper for late responses than for acute reactions. It has been suggested (Thames

et al, 1982) that this is a consequence of an intrinsic difference in the shapes of the clonogenic survival curves of the target cells responsible for these effects (i.e. the intrinsic radiosensitivities) and the delayed response of these organs to radiation insult simply reflects the slow kinetics of renewal of parenchymal cells (Withers et al, 1980). On the basis of this hypothesis one would expect the time for development of functional impairment to be dose-independent.

The viewpoint that the differences in fractionation reponse between acute and late responders are due to differences in the intrinsic radiosensitivities of target cells has been challanged by Zeman and Bedford (1984). These authors have suggested that cell cycle redistribution may be a major contributory factor. The radiosensitivity of a cell depends on which point in the cell cycle it is in. For instance, cells in the mitotic or M phase are more sensitive to radiation than those in the DNA synthetic S phase. Following irradiation of a population of cells the "survivors" (i.e. those fully clonogenic) will contain a higher proportion of cells in resistant phases than the original sample. However over a period comparable with the cell cycle time the synchrony of the cell population is lost due to variations in cycle times. A cell population actively involved in redistribution would be expected to display an enhanced radiosensitivity relative to what it would be like if redistribution did not occur. The effects of redistribution would be expected to be greater in acute

effects target cells than late responding ones due to the shorter cell cycle durations of the former. Another hypothesis to explain the differences between acute and late responders has been advanced by Schultheiss et al (1987). These authors suggest that the greater the amount of heterogeneity in cellular radiosensitivity parameter values the more apparently "flat" the response curve for tissue function will be. This could distinguish two different cell lineages even if the average radiosensitivity parameter values are the same. The apparently flatter dose-response curves for acute responding tissues may therefore be due to a greater amount of heterogeneity.

An alternative suggestion is that the late response is governed by the "flexible" or F-type organizational structure of the target cell lineage (Michalowski, 1981). On the basis of this hypothesis the behaviour of a late responding tissue following radiation insult may be summarized as follows :- Cells capable of both division and physiological function will die and be removed at the end of their lifetime. A dose-dependent proportion of the remaining cells will have been rendered non-viable by radiation. On receiving a homeostatic signal for compensatory proliferation these will attempt to divide in order to restore tissue function. In this way latent radiation damage may be expressed as an "avalanche" of abortive mitoses, driven by the increasing strength of the signal for cell recruitment generated in response to the dwindling population size. Post irradiation stimulation of

proliferation would lead to a potentially catastrophic failure of mitoses. This is in contrast to an H-type tissue where stimulated proliferation would be beneficial. For a F-type tissue the behaviour of cells rendered incapable of full clonogenicity but with a limited division potential will be an important factor in the functional dose response curve. "Doomed" cell proliferation of only one or two generations following irradiation may preserve the functional integrity of the tissue. There will consequently be a dissociation between clonogenic and functional endpoints. The functional dose-response curve will be "curvier" than for an H-type tissue, even if the underlying intrinsic clonogenic radiosensitivity of the constituent cells are the same. For an F-type organ the time to expression of radiation damage will be inversely dose-dependent (Michalowski, 1981). This was illustrated by the mathematical model study of Wheldon et al (1982). Wheldon and Michalowski (1986) have suggested that a spectrum of proliferative organizations may occur in different organs from pure H-type to pure F-type and HF hybrids between these extremes. HF hybrids will also possess some of the pure F-type characteristics such as the "avalanche" effect and the dose-dependent latency of radiation damage.

Inversely dose-dependent times to functional impairment have been observed clinically and experimentally in animals (Focht et al, 1966; Phillips and Roth, 1973; van der Kogel and Barendsen, 1974; Madrazo and Churg, 1976; White and Hornsey, 1978; Masuda et al, 1980; Schultheiss

et al, 1984, Lebesque et al, 1986). However Thames et al (1986) concluded that differences in post irradiation proliferative behaviour could not explain the differences in fractionation sensitivity between acute and late responding tissues. Presently there is no conclusive proof of the correctness of any of the theories of why acute and late responding tissues differ in their fractional dose-dependence. The HF approach appears to be most amenable to experimental testing and conceivably this may be the first hypothesis to be accepted or rejected.

1.4 ACCELERATED AND HYPER-FRACTIONATION

From the perspective of isoeffect models, late responses have an increased dependence on the size of dose fractions in comparison with acute reactions, and have little dependence on the timescale of a radiotherapy treatment for all reasonable durations. Lung is, to some extent, exceptional in that a phenomenon called "slow repair" has been observed (Field et al, 1976). The nature of this is uncertain. It appears to be different from the compensatory proliferation seen in acute tissues, although it may be a protracted form of this. In clinical practice late effects are generally dose-limiting. This is because acute effects can usually be seen developing and fractionation schedules modified (e.g. by inserting a break in treatment) to limit them. Conversely, late effects are not apparent sometimes for years and their severity cannot be predicted by the severity of acute effects. With isoeffect models like the linear-quadratic (see Chapter 3) late effects can be anticipated and

attempts have been made to improve the therapeutic ratio between radiation effects on tumours and those on late responding normal tissues. Hyperfractionation, or using a schedule with a large number of small fractions enables higher doses to be given for the same late toxicity (Douglas, 1982; Thames et al, 1983). It has been suggested that tumours have less fraction size dependence (Williams et al, 1985) and consequently will be damaged more by hyperfractionation. Accelerated fractionation, or giving the treatment in a shorter time than conventional, has also been suggested as a possible way to increase the therapeutic ratio (Thames et al, 1983). This is because tumour cells are expected to have short intermitotic intervals (Steel, 1977; Trott and Kummermehr, 1985). Shortening the treatment time may thus enable fewer potential tumour doublings. These arguments are largely qualitative and open to criticism on the grounds that tumours are not all similar. In a later chapter information for specific normal tissues and tumour types is used to derive optimal fractionation schedules. Some of these schedules have elements of both accelerated and hyper-fractionation.

2.0 HUMAN TUMOUR RADIOBIOLOGY

For the vast majority of mammalian cells the ability to replicate through mitotic division is the function most sensitive to radiation dose. This property can be permanently lost by otherwise apparently normal, intact cells which remain capable of protein synthesis. The ability of tumours to proliferate indefinitely, without reference to homeostatic control, provides one of their main life-threatening qualities. The aim of radiotherapy is to remove the ability of tumour cells to replicate i.e. to sterilize them. In clinical radiotherapy, it has long been common knowledge that different human tumours respond very differently to the same treatment strategy. In part, this could be ascribed to differences of tumour cell kinetics and architecture, leading to different rates of cell removal and hence visible tumour shrinkage (Steel, 1977; Stephens and Steel, 1980). However clinical observation also extends to the variation which exists in "radiocurability" or "local controlability" (Rubin et al, 1974) irrespective of the rate of tumour shrinkage following treatment (Suit et al, 1965), although there may be an inverse correlation between regression and the probability of recurrence (Barkley and Fletcher, 1977). For several decades, this variable radiocurability, presumably reflecting differences in clonogenic cell survival in different tumours, has been attributed to mechanisms which are based on the relative radioresistance of hypoxic cells. A major part of applied radiobiology has been devoted to the search for ways of overcoming or

circumventing the "hypoxic cell problem" in radiotherapy (Adams, 1973; Duncan, 1973; Watson et al, 1978). Undoubtedly, the upsurge of interest in the radiobiology of hypoxic cells owed much to the seminal observations of Gray et al (1953) and Thomlinson and Gray (1955) and their conclusions on the likely existence of hypoxic cells in human tumours. However, one component in the collective decisions of radiobiologists to focus on hypoxia was the lack of evidence for any convincing differences in the radiosensitivities of tumour cells in vitro (e.g. Nias, 1974; Weichselbaum et al, 1980). This perhaps made it seem unlikely that the variability of tumour response in the clinic reflected a corresponding variability in the "intrinsic" radiosensitivity of clonogenic cells.

In recent years, several lines of investigation have resulted in new evidence which justifies reconsideration of the extent to which variation in radiocurability might be ascribed to variations in the sensitivities of clonogenic cells in different tumour types. Fertil and Malaise in 1981, reviewed the available evidence on the intrinsic radiosensitivities of human tumour cells in vitro, demonstrating that the cell survival curves reported differed significantly from one tumour line to another (this being especially true of the low dose region of the survival curve) and that the estimated survival following a dose of 2 Gy correlated well with clinical radioresponsiveness. Recently they have confirmed and extended these observations (Fertil and Malaise, 1985). Deacon et al (1984) have come to the same conclusions.

Rofstad and Brustad (1987) found that the radioresponsiveness of human melanoma xenografts grown in nude mice was positively correlated with the initial slopes of the in-vitro cell survival curves. There was no relationship between the radioresponsiveness and any known growth or microenvironmental parameter. These authors strongly supported the suggestions of Fertil and Malaise and Deacon et al that the clinical radioresponsiveness may be positively correlated to the initial slope of the in-vitro cell survival curve. Studies on the radiation response of human tumour spheroids have likewise indicated suggestive correlations between clinical radioresponsiveness and the response to fractionated irradiation of spheroids derived from the different tumour types (Wheldon et al, 1985(a); Evans et al, 1986). In addition to these investigations of "intrinsic" cellular radiosensitivity, Weichselbaum and co-workers have reported a series of studies indicating important differences in the capacity of human tumour cells in vitro to undertake repair of potentially lethal damage. Here too, the observations correlated with clinical radioresponsiveness (Weichselbaum et al, 1982; Weichselbaum and Little 1982 (a) & (b); Guichard et al, 1984).

Taken collectively, these observations suggest that at least some of the mechanisms responsible for variability of tumour response in the clinic are operative at the cellular level and hence may be amenable to experimental investigation in vitro. Conversely, in vitro studies may

be capable of providing cellular radiosensitivity data which are applicable to clinical treatment. The work described in a later chapter on the subject of optimal scheduling proceeds on the assumption that this is the case. It follows that in vitro radiation survival curves of human tumour cells are the basic data required. In order to approach this subject in a scientific way it is essential to have a method or methods of quantitating the effects of radiation on living cells.

The ability of cells to proliferate indefinitely can only presently be assessed by observing whether or not they do so when placed in a permissive environment. Experimental data generally consists of paired information, given dose and the associated proportion of "surviving" cells. Here survival has the restricted meaning of retention of the capacity to give rise to a colony of cells (e.g. Alper, 1979). The most convenient way of displaying cell survival data is in the form of a graph of the logarithm of the surviving fraction as a function of radiation dose. The logarithmic scale is neccessary to encompass the range of surviving fractions commonly produced in experimental studies. It also reflects the fact that the surviving fraction has a relationship with dose which is essentially exponential.

When radiation interacts with matter energy is deposited in discrete ionization events along the path of the ionizing particle. With biological material some of these ionization events will occur outside cells or in a non-critical region within cells. These will not give rise

to cell sterilization. However other events will produce damage in critical cellular targets, possibly leading to death (Elkind, 1980). The concept of intracellular targets has been dominant in radiobiology for many years and all models for radiation effects on cells are based on this.

2.1 CELL SURVIVAL EQUATIONS

2.1.1 EXPONENTIAL

Pure exponential survival curves are unusual but not absent in the study of mammalian cells (Alper, 1979). Inactivation curves for prokaryotic cells, viruses and proteins are almost invariably exponential in form. Historically the explanation for this type of curve has been provided by assuming that critical targets required only a single "hit". Simple Poisson statistics then determine the survival curve. If a dose D_0 is defined as the mean inactivation dose i.e. it gives an average number of one critical hit per cell then a proportion e^{-1} cells will have experienced no hits and will therefore survive. The surviving fraction after a dose, d will be given by

$$S(d) = e^{-d/D_0} \qquad (2.1)$$

This is the simplest form of survival curve equation. Almost all survival curves for mammalian cells exhibit a "shoulder" region at low doses. This indicates that the effectiveness of cell killing by low doses is less than that for high doses. Survival curve equations must be able to describe this shape. The first attempts to describe shouldered survival curves mathematically were also based on the Poisson statistics of ionization but with the

assumption of either a multiplicity of critical targets which all had to be hit or a target which had to be hit more than once to sterilise the cell.

2.1.2 MULTITARGET

It is postulated that a cell contains n sensitive targets, all of which must be inactivated or "hit" in order to achieve sterilisation (Alper, 1979). It follows that the probability of hitting one target is given by

$$P_1 = (1 - e^{-d/D_0}) \qquad (2.2)$$

That of hitting n independent targets is therefore

$$P_n = (1 - e^{-d/D_0})^n \qquad (2.3)$$

and the probability of a cell surviving

$$S(d) = 1 - (1 - e^{-d/D_0})^n \qquad (2.4)$$

The slope of the survival curve is given by

$$\frac{d}{dd} S(d) = -\frac{n e^{-d/D_0}}{D_0} (1 - e^{-d/D_0})^{n-1} \qquad (2.5)$$

It can be seen that at low doses

$$\lim_{d \to 0} \left[\frac{d}{dd} S(d) \right] = 0 \qquad (2.6)$$

This means that the initial slope of the survival curve is zero.

At high doses

$$\lim_{d \to \infty} \left[\frac{d}{dd} S(d) \right] = -\frac{n}{D_0} e^{-d/D_0} \qquad (2.7)$$

$$S(d) \doteqdot n e^{-d/D_0} \qquad (2.8)$$

Therefore at high doses the survival curve approximates to a pure exponential. On a log-linear plot it will appear as a straight line of gradient $-1/D_0$. If the terminal part of the curve is extrapolated back to the log(S) axis it will intersect at a value log(n). n is known as the "extrapolation number" (Alper et al, 1960).

Alternatively it can be assumed that there are μ separate regions in a cell and deactivation of any one of these is lethal. Each region behaves as if it were composed of r individual targets. The survival curve equation for this case is given by

$$S(d) = \left[1 - (1 - e^{-d/D_0})^r\right]^{\mu} \qquad (2.9)$$

This equation again specifies a survival curve with an initial slope of zero. At high doses the curve approximates to an exponential with an extrapolation number of r^{μ}.

It is possible to use an alternative parameter to describe this form of survival curve rather than the extrapolation number. This is the "quasi-threshold dose" denoted Dq (Alper et al, 1962). It corresponds to the intercept on the dose axis of the extrapolation of the terminal straight line portion of the survival curve. It is therefore not independent of n. They are interchangeable and are related by

$$D_q = D_0 \ln(n) \qquad (2.10)$$

By virtue of their mathematical form the multitarget equations have an initial slope of zero. This means that the first increment of dose will not kill any cells. Experimentally this is not usually found to be the case (see Alper, 1975). Most survival curves have non-zero initial slopes. The biological implication of this from the perspective of the theoretical background to the multitarget formulae is that there is some component of "single-hit" damage i.e. deactivation of a critical target by only one "hit" is sufficient for cell sterilisation.

This observation can be built into the mathematical formulae simply

$$S(d) = e^{-d/D_1}\left(1 - \left(1 - e^{-d/D_2}\right)^n\right) \qquad (2.11)$$

At low doses

$$S(d) = e^{-d/D_1} \qquad (2.12)$$

At high doses

$$S(d) = n \exp - \left(\frac{D_1 + D_2}{D_1 D_2} d\right) \qquad (2.13)$$

The observed final slope is related to the separate slope components by

$$D_0 = \frac{D_1 D_2}{D_1 + D_2} \qquad (2.14)$$

2.1.3 LINEAR QUADRATIC

In recent years the linear-quadratic model has come to pre-eminence due to its use as a normal tissue isoeffect relationship; however the use of a quadratic function of dose to describe radiation effects has a longer history. Several theoretical models of the mechanism of radiation damage to living cells predict that the surviving fraction of cells following a single dose, d, will be given by the relationship

$$S(d) = e^{-(\alpha d + \beta d^2)} \qquad 2.15$$

where S(d) is the fraction of cells surviving after a dose,d and α and β are constants. The linear-quadratic cell survival model has been proposed on the basis of the theory of "dual radiation action" by Kellerer and Rossi (1972) and also on the molecular model of Chadwick and Leenhouts (1973) which assumed that cell death was due

to double strand breaks in DNA and that low LET radiation could cause these lesions due to the interaction of two single strand breaks in complementary strands in close proximity.

2.2 DISCUSSION

As empirical formulae for the construction of survival curves equations 2.4, 2.11 & 2.15 have been used successfully to fit experimental data. Opinions vary on whether a better fit is provided by the multitarget or quadratic function. Certainly in some cases it appears that the quadratic fit is superior, especially at low doses, but there are also cases where the terminal part of the curve does not appear to possess continuous curvature. There is a body of evidence from split dose studies which suggests that survival curves have finite final slopes. Using CHO cells Elkind & Sutton (1959 & 1960) demonstrated that when cells were exposed to a radiation dose and then given a sufficient interval for full recovery, the survival curves following a second dose had final slopes that were parallel to single dose curves. Also the difference between the two doses (one given as a single exposure and the other being the sum of the split-dose components) required for the same effect was independent of the size of the conditioning dose once it was higher than a minimum value. This is contrary to what would be expected for continuously bending survival curves. Although determining whether quadratic or multitarget functions best describe cell survival may seem important in the understanding of underlying mechanism, it

is not possible to decide on the basis of experimental data with it's associated uncertainties. Alper (1980) has pointed out that any curve passing through the origin can be fitted by the polynomial

$$Y = Ax + Bx^2 + Cx^3 \qquad (2.16)$$

provided there is a non-zero initial slope and also that the slope does not become obviously straight over the observed dose range. The first 2 terms will suffice if the degree of curvature is not too great. Caution is therefore nec essary in attempting to deduce underlying mechanisms from survival data. Both the multitarget and linear-quadratic models interpret the shoulder region of survival curves as being due to the accumulation of radiation damage. In the multitarget format damage is accumulated as independent targets are hit. This damage is "sub-lethal" in that only by hitting the last remaining target is it translated into lethality. In the linear-quadratic model the βd^2 term is due to the interaction of two "sub-lethal" lesions either of which in isolation will not result in sterilisation. The use of the LQ model as a normal tissue isoeffect relationship will be reviewed in the next section, where it will be seen that a mathematical representation of repair, operating on sub-lethal lesions, can be incorporated. Nevertheless the LQ model is still essentially an accumulation model. Multitarget and LQ equations have been very successful in empirically fitting survival data, for which purpose they are still used to the virtual exclusion of all other formulae. However conceptually, from the point of view of

underlying mechanism, opinion is moving away from them and is turning to a class of models known as repair models.

2.3 REPAIR MODELS

The vast amount of DNA in the genome of mammalian cells is replicated every cell cycle. There is thus an enormous potential for errors in replication, even before considering the damage which environmental factors could possibly cause. It is therefore not surprising that cells have highly developed systems for the detection and repair of damaged DNA. Any model of radiation sterilisation of cells would therefore, appear inadequate if it did not include repair as an important factor. Radiation can cause a variety of lesions in DNA including double strand breaks (DSB), single strand breaks, base damage, DNA-DNA and DNA-protein cross links (Cole et al, 1980). The most widely held view at present is that it is DSB which are the critical lesion from the point of view of viability (Painter, 1980; Ward, 1986). However there is considerable evidence that most of the induced DSB can be enzymatically repaired (Elkind, 1985; Collins, 1987; Teoule, 1987) an effect not taken account of in, for example, derivations of the linear-quadratic model of Chadwick and Leenhouts. There are fundamental differences between accumulation models and repair models. The resultant survival curve is assumed in the case of accumulation models to be due to a base line of irrepa rable damage from where processes of damage accumulation further reduce survival to the observed survival curve. In the case of repair models a base line of potentially lethal damage is subject to

repair processes which increase the survival probability to the level of the resultant survival curve. Single hit inactivation is the basic mechanism of cell sterilisation in repair models.

One type of repair model explains survival curve shapes by a postulated repair process which operates most efficiently at low doses but becomes increasingly ineffectual as the dose increases. This is a repair-saturation model (Laurie et al, 1972, Goodhead, 1980). Haynes (1966) description of survival for the repair proficient bacterial strain E.coli B/r after irradiation by u.v. light is consistent with a repair saturation model

$$S(d) = \exp\left[-kd + \alpha\left(1 - e^{-\beta d}\right)\right] \qquad (2.17)$$

In this equation the -kd term represents the linear induction of radiation damage which is partially offset by the repair term $\alpha(1 - e^{-\beta d})$. This initially increases with dose but asymptotically approaches a cut-off value of α representing the saturation of repair capacity. Models of this type can effectively explain the shapes of survival curves. Another repair model is the repair-misrepair or RMR model of Tobias et al (1980). Repair enzymes can begin to operate only after the initial physical and chemical processes are over. These processes leave a quantity of "uncommited" lesions, U, which are subject to repair processes. Repair can either be linear or quadratic so that

$$\frac{d}{dt} U(t) = -\lambda U(t) - k U^2(t) \qquad (2.18)$$

Each of the linear and quadratic processes can then either

yield "eurepaired states" (i.e. perfect repair) or "misrepaired states". In a simple case of the RMR model it can be assumed that linear repair is always "eurepair", interpreted as e.g. correctly rejoining broken DNA. Quadratic repair is always misrepair, which can be interpeted as the joining of pieces of broken DNA which don't belong together. Misrepair leads to lethality or mutation. Survival is determined by the competition between eurepair and misrepair. This procedure leads to an equation for surviving fraction

$$S(d) = \exp\left[-\alpha d \left(1 + \frac{\alpha d}{\epsilon}\right)^{\epsilon}\right] \qquad (2.19)$$

assuming enough time for full repair and where

ϵ = repair ratio = λ/κ

α D is the postulated linear relationship between dose and DNA strand breaks. This equation appears to fit experimental data on lethal effects of low LET radiation well. More complex equations can be derived from the RMR model depending on the nature of the assumptions regarding repair and on the post-irradiation time available for repair. Another repair model is the lethal and potentially lethal (LPL) model of Curtis (1986). This model incorporates a number of mechanisms such as the creation of irrepairable lesions, lesion repair by first order kinetics, binary misrepair and the existence of two different types of repairable lesion.

There is no explicit necessity for repair saturation in the RMR or LPL models although such a mechanism could be accomodated in the equations. These equations can describe a wide range of experimental results very well. As has

been pointed out several times, it is not possible to decide between models on the basis of curve fitting. The models based on repair processes do, however, seem to be more biologically realistic and one would anticipate that the future development of cell survival models will owe more conceptually to these than to the "physical" models of the multitarget or linear quadratic type.

2.4 THE LOG CELL KILL FUNCTION

It will be useful to define here a quantity which will be used in subsequent chapters to describe the effects of radiation on tumour cells. This is the "log cell kill" function.

$$T = -\log [S(d)]$$

i.e. the negative logarithm of the surviving fraction. This is a monotonically increasing function of tumour cell sterilisation. When base 10 logarithms are used, T represents the number of decades of reduction of tumour cells. However use will also be made of base e logarithms when mathematical convenience dictates.

2.5 TUMOUR KINETICS

2.5.1 REPOPULATION

The number of surviving tumour cells following a fractionated course of radiation treatment depends not only on dose-associated parameters but also on time-associated ones. This is due mainly to the repopulation of tumour cells.

Studies on experimental animals have revealed a characteristic growth pattern for transplanted tumours

(reviewed by Steel, 1977). At small volumes (depending on species) growth is rapid and exponential. This corresponds to the "silent interval" (Brues et al, 1939) where the existence of a tumour may be inferred but no detectable mass is observable. Tumours at this stage are avascular and essential nutrients are available only through diffusion from outside. As the tumour mass increases it as been observed that tumours can produce a substance - tumour angiogenesis factor (Folkman, 1976; Walker et al, 1984; Buki and Seppa, 1985) which stimulates the neovascularization of the tumour i.e. blood vessels form to supply the tumour with nutrients. As tumours become larger the growth slows down tending towards some asymptotic limit which is usually not reached because of either a) death of host or b) size reduction after treatment or cure. Mathematically the macroscopic growth curve follows a "decelerating" pattern which appears to be well described by the Gompertz equation (Winsor, 1932). This suggests a composite Gompertz-exponential or "Gomp-ex" growth curve (Steel, 1977) with tumours in the microscopic size range following rapid exponential growth kinetics. Little is known about the regeneration of bulk tumours following radiation treatment. There is no obvious reason why repopulating tumour cells should follow the same growth curve as the original untreated tumour cells. It is possible that a pre-existing blood capillary network may support growth up to the immediately pre-treament size. However, radiation damage to the vasculature may restrict

the regeneration. Also repopulating tumour cells will experience a different microenvironment composed of killed and degenerating cells.

The potential doubling time or Tpot is the time required for a cell population to double if there are no cells lost. Cells may be lost from the proliferating tumour population by maturation or differentiation, by death or by emigration from the tumour mass. The potential doubling time and the actual population doubling time, Td together give the cell loss factor, ϕ

$$\phi = 1 - \frac{T_{pot}}{T_D} \qquad (2.21)$$

Trott and Kummermehr (1985) reported their studies of three mouse tumours. In two out of three cases the average repopulation rates following radiation treatment were equal to the potential doubling rates. They also referred to three sets of clinical data :

1/ The data of Maciejewski et al (1983) suggests that the time dependence of the 50% tumour control dose for carcinoma of the larynx is consistent with a repopulation doubling time of 4 days, equal to the potential doubling time calculated for squamous cell carcinoma of the head and neck.

2/ The data of Friedman et al (1967) is consistent with a dose equivalent of 0.3 Gy per days repopulation. This is compatible with the calculated Tpot.

3/ There is a correlation between measured Tpots for Burkitt's lymphoma and the clinical data of Norin and Onyango (1977) relating the complete response rate to the interfraction interval.

Trott and Kummermehr conclude that "the cell production rate of the tumour before treatment may be an indicator of the repopulation potential and might therefore serve as a guide to selecting tumours for accelerated fractionation schedules."

The in-vitro data of Wheldon et al (1985(a)) on human neuroblastoma multicellular tumour spheroids (MTS) indicates that spheroid regrowth following radiation is exponential and equal to the original growth rate. As MTS may be a suitable in-vitro model of avascular micrometastases in-vivo this is especially relevant in the context of microscopic systemic disease, the prime candidate for systemic radiation therapy. Other data germane in this context include :

1/ For paediatric solid tumours (an important patient group for systemic radiation therapy) the likelihood of fast growth kinetics is supported by the the occurence of palpable tumours in very young children below the age of one year (Voute et al, 1986; Tournade et al, 1986; Barrett, 1986). For example, neuroblastoma belongs to a group of paediatric tumours for which the volume doubling time, estimated in the clinical size range, is of the order of 20 days, considerably less than the estimated volume doubling time of various adult tumours of comparable size. Measurements on the volume doubling time of Ewing's sarcoma classify them as the fastest growing sarcomas and are also of the order of 20 days. Since such estimates apply to tumours in the Gompertzian growth range, it is likely that the rate of growth of microscopic

populations is considerably more than this.

2/ Some detailed studies of cell population kinetics have been made on neoplasms for which systemic radiotherapy is used or being considered. Estimates of the intermitotic interval for acute leukaemias indicate a rapidly dividing population. The median of 6 independent series of measurements of intermitotic time was approximately 2.5 days (Steel, 1977). The Tpots for paediatric tumours classify them as one of the fastest growing groups. The median of 5 measurements of Tpot for neuroblastoma has been reported as 5.2 days (Aherne and Buck, 1971). These values may provide realistic estimates of the population doubling time of clonogenic cells in metastases and of the primary tumour when it is small or regrowing after therapy.

Steel (1977) concluded from data on labelled mitoses curves for a variety of human tumours that "In human tumours, even more than in tumours of laboratory animals, there is no clearly defined "cell cycle", but a broad band of intermitotic times whose median value (in cases where it has been reliably determined) has been in the region of 2-3 days."

For radical systemic treatment with, hopefully, patients in clinical remission only low levels of diffuse or metastatic disease are present. In these circumstances it is reasonable to assume that the proliferation of tumour cells will be fast and exponential.

Measurements of tumour cell kinetics have suffered from their extremely laborious and time-consuming nature. A new

technique with potential to dramatically reduce the time required and increase the quantitative resolution of cell kinetic measurements has been described (Danova et al, 1987; Wilson et al (1988). This approach is based on the incorporation of the thymidine analogue bromodeoxyuridine (BrdU) into DNA. A monoclonal antibody raised against BrdU integrated in DNA and flow cytometric techniques of measuring it enable rapid (within 24 hours) estimation of the cycling fraction, cell cycle time and the durations of the individual phases of the cell cycle. The next few years are likely to see the application of this method to the clinic where it may be useful as a predictive assay of tumour response.

2.5.2 REDISTRIBUTION

Cell cycle redistribution was briefly mentioned in chapter 1 with reference to the hypothesis of Zeman and Bedford (1984) on its likely effects in normal tissues. Cellular radiosensitivity is dependent on cell cycle phase. Cycling cells surviving a dose of radiation tend to be synchronised in resistant phases of the cycle. This effect may be enhanced by radiation-induced blocks in cell cycle progression at the G1/S and G2/M interfaces (Denekamp, 1986). However the synchronisation does not last long due to variations in the speeds with which individual cells progress round the cycle. This breakdown in synchronisation is called redistribution. Redistribution may be an important factor determining the responses of cells which are actively progressing through the cycle. It will have the effect of proportionately re-sensitising

cells which have fast cycling times. This will work in an opposite sense to repopulation processes in terms of its effects on the cellular population size following a fractionated course of radiation treatment. One would expect that cells in a non-cycling "G0" state will not be affected by redistribution. However following radiation insult these cells may be recruited into the cycling population and, hence, be subject to the effects of redistribution.

2.5.3 REOXYGENATION

At the beginning of this chapter the "problem of hypoxic cells" was alluded to. The oxygen effect was noted as early as 1921. Briefly it has been found that the dose required for a certain level of survival is about three times greater under hypoxic conditions than it is under fully oxygenated conditions (Hall, 1978). The ratio of hypoxic to oxic doses needed to achieve the same biological effect is the same at all survival levels and is called the oxygen enhancement ratio (OER). Thomlinson and Gray in 1955 reported the histology of some bronchial carcinoma specimens where they found a characteristic pattern of viability and necrosis. Regions of viable tumour cells were seen around blood vessels, but further away from the supply of nutrients were large areas of necrotic tissue. They deduced the existence of a hypoxic region between the anoxic necrotic areas and the fully oxygenated tumour cells and suggested that the presence of a relatively small proportion of hypoxic cells could limit the success of radiotherapy in some clinical

circumstances. There is a body of evidence for the existence of hypoxic cells in human tumours (Glassburn, 1977; Thomlinson et al, 1976; Fowler, 1983) but it is a moot point whether they affect radiocurability when standard fractionated treatment schedules are used which allow reoxygenation. Following irradiation there are several possible mechanisms of reoxygenation, none of which excludes the others (Denekamp, 1986). It is possible that radiation-damaged cells respire more slowly and thus use less oxygen. This may allow oxygen to diffuse further. When the lethally irradiated cells die and are removed by lysis, previously hypoxic cells may move back to the oxygen-rich region. Also recirculation of blood through vessels which were previously closed may facilitate reoxygenation. Given all the radiobiological effort in investigating hypoxia, there is no doubt that clinical approaches based on countering "the problem of hypoxic cells" have been disappointing. The treatment of systemic microscopic disease is probably the least likely clinical situation where hypoxia may be a contributory factor to treatment failure.

3.0 THE LINEAR-QUADRATIC ISO-EFFECT MODEL FOR NORMAL TISSUES

Various theoretical models of the effects of radiation on cells predict linear-quadratic survival curves as described in Chapter 2. Empirically there appears to be a satisfactory agreement between a quadratic equation and experimental data for single dose survival curves for, at least, some of the dose range including what, up till now, has been the dose range of clinical interest (e.g. Steel et al, 1987). Although it is a matter of some controversy as to whether the prime factor determining survival of parenchymal cells is radiation damage to the vasculature or clonogenic sterilisation of the parenchymal cells themselves it seems reasonable to assume that there is a one-to-one correspondence between the level of depletion of parenchymal or stromal cell number and the impairment of physiological function of an organ or tissue (Thames et al, 1982; Withers et al, 1983). This assumption is central to the use of the linear-quadratic equation, and in fact any cell survival equation, as the basis of a normal tissue isoeffect model.

3.1 FRACTIONATED RADIATION SCHEDULES

Douglas and Fowler (1976) used a quadratic function of dose,

Effect, $E = n(\alpha d + \beta d^2)$ (3.1)

to analyse the effects of fractionated radiation on mouse skin reactions. These authors originated the so-called " Fe plot ", a plot of reciprocal total dose against dose

per fraction for a set of fractionation patterns judged to be isoeffective. If the quadratic equation was a realistic description of what was happening this plot will produce a straight line. The ratio of intercept on the reciprocal dose axis to slope is the ratio α/β . Alternatively, as pointed out by Michael (1985) the intercept on the dose per fraction axis is also α/β . In 1982 Thames et al used a quadratic equation as the basis for an isoeffect model which distinguished between acute and late normal tissue reactions. Late reactions had a consistently lower α/β ratio of around 3 Gy in comparison to acute reactions, which had a characteristic α/β ratio of around 10 Gy This suggested that the underlying cell survival curve for the target cells was more " curvy " than for those responsible for acute effects. In their theoretical development of the model these authors assumed:

1/ The same level of cell killing results from each successive dose i.e. equal effects per fraction.

2/ The effects of proliferation were negligible, therefore the surviving fraction after N successive doses was simply $(S(d))^N$.

3/ The biological effect is determined exclusively by the survival level of the target cells.

The level of biological effect, E was written as

$$E = - \ln \left[S(d)\right]^N \qquad (3.2)$$

as the survival equation used was

$$S(d) = e^{-(\alpha d + \beta d^2)} \qquad (3.3)$$

This enabled the relationship

$$\frac{1}{D} = \frac{\alpha}{E} + \frac{\beta}{E}\alpha \qquad (3.4)$$

to be obtained which is equivalent to the equation used by Douglas and Fowler (the basis of the Fe plot). Simultaneously, Barendsen (1982) proposed a linear-quadratic model for isoeffective dose prediction. Barendsen's approach, although computationally identical to Thames et al, differed conceptually in that he invoked a specific " micro-theory " to explain the quadratic dependence. A number of assumptions were made, specifically:

1/ Radiation causes cell sterilisation by the induction of lesions. Some of these lesions are produced linearly with dose (single hit damage) while others are produced by the interaction of two " sub-effective " lesions. Lethal lesions produced by sub-effective lesion interaction increase as the square of the dose.

2/ Sub-effective lesions must be produced close enough together in space and time in the same cell. In the case of low LET radiation these will be produced independently by different ionising particles.

3/ Sub-effective lesions remain available for interaction for only a limited time after their induction. Although Barendsen proposed an exponential decay in the capacity for sub-effective lesion interaction with a half-time of between 0.5 and 2 hours this theme was not fully developed until Dale's modification to the linear-quadratic model in 1985.

4/ In a similar fashion to Thames et al, (1982) Barendsen assumed that equal dose fractions are equally

effective, independent of the preceding or following fractions.

5/ The response of an organized tissue is dependent on the average number of lesions in the constituent cells. This number depends on what level of response is considered but, if the relationship between tissue response and surviving fraction is unknown, a response level of Fr, say, can be used without reference to a survival curve.

6/ proliferation effects must be allowed for each type of tissue on an individual basis.

Barendsen introduced a useful quantity which he called the " relative effectiveness per unit dose" or RE. This follows simply from

$$F_r = (\alpha d + \beta d^2) \qquad (3.5)$$

for a single dose of size d,(in Barendsen's notation. α =a1; β =a2).

For n fractions giving rise to the response level of interest

$$F_r = n(\alpha d + \beta d^2)$$

$$= nd(\alpha + \beta d) \qquad (3.6)$$

RE is defined as

$$RE = \frac{F_r}{n\alpha d} = \left[1 + \frac{d}{(\alpha/\beta)}\right] \qquad (3.7)$$

It should be noted that nd is simply the total dose D .

Barendsen defined the " extrapolated tolerance dose" ETD

$$ETD = \frac{F_r}{\alpha} \qquad (3.8)$$

The term "tolerance" may perhaps convey the wrong impression as these relationships would also hold for

sub-tolerance levels of radiation damage. He also used the term " extrapolated response dose" ERD and this is the term used in this work.

$$ERD = RE \times D \qquad (3.9)$$

The ERD can be thought of as the dose required for a particular biological effect if the radiation is given as an infinite number of infinite simaly small fractions

i.e. $\lim_{n\to\infty : d\to 0} (nd)$

Equation (3.9) can be used to examine the conditions for equivalences between schedules. Two schedules are isoeffective for a particular biological effect characterised by α/β if their ERDs are equal

$$ERD_1 = ERD_2 \qquad (3.10)$$

i.e.

$$D_1 \times RE_1 = D_2 \times RE_2 \qquad (3.11)$$

For fractionated radiotherapy

$$RE = \left[1 + \frac{d}{(\alpha/\beta)}\right]$$

Therefore

$$n_1 d_1 \left[1 + \frac{d_1}{\alpha/\beta}\right] = n_2 d_2 \left[1 + \frac{d_2}{\alpha/\beta}\right] \qquad (3.12)$$

So, for example, if it is desired to give a radiotherapy schedule with fraction size d_2 to produce an equivalent effect (α/β ratio) to a schedule of n_1 fractions of size d_1, the appropriate number of fractions n_2 is found from

$$n_2 = \frac{n_1 d_1 \left[1 + d_1/(\alpha/\beta)\right]}{d_2 \left[1 + d_2/(\alpha/\beta)\right]} \qquad (3.13)$$

Alternatively if it is decided to choose a certain number

of fractions, n_2, d_2 is found by rearranging equation (3.12)

$$\frac{n_2 d_2^2}{\alpha/\beta} + n_2 d_2 = n_1 d_1 \left[1 + \frac{d_1}{\alpha/\beta} \right]$$

Therefore

$$d_2^2 + \alpha/\beta \; d_2 - \alpha/\beta \; \frac{n_1}{n_2} \; d_1 \left(1 + \frac{d_1}{(\alpha/\beta)} \right) = 0$$

It now follows from the formula for the roots of a quadratic equation that

$$d_2 = \left[\sqrt{(\alpha/\beta)^2 + 4 \; \frac{n_1}{n_2} \; d_1 \left(\frac{\alpha}{\beta} + d_1 \right)} \; - \alpha/\beta \right] / 2 \qquad (3.14)$$

The negative root is obviously not meaningful in this context and is ignored.

Withers et al (1983) have pointed out that no single isoeffect curve can describe the dose-fractionation response of all tissues. It follows that, in order to use these equations to derive isoeffective fractionation schedules for a particular biological effect it is neccessary to know the characteristic α/β ratio for that effect. Published values of α/β ratios cover quite a large range (e.g. Barendsen, 1982; Fowler, 1984) depending on the organ system studied. However the consensus view at present appears to be that there is a broad category of "late responders" with α/β ratios in the range of about 1 Gy to 5 Gy and a broad category of "early responders" with α/β ratios in the range of about 6 Gy to 14 Gy (e.g. Fowler, 1984). Taking a value of 3 Gy as representative of late effects and a value of 10 Gy as representative of acute effects is a reasonably justifiable position. In the treatment of systemic malignancy, the theme of this thesis, radiation induced

pneumonitis is a particularly important toxicity. For this biological endpoint, which is described in the next chapter, several studies in mice have indicated that an α/β of 3 Gy is a good predictor of isoeffective schedules (Parkins et al, 1985; Parkins and Fowler, 1985; Vegnesa et al, 1985; Travis and Tucker, 1986).

3.2 CONTINUOUS RADIATION SCHEDULES

Dale (1985) used the same microdosimetric assumptions as Barendsen (1982) and a similar theoretical development to the "accumulation" model of Roesch (1978) in his extension of the linear quadratic model to continuous radiation exposures at constant or exponentially decaying dose-rates. He assumed that sub-lethal damage repairs exponentially with a time constant, μ , or, more accurately, that the probability of a sub-effective lesion existing decays exponentially with a decay constant, μ . Dale produced an equation for the RE of a continuous radiation exposure at a constant dose-rate, r, over a time, T

$$RE = 1 + \frac{2r}{\mu(\alpha/\beta)}\left[1 - \frac{1}{\mu T}\left(1 - e^{-\mu T}\right)\right] \quad (3.15)$$

This finding of Dale is computationally identical to that of Thames' (1985) incomplete repair (IR) model when applied to a linear-quadratic survival equation for continuous low dose-rate exposures. Thames' model, in turn, is a generalisation of Oliver's (1964) " dose-equivalent of incomplete repair" model to multifractionated and continuous radiation exposures. This approach does not invoke a "microtheory" but instead makes

a more empirical assumption. Following sufficient time for complete repair after a single radiation dose the survival curve for a second dose will retrace that of the first. If, however, repair is not complete the second survival curve will retrace the first starting from a non-zero dose value. This value is θ d where $\theta = \exp(-\mu \Delta T)$ where T is the time between doses and μ is the repair constant. Both this analysis and that of Dale yield conclusions which can be expressed as equation (3.15). Dale has shown that when fractionated high dose-rate and continuous low dose-rate exposures are considered, in order for the same dose to be of equal biological effect the following condition must be satisfied

$$n = \frac{\mu T}{2\left[1 - \frac{1}{\mu T}(1 - e^{-\mu T})\right]} \qquad (3.16)$$

This is also the general equation for equating acute and protracted treatment schedules derived by Liversage (1969). Dale examined the correspondence between the predictions when equation (3.15) was used and doses which were found clinically to be equivalent in the treatment of carcinoma of the cervix given as both fractionated high dose-rate and continuous low dose-rate exposures. He found that an α/β ratio of 2.5 Gy and a μ value of 0.46 hr-1 (corresponding to a repair half-time of 1.5 hours) gave a good fit to the clinical data. Using the NSD, CRE and TDF models in their fractionated and continuous forms (and including experimentally derived normalisation factors where neccessary) to predict the isoeffective doses led to a gross overestimate of the

equivalent fractionated dose. Thames et al (1984) found that for three acutely responding tissues in the mouse (jejunum, colon and bone marrow) repair half-times ranged from 0.3 to 0.9 hours. For mouse lung, using the LD50 for pneumonitis endpoint and also for rat spinal cord, they estimated a repair half-time of 1.5 hours. Thames (1985) has shown that the IR model, which is computationaly identical to the Dale model, is also equivalent to the LPL model of Curtis (1986) for fraction sizes of the order of 4 Gy or less and for dose-rates of the order of 0.2 Gy per minute or less. There is thus intermodel correspondence in the clinical dose and dose-rate range.

3.3 EXPONENTIALLY DECAYING DOSE-RATES

In his 1985 paper Dale also addressed the situation of exponentially decaying dose-rates. For irradiation of time, T with a decaying dose-rate of decay constant, λ the full expression for the RE is

$$RE = 1 + \frac{2 r_0 \lambda}{(\mu+\lambda)\alpha/\beta} \left[\frac{\frac{1}{2\lambda}(1-e^{-2\lambda T}) - \frac{1}{(\mu+\lambda)}(1-e^{-(\mu+\lambda)T})}{(1-e^{-\lambda T})} \right] \qquad (3.17)$$

Where r_0 is the initial dose-rate.

If the irradiation time is long enough that the dose-rate decays effectively to zero the equation for RE simplifies to

$$RE = 1 + \frac{r_0}{(\mu+\lambda)\alpha/\beta} \qquad (3.18)$$

3.4 REPAIR KINETICS

Currently the most-used models for the interpretation of

experimental fractionation (when repair is not complete) and low dose-rate studies are the Thames (IR) model, the Dale (LQ) model and the Curtis (LPL) model. These formulations are based on monoexponential repair kinetics. Recently this tenet has come under attack from several experimental studies. Stephens et al (1987) found that for 3 mouse tumour cell lines and for 1 human tumour xenograft (HX34 melanoma) irradiated in-vitro the data were well fitted by both the IR and LPL models. However for 2 of the 4 lines the estimated repair half-time based on low dose-rate exposures was a factor of 10 less than that derived from split dose experiments (0.1 hour versus >1.0 hour). These authors suggested that this could be due to temperature factors in the experimental procedure. Alternatively they pointed out that it could be due to the existence of 2 components of repair rather than a single exponential. Steel et al (1987) stated that there was a systematic tendency for split dose half-times to be longer than those derived from analysis of the dose-rate effect. It has been suggested (Ang et al, 1985) that for mouse mucosa the half-time of repair could depend on the fraction size. Dale et al (1988) in a re-examination of the data of Huczkowski and Trott (1984 & 1987) relating to jejunal crypt survival in C3H mice following fractionated total body irradiation, found that the repair rate appeared to slow down as the dose-rate increased. In contrast Scalliet et al (1987) found, using Dales' LQ model, that the half-time of repair in the mouse mucosa system remained constant over the range of dose-rates

investigated (642 Gy hr-1 to 1.5 Gy hr-1). Braby et al (1980) found the repair rates for the eukaryotic algae Chlamydomonas reinhardi to be dependent on temperature but no significant differences were found between split-dose and dose-rate based techniques of measurement. It is shown in the appendix to this chapter that the isoeffect data of Paterson and Green as embodied in the continuous CRE formula is consistent with a (α/β = 3 Gy : $T_{1/2}$ = 1.5 hour) quadratic model tissue with monoexponential repair. The situation is therefore in a state of flux, new experimental data may show deficiencies in the current models which will suggest ways in which they should be revised. In the meantime the LQ model is one of the best available and will be used in the following chapters as the basis of analysis of radiation damage to normal tissues.

3.5 APPENDIX

COMPARISON OF THE PREDICTIONS OF THE LQ MODEL AND THE CRE MODEL FOR CONTINUOUS IRRADIATION

3.5.1 CONSTANT DOSE-RATES

Returning to equation (3.10) the condition for equivalence between two radiotherapy schedules 1 and 2 is that their ERDs are equal.

i.e.

$$ERD_1 = ERD_2$$

This relationship can be used, together with equation (3.15) to compare the predictions of the linear quadratic model with those of the continuous CRE formula. The ERD for a continuous radiation exposure at a constant

dose-rate, r, for a time, T, is given by

$$ERD = rT\left[1 + \frac{2r}{\mu(\alpha/\beta)}\left[1 - \frac{1}{\mu T}\left(1 - e^{-\mu T}\right)\right]\right] \quad (3.19)$$

For example schedule 1 is chosen to be 60 Gy in 7 days, a fairly standard doseage for low dose-rate treatments (Paterson, 1948). A different irradiation time for schedule 2 can be chosen and the dose-rate, and hence the total dose which is required for the two schedules to be isoeffective, calculated using the above equation. By varying the irradiation times for schedule 2 a curve of isoeffective dose as a function of irradiation time can be produced. Estimates of α/β = 3 Gy: $T_{1/2} = \ln 2/\mu$ = 1.5 hours and α/β = 10 Gy: $T_{1/2} = \ln 2/\mu$ = 1.0 hours are taken for late and acute responding tissues respectively. One may expect that the resulting curves will be valid for these tissues if the model is an adequate description. The derived curves are shown in Fig (3.1) together with the CRE isoeffect curve based on the combined clinical experience of Paterson and Green (Kirk et al, 1972). It can be seen that the "late effects" and CRE curves correspond closely while the "acute effects" and CRE curves do not. Bearing in mind that the continuous LQ formula is a theoretically derived construct while the CRE is simply a mathematical description of a clinical isoeffect, the inter-model correspondence is encouraging. No cellular repopulation factor is incorporated in the continuous LQ model. For this reason the behaviour of "real" acute responses at long irradiation times is likely to deviate from the plotted curve. Repopulation will have

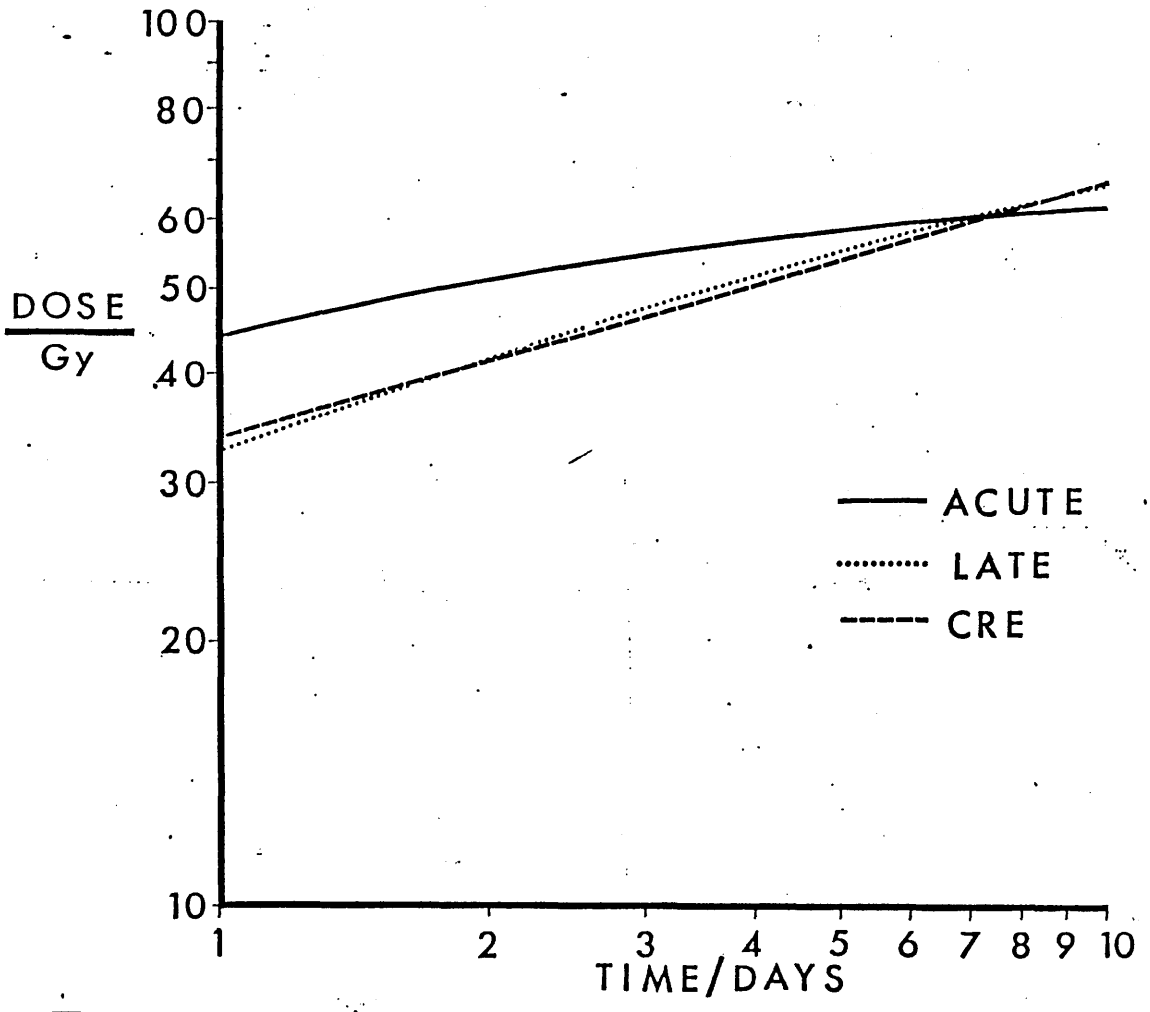

Fig 3.1
Calculated doses isoeffective to 60 Gy given at a constant dose-rate in a total time of 7 days using the LQ model for acute and late effects and the CRE model.

the consequence of increasing the isoeffective dose as the irradiation time increases, thus bringing the acute effects curve closer to those for the CRE formula and late effects. Although not shown in Figure 3.1, the late-isoeffective dose curve does not increase indefinitely as the irradiation time increases. Instead it approaches a plateau dose of appromimately 90.5 Gy asymptotically. This means that the CRE and the late effects LQ formulae become incompatible at very long irradiation times where, unfortunately, there is no relevant isoeffect data.

3.5.2 EXPONENTIALLY DECAYING DOSE-RATES

For the case of dose-rates which deacy exponentially to zero, again the predictions of the linear-quadratic model can be compared with those of the CRE formula. From Kirk et al, 1973, the total dose D_2 given as an exponentially decaying dose-rate with decay constant λ_2 which is equivalent to a dose D_1, given at an exponentially decaying dose-rate with time constant λ_1 is

$$D_2 = \left(\frac{\lambda_1}{\lambda_2}\right)^{0.29} D_1 \qquad (3.20)$$

For the LQ model, using equations (3.10), (3.11) and (3.18) together with the formula for total dose for an exponentially decaying dose-rate

$$D = r_0/\lambda \qquad (3.21)$$

It is staightforward to show that for equivalence

$$D_2 = \frac{\left[\sqrt{\left[(\mu+\lambda_2)\alpha/\beta\right]^2 + 4\lambda_2(\mu+\lambda_2)\alpha/\beta\, ERD_1} - (\mu+\lambda)\alpha/\beta\right]}{2\lambda_2} \qquad (3.22)$$

A schedule of 60 Gy total dose given by a dose-rate which decays exponentially to zero with an effective half-time of 5 days has been taken arbitrarily as a reference. Figure 3.2 shows the predicted isoeffective dose curves, based on equation (3.20) for the CRE formula and equation (3.22) for the LQ model, as a function of the effective radionuclide half-time. This is a somewhat unusual index of comparison given that for any implant or insertion using radioactive sources the decay half-time remains constant. However, as will be described in a later chapter, for biologically targeted radiotherapy (BTR) using radionuclides linked to molecules such as monoclonal antibodies or meta-iodobenzylguanidine (mIBG) this comparison is highly relevant. Parameter values used were α/β = 3 Gy : $T_{1/2}$ = 1.5 hours for late effects and α/β = 10 Gy : $T_{1/2}$ = 1.0 hours for acute effects. It can be seen that all three curves are quite different although the CRE curve is closer to the late effects quadratic than it is to the acute effects quadratic. Good curve matches can be obtained by using α/β = 3 Gy : $T_{1/2}$ = 4 hours or alternatively α/β = 1 Gy : $T_{1/2}$ = 1.5 hours. This analysis indicates that the CRE formula describes a tissue response which has a greater dose-rate effect than is expected for an α/β = 3 Gy : $T_{1/2}$ = 1.5 hour LQ model tissue. The discrepancy is somewhat surprising given the correspondence for the constant dose-rate case. There is no available clinical or experimental data which can be used to resolve this inconsistency. Both CRE and LQ formulations reduce to their constant dose-rate forms if

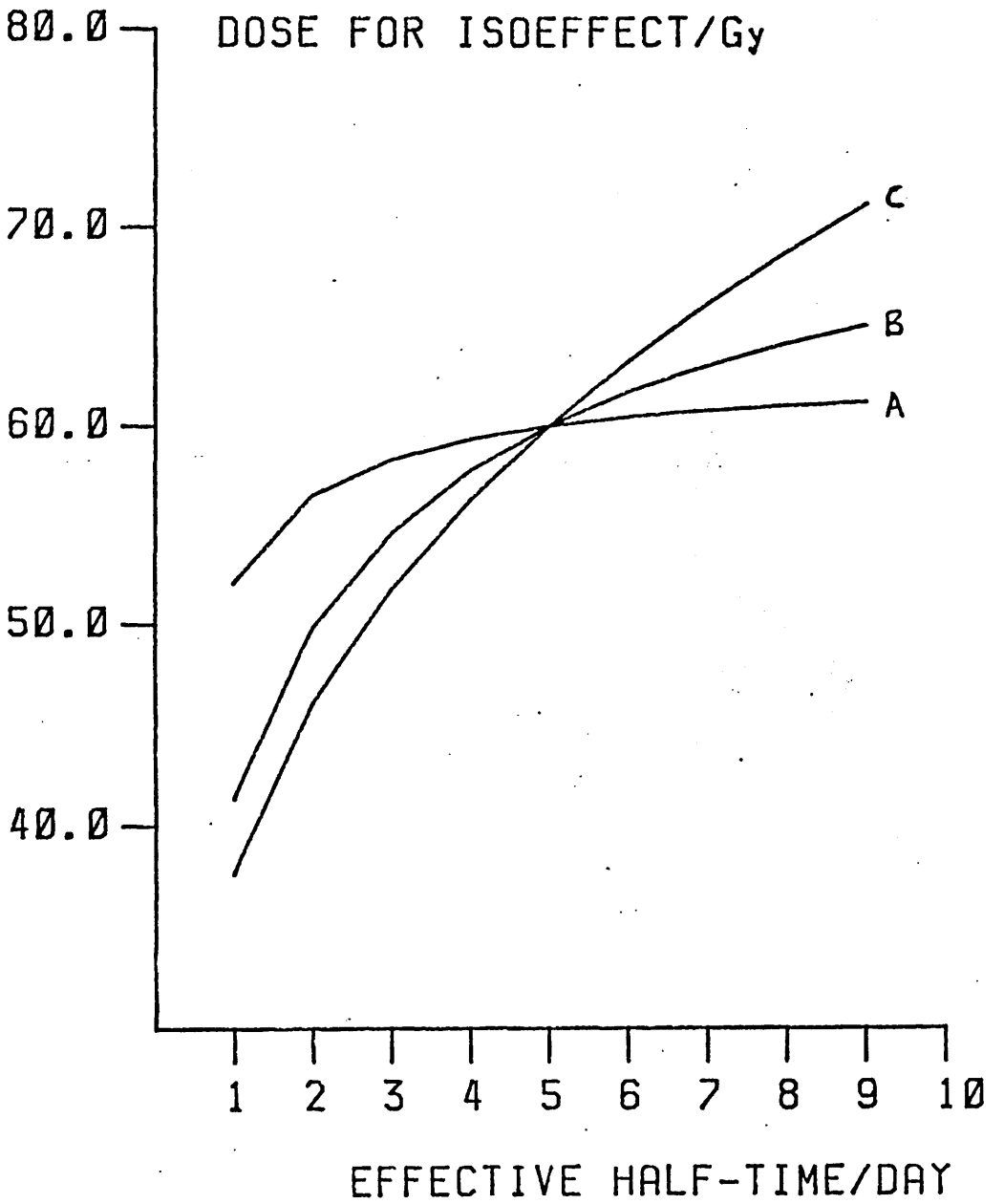

Fig 3.2
Calculated doses isoeffective to 60 Gy given by an exponentially decaying dose-rate with 5 day half-time.
A = LQ model-acute effects
B = LQ model-late effects
C = CRE model

the decay half-time tends to infinity. The LQ version however, has the advantage that the equations reduce to their fractionated form for very short half-times. The CRE equations do not. Another observation on the CRE formula is that, similarly to the constant dose-rate case, as the half-time increases there is no region of dose-rate independence. Any doubling of the effective half-time will always increase the predicted equivalent dose by 22%. This is not the case for the LQ model. As the half-time becomes very large, doubling it has no significant effect on the predicted equivalent dose. Again attention is drawn to the lack of any repopulation factor in the LQ model. This will be of importance for acute effects and it is undoubtedly a deficiency of the LQ model that it does not take account of this factor. Neither the LQ nor the CRE formulae are based on clinical data. Both are extrapolations. The LQ formula, as derived by Dale (1985), is based on the same "microtheoretical" assumptions as the constant dose-rate version. The CRE formula of Kirk et al (1973) is a more empirical derivation from the constant dose-rate equation. It should be pointed out that the discrepancy between the models is produced in the mathematical manipulations and not in any underlying biological database.

4.0 TOTAL BODY IRRADIATION

After many years of the clinical application of TBI there is still some controversy as to the rationale for its use. In this section an attempt is made to state the rationale for TBI and marrow transplantation in the context of the treatment of systemic malignant disease.

TBI is a form of systemic treatment. As such, it has similarities and differences to chemotherapy. Both are cytotoxic in a general sense and produce damage to "target" cells and "non-target" cells. This means that for both modalities the limitation on dose is provided by the tolerance of normal organs and tissues. Different chemotherapeutic agents give rise to different patterns of toxicity e.g. methotrexate dose is limited by effects such as myelosuppression, mucositis and renal and liver dysfunction, adriamycin dose can be limited by myelosuppression, cardiomyopathy, encephalopathy and tissue necrosis (Olive and Peeters, 1986). If these problems could be overcome, particularly myelosuppression, doses could be escalated until other organ toxicities became prominent. As a systemic cytotoxic therapy this principle applies equally to TBI which has its own pattern of toxic side-effects. Dose escalation is desirable if this produces a greater effect on the target cells i.e. if there is a dose-response curve. Conceivably, chemotherapeutic drugs may not always have a simple dose-response curve and dose escalation may not always be useful. This could happen with, for example, cycle specific drugs which may kill all cycling cells. Further

increasing the dose will not cause a greater amount of cell kill. With radiation, although there are variations in radiosensitivity between cells in different phases of the cycle and with cells in the G0 state, increasing the dose invariably increases the biological effect. In the absence of haematological reconstitution the limit on TBI dose is set by the blood forming cells of the bone marrow. Beyond doses of about 2 Gy to the whole body the incidence of marrow failure increases rapidly (UNSCEAR, 1982). If the bone marrow can be replaced after TBI it is no longer dose limiting since the functioning marrow after TBI is not the irradiated marrow which was present during TBI. This procedure enables the dose to be increased to what is recognised from accident data to be well over the lethal limit (Nenot, 1987). This is the primary rationale for the combination of TBI with bone marrow transplantation. There is also the problem of "sanctuary sites". The central nervous system (Inati et al, 1983; Schweinle and Alperin, 1980) and the testes (Mahoney et al, 1981; Stoffel et al, 1975) are protected to some extent from the effects of chemotherapeutic drugs, but can be reached by the use of externally applied radiation beams. Another widely known phenomenon is the emergence of drug resistant tumour cell populations following treatment with a single chemotherapeutic agent (Curt et al, 1984; Goldie and Coldman, 1984). This provides one of the rationales for multi-agent chemotherapy which has proved so successful in the treatment of diseases like Hodgkin's lymphoma. In the laboratory the development of radiation resistant clones

is much rarer and for this reason TBI has a potential advantage over chemotherapy. The disadvantages of TBI are due to its late effects, which tends to be more severe than that of chemotherapy. This aspect will be discussed later. The above considerations lead to the conclusion that TBI is another systemic cytotoxic agent with some potential advantages and disadvantages in comparison to chemotherapy. The purpose of TBI is to sterilize target cells and dose escalation, beyond that afforded by marrow rescue, is limited by the characteristic pattern of toxic side-effects. In the case of neoplastic disease the fundamental goal of radical therapy is the elimination of every tumour cell. The "target" cells for TBI are therefore tumour cells and the use of marrow rescue means that more tumour cells can be sterilized. It follows that any malignant disease which is widespread may be a candidate for TBI. In other words there is no a priori reason why TBI should not be considered when deciding how a particular malignant condition should be treated. Of course there may be good reasons for deciding not to use it.

Ablation of existing bone marrow has been quoted as a primary objective of TBI (e.g. Quast, 1987). This assertion bears some examination. For non-malignant haemopoietic disorders, marrow ablation and replacement by healthy cells is the basis of the therapeutic procedure. Is there any reason to disbelieve this for malignant conditions ? In order to answer this question it is instructive to answer two further ones. If TBI produced

marrow ablation but did not sterilize tumour cells, would it be used? Additionally, if TBI sterilized tumour cells but did not ablate normal marrow, would it be used? It is suggested that the answers to these questions are no and yes, respectively. Historically leukaemia, a malignant haemopoietic condition, was the first neoplastic disease to be treated with TBI and marrow transplantation (Thomas et al, 1975). It was perhaps natural in this context to think that the purpose of TBI was to ablate the leukaemic marrow. It is only a small step for this to be conceptually identified as ablation of normal marrow. The distinction may not be very important in the case of leukaemia where the radiation sensitivities of leukaemic and normal haemopoietic cells appear to be broadly similar. However for other neoplastic diseases where TBI and marrow rescue may have a role the differences can be greater. Thinking of marrow ablation as an objective of TBI may therefore lead to the mis-identification of the appropriate target cell population and a consequent reduction in the potential therapeutic ratio. For this reason it appears to be an error to consider marrow ablation an objective of TBI. It should rather be thought of as a side-effect.

Another aspect of the use of TBI is the severe immune suppression which it produces. This is due to the sterilization of normal radiosensitive lymphocyte populations. It is useful to examine critically to what extent immune suppression should be regarded as a goal of TBI.

There are three categories of marrow transplant. In syngeneic transplants the donor marrow is provided by a genetically identical twin of the recipient. Statistically this accounts for only a small proportion of cases. There is no incompatibility between graft and host and a host versus graft response is lacking as is any graft-versus-host reaction. The immune supression caused by TBI in this context can only be regarded as an unwelcome side-effect as it reduces the patients ability to respond to infection.

In autologous transplants the incoming graft has been removed from the patient prior to intensive chemoradiotherapy and returned following it. This procedure has the potential disadvantage that any tumour cells removed with the marrow will be re-introduced at the time of infusion. For some categories of disease it is possible to purge the marrow graft of tumour cells ex-vivo. In principle this seems a logical and sensible thing to do, although as yet opinion is divided as to whether it has any clinical relevance (Philip et al, 1986; Glorieux et al, 1986; Prindull, 1986). Obviously for autologous transplants there are no histocompatibility problems. Again, in this context TBI-induced immune suppression is apparently unavoidable but not desirable.

In allogeneic transplants the incoming marrow graft is provided by a genetically non-identical donor. Ideally the donor should be a sibling who has inherited the same histocompatibility antigens as the recipient. However, even with perfect HLA matching host-graft reactions can

occur (Yunis et al, 1983). Immune suppression of the recipient is neccessary to enable the graft to "take", otherwise it will be rejected. The withdrawal of the immune defence system of the recipient leaves him open to opportunistic infections e.g. pneumocytis carinii, etc in a similar way as patients with AIDS. There is however an additional problem posed by the immune competence of the incoming graft which can attempt to "reject" the recipient. This is graft-versus-host disease (GvHD), an often fatal complication of allotransplantation (Sullivan and Parkman, 1983). The reaction may be mediated by donor T-lymphocytes which are cytotoxic to host cells (Tsoi et al, 1983). Many attempts have been made to reduce the incidence of GvHD (e.g. Ramsay et al, 1982; Thierfelder et al, 1981; Trigg et al, 1985) but, with the exception of one series (Prentice et al, 1982) using marrow depleted of T-cells by the monoclonal antibody OKT3, these have not been encouraging. It was hoped it may have been possible to rescue with histoincompatible marrow after T-cell depletion as is possible in mice (Vallera et al, 1981). This prospect now seems less promising and it was recently recommended that T-cell depletion should not be pursued in standard-risk patients (Maraninchi et al, 1987).

The major problem of this approach stems from the apparent antileukaemic effect produced by the allograft (Weiden et al, 1979 & 1981). Current techniques of T-cell depletion for reduction of GvHD concomitantly reduce the level of antileukaemic effect (Apperley et al, 1986; Helenglass et

al, 1987). Ideally it seems that one would like a controllable GvH reaction. At present it is unknown whether the sub-population of cells which mediate the antileukaemic effect is the same as that responsible for GvHD. If it is then the antileukaemic effect may be due to the general increase in the cytotoxicity of the treatment. Alternatively, if the sub-populations are distinct it may be possible to increase the therapeutic ratio by selective elimination of the cells responsible for GvHD. The antileukaemic effect of allogeneic transplantation may provide a rationale for the ablation of pre-existing functional marrow, but as previously observed, it seems counter-productive to concentrate on the effectiveness of TBI for marrow sterilisation, rather than in its role as a antitumour agent.

TBI is a non-specific cytotoxic agent. For all organs except bone marrow, transplantation is not a practical option. Consequently the escalation of dose will increase the radiation damage in all the organs and tissues of the body eventually limiting the possible TBI dose. Clinically observable toxicities include damage to the liver (veno-occlusive disease) (Shulman et al, 1980; Woods et al, 1980; McDonald et al, 1985), kidneys (Tarbell et al, 1987(a)) also in the heart, GI tract and the CNS (Bortin et al, 1983). Latent effects in children can be expressed as growth abnormalities (Barret et al, 1987) and intellectual impairment (Meadows et al, 1981). However it is generally accepted that the most important dose limiting organ for TBI with marrow rescue is the lung

where radiation damage is manifest as interstitial pneumonitis.

4.1 RADIATION DAMAGE TO LUNG

Interstitial pneumonitis (IP) indicates a tissue reaction with infiltration and thickening in the walls of the alveoli (Cardozo and Hagenbeek, 1985). Clinical symptoms include dyspneoa, tachypneoa, pyrexia, non-productive cough and cyanosis. Chest X-rays often show bilateral diffuse shadowing. Lung function studies indicate a reduction in lung volume and a low diffusing capacity (Depledge et al, 1983). IP has been reported in up to 50% of all marrow transplants, approximately 50% of these cases are lethal. IP occurs most often between 30 and 100 days after transplant (Cardozo and Hagenbeek, 1985).

There are a number of factors associated with IP including infectious agents (e.g. viruses such as CMV), grade of GvHD, type of chemotherapy (e.g. busulphan) and radiation parameters (e.g. dose-rate and fractionation). Radiation damage to lung certainly appears to be a predisposing factor, at least, in the aetiology of IP. This section will deal with the relationship between radiation parameters and IP, although it should be realised that it is somewhat unrealistic to focus on one variable in isolation.

In 1978 Fryer et al published a study describing the toxic effects of large single doses of radiation given to the entire lung during upper half body irradiation for metastatic disease. In 1981 van Dyk et al re-evaluated this data using a more accurate method of calculating lung

dose. Dose-rates ranged from 0.5 to 4 Gy per minute. These authors found that the incidence of IP was a steeply rising sigmoid function of dose with the onset of IP occurring at a dose level of about 7.5 Gy. Keane et al in 1981 compared the incidences of IP from a number of centres using allogeneic bone marrow transplant and all giving a nominal TBI dose of 10 Gy with those from centres using a lower TBI dose. The higher TBI doses were associated with a greater incidence of idiopathic IP. For the low dose group, based on published data on the specific irradiation procedures used, these authors estimated the actual dose to lung for each of the centres. They used a probit regression analysis to relate the crude incidence of IP to absolute dose in lung. A clear dose-response relationship was found which suggested that the development of IP in patients receiving allogeneic transplants was directly related to lung dose. Several studies since have shown that, over a more protracted timescale there is a dimunition of lung function (Depledge et al, 1983). Some of these changes were associated with previous IP and some were not (Springmeyer et al, 1983). Based on an accumulation of radiobiological knowledge and also specifically designed animal experiments it is apparent that total dose by itself is not an independent parameter but has to be considered in association with the dose-rate (Depledge and Barrett, 1982) and fractionation details. Peters et al in 1979 argued that multifractionated TBI should provide a higher therapeutic ratio than single fraction TBI. This is because

haemopoietic cells have a lesser repair capacity than dose-limiting organs such as lung. There is a body of data from animal experiments which illustrates the dependence of lung damage on radiation dose-rate and the sizes of the individual fractions (e.g. Cardozo et al,1985; Travis et al, 1986). Analyses of such data can yield parameters such as α/β ratios and repair half-times for the LQ model (Parkins and Fowler, 1985; Vegnesa et al, 1985). Typical parameter values are α/β = 3 Gy and repair half-time = 1.5 hours.

4.2 DISEASE CATEGORIES

In this section some features of neoplastic diseases which are currently treated, or could be treated in the future, with systemic radiotherapy are reviewed. These include a high proportion of childhood cancers.. This reflects both the relative ease of marrow transplantation in children in comparison with adult patients and also the relative radiosensitivity of some paediatric neoplasms.

4.2.1 LEUKAEMIA/LYMPHOMA

By far the largest number of patients treated with TBI and bone marrow transplantation for a malignant condition are suffering from leukaemia of some form. Leukaemia literally means "white blood" and the leukaemias are a group of conditions where there is a progressive uncontrolled proliferation of white blood cells. Two main varieties of leukaemia are recognised; one involving the lymphocytic series (lymphocytic leukaemias) and the other involving myeloid cells which normally proliferate and mature in the

bone marrow (myeloid or granulocytic leukaemias). Both these versions can occur in acute or chronic form. Contrary to popular belief leukaemia is a disease of old age with the incidences of all forms rising to a peak at about 80 years of age. There is a smaller peak in childhood and adolescence for the acute leukaemias and patients considered for TBI and marrow transplant are usually from this group. Acute lymphocytic leukaemia (ALL) is the most frequent malignancy in childhood comprising 30% of all neoplasms. In 1965 fewer than 1% of children with ALL were reported to be long term survivors (Burchenal and Murphy, 1965). Nowadays approximately 50% - 75% of patients will have an unmaintained remission of longer than 5 years and a large majority of these will actually be cured (Moe, 1984). TBI with marrow transplantation has a role for patients with ALL in 2nd or subsequent remission and, more controversially, for high risk patients in first remission. For patients transplanted in 2nd or subsequent remission 20% - 40% 5 year survivals have been reported with leukaemic relapse being the main problem (Thomas et al, 1983(a)). There is a risk of children with ALL developing meningeal leukaemia. This may be from 66% to 5% depending on whether CNS prophylaxis, including cranial irradiation, is used (Littman et al, 1987). However, cranial irradiation is a major factor in the development of intellectual impairment and neuropsychological dysfunction (Meadows et al, 1981; Pfefferbaum-Levine et al, 1984). For this reason the use of intrathecally administered antibody-conjugated

radionuclides is under consideration. It may be possible with this approach to preferentially irradiate the cerebro-spinal fluid without irradiating the underlying brain and spinal cord. This would be expected to significantly reduce the side-effects. The same approach may be suitable for leptomeningeal malignancy derived from other tumours, such as medulloblastoma, glioblastoma and neuroblastoma (Lashford et al, 1988). Acute nonlymphocytic leukaemia (ANL) is less frequent than ALL. It constitutes about 20% of childhood acute leukaemia (Choi and Simone, 1976). TBI and marrow transplantation appears to have a more definitive role than for ALL (Willoughby, 1986). 70% - 80% of children can be brought into complete remission (CR) with current induction chemotherapy. Clinical studies with TBI and marrow transplantation have produced encouraging results (Sanders et al, 1981; Kersey et al, 1982; Forman et al, 1983; Dinsmore et al, 1984) with low relapse rates and 50% - 60% disease free survivals at 1 - 2 years. However there is evidence that as many as 25% of patients may relapse up to as much as 6 years after transplant (Thomas et al, 1983(b)). Recurrence rates are higher for patients transplanted in relapse or 2nd or subsequent remission (Applebaum et al, 1983). Chronic leukaemia accounts for only approximately 2% - 5% of childhood leukaemias. This is of the granulocytic or myelocytic variety and it has been suggested that childhood chronic lymphocytic leukaemia does not occur (Schaison and Castro-Malaspina, 1986). Philadelphia chromosome positive (Ph'+) CML

occurs predominately in adults, usually presenting in middle age. Systemic chemoradiotherapy and marrow transplantation offers a possible improvement in the treatment of these patients, at least in the chronic phase of the disease (Feter et al, 1982; Goldman, 1987). A very rare juvenile form of CML occurs in children under the age of 4. It is Ph'- and has different clinical features from Ph'+ CML. Marrow transplantation may have some potential in this disease category (Sanders et al, 1979).

Lymphoma is the third most common malignant diagnosis in children following acute leukaemia and brain tumours. About 45% of patients have Hodgkin's lymphoma, the remainder have a diverse group of diagnoses collectively referred to as non-Hodgkins lymphoma (NHL) (Young and Miller, 1975). The peak incidence of Hodgkin's lymphoma is in the third decade of life (Thompson, 1977). Improvements in the treatment of Hodgkin's lymphoma in the last 20 years have been dramatic. Today five-year survival figures of over 80% are common (Fowler, 1983). Children with Hodgkin's have a better than 90% likelihood of cure if they are treated in a specialist centre (Donaldson, 1986). TBI and marrow transplantation may have a role in relapsed or non-responding Hodgkin's. Dicke and Spitzer in their 1986 review recommend a group of categories of Hodgkin's and non-Hodgkin's lymphomas where high dose cytoreductive therapy and marrow support should be considered. The cytoreduction could conceivably include TBI. Goldstone (1986) reported a study of 44 patients

undergoing autologous marrow transplantation for NHL in first complete or partial remission. The long-term disease-free survival was 65%. Verdonck et al in 1987 reported 20 patients with high grade NHL treated with high dose cyclophosphamide and TBI followed by autologous transplant. Two of five patients treated in partial remission became long term disease-free survivors. Seven of ten treated in complete remission were in unmaintained remission, four with long follow up.

4.2.2 NEUROBLASTOMA

Neuroblastoma (NBL) is the most common extracranial solid tumour of childhood accounting for about 10 % of paediatric cancers. Although NBL is a clinically radiosensitive tumour (D'Angio and Evans, 1983) it is characterised by early dissemination and at least 50 % of children with NBL are first diagnosed with advanced disease. CR rates of Evans stages III and IV NBL have been quoted as 30-40% (Dicke and Spitzer, 1986). They are higher in Europe (65 - 80 %) but overall survival is around 20 % (Barrett, written communication, 1987). The use of high dose TBI with marrow rescue following intensive induction chemotherapy is made more attractive by the availability of ex-vivo methods of separating neuroblastoma cells from haemopoietic cells (Kemshead et al, 1985(a) ; Treleaven et al, 1984) thereby allowing the use of autologous marrow. Philip et al (1985) reported preliminary results of a study of high dose cytotoxic therapy and autologous bone marrow transplantation (ABMT) using purged marrow on 16 children, 13 of whom received

TBI. The mean follow up for the entire group was short (5 months) but a sub-group consisting of 10 stage IV patients had a mean follow up of 18 months with 8/10 survivors. The authors compared this with their previous experience with non-ABMT treatment where survival at 18 months was 24%. Other groups have embarked on supralethal therapy with marrow reconstitution for advanced NBL. Although the results are encouraging, the toxicity of treatment is great (D'Angio et al, 1985). NBL is a particulary interesting neoplasm from the points of view of molecular biology and its potential for biological targeting by cytotoxic modalities. Amplification of the N-myc oncogene has been reported to be directly related to disease stage and prognosis (Brodeur et al, 1984; Seeger et al, 1985; Tsuda et al, 1987). Due to the sythesis of epinephrine and nor-epinephrine by tumour cells some NBLs can be targeted with the catecholamine precursor molecule meta-iodobenzylguanidine (mIBG) (Treuner et al, 1984; Gerrard et al 1987). Biologically targeted radiotherapy (BTR) may have the potential to increase the tumouricidal effect while reducing the treatment toxicity. mIBG is to be the subject of a forthcoming clinical trial organised by the United Kingdom Childrens Cancer Study Group (UKCCSG) Also a number of monoclonal antibodies (moabs) have some specificity for NBL. In particular the moab UJ13A has been used for both scintigraphic imaging (Horne et al, 1985) and radioimmunotherapy (Kemshead et al, 1985(b)). NBL is therefore one of the prime candidate neoplasms for BTR.

4.2.3 EWING'S SARCOMA

Ewing's sarcoma is a malignant disease of bone. It accounts for 10% - 15% of all primary malignant bone tumours (Huvos, 1979). Ewing's sarcoma rarely occurs under the age of 5 years or over the age of 30 years, the peak incidence is between 10 and 15 years (Glass and Fraumeni, 1970). Approximately 20% of patients present with clinically detectable metastases, of these 50% have lung metastases and about 40% have multiple bone involvement and/or diffuse bone marrow involvement (Jurgens et al, 1986). Before adjuvant chemotherapy the 5-year survival rates were in the dismal range of 5% - 10% due to rapid systemic spread of the disease (Falk and Alpert, 1967; Phillips and Higinbotham, 1967). Ewing's sarcoma is clinically radiosensitive (Razek et al, 1980; Perez et al, 1981; Thomas et al, 1984) although there is a definite risk of local failure following radiation (Donaldson and Hendrickson, 1983). The combination of local control with radiotherapy and/or surgery in association with systemic multidrug chemotherapy has improved disease free survival to within the range 50% - 60%. The use of TBI followed by autotransplant is the subject of current clinical trials (Kinsella et al, 1984).

4.2.4 OTHER TUMOURS

Several other neoplastic diseases are candidates for high dose cytotoxic therapy and marrow rescue. Whether or not TBI is incorporated depends on the efficacy of available

chemotherapeutic agents and the radiosensitivity of the tumour. Examples of neoplasms where there is a potential role for TBI and marrow rescue are :

1/ Small cell bronchogenic carcinoma or oat cell carcinoma of lung. CR rates of 60% are achievable but few are maintained, relapses continuing to occur after 2 years of disease free survival. Contamination of bone marrow and peripheral blood with tumour cells is a frequent occurrence (Dicke and Spitzer, 1986). High dose chemotherapy, chest irradiation and autotransplant have shown some encouraging results (Spitzer et al, 1985).

2/ Breast cancer is highly prone to metastatic spread. Intensification therapy of multiple chemotherapeutic drugs, each course in conjunction with marrow rescue has been proposed (Dicke and Spitzer, 1986). Breast cancer is a heterogeneous disease but radiation is a current mainstay of local treatment, both as external beam therapy and also delivered via interstitial implantation.

3/ Malignant melanoma is prone to dissemination and although widely believed to be uniformly radioresistant actually appears to have a wide spectrum of radiosensitivity both clinically (Habeshaw and Wheldon, 1984) and experimentally in vitro (Rofstad, 1986). Local radiotherapy certainly has a role in disease management (Overgaard, 1986). At least some widespread melanomas may therefore be candidates for TBI and marrow transplant. The availability of anti-melanoma antibodies means that there is also a potential for the use of biologically targeted radiotherapy (Larson, et al, 1983)

4/ For ovarian cancer the cure rate can be high for minimal disease. However for extensive disease only low levels of cure are acheived. Intensification of chemotherapy has been proposed (Dicke et al, 1984). Ovarian cancer is also high on the list of candidates for biologically targeted radiotherapy and some encouraging results have been obtained with intraperitoneal infusions of monoclonal antibody conjugated radionuclides (Epenetos, 1987).

These examples suffice to show the possible applications of systemic chemo-radiotherapy and marrow rescue to the treatment of widespread malignant disease.

4.3 PHYSICAL ASPECTS OF TBI

TBI is a systemic therapy and contrasts with the usual techniques of radiotherapy where the object is to deliver a high dose of radiation to a specified, generally small, volume while at the same time reducing the dose to regions outwith the treatment volume to a minimum. Conventionally for TBI the treatment volume encompasses the whole body. This gives rise to a number of physics and dosimetry problems. Very large radiation field sizes are required typically between 1.5 and 2 metres in length. There is a wide spectrum of TBI techniques in use (Quast, 1987). A few centres have dedicated TBI facilities available (e.g. Leung et al, 1981) but, with the proliferation of centres now performing TBI, in most cases a conventional cobalt machine or linac is used (Miller et al, 1976; Aget et al, 1977; Goolden et al, 1983). In order to achieve the large field sizes it is nec essary to use a very long treatment

distance (4 metres or therabouts). Due to the inverse square dependence of radiation dose-rate on distance comparatively low dose-rates are common. This has consequences from a radiobiological point of view. Whole body dosimetry is a notoriously complex subject; many factors contribute to this (van Dyk, 1987). The transition from standard radiotherapy beam measurements to the TBI situation requires investigation of dose build-up distances, the validity of the inverse square relationship and the modifying effects of attenuating filters. There are wide variations in body contours, for example from the neck to the pelvis. Density fluctuations also occur, the most obvious being in the region of the lungs. These fluctuations are age-dependent and also depend on the treatment position (Lagrange et al, 1987). Patient orientation with respect to the treatment fields (Shank, 1983) and the number of fields used (Doughty et al, 1987) affect the resultant dose distribution as does the proximity of structures such as floors and walls in the treatment room. With all these factors in operation it is no surprise that a great deal of the efforts of physicists have been devoted towards achieving an accurate, controllable and uniform dose distribution. This has been with the aid of CT scanners, complex dose calculation algorithms and measurements of actual dose delivered either using TLDs or in real-time with diode detectors. Dose uniformity can be improved by the liberal use of tissue-equivalent bolus material and individualised beam

compensators. A question which is seldom asked is - How important is dose uniformity ? If it is imagined that a biologically targeted radiotherapy (BTR) technique produced a uniform dose throughout the body, then it would be considered a failure. In the BTR situation it is easy to see that what is required is higher radiation doses where there are concentrations of tumour cells. If this logic is applied to external beam TBI, in the absence of information about the concentrations of tumour cells, it is less clear what the conclusions should be. Increasing the dose to all regions which can tolerate it may be a way, but "tolerance" is only a term for a certain level, conventionally 5%, of serious side-effects. Many centres currently shield lung from the full TBI dose. It seems reasonable to ask; if lung is shielded what constitutes the next limitation on how high the dose can go ? and, is it worth increasing the dose to the rest of the body ? It is likely that organs such as liver or kidney may be the next most vulnerable to TBI. Can these be shielded and the dose increased still further ? This philosophy could be described as differential shielding and would result in a situation where there were a variety of dose prescriptions, one for each individual region of the body. Techniques such as localised radiotherapy boosts to the CNS and testes are frequently employed to reduce the risk of relapse at these sites. They are also explicit deviations from uniformity. These illustrations indicate that perhaps dose uniformity should not be the goal of TBI treatment planning and rather that an engineered

non-uniformity may be better. Biologically targeted radiotherapy may be the only practical way in which a useful engineered non-uniformity may be achieved. This will be the subject of the concluding three chapters of this thesis.

5.0 THE IN-VITRO RADIOSENSITIVITIES OF HUMAN LEUKAEMIA/LYMPHOMA, NEUROBLASTOMA AND EWING'S SARCOMA CELLS

Data on the radiosensitivities of human tumour cell lines are often published in the scientific and clinical literature. A computerised literature search of papers published up till the end of 1987 was used to gather the survival curve information reported in this thesis. These data have been used to derive representative survival curves for some forms of human cancer which are currently being treated or are proposed to be treated by high dose TBI and bone marrow transplantation. The justification for following this approach is provided by the work of Fertil and Malaise (1981 & 1985), Deacon et al (1984) and Rofstad and Brustad (1987) who have all reported a correlation between the in vivo qualities of radiocurability and controlability and the in vitro radiation responses of relevant tumour cell lines. Where a number of data on tumour lines of the same type are available the median values of the radiosensitivity parameters can be derived. In order to do this reasonably, some selection rules are neccessary.

5.1 SELECTION RULES FOR MEDIAN SURVIVAL CURVE PARAMETERS

The most justifiable position to take, appears to be the following one. The independent variable is the patient from whom a cell line or lines was derived. In effect this means that multiple measurements on a cell line or lines from the same patient should be pooled before a set of

pooled measurements are combined to yield median values. This approach has the disadvantage that the principle that multiple measurements on one line increase the precision of the estimate is not allowed for i.e. there is no weighting of individual data on the basis of repeat measurements. Although this is not ideal it is preferable to the alternative position. If individual measurements rather than individual patients are taken as the independent variable then a disproportionate amount of experimental data for one or two cell lines will produce a biased estimate of the median. The procedure adopted here is thus:

1/ Pool the radiosensitivity data from the cell line or lines derived from each individual patient.

2/ Use the pooled data to derive median parameter values.

5.2 IN-VITRO DATA

5.2.1 LEUKAEMIA/LYMPHOMA

The complete database for human leukaemia/lymphoma is presented in Tables (I, II and III) segregated into the three categories of lymphocytic and nonlymphocytic leukaemia and lymphoma. Weichselbaum et al (1981) reported the radiosensitivities of 4 human leukaemic cell lines, HL-60 (promyelocytic leukaemia), K562 (erythroleukaemia), 45 (acute lymphocytic leukaemia) and 176 (acute monomyelogenous leukaemia). Survival was assayed by a colony counting technique. The data of Johansson et al (1981) on 4 B-cell lymphomas was arrived at by means of a " growth extrapolation" procedure. This involved measuring the concentrations of tumour cells in

LYMPHOCYTIC LEUKAEMIA

DESIGNATION	TYPE	DO	n	REFERENCE
45	ALL	1.47	1.1	Weichselbaum, 1981
K45	T-cell	0.61	0.80	Fitzgerald, 1986
Reh	non-B,non-T	0.53	13.9	Lehnart, 1986
MOLT-4	T-cell	0.49	0.92	Szekely, 1985
MOLT-4	T-cell	1.30	1.5	Seshadri, 1985
JM (Jurkat)	T-cell	2.30	1.6	Seshadri, 1985
CCRF-CEM	T-cell	1.16	1.1	Seshadri, 1985
APHID-D2	T-cell	1.13	1.4	Seshadri, 1985
RCH-ACV	pre-B-cell	0.92	1.0	Seshadri, 1985

TABLE I

LYMPHOMA

DESIGNATION	TYPE	DO	n	REFERENCE
P3HR-1	B-lymphocytic	1.3	1.2	Johansson, 1982
U715	B-lyphocytic	1.6	1.0	Johansson, 1982
U698	B-lymphocytic	1.8	1.2	Johansson, 1982
SU-DHL-4	DHL (B-origin)	1.4	1.2	Johansson, 1982
JY	B-lymphocytic	0.61	2.13	Fitzgerald, 1986
Daudi	B-lymphocytic	0.57	1.25	Fitzgerald, 1986
U937	histio/monocytic	0.88	4.60	Fitzgerald, 1986
HRIK	B-lymphocytic	1.50	1.1	Seshadri, 1985
FMC-HU-1B	B-lymphocytic	0.90	1.4	Seshadri, 1985

DHL = diffuse histiocytic lymphoma

TABLE II

NONLYMPHOCYTIC LEUKAEMIA

DESIGNATION	TYPE	DO	n	REFERENCE
HL60	promyelocytic	1.17	1.3	Weichselbaum, 1981
HL60	APL	0.83	2.5	Rhee, 1985
HL60	monomyeloid	1.25	2.2	Fitzgerald, 1986
HL60	APL	1.37	1.1	Lehnart, 1986
K562	erythroleukaemia	1.65	1.4	Weichselbaum, 1981
K562	erythroleukaemia	1.41	1.1	Fitzgerald, 1986
K562	CML	1.39	4.5	Lehnart, 1986
KG1	monomyeloid	1.25	2.2	Fitzgerald, 1986
KG+	erythroleukaemia	0.64	3.45	Lehnart, 1986
176	AML	0.76	4.0	Weichselbaum, 1981
1	AMML (BM)	2.10	1.3	Ozawa, 1983
2	APL (BM)	1.80	1.2	Ozawa, 1983
2	APL (PB)	1.95	1.0	Ozawa, 1983
3	AMML (PB)	1.90	1.7	Ozawa, 1983
3	AMML (PB)	1.85	1.1	Ozawa, 1983
4	AMML (BM)	1.40	1.4	Ozawa, 1983
4	AMML (PB)	1.30	1.3	Ozawa, 1983
4	AMML (PB)	1.00	1.1	Ozawa, 1983
5	AML (PB)	1.35	1.3	Ozawa, 1983
6	AML (PB)	1.20	1.3	Ozawa, 1983
7	AML (BM)	0.90	1.2	Ozawa, 1983
8	AMML (PB)	0.70	1.2	Ozawa, 1983

TABLE III

9	AMML (PB)	0.70	1.1	Ozawa, 1983
10	AMML (PB)	0.55	1.0	Ozawa, 1983
11	AML (PB)	0.50	1.0	Ozawa, 1983
12	AML (PB)	0.30	1.0	Ozawa, 1983
CS	ANL	0.63	0.86	Kimler, 1985
CS	ANL	0.52	0.97	Kimler, 1985
GH	ANL	0.41	0.93	Kimler, 1985
GH	ANL	0.61	0.98	Kimler, 1985
JR	ANL	0.68	0.91	Kimler, 1985
TC	ANL	0.68	1.01	Kimler, 1985
GS	ANL	0.72	0.84	Kimler, 1985
HF	ANL	0.56	1.11	Kimler, 1985
JL	ANL	0.48	1.07	Kimler, 1985
LJ	ANL	0.95	1.29	Kimler, 1985

APL = acute promyelocytic leukaemia

CML = chronic myeloid leukaemia

AMML = acute myelomonocytic leukaemia

AML = acute myeloblastic leukaemia

ANL = acute nonlymphocytic leukaemia

BM = bone marrow sample

PB = peripheral blood sample

TABLE III (CONTD)

suspension culture 3 to 4 times per week. Repeated dilutions were made to keep the concentrations between 10E5 and 10E6 cells per ml. The increase in cell number following varying degrees of dose-dependent inhibition stabilised to an exponential function of time. These exponential growth curves when extrapolated back to zero time yield estimates of the apparent number of initial clonogens following radiation insult. Split-dose experiments confirmed that these cell lines had little repair capacity as suggested by the low values of extrapolation number found. Ozawa et al (1983) reported the radiation sensitivities of leukaemic progenitor cells in 12 cases of acute nonlymphocytic leukaemia utilising a colony counting assay. They found D0 values ranging from 0.3 Gy to 2.1 Gy with a maximum observed extrapolation number of 1.7. Kimler et al (1985) published the results of their analysis of the radiosensitivities of human nonlymphocytic leukaemia cells. Bone marrow samples were obtained from patients prior to treatment. Survival was assayed by colony counting. These authors found that the leukaemic colony forming cells had survival curves with steep slopes and no shoulders. They also found normal bone marrow cell radiosensitivity to be high, although not as high as for the leukaemic samples. Seshadri et al (1985) reported the radiation sensitivities of two human B-lymphocytic lymphoma lines HRIK and FMC-HU-1B, four T-cell acute lymphocytic leukaemias; MOLT-4, JM (Jurkat), CCRF-CEM and APHID-D2 and one pre-B-acute lymphocytic leukaemia RCH-ACV. These authors used a clonogenic end

point and found D0 values in the range 0.90 Gy to 2.3 Gy with extrapolation numbers ranging from 1.6 to 1.0. They singled out the T-cell ALL line JM (Jurkat) as being radioresistant (D0 = 2.3 Gy : n = 1.6) while all the others were said to be similar to previously studied nprmal T-lymphocytes. Rhee et al (1985) studied the response of the acute promyelocytic cell line HL-60 to fractionated irradiation and single irradiations at different dose-rates. They found a substantial split-dose repair capacity and a dependence of the observed D0 on dose-rate ranging from 0.76 Gy at 1.1 Gy/min to 1.20 Gy at 0.03 Gy/min. The survival curve parameters of D0 = 0.83 : n = 2.5 which were reported for a dose-rate of 0.78 Gy/min are taken for the purposes of the derivation of the median. Szekely and Lobreau (1985) reported a high radiosensitivity for the T-cell acute lymphocytic leukaemia cell line MOLT-4 (D0 = 0.49 Gy : n = 0.92). No split dose recovery was seen as predicted by the exponential nature of the survival curve. The data of Fitzgerald et al (1986) for the human B-cell lymphoma lines JY and Daudi, the histiocytic/monocytic lymphoma line U937 and the leukaemic lines K45 (T cell), K562 (erythroleukaemia), HL60 and KG1 (monomyeloid) were derived using a colony counting procedure. These authors used 3 different dose-rates of 0.05 Gy/min, 0.20 Gy/min and 2.00 Gy/min on each of the cell lines. They found that for 6/7 of the human cell lines examined there was no significant dose-rate effect. However for 1 line (U937) a clear dose-rate effect was seen. For the purposes of the

derivation of the median parameters, the result from the 2.00 Gy/min experiments on this line are used. Lehnart et al (1986) reported the radiosensitivities of 5 human leukaemic cell lines, Reh (non-T, non-B ALL), NALM-6 (B-cell ALL), HL60 (acute promyelocytic leukaemia), KG+ (erythroleukaemia) and K-562 (chronic myelocytic leukaemia) based on a colony counting assay. These authors used both linear quadratic and multitarget functions to describe the resultant survival curves. The linear quadratic gave a better fit to the data. Multitarget functions appeared to be innappropriate, as the survival data was of the continuously bending variety. However, in order to define median radiosensitivity parameters the multitarget descriptions must be used. This is because the data from all the other groups were analysed using multitarget functions and it is not possible to define medians when two different representations are used. The quantity of mean inactivation dose, as defined by Kellerer and Hug (1972) and subsequently used by Fertil et al (1984) is a possible way to compare survival curves described by different mathematical functions, but this suffers from the disadvantage that the "fine structure" of the curve shape is lost. The high extraploation numbers and very low D0 values found for two of these lines (Reh, KG+) may be partially explained by the innappropriateness of the curve fit.

Several cell lines were the subject of studies by more than one group. Line HL60 was used by Weichselbaum et al,

Rhee et al, Fitzgerald et al and Lehnart et al and with the exception of Rhee et al so was line K562. Line K45 of Fitgerald et al is the same as 45 of Weichselbaum et al. Line KG1 of Fitgerald et al is equivalent to line KG+ of Lehnart et al. Finally, some of the data of Ozawa et al and Kimler et al involve repeat measurements on the same sample or on samples from different sites (e.g. peripheral blood versus bone marrow). The intercomparison of derived radiosensitivity parameters for these lines is shown in Table (IV).

There is a degree of variation between measurements on equivalent tumour lines. The lines where the DOs measured by different groups differ by a factor of 2 or more are 45/K45, MOLT-4 and KG1/KG+. The differences in the last line may be partly due to the innappropriateness of a multitarget fit to the data of Lehnart, et al as described above. This line is also classified differently by the two experimental groups. Lehnart's value of 4.5 for the extrapolation number of line K562 contrasts with the measurements of the other two groups as does the histological classification. It appears that there are greater differences between established cell lines than between fresh patient material, but this may be deceptive. Intercomparisons between established cell lines are between different laboratories, while the multiple measurements on fresh patient samples were carried out in the same labs using the same techniques and subject to the same sources of error. It is not possible to say to what extent these differences are due to true variations in the

INTERCOMPARISON OF LEUKAEMIC CELL LINES OF COMMON ORIGIN

DESIGNATION	TYPE	DO	n	REFERENCE
45	ALL	1.47	1.1	Weichselbaum, 1981
K45	T-cell ALL	0.61	0.80	Fitzgerald, 1986
HL60	promyelocytic	1.17	1.3	Weichselbaum, 1981
HL60	APL	0.83	2.5	Rhee, 1985
HL60	monomyeloid	1.25	2.2	Fitzgerald, 1986
HL60	APL	1.37	1.1	Lehnart, 1986
K562	erythroleukaemia	1.65	1.4	Weichselbaum, 1981
K562	erythroleukaemia	1.41	1.1	Fitzgerald, 1986
K562	CML	1.39	4.5	Lehnart, 1986
MOLT-4	T-cell ALL	1.30	1.5	Seshadri, 1985
MOLT-4	T-cell ALL	0.49	0.92	Szekely, 1985
KG1	monomyeloid	1.25	2.2	Fitzgerald, 1986
KG+	erythroleukaemia	0.64	3.45	Lehnart, 1986
2	APL (BM)	1.80	1.2	Ozawa, 1983
2	APL (PB)	1.95	1.0	Ozawa, 1983
3	AMML (PB)	1.90	1.7	Ozawa, 1983
3	AMML (PB)	1.85	1.1	Ozawa, 1983
4	AMML (BM)	1.40	1.1	Ozawa, 1983
4	AMML (PB)	1.30	1.3	Ozawa, 1983
4	AMML (PB)	1.00	1.0	Ozawa, 1983
CS	ANL	0.63	0.86	Kimler, 1985
CS	ANL	0.52	0.97	Kimler, 1985
GH	ANL	0.41	0.93	Kimler, 1985
GH	ANL	0.61	0.98	Kimler, 1985

TABLE IV

cell lines as they have evolved. Some offshoots may have become radiation sensitive, others more resistant. Alternatively different experimental techniques may be the cause of the variations, or a combination of the two. Had there been some intergroup comparisons of fresh patient material, the sources of variation may have been more clearly seen.

The intrinsic radiosensitivity of lymphocytes in chronic lymphocytic leukaemia was the subject of a study reported by Thomson et al in 1985. These authors examined the radiation responses of lymphocytes from 18 patients. Apparent survival was assayed by determining the number of intact cells exhibiting nuclear pyknosis, which was a function of both dose and the time elapsed since irradiation. They found that the survival levels of 14/18 could be described by a function

$$S(d,t) = \exp\left(-\frac{d}{D_0}\right) + \exp(-\beta t)\left(1 - \exp\left(-\frac{d}{D_0}\right)\right) \quad (5.1)$$

Where d is the dose, t is the time elapsed since irradiation, DO is the exponential survival curve parameter describing radiosensitivity and β is the first order constant governing cell death. The computed values of DO for these 14 cases ranged from 0.31 Gy to 1.28 Gy with a mean value of 0.88 Gy and standard deviation of 0.27 Gy. For the remaining 4 samples atypical responses were observed. Two behaved as if there were 2 sub-populations of differing radiosensitivity. One behaved as if there were a single responding sub-population (DO ~ 2.2 Gy) co-existing with extremely radioresistant cells

which were not affected by the highest radiation dose used (5 Gy). The last sample's behaviour could be explained by either a single population of radiosensitive cells with a very slow death rate or alternatively by a mixed population of radiosensitive and radioresistant cells in indeterminate proportions. It should be noted that the assay used could not assess clonogenic potential. These data are therefore not directly comparable with the conventional survival curves reported above and are not used in the estimation of median radiosensitivity parameters. CLL is a condition not presently considered for TBI and marrow rescue, but it is interesting to note that, for the most part, the responses are suggestive of high radiosensitivity and no shoulder on the part of CLL lymphocytes.

The database consists of 54 sets of radiation survival measurements made on cell lines derived from 40 individual patients. Multiple measurements on the same cell line have been pooled as described previously and the pooled data is shown in Table V and plotted as a scatter diagram in fig (5.1). This diagram shows some degree of heterogeneity but, in general, the values of the extrapolation number, n are clustered around 1 indicating a characteristic lack of shoulder. The median value of n is 1.2. The values of D0 are typically below 1 Gy, the median is 0.9 Gy. This indicates that the radiosensitivities are relatively high in comparison with other tumour types. Taking both parameters together, for the most part, the leukaemias exhibit high radiosensitivity and little or no capacity to

POOLED DATA FOR LEUKAEMIC CELL LINES

DESIGNATION	DO	n	REFERENCE
HL60	1.21	1.75	W,F,L,R
K562	1.41	1.4	W,F,L
45/K45	1.04	0.95	W,F
KG1/KG+	0.95	2.83	F,L
176	0.76	4.0	W
Reh	0.53	13.9	L
P3HR-1	1.3	1.2	J
U715	1.6	1.0	J
U698	1.8	1.2	J
SU-DHL-4	1.4	1.2	J
JY	0.61	2.13	F
Daudi	0.57	1.25	F
U937	0.88	4.60	F
MOLT-4	0.9	1.21	S1,S2
JM(Jurkat)	2.3	1.6	S1
CCRF-CEM	1.16	1.1	S1
APHID-D2	1.13	1.4	S1
RCH-ACV	0.92	1.0	S1
HRIK	1.50	1.1	S1
FMC-HU-1B	0.90	1.4	S1
1	2.1	1.3	O
2	1.88	1.1	O
3	1.88	1.4	O
4	1.30	1.3	O

TABLE V

5	1.35	1.3	O
6	1.20	1.3	O
7	0.90	1.2	O
8	0.70	1.2	O
9	0.70	1.1	O
10	0.55	1.0	O
11	0.50	1.0	O
12	0.30	1.0	O
CS	0.58	0.92	K
GH	0.51	0.96	K
JR	0.68	0.91	K
TC	0.68	1.01	K
GS	0.72	0.84	K
HF	0.56	1.11	K
JL	0.48	1.07	K
LJ	0.95	1.29	K

KEY TO REFERENCES

W - Weichselbaum et al, 1981

F - Fitzgerald et al, 1986

L - Lehnart et al, 1986

R - Rhee et al, 1985

J - Johansson et al, 1982

S1 - Seshadri et al, 1985

S2 - Szekely et al, 1985

O - Ozawa et al, 1983

K - Kimler et al, 1985

TABLE V (CONTD)

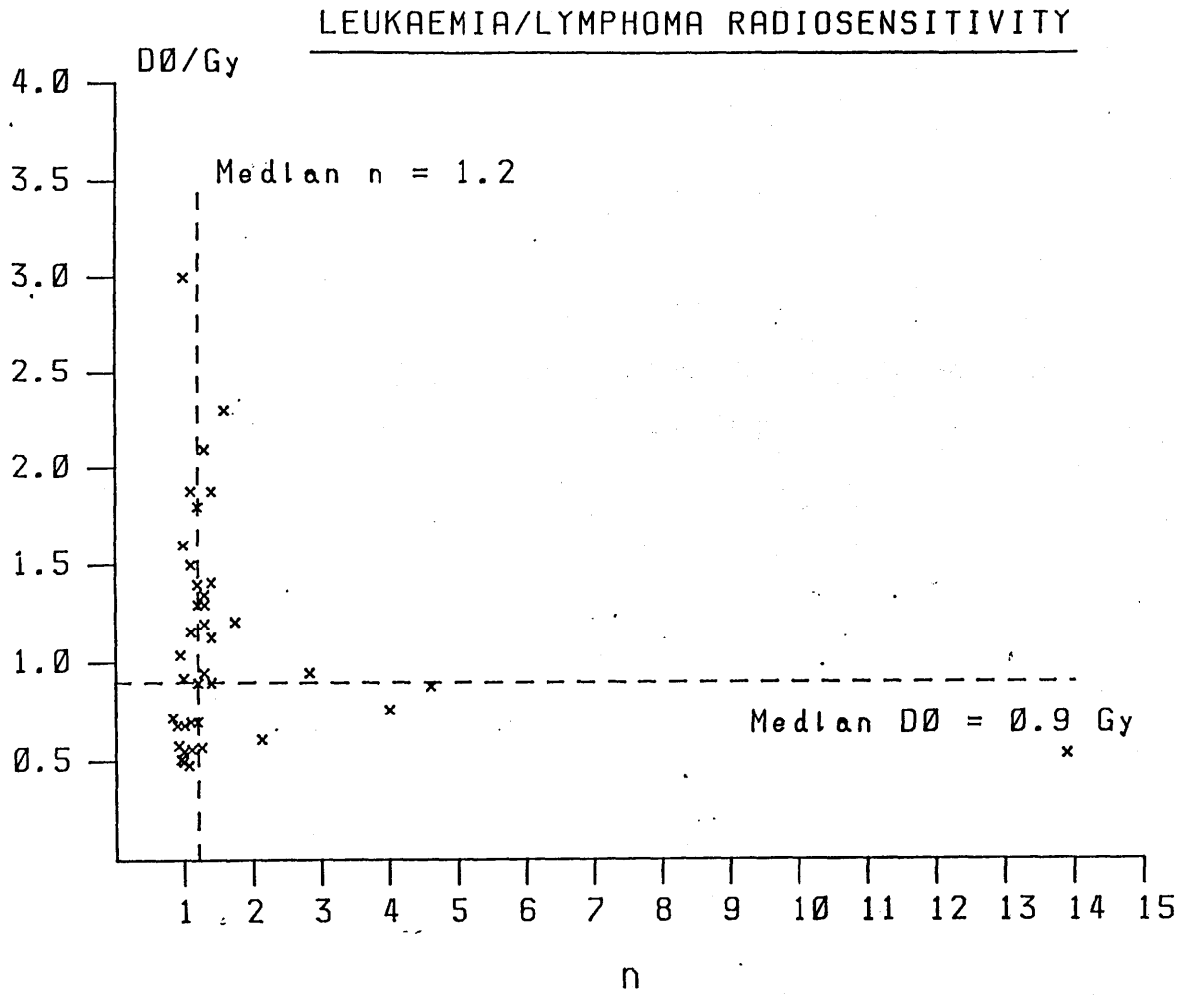

Fig 5.1 Scatter diagram of the values of the survival curve parameters DO and n found for human leukaemia/lymphoma. The values of DO are typically low (median 0.9 Gy), those of n are clustered around 1.0 (median 1.2)

repair radiation damage. They consequently would not be expected to display a significant fraction size or dose-rate effect. Where this prediction was tested (Johansson et al, Fitzgerald et al, Szekely and Lobreau) it was usually found to be true, although the studies of Rhee et al on HL-60 and Fitzgerald et al on U937 should be borne in mind.

As the best estimate of a representative survival function the median radiosensitivity parameters D0 and n are used in an unmodified multitarget equation viz.

$$S(d) = 1 - \left(1 - \exp\left(-\frac{d}{0.9}\right)\right)^{1.2} \qquad (5.2)$$

This function is used in the calculations of the effects of different fractionation schedules on leukaemia/lymphoma.

5.2.2 NEUROBLASTOMA

The in-vitro radiosensitivity database for neuroblastoma presently consists of measurements from cell lines derived from 8 different patients. The line NB-100 reported by Evans et al, 1986 is the same as CHP-100 (Bellamy et al, 1984; Schlesinger et al, 1976). Cells were irradiated as exponentially growing monolayer cultures (NB1, Ohnuma et al, 1977; CHP-100, Lan-1, Bellamy et al, 1984; Lan-1, Weichselbaum et al, 1980, SK-N-SH, Marchese et al, 1987) as single cell suspensions (HX138, Deacon et al, 1985a; HX142, Deacon et al, 1985b) and as multicellular tumour spheroids (HX138, Deacon et al, 1985a; NB1-G, NB2-G, Wheldon et al, 1985a; NB-100, Lan-1, Evans et al, 1986). Ohnuma et al (1977) found an unshouldered survival curve

for NB-1 with a DO of 1.13 Gy and suggested that this indicated the absence of sub-lethal damage repair, although this was not tested experimentally. Weichselbaum et al (1980) reported the radiation sensitivity of the human neuroblastoma line Lan-1 passaged over 100 times. They found DO to be 1.49 Gy, not significantly different from the DOs of the 8 other tumour types examined but the extrapolation number of 1.2 was one of the two smallest. The lines Lan-1 and CHP-100 were included in the study of Bellamy et al (1984) on the sensitivities of human and murine tumour cell lines to radiation and drugs. These authors found Lan-1 to be very sensitive to radiation (DO = 0.66 Gy) in contrast to the data of Weichselbaum (DO = 1.49 Gy). Examination revealed differences in morphology and surface markers between the two strains and Bellamy et al suggested that their Lan-1 was identical to the original reference (Seeger et al, 1977) whereas that used by Weichselbaum had altered characteristics. They also found the line CHP-100 to be radiosensitive (DO = 0.79 Gy) as was another neuroblastoma line TR-14A, data for which was not reported. Deacon et al (1985a) studied the radiation response of the human neuroblastoma line HX138 in-vitro with single cell suspension and multicellular tumour spheroids (MTS) and also in-vivo as xenografts in immune suppressed mice. Radiation response was assessed by growth delay for spheroids and xenografts and by clonogenic assay for all experimental systems. A high degree of radioresponsiveness was observed in the growth delay experiments. Clonogenic assay produced

shoulderless survival curves. The cell suspension system yielded a DO of 0.9 Gy under oxic conditions. The mean DO from the MTS experiments was 1.26 Gy while for cells from xenografts irradiated in oxic conditions the DO value was 1.51 Gy. Sub-lethal damage repair capacity assessed in the suspension experiments was modest. PLDR following a 24 hour delay in plating with the MTS experiments had the effect of increasing the DO value by a factor of approximately 1.4. No PLDR was seen in the xenograft experiments. The authors concluded that this tumour line was intrinsically highly radiosensitive with a limited repair capacity. In another paper Deacon et al (1985b) reported on the line HX142. Irradiated in suspension culture this yielded a survival curve with n = 2.3 and DO = 0.74 Gy. Xenograft experiments produced estimates of 1.4 for n and 1.43 Gy for DO. Again they concluded that neuroblastoma cells were intrinsically highly radiosensitive. Wheldon et al (1985a) reported the radiosensitivities of two neuroblastoma lines designated NB1-G and NB2-G grown as MTS. Clonogenic surviving fraction was estimated for each dose level by extrapolation of the regrowth curve to zero time. The consequent survival curves indicated radiosensitivity with little capacity for the accumulation and repair of sub-lethal damage (DO = 1.04 Gy : n = 1.18 for NB1-G, DO = 0.81 Gy : n = 1.36 for NB2-G). In a subsequent paper (Wheldon et al, 1986) split dose experiments were carried out on the line NB1-G showing no significant difference in the effectiveness of single compared to split dose

irradiation. Another paper (Wheldon et al, 1987) examined the effect on NB1-G spheroids of fractionated regimes calculated to be isoeffective for late responding normal tissues. As this constituted a test of some of the predictions to be described later in this thesis discussion of this experiment will be deferred until then. Evans et al (1986) examined three human neuroblastoma lines Lan-1, NB-100 and NB-134 grown as MTS. Data were presented only for Lan-1 and NB-100 in the form of regrowth curves of spheroid diameter versus time for a variety of single doses. The neuroblastomas were found to be significantly more radiosensitive than other tumour lines examined (Yuhas, written communication, 1984). In order to derive survival curve parameters, diameters have been converted to log(volume) assuming spherical geometry and apparent surviving fractions calculated by back-extrapolation of the exponential portion of the regrowth curve as described by Wheldon et al, 1985. The transformed growth curves of Evans et al are reproduced in figures 5.2 & 5.3 along with the construction lines used to derive apparent clonogenic surviving fraction. It can be seen from the figures that this approach seems more valid for the Lan-1 data than for the NB-100 data. However both sets and the survival curve parameters derived from them are included in the review. Marchese et al (1987) presented survival curves for a human neuroblastoma line SK-N-SH growing in both exponential and density-inhibited plateau phase culture for both immediate and 6 hour delayed plating. For consistency with the other data the

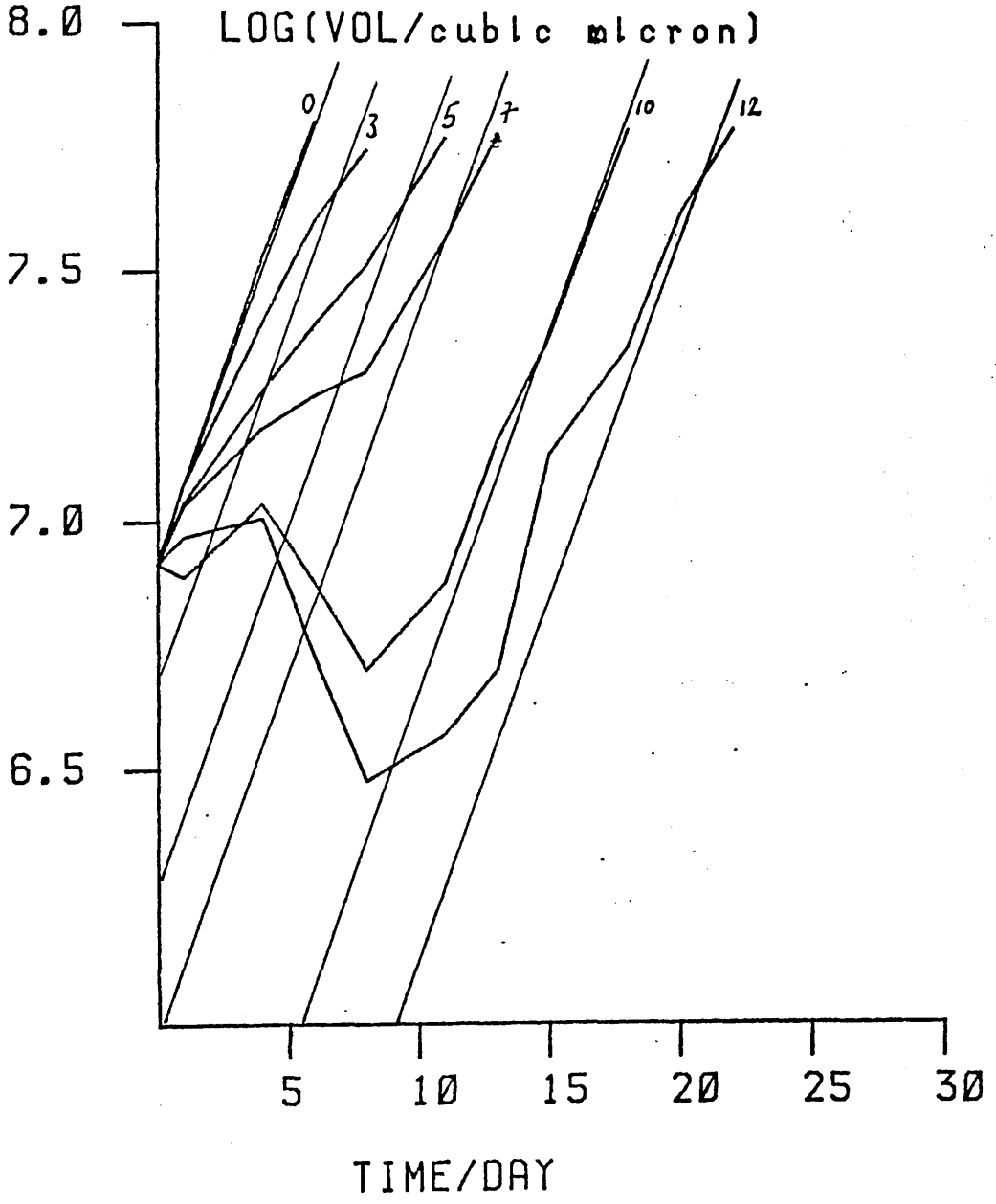

Fig 5.2 Regrowth curves for human neuroblastoma MTS line LAN-1 after Evans et al, 1986. The numbers refer to the given doses for the corresponding regrowth curve. Diameter values have been converted to log (volume). The straight lines are constructions used to estimate clonogenic surviving fraction based on the method of Wheldon et al, 1985.

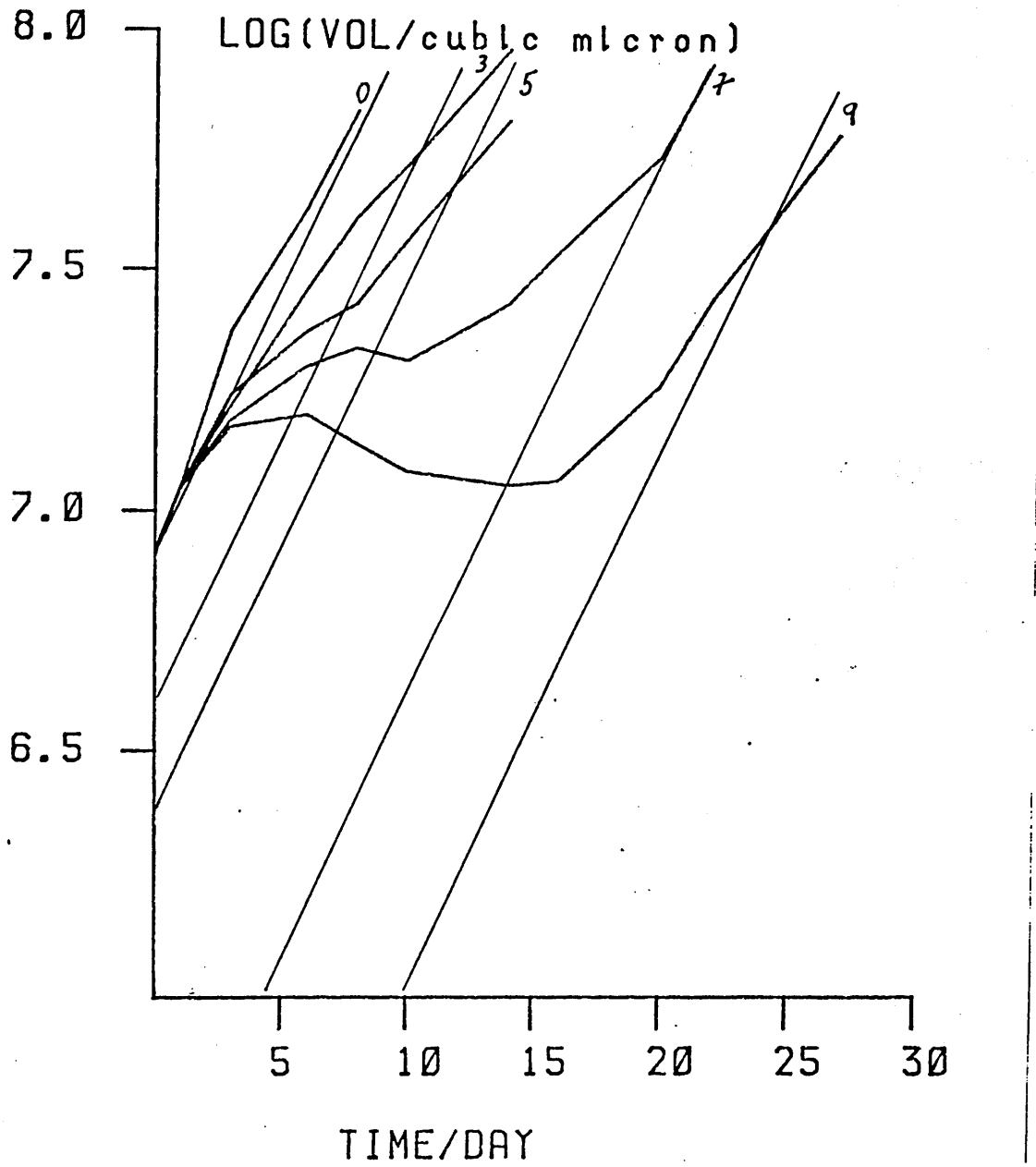

Fig 5.3 Regrowth curves for human neuroblastoma MTS line NB-100 after Evans et al, 1986. The numbers refer to the given doses for the corresponding regrowth curve. Diameter values have been converted to log (volume). The straight lines are constructions used to estimate clonogenic surviving fraction based on the method of Wheldon et al, 1985.

exponential phase results have been used here (this is the closest to the clinical situation with disseminated microscopic disease, plateau phase may be more representative of bulk disease). The data for immediate plating was selected, although it is apparent that there is little difference between the survival curves for immediate and 6-hour delayed plating for exponential phase culture. The survival curve was redrawn to yield the radiosensitivity parameters. This study indicated that the line SK-N-SH had a high capacity for PLDR in plateau phase.

Survival curve data are shown in Table VI and plotted as a scatter diagram in figure (5.4). The median values of the multitarget parameters n and D0 derived in accordance with the rules described earlier were: median n = 1.75; median D0 = 1.06 Gy. These data describe small shouldered radiosensitive cells, although not so radiosensitive as the leukaemias. Rather than take these at face value and use a standard multitarget equation to describe radiation sterilisation an attempt has been made to derive a "best estimate" of the median survival curve with a non-zero initial slope for this group of neuroblastoma cell lines. The "best estimate" was arrived at by the following procedure. For all the survival curves surveyed the upper limit on the initial slope was the gradient of the straight line joining the origin and the first data point. For pure exponential curves (NB1, HX138) the initial slope is simply equal to the final slope. For the line HX142 an actual survival curve was not available, only the

NEUROBLASTOMA RADIOSENSITIVITY

DESIGNATION	DO	n	REFERENCE
NB1	1.13	1.0	Ohnuma, 1977
LAN-1	1.49	1.20	Weichselbaum, 1980
LAN-1	0.66	2.13	Bellamy, 1984
LAN-1	1.78	5.4	Evans, 1986
CHP-100	0.79	3.12	Bellamy, 1984
NB-100	1.19	15.8	Evans, 1986
HX138(sus)	0.9	1.0	Deacon, 1985(a)
HX138(sph)	1.26	1.0	Deacon, 1985a
HX142	0.74	2.3	Deacon, 1985b
NB1-G	1.04	1.18	Wheldon, 1985a
NB2-G	0.81	1.36	Wheldon, 1985a
SK-N-SH	1.29	3.0	Marchese, 1987

TABLE VI

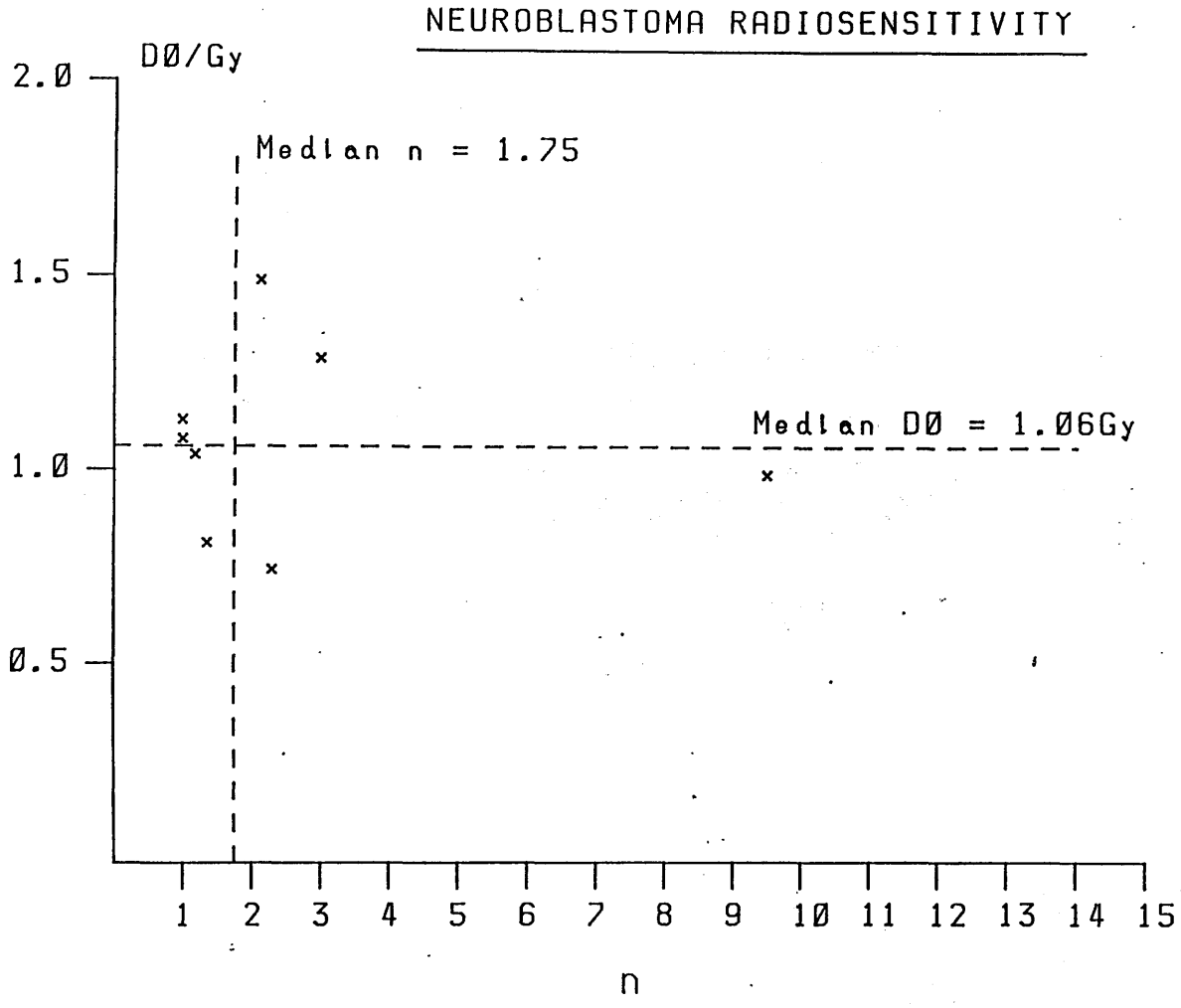

Fig 5.4 Scatter diagram of the values of the survival curve parameters DO and n found for human neuroblastoma. The values of DO are typically low (median 1.06 Gy), although not as low as found for leukaemia/lymphoma. The values of n are also low (median 1.75)

parameters n and D0, the initial slope was taken as 0 i.e. $D1 \rightarrow \infty$ in the equation

$$S(d) = \exp\left(-\frac{d}{D_1}\right)\left(1 - \left(1 - \exp\left(-\frac{d}{D_2}\right)\right)^n\right) \qquad (5.3)$$

The values of these initial slopes are shown in table VII. The median was D1 = 2.04 Gy. The second component of slope D2 was calculated from the relationship

$$D_2 = \frac{D_0\, D_1}{(D_1 - D_0)} \qquad (5.4)$$

where D0 is the median value of the final slopes.

Therefore D2 = 2.21 Gy.

Two equations were then constructed

$$S(d) = 1 - \left(1 - \exp\left(-\frac{d}{1.06}\right)\right)^{1.75} \qquad (5.5)$$

$$S(d) = \exp\left(-\frac{d}{2.04}\right)\left(1 - \left(1 - \exp\left(-\frac{d}{2.21}\right)\right)^{1.75}\right) \qquad (5.6)$$

As a best estimate of the true survival curve for all doses the mean on a log-linear scale of these two equations was taken

$$\ln\left[S(d)\right] = \frac{1}{2}\ln\left(1 - \left(1 - e^{-\frac{d}{1.06}}\right)^{1.75}\right) + \frac{1}{2}\ln\left\{e^{-\frac{d}{2.04}}\left(1 - \left(1 - e^{-d/2.21}\right)^{1.75}\right)\right\} \qquad (5.7)$$

i.e. the geometric mean in terms of surviving fractions. This modified survival curve equation was used in the calculations of the effects of different fractionation schedules on neuroblastoma.

5.2.3 EWING'S SARCOMA

The database on the in-vitro radiosensitivity of Ewing's Sarcoma is rather limited. Only two cell lines, designated 5838 and 4573 have been the subject of studies aimed at producing survival curves (Kinsella et al, 1984). The survival data were analysed using a multitarget function to yield n = 6.0 : D0 = 1.26 Gy for the line 5838 and n =

NEUROBLASTOMA INITIAL SLOPES

DESIGNATION	D1	REFERENCE
NB1	1.13	Ohnuma, 1977
LAN-1	2.05	Weichselbaum, 1980
LAN-1	1.80	Bellamy, 1984
LAN-1	5.5	Evans, 1986
CHP-100	25.6	Bellamy, 1984
NB-100	4.2	Evans, 1986
HX138(sus)	0.9	Deacon, 1985a
HX138(sph)	1.26	Deacon, 1985a
HX142	∞	Deacon, 1985b
NB1-G	1.47	Wheldon, 1985a
NB2-G	2.64	Wheldon, 1985a
SK-N-SH	2.03	Marchese, 1987

TABLE VII

3.2 : D0 = 2.04 Gy for line 4573. On the basis of these data the authors rec ommended a schedule of 2 fractions of 4 Gy for TBI. It is apparent from examination of the survival data that a continuously bending function with a non-zero initial slope would give a better fit. The parameters which fit a linear-quadratic to the survival data are α = 0.16 Gy-1 : β =0.051 Gy-2 for line 5838 and α = 0.25 Gy-1 : β = 0.01 Gy-2 for line 4573 (Wheldon et al, 1985b). It is interesting to note that the α/β ratios for the two lines (3 Gy for 5838 and 25 Gy for 4573) are very dissimilar suggesting that their fractionation dependence and, by implication, their dose-rate dependence are also dissimilar. This is another example of the heterogeneity of tumour radiosensitivity within the same clinical entity. Due to the scarcity of data it is not possible to come to any conclusions regarding the radiation characteristics of a "typical" Ewing's Sarcoma cell line. In the subsequent analysis both sets of α and β are used individually. The different LQ parameters will lead to different predictions of optimal fractionation schedules for the two lines as will be seen later.

The survival equations which will be used to describe Ewing's Sarcoma are therefore;

Line 5838 $$S(d) = \exp(-0.16d - 0.051d^2) \quad (5.8)$$

Line 4573 $$S(d) = \exp(-0.25d - 0.01d^2) \quad (5.9)$$

6.0 FRACTIONATED VERSUS LOW DOSE-RATE TOTAL BODY IRRADIATION : RADIOBIOLOGICAL CONSIDERATIONS IN THE SELECTION OF TREATMENT SCHEDULES

As has been described in previous chapters the major dose-limiting constraint for TBI with marrow rescue is lung where radiation damage is manifest as interstitial pneumonitis. It is widely recognised that fractionating the dose or reducing the dose-rate enables a substantial increase in total dose for the same level of toxicity. In 1979 Peters et al suggested that this phenomenon when considered alongside the shapes of murine haemopoietic cell radiation survival curves provided a rationale for using fractionated TBI schedules for bone marrow transplantation rather than a single fraction given at a high dose-rate. Similar arguments can be advanced for low dose-rate TBI. Song et al (1981) challenged these suggestions stating that in their judgement the therapeutic gain due to fractionation was overestimated by other investigators. This prompted a correspondence (Peters and Withers, 1982; Song et al, 1982) where the two sides of the disagreement were set out. The crux of the problem relates to the repair capacities of the target cells for TBI. In the previous chapter the in-vitro radiosensitivities for human leukaemias and neuroblastomas were reviewed. The conclusion was that these have, typically, little repair capacity as evidenced by the lack of a shoulder on the survival curves, although there were exceptions with significant repair capacity. However, on the whole, this work supports the argument of Peters et al over that of Song et al. For these tumour types cell

survival after fractionated TBI would be little influenced by fraction size and consequently the increase in total dose brought about by fractionation or dose-rate reduction will provide a corresponding proportional increase in log cell kill (i.e. the number of decades of reduction of clonogenic tumour cells). Compared to a single high dose-rate fraction the irradiation time required for a low dose-rate exposure is longer. For fractionated schedules it is longer still. The proliferation effects therefore favour single, high dose-rate fractions but, as will be shown in the next chapter, the magnitude of these effects is not large enough to change the above conclusions. The work reported in this chapter therefore starts from the premise that fractionated or low dose-rate TBI is more appropriate for the treatment of radiosensitive tumours like leukaemia and neuroblastoma which have low capacities for repair of sub-lethal damage.

6.1 EQUIVALENCE CONDITIONS FOR FRACTIONATED AND LOW DOSE-RATE TBI

In this section Dale's extension of the LQ model to low dose-rates will be used in order to calculate the dose-rates required to make continuous irradiation treatment therapeutically equivalent to fractionated treatment. This will enable some conclusions to be drawn with respect to which of these 2 modalities is preferable. The response of lung to fractionated radiation is characterised by a large dependence on fraction size and almost independence of the overall treatment time (Travis et al, 1983; Wara et al, 1973). In terms of the LQ model

the characteristic value of α/β is low, most estimates being around 3 Gy. Based on the radiobiological characteristics of human leukaemias and neuroblastomas, to a first approximation and in the absence of proliferation effects, the sterilisation of tumour cells will depend only on total dose (i.e. independent of fractionation and dose-rate). In his 1985 paper Dale derived an equation describing the parameters of fractionated and continuous low dose-rate schedules of equal total dose which should also be of equal biological effectiveness. This was arrived at by equating separately the linear and quadratic components of damage. The relationship is independent of α/β ratios and can be written as

$$N = \frac{\mu T}{2\left\{1 - \frac{1}{\mu T}\left(1 - \exp(-\mu T)\right)\right\}} \qquad (6.1)$$

Where N is the number of fractions in the fractionated schedule and T is the irradiation time of the continuous schedule. The parameter μ is the first order time constant for the decay of the capacity of sub-effective lesions to interact. This is usually interpreted as repair of sub-lethal damage. The equation is identical to the general formula of Liversage (1969). Solutions to this equation will satisfy the dual criteria that:

1/ They will be biologically equivalent for a tissue with repair constant μ

2/ They will be biologically equivalent for tumour cells having low capacities for SLD repair (same total dose).

The schedules with the required parameters will thus be interchangeable and the choice of which type to use for

TBI can be determined by convenience. This equation can be solved for T for different values of N and μ, corresponding to different numbers of fractions in the fractionated schedule and different repair constants. The alternative quantity of repair half-time $T_{1/2}$ is given simply by

$$T_{1/2} = \frac{\ln 2}{\mu} \qquad (6.2)$$

Due to the appearance of the term exp($-\mu T$) equation (6.1) must be solved by a numerical approach. A computer-based method of false position has been used. Figure 6.1 shows how T varies as a function of N for different values of the repair half-time, $T_{1/2}$.

For lung the repair half-time has been estimated as 1.5 hours (Thames et al, 1984). The use of the analysis is illustrated in the following example.

6.1.1 APPLICATION TO FRACTIONATED AND PROTRACTED TBI

A TBI schedule of 6 fractions of 2 Gy (i.e. a total dose of 12 Gy) is currently used in a number of centres for the treatment of leukaemia (Clift et al, 1982; Goolden et al, 1983). By examination of figure 6.1 it can be seen that the irradiation time required for a continuous low dose-rate exposure to achieve the same total dose (and hence anti-leukaemic effect) and also the same level of lung damage is 23.6 hours (corresponding to a dose-rate of 0.51 Gy hr-1). In the next chapter it is suggested that 10 fractions of 1.4 Gy (i.e. a total dose of 14 Gy) can be an optimal fractionation schedule in some circumstances. For this schedule figure 6.1 and thus equation (6.1) predicts an isoeffective irradiation time

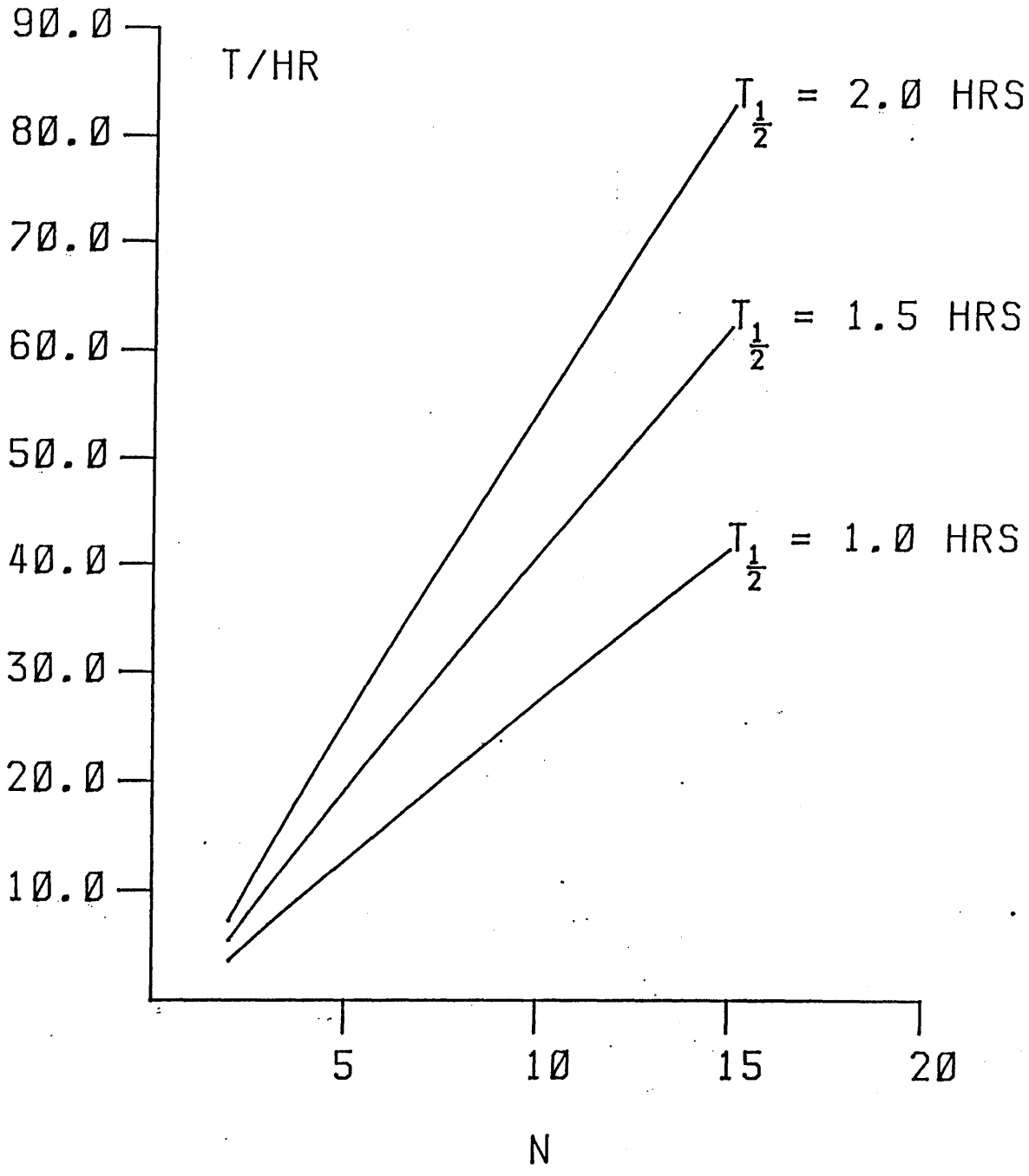

Fig 6.1 T is the irradiation time for a single low dose-rate exposure to produce the same biological effect as a fractionated schedule of the same total dose given in N fractions. The relationship is shown for different assumed values of $T_{1/2}$, the half-time for repair of radiation damage.

of 41.0 hours (corresponding to a dose-rate of 0.34 Gy hr-1). It is apparent that these times are unlikely to be acceptable for practical reasons.

When the term exp($-\mu T$) can be neglected (i.e. << 1) approximately when $\mu T > 5$ or in this example T > 10 hours equation (6.1) can be reduced to

$$N = \frac{(\mu T)^2}{2(\mu T - 1)}$$

Inverting and separating the terms gives

$$\frac{1}{2N} = \frac{1}{\mu T} - \left(\frac{1}{\mu T}\right)^2$$

therefore

$$\frac{1}{\mu T} = \frac{1 - \sqrt{1 - 2/N}}{2}$$

rejecting the alternative root which violates the condition $\mu T > 5$. Finally

$$T = \frac{2}{\mu\left(1 - \sqrt{1 - 2/N}\right)} \qquad (6.3)$$

T may then be found directly from the above equation. From figure 6.1 it can be seen that this may be used for values of N of 3 or more.

It should be noted that equivalence can only be achieved for one value of the repair kinetic parameter μ . This value determines the irradiation time and hence dose-rate which must be used. Taking the previous example and using a repair half-time of 1 hour the required irradiation times are 15.7 hours (equivalent to 6 fractions of 2 Gy) and 27.3 hours (equivalent to 10 fractions of 1.4 Gy). For a repair half-time of 2 hours the times are 31.4 hours (equivalent to 6 fractions of 2 Gy) and 54.7 hours

(equivalent to 10 fractions of 1.4 Gy). This information is presented in Table VIII.

	EQUIVALENT SCHEDULE	
REPAIR HALF-TIME/HRS	6 X 2 Gy	10 X 1.4 Gy
1.0	15.7 hr	27.3 hr
1.5	23.6 hr	41.0 hr
2.0	31.4 hr	54.7 hr

Irradiation times required for single fractions.

TABLE VIII

In terms of the LQ model equation (6.1) must be satisfied in order that the non-linear components of radiation damage from fractionated and continuous schedules be equal. There is some evidence that the timescale of repair may vary from tissue to tissue with acute responders having shorter times (Thames et al, 1984). This means that equation (6.1) will not be satisfied for all tissues. If a value appropriate for lung is taken the fractionated schedule will produce a greater amount of quadratic damage in acute responders than the continuous schedule. This would not be expected to be a large effect because for high values of α/β (~10 Gy) the quadratic damage will be relatively less important. The irradiation time required for the continuous schedule is dependent on the number of fractions in the fractionated schedule. It follows that hyperfractionated schedules require a longer irradiation time for an equivalent continuous exposure and

will be consequently more difficult to mimic radiobiologically. This point was illustrated in the numerical example.

6.2 PROLIFERATION EFFECTS

Up till now the assumption has been made that proliferation effects are negligible. This enabled the Liversage formula (equation (6.1)) which applies for equal total doses to be used. In reality tumour cell proliferation is an important process and may in some sorts of treatment be one of the major factors determining cure (e.g Trott and Kummermehr, 1985). For TBI, proliferation effects may be expected to be important for fractionated schedules but probably negligible for continuous low dose-rate irradiations (even those lasting an entire day). This means that, from the point of view of tumour cells, fractionated schedules must have a greater total dose than single irradiations to produce the same level of sterilization. The difference in dose is that required to counteract the growth in the tumour cell population over the duration of the fractionated schedule. For example if a TBI schedule of 5 days duration is being used to treat a tumour cell population with a doubling time of 2 days, 2.5 doublings will occur over this time (i.e. 0.75 logs). The total dose of the fractionated schedule will have to be higher by the equivalent of 0.75 logs of tumour cell reduction. The possible dose reduction for a single exposure compared to a fractionated schedule depends on the radiosensitivity of the target tumour cells. Based on the information contained in chapter 5 for

human leukaemias and neuroblastomas a reasonable approximation of a "typical" survival curve would be shoulderless and would have a D0 of about 1 Gy. In order to arrive at a mathematical equivalence condition to describe this situation it is necessary to use some of the LQ model quantities described in chapter 3.

For a continuous low dose-rate schedule to be equivalent to a fractionated schedule we require

$$RE_f \times D_f = ERD_f = ERD_c = RE_c \times D_c \qquad (6.4)$$

REf is simply (1+d/(α/β)) where d is the dose per fraction of the fractionated schedule.

$$RE_c = 1 + \frac{2R}{\mu(\alpha/\beta)}\left\{1 - \frac{1}{\mu T}(1 - exp(-\mu T))\right\} \qquad (6.5)$$

Where R is the dose-rate, T is the irradiation time and μ is the time constant for repair of radiation damage. As Dc = RT, equation (6.4) may be written as

$$D_f\left(1 + \frac{d}{\alpha/\beta}\right) = D_c + \frac{2D_c^2}{\mu T(\alpha/\beta)}\left\{1 - \frac{1}{\mu T}(1 - exp(-\mu T))\right\} \qquad (6.6)$$

which can be expressed as

$$\frac{2D_c^2}{\mu T(\alpha/\beta)}\left[1 - \frac{1}{\mu T}(1 - exp(-\mu T))\right] - \left[D_f - D_c + \frac{D_f}{N(\alpha/\beta)}\right] = 0 \qquad (6.7)$$

It is reasonably simple to solve this equation for T numerically by computer using the method of false position. This approach is used in the following examples.

6.2.1 APPLICATIONS

For lung the α/β ratio has been estimated at around 3Gy. Taking the same two schedules as in the previous example we will look at the predicted irradiation times

for equivalent effects on lung tissue and tumour cells. A schedule of 6 fractions of 2 Gy, two fractions per day takes approximately 2.5 days to complete. For a tumour cell population doubling time of 2 days this corresponds to 1.25 doublings or 0.38 logs. The dose needed to reduce the surviving fraction by this amount is 0.87 Gy therefore the total dose of the single irradiation can be reduced by this amount i.e. to 12-0.87 = 11.13 Gy. Substituting this value into equation (6.7) and solving gives a T of 17.68 hours

This value along with the corresponding values for tumour doubling times of 4 days and 20 days are shown in Table IX. For a schedule of 10 fractions of 1.4 Gy, twice daily the duration of treatment is approximately 4.5 days. This corresponds to 2.25 doublings of a tumour cell population with a doubling time of 2 days (i.e. 0.68 logs). The required dose for this level of sterilisation is 1.56 Gy. Consequently the single fraction need only have a total dose of 14-1.56 = 12.44 Gy. Solving equation (6.7) for this leads to a predicted irradiation time of 25.22 hours. The corresponding times for 4 and 20 day doubling times are also shown in Table IX. It should be noted that this argument neglects any effects of tumour cell cycle redistribution, which would be expected to favour the longer (fractionated) schedule. The irradiation times of Table IX should therefore be considered as lower limits.

	EQUIVALENT SCHEDULE	
DOUBLING TIME/DAYS	6 X 2 Gy	10 X 1.4 Gy
2	Dose = 11.13 Gy Irr.time = 17.68 hr	Dose = 12.44 Gy Irr.time = 25.22 hr
4	Dose = 11.57 Gy Irr.time = 20.49 hr	Dose = 13.22 Gy Irr.time = 32.16 hr
20	Dose = 11.91 Gy Irr.time = 22.91 hr	Dose = 13.84 Gy Irr.time = 38.99 hr

Equivalent total doses and necessary irradiation times for single fractions to be equivalent to the indicated fractionated schedules for both tumour cells with doubling times shown and a LQ model tissue with α/β = 3Gy and $T_{1/2}$ = 1.5 hours.

TABLE IX

It should be noted that where the term $\exp(-\mu T)$ can be neglected an analytical solution for T is possible. In this case equation (6.6) reduces to

$$D_f\left(1+\frac{d}{\alpha/\beta}\right) = D_c + \frac{2D_c^2}{\mu T(\alpha/\beta)}\left(1-\frac{1}{\mu T}\right)$$

rearranging this

$$\frac{2D_c^2}{\alpha/\beta}\left[\frac{1}{\mu T}-\left(\frac{1}{\mu T}\right)^2\right] = D_f - D_c + \frac{D_f d}{\alpha/\beta}$$

therefore

$$\left(\frac{1}{\mu T}\right)^2 - \frac{1}{\mu T} + \frac{D_f - D_c + D_f d/\alpha/\beta}{2D_c^2/\alpha/\beta} = 0$$

from the roots of a quadratic equation

$$\frac{1}{\mu T} = \left(1-\sqrt{1-2\left(\frac{\alpha/\beta\,(D_f-D_c)+D_f^2/N}{D_c^2}\right)}\right)/2$$

rejecting the other root as in applicable, finally

$$T = \frac{2}{\mu\left(1 - \sqrt{1 - 2\left(\frac{\alpha/\beta\,(D_f - D_c) + D_f^2/N}{D_c^2}\right)}\right)} \qquad (6.8)$$

In this equation it should be noted that if $D_f \rightarrow D_c$ the expression reduces to equation (6.3). The main potential radiobiological advantage low dose-rate irradiation has over fractionated radiation treatment is the shorter overall time. Two characteristics of the tumour cell population will determine the importance of the difference in treatment time. The first is the proliferation kinetics of the tumour cells. Table IX illustrates that more slowly growing tumour cell populations imply that higher total doses given at a low dose-rate are necessary in order to match the sterilization produced by the fractionated treatment. This, in turn, implies longer irradiation times in order to limit lung damage. Conversely, for fast growing populations more doublings are spared and the number of tumour cells which must be sterilized is less. Consequently, in this case, less dose is necessary for an equivalent tumour effect. The other factor of importance is the radiosensitivity of the tumour cells. This determines the dose-equivalent of a population doubling. For example, if we have two cell populations; P1 and P2, possessing shoulderless survival curves with associated D0 values; D1 and D2, where D2 = 2 X D1, then the amount of dose required to reduce the number of cells in P2 by a particular amount is twice that required for P1. It is apparent how this factor will affect the importance of the number of doubling times saved.

It is possible to set limits on the parameters a tumour cell population must have in order to make low dose-rate irradiation better than fractionated treatments. Less dose is given by the single exposure. Because of this fewer cells are sterilized by radiation. For cells with shoulderless survival curves the proportional difference is

$$F_d = \exp(-\Delta/D_0)$$

where Δ is the difference in dose between the two techniques. Also, because the low dose-rate exposure is completed in a shorter time there is less proliferation throughout the duration of treatment. The proportional difference here is

$$F_p = \exp(\tau \ln 2 / TD)$$

where τ is the difference in total time between the two techniques and TD is the tumour population doubling time. The approximation can be made that τ is equal to the duration of the fractionated treatment schedule. For both techniques to have equal effects on the tumour cell population we require

$$F_d \, F_p = 1$$

i.e.

$$TD = \frac{\tau D_0 \ln 2}{\Delta} \qquad (6.9)$$

It can be seen that the required tumour doubling time for equal effect is dependent on the values of D0, τ and Δ. If, on grounds of practicality, we take the upper limit on how long a single exposure could last as 10 hours and require that it must be isoeffective for lung with a fractionated schedule of 6 fractions of 2 Gy in 2.5 days

it can be shown by solving equation (6.7) that the total dose is 9.6 Gy (i.e. at 0.96 Gy/hr). It is necessary for equivalence that the population increase over 2.5 days be equal to the sterilization produced by (12 - 9.6) = 2.4 Gy. For cells with shoulderless survival curves and a DO of 1 Gy this dose is equivalent to 1.04 logs or 3.5 doublings and requires that the tumour cells have a doubling time of less than 18 hours. Required doubling times for other values of DO and other fractionated schedules may be found from equation (6.9) and some of these are shown in Table X.

	FRACTIONATED SCHEDULE		
DO/Gy	3 X 3.32 Gy	6 X 2 Gy	10 X 1.4 Gy
0.5	8.7 days	8.7 hrs	8.5 hrs
1.0	17.3 days	17.3 hrs	17.0 hrs
1.5	26.0 days	26.0 hrs.	25.5 hrs
2.0	34.7 days	34.7 hrs	34.0 hrs

Required doubling times for tumour cell populations in order that the effects of both the indicated fractionated schedule and also a continuous low dose-rate exposure of 9.6 Gy in 10 hours are equal

TABLE X

The examples in the Table show that there is little possibility of a low dose-rate exposure of 10 hours or less being better than a fractionated schedule consisting of 6 or more fractions. Only for very fast growing tumour

cell populations is the low dose-rate treatment better. It is also apparent from Tables VIII and IX that the irradiation times calculated for equivalent biological effects on lung tissue and tumour cells are usually very long. For any foreseeable clinical scenario they are almost certainly too long to be practicable.

Given that fractionated schedules are likely to be better than single low dose-rate exposures and also that these are better than single high dose-rate exposures, it may seem plausible that combining the two modalities i.e. using a fractionated low dose-rate schedule would give the best of both worlds and may be an optimal method of treatment. In fact, this is not the case, as the following analysis will show.

6.3 FRACTIONATED LOW DOSE-RATE SCHEDULES

Equation (6.7) gives the condition for a single low dose-rate exposure of dose Dc to be equivalent for an LQ model tissue to a fractionated schedule of total dose Df in N fractions. This equation can be modified to answer the current question if we put

Df = df, the fraction size of a single high dose-rate schedule

and N = 1

Equation (6.7) can be rewritten

$$\frac{2D_c^2}{\mu T(\alpha/\beta)}\left\{1-\frac{1}{\mu T}(1-\exp(-\mu T))\right\}-\left[d_f-D_c+\frac{d_f^2}{\alpha/\beta}\right]=0 \quad (6.10)$$

Say we want to take advantage of the "dose-rate" effect in

order to increase the given dose by x%

i.e. Dc = df(1 + x/100)

By substituting the appropriate values into equation (6.10) and solving by numerical iteration the required irradiation times may be found.

6.3.1 APPLICATIONS

1/ We start with a relatively high dose, given at a high dose-rate, 6.5 Gy say, and ask the question. What irradiation time and dose-rate is required in order that this dose can be increased by 5%, which is probably at the limit of clinical detectability, and still have the same biological effect on (a) - late responding and (b) - acute responding tissues.

In equation (6.10) df = 6.5 Gy : Dc = 6.825 Gy

(a) - Late effects

Taking α/β = 3 Gy and $T_{1/2} = \ln 2/\mu$ = 1.5 hours

The necessary irradiation time is 0.81 hours i.e. at a dose-rate of 8.4 Gy/hr (14 cGy/min). This is still quite a high dose-rate, well within the clinical TBI range.

(b) - Acute effects

Taking α/β = 10 Gy and $T_{1/2}$ = 1.0 hours

The irradiation time is again 0.81 hours i.e. a dose-rate of 8.4 Gy/hr.

So a 5% increase in dose for the same toxicity, compared to a high dose-rate fraction, is certainly possible without any significant logistic problems for a baseline fraction of 6.5 Gy.

2/ Fraction size = 2 Gy

Again calculating the required irradiation time for a possible 5% dose increase.

In equation (6.10) df = 2 Gy : Dc = 2.1 Gy

(a) - Late effects

α/β = 3 Gy : $T_{1/2}$ = 1.5 hours

The required irradiation time is 1.2 hours at a dose-rate of 1.8 Gy/hr. This dose-rate is at the lower end of the clinical TBI range.

(b) - Acute effects

α/β = 10 Gy : $T_{1/2}$ = 1.0 hours

The required irradiation time here is 1.85 hours at a dose-rate of 1.1 Gy/hr. This dose-rate is beyond the lower limit of TBI schedules used clinically so far.

These values indicate that the required dose-rates may be a good deal lower for smaller fractions than for larger ones. This is made clearer by Table XI where these values together with the corresponding ones for a fraction size of 1.4 Gy and different levels of dose enhancement are shown.

	DOSE INCREASE			
BASELINE FRACTION	5%	10%	15%	20%
6.5 Gy	L:8.43 A:8.48	L:4.35 A:4.19	L:2.98 A:2.73	L:2.29 A:1.97
2.0 Gy	L:1.76 A:1.13	L:0.87 A:0.44	L:0.57 A:0.17	L:0.41 A: NP
1.4 Gy	L:1.02 A:0.54	L:0.49 A:0.15	L:0.30 A: NP	L:0.21 A: NP

Dose-rates in Gy/hr required to enable an increase in total dose by the specified amount for 3 different baseline fraction sizes. NP denotes not possible.

TABLE XI

It should be noted from table XI that :

1/ In order to affect the dose increases very low dose-rates are required for standard fraction sizes

2/ Acute effect equivalence requires lower doses than late effects

3/ Some levels of enhancement are impossible for acute effects. This is because the ERD values of the high dose-rate baseline fractions are lower than the doses required for the low dose-rate fractions. This is also true of late effects at higher levels of enhancement. The maximum enhancements for late effects are 67% for 2 Gy fractions and 47% for 1.4 Gy fractions. This corresponds to dose-rates tending towards zero and consequently having irradiation times tending towards infinity.

The work of Tarbell et al (1987b) is relevant to the

above considerations. These authors found that, for mice, using fractionated or low dose-rate irradiation did not reduce the effectiveness of haemopoietic stem cell sterilisation in comparison to high dose-rate single fractions, indeed there was a lower apparent DO value associated with fractionated radiation, which could be due to the effects of tumour cell cycle redistribution as mentioned previously. Toxic side-effects for thoracic irradiation however, were dependent on the method of delivery. Two toxicity endpoints were used. The first was the LD 50/30 endpoint i.e mortality at 30 days post-irradiation. The second endpoint was the LD 50/30-180 i.e. mortality between 30 and 180 days post-irradiation. The actual mechanisms of death were not specified but, loosely, we can think of the LD 50/30 as acute toxicity and the LD 50/30-180 as late toxicity. Several different irradiation protocols were used; Single doses at both high (48 Gy/hr) and low (3 Gy/hr) dose-rates and multifractionated schedules of 2 or 1.2 Gy fractions at both dose-rates. The authors found that for the LD 50/30 endpoint there was a highly significant dose-rate effect when single fractions were used; 14.88 Gy for high dose-rate (HDR), 24.87 Gy for low dose-rate (LDR). Fractionation produced a higher LD 50/30 than the single LDR exposure (approximately 30 Gy) but there was no discernible dose-rate effect within the fractionated schedules. In the context of the previous analysis these observations are not surprising, especially for an acute

endpoint. It was seen that dose enhancement by dose-rate reduction is more difficult for acute effects than for late effects, but becomes easier the higher the baseline dose used. Tarbell et al used a HDR baseline dose of 14.88 Gy. For the fractionated schedules the baseline doses were 2 Gy and 1.2 Gy where, it would be anticipated, much lower dose-rates are required to show a dose enhancement. For the LD 50/30-180 endpoint again a highly significant dose-rate effect was seen with single fractions; 12.99 Gy for HDR against 22.47 for LDR.

Fractionation brought about an even greater increase; to 32.66 Gy for once-daily HDR fractions of 2 Gy; to 24.1 Gy for twice-daily HDR fractions of 2 Gy with 6 hours between fractions; to 28.42 Gy for twice-daily LDR fractions of 2 Gy with 6 hours between fractions; and to approximately 28.4 Gy for three times daily doses of 1.2 Gy with four hours between fractions independent of dose-rate. The differences in the isoeffective doses between HDR and LDR fractions of 2 Gy is surprisingly great, given the previous analysis. Although it would be predicted to be easier to achieve an enhancement for late effects compared to acute effects, the size of the difference (18%) is greater than expected. In these experiments fractionation appeared to be better than low dose-rate. This may be because the "low dose-rate" used (3 Gy/hr) is not low enough. The difference between the LD 50/30-180 for once and twice daily fractions of 2 Gy is interesting. It is not possible to say if this is due to incomplete repair

for the twice daily treatment or to proliferation effects in some important target cell population. Had the 1.2 Gy fractionation experiments been performed for different interfraction intervals this may have been clarified somewhat. It does appear, judging by the scale of the effect, that proliferation effects are responsible.

6.4 DISCUSSION

It must be emphasised that these conclusions apply to the treatment of radiosensitive neoplasms which have low SLD repair capacities like leukaemia and neuroblastoma by high-dose TBI followed by bone marrow rescue. Here dose-limiting toxicities are highly dependent on fraction size or dose-rate / irradiation time and these factors can be manipulated to increase the therapeutic ratio. For low dose TBI without marrow transplantation failure of the haemopoietic system would be dose-limiting and, in this context, a substantial increase in total dose via fractionat ion or dose-rate reduction would not be predicted. Some neoplasms where high dose TBI has been used can have radiation survival curves which are not shoulderless and radiosensitive (Kinsella et al, 1984). In these cases the choice of fractionation or low dose-rate may be less clear cut and, as will be seen in the next chapter, in some circumstances neither may be appropriate.

To summarize this chapter; for the treatment of radiosensitive neoplasms by TBI followed by bone marrow rescue, all the benefits of fractionation can be achieved

by using low dose-rate irradiation. However, in practice, the irradiation times required for low dose-rate schedules are likely to render them impracticable. Fractionated radiation appears, therefore, to be a better way of exploiting the potential advantages inherent in the different radiobiological properties of neoplastic cells and lung.

7.0 OPTIMAL SCHEDULING OF FRACTIONATED TBI WITH BONE MARROW RESCUE

In this chapter some aspects of the theoretical optimisation of TBI treatment will be described. Use will be made of the LQ model as described in chapter 3 to describe the radiation response of normal tissues. The human tumour radiosensitivity data of chapter 5 will be used to describe tumour effects. Mathematical expressions for the effects of radiation on normal tissues and those for the effects of radiation on tumour cell survival can be combined to yield optimal dose sizes and schedules. The first part of this chapter will deal with what could be described as analytical optimization. Although this approach is not directly applicable to the clinical situation it is a useful exercise and, in some circumstances, does provide an insight into the reasons why optimal schedules take the forms they do.

7.1 ANALYTICAL OPTIMIZATION

Analytically it is possible, in some circumstances, to derive the optimal size of dose to maximise the therapeutic ratio. For this approach the effects of cellular proliferation must be ignored.

If we can write an expression which represents the effects of a dose of radiation, d, on tumour cells and also an expression which represents normal tissue damage then it is possible to derive therapeutically optimal doses. This follows simply from the following analysis :

Tumour effect of dose, d = T(d) = T

Normal tissue effect = N(d) = N

A measure of the therapeutic ratio, R is given by

R(d) = T(d)/N(d) or for convenience R = T/N

The value of R will depend on the dose. Some doses will give a high value of R, others a low value. However, one value of d will produce a maximum value of R and this will be the optimal dose size in the absence of the effects of cellular proliferation.

For the optimal dose size

$$R' = 0$$

and

$$R'' < 0$$

where R' denotes the derivative of R with respect to d and R" denotes the second derivative of R with respect to d.

As R = T/N

$$R' = \frac{T'N - N'T}{N^2}$$

Therefore the requirement for R' = 0 is

$$T'N - N'T = 0 \qquad (7.1)$$

The requirement for this stationary point of R(d) to be a maximum is R" < 0

$$R'' = \frac{N^2(T''N - N''T) - 2NN'(T'N - N'T)}{N^4}$$

As T'N - N'T = 0 at a stationary point this expression reduces to

$$R'' = \frac{T''N - N''T}{N^2}$$

And the condition R" < 0 is given by

$$T''N < N''T \qquad (7.2)$$

Substitution of expressions for tumour and normal tissue effects into equations (7.1) and (7.2) enable optimal dose sizes to be calculated. This will be done for two cases :

1/ Tumour effects are given by a log cell kill value from a multitarget equation with zero initial slope. Normal tissue effects are given by a LQ equation

$$T(d) = -\ln\left\{1 - (1 - \exp(-d/D_0))^n\right\}$$

$$N(d) = \alpha d + \beta d^2 = \alpha\left(d + \frac{d^2}{\gamma}\right)$$

where $\gamma = \alpha/\beta$

It then follows that

$$T' = \frac{n e^{-d/D_0}(1 - e^{-d/D_0})^{n-1}}{D_0\left(1 - (1 - e^{-d/D_0})^n\right)}$$

$$T'' = \frac{n e^{-d/D_0}(1 - e^{-d/D_0})^{n-2}\left\{n e^{-d/D_0} - (1 - (1 - e^{-d/D_0})^n)\right\}}{D_0^2\left(1 - (1 - e^{-d/D_0})^n\right)^2}$$

$$N' = \alpha(1 + 2d/\gamma)$$

$$N'' = 2\alpha/\gamma$$

Substituting these expressions into equations (7.1) and (7.2) enables α to be cancelled out. The optimal dose

size then depends only on the n and D0 of the tumour cells and the α/β ratio of the normal tissue (denoted by γ).

Example Human Leukaemia/Lymphoma

From chapter 5 the median values of the parameters n and D0 for human leukaemia/lymphoma were found to be

$$n = 1.2 \; ; \; D0 = 0.9 \text{ Gy}$$

For lung the characteristic α/β ratio is 3 Gy.

These values are used to evaluate the optimal dose size. A numerical solution to equation (7.1) is required and a computer program was written to perform this task using a "false-position" approach.

The optimal dose size for the above values of n, D0 and α/β is 0.46 Gy. For the same values of n and D0 but an α/β ratio of 10 Gy (more descriptive of an acute responding normal tissue) the optimal dose size is 1.09 Gy.

These dose values illustrate that, in this case, hyperfractionation is an approach more likely to increase the therapeutic ratio with respect to late responding tissues rather than acute responders.

2/ Tumour effects are given by a log cell kill value derived from a multitarget equation with a non-zero initial slope, the so-called two-component or TC equation. Normal tissue effects are again given by a LQ equation. In this case

$$T(d) = -\ln\left[e^{-d/D_1}\left(1-\left(1-e^{-d/D_2}\right)^n\right)\right]$$

It then follows that

$$T' = \frac{\left(\dfrac{e^{-d/D_1}\left(1-\left(1-e^{-d/D_2}\right)^n\right)}{D_1} + \dfrac{n e^{-\left(\frac{D_1+D_2}{D_1 D_2} d\right)}\left(1-e^{-d/D_2}\right)^{n-1}}{D_2} \right)}{e^{-d/D_1}\left(1-\left(1-e^{-d/D_2}\right)^n\right)}$$

$$T'' = \left[\left(\frac{e^{-d/D_1}\left(1-\left(1-e^{-d/D_2}\right)^n\right)}{D_1} + \frac{n e^{-d/D_2}\left(1-\left(1-e^{-d/D_2}\right)^{n-1}\right)}{D_2} \right)^2 \right.$$

$$+ e^{-d/D_1}\left(1-\left(1-e^{-d/D_2}\right)^n\right) \times$$

$$\left\{ n e^{-\left(\frac{D_1+D_2}{D_1 D_2} d\right)}\left(1-e^{-d/D_2}\right)^{n-2}\left((n-1)e^{-d/D_2} - \frac{D_1+D_2}{D_1 D_2}\left(1-e^{-d/D_2}\right)\right) \right.$$

$$\left. \left. - e^{-d/D_1}\left(\frac{1-\left(1-e^{-d/D_2}\right)^n}{D_1} + \frac{n e^{-d/D_2}\left(1-e^{-d/D_2}\right)^{n-1}}{D_2} \right) \right\} \right]$$

$$\frac{}{\left[e^{-d/D_1}\left(1-\left(1-e^{-d/D_2}\right)^n\right) \right]^2}$$

Again α can be cancelled on substitution of these expressions into equations (7.1) and (7.2). Now the optimal dose size depends only on the parameters n, D1 and D2 of the tumour cells and the α/β ratio of the normal tissue.

Example Human Neuroblastoma

From chapter 5 a representative set of values of n, D1 and D2 for human neuroblastoma were found to be :

n = 1.75 ; D1 = 2.04 Gy ; D2 = 2.21 Gy

Numerical solutions to the equations are again required and the computer program referred to previously was used to calculate these. For a normal tissue α/β ratio of 3 Gy the optimal dose size is 0.39 Gy. The optimal dose size consistent with an α/β ratio of 10 Gy is 1.69 Gy. This example again illustates the increased effectiveness of hyperfractionation as a therapeutic strategy with respect to late responding rather than acute responding tissues.
The best estimate of a "typical" neuroblastoma survial curve was a composite of the above TC equation and a zero initial slope equation. The analytical optimal dose size found here refers to the TC equation component only and, therefore, is not applicable to the composite equation.
The approach described above, although illustrative of the principle of optimal scheduling is not particularly useful in practice. There are three main reasons for this :
1/ Using a quadratic equation as the mathematical representation of tumour effects allows only two possible optimal dose values :- zero and infinity.
2/ Proliferation is ignored which means that it is not suitable for schedules of more than one fraction.
3/ There is no intuitive feeling for how much better the optimal dose size is as opposed to some other dose.
For these reasons this approach will not yield true optimal schedules. In the next section a model will be presented which overcomes the objections listed above and enables optimal fractionation schedules to be derived.

7.2 A MODEL FOR THE OPTIMAL SCHEDULING OF FRACTIONATED TBI WITH BONE MARROW RESCUE

The approach adopted here is as follows :
A clinically acceptable treatment schedule is taken. A set of schedules can then be calculated using the LQ model which should all be isoeffective for some dose-limiting side-effect. The effects on a tumour cell population of these schedules can then be calculated. In general these will not be the same and the schedule which produces the smallest surviving fraction of tumour cells is the optimal schedule. In the case of TBI with marrow rescue, as described previously, the primary dose-limiting toxicity is due to lung damage in the form of radiation-induced pneumonitis. A variety of treatment schedules have been clinically used, however one which has been employed in a number of centres is a fractionated course of treatment consisting of 6 fractions of 2 Gy, total dose 12 Gy. This schedule has been given either as 1 fraction per day or as 2 fractions per day. From the point of view of lung toxicity there should be no significant difference between these as long as sufficient time is allowed between successive fractions for effectively full repair of repairable radiation damage. Additionally, in the case of lung, one would expect proliferation-associated factors to be irrelevant for the timescale of these treatments (1 week or therabouts). Figure 7.1 shows the set of isoeffective schedules for fraction numbers from 1 to 100 which are equivalent to 6 fractions of 2 Gy on the basis

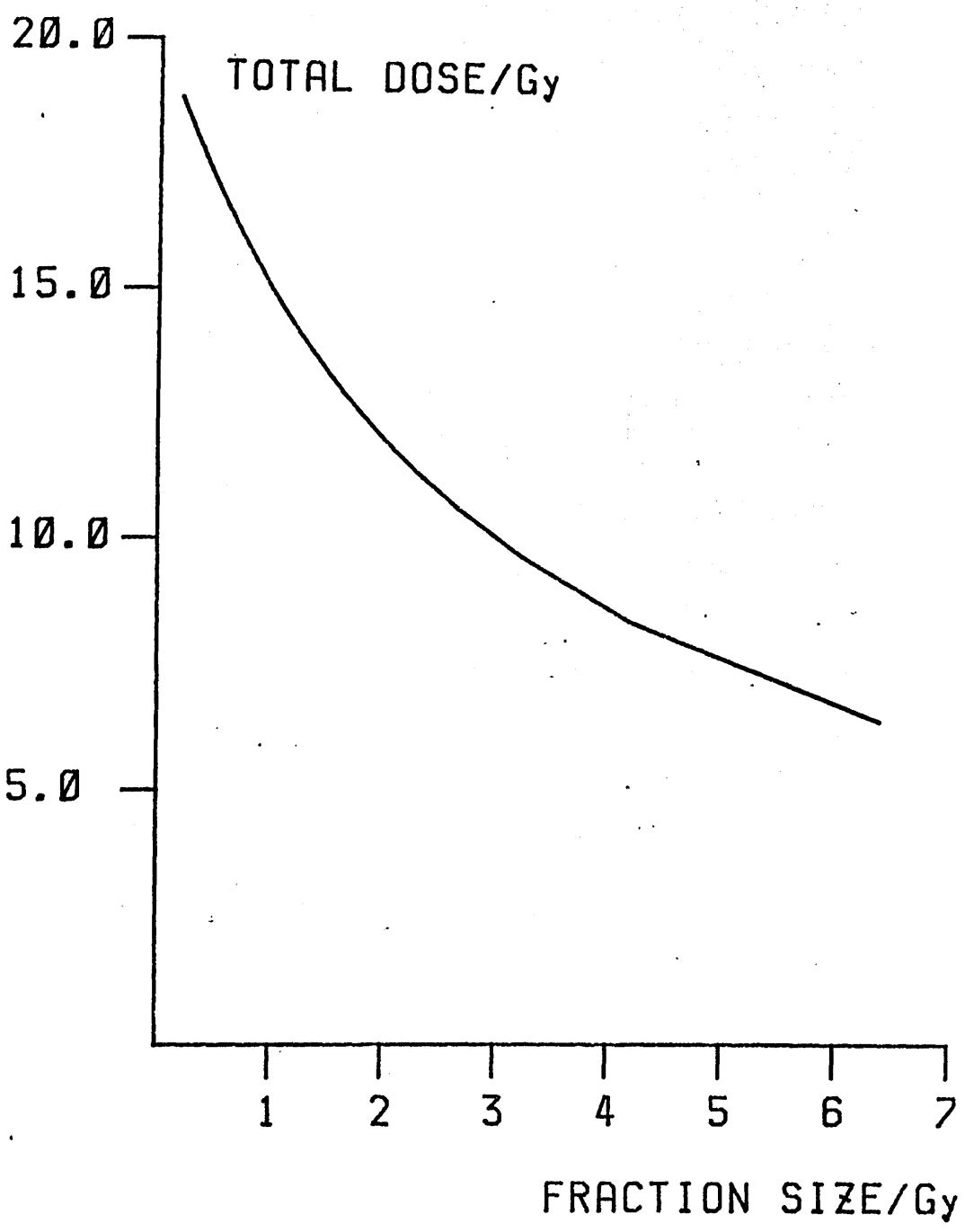

Fig 7.1 Total dose as a function of fraction size calculated to have the same effect on lung as 6 fractions of 2 Gy. (Based on the LQ model with α/β = 3 Gy)

of the LQ model with α/β = 3 Gy. As was pointed out in chapter 3 there is some doubt as to the LQ models' validity at small fraction sizes. However for the purposes of this analysis the isoeffective schedules are taken at face value. The next step in the optimization process is to rank these schedules in terms of their effects on tumour cells. In this regard there are three variables of importance :

1/ The intrinsic radiosensitivity of the tumour cells in question expressed as the single dose survival curve equation for S(d)

2/ The proliferation rate of the tumour cells described by a population doubling time and assuming exponential growth kinetics.

3/ The temporal distribution of the dose fractions i.e. the number of fractions given per day. As described in chapter 3 a minimum interfraction interval of 6 hours is believed to be necessary for essentially full repair of repairable radiation damage and this interval is assumed for all schedules considered. This means that the theoretical maximum number of fractions per day is 4.

Taking these three factors into consideration an equation can be written for the effect on tumour cells after a fractionated course of radiotherapy

$$S = \left[S(d)\right]^N e^{\lambda T}$$

where T is the duration of the schedule and

$$\lambda = \ln 2 / TD$$

where TD is the tumour cell population doubling time.

It is convenient here to use the "log cell kill" function representing the number of decades of reduction of tumour cells

$$K = -\log_{10}(S)$$
$$= -\left\{N \log_{10}[S(d)] + \log_{10}\left[e^{T\ln 2/TD}\right]\right\}$$
$$= -N \log_{10}[S(d)] + 0.301\ T/TD$$

The allowable combinations of N and d are set by the normal tissue isoeffect model and the reference schedule chosen. These are shown in Fig 7.1. Fractions are taken to be delivered at a sufficiently high dose-rate that its exact value has little influence on cell killing. In this way the dose-rate factor is removed from the analysis. Finally the object is to maximize K(N,d,S(d),T,TD). The schedule which does this is the optimal schedule. The relationship between the calculated optimal schedules and the parameters S(d), T and TD is the subject of the next section.

7.3 HUMAN LEUKAEMIA/LYMPHOMA

From chapter 5 the equation used to describe the surviving fraction of human leukaemia/lymphoma cells following a dose d is

$$S(d) = 1 - \left(1 - e^{-d/0.9}\right)^{1.2}$$

In the computer calculation the tumour doubling times are allowed to have the values of 2, 4 and 20 days. 2 days represents a fast growing tumour cell population and 20 days represents a rather slowly growing one. Schedules can be given at 1, 2, 3 or 4 fractions per day. This enables the advantages of accelerated fractionation to be

assessed. Schedules are also considered in which treatment is given only on conventional working days (i.e. Monday to Friday) and during conventional working hours (i.e. 9 a.m. to 5 p.m.). The latter factor means that only once or twice daily fractionation schedules are considered, due to the minimum requirement of 6 hours between fractions. This enables the effects of "social" constraints to be assessed. Figures 7.2-7.7 plot the tumour log cell kill against fraction size for all these situations.

7.3.1 RESULTS OF COMPUTER SIMULATION

It is immediately apparent from examination of figures 7.2-7.7 that they are all of a similar shape. When moving from schedules with large fraction sizes to schedules with small ones there is first an increase in the tumour effect (and thus the therapeutic ratio) until a maximum is reached. This is because the schedules are becoming more efficient at exploiting the different radiobiological parameters of the tumour cells and lung. Thereafter the therapeutic effect decreases owing to the effects of cellular proliferation. The fraction sizes of the optimal schedules are the dose values corresponding to the turning points of the curves. Examining any of figures 7.2-7.7 shows that the tumour cell population doubling time is a factor of importance in determining the optimal schedule. Fast doubling times lead to predictions of optimal schedules which have a relatively fewer number of relatively larger fractions than those predicted for slow doubling times. This is not difficult to understand,

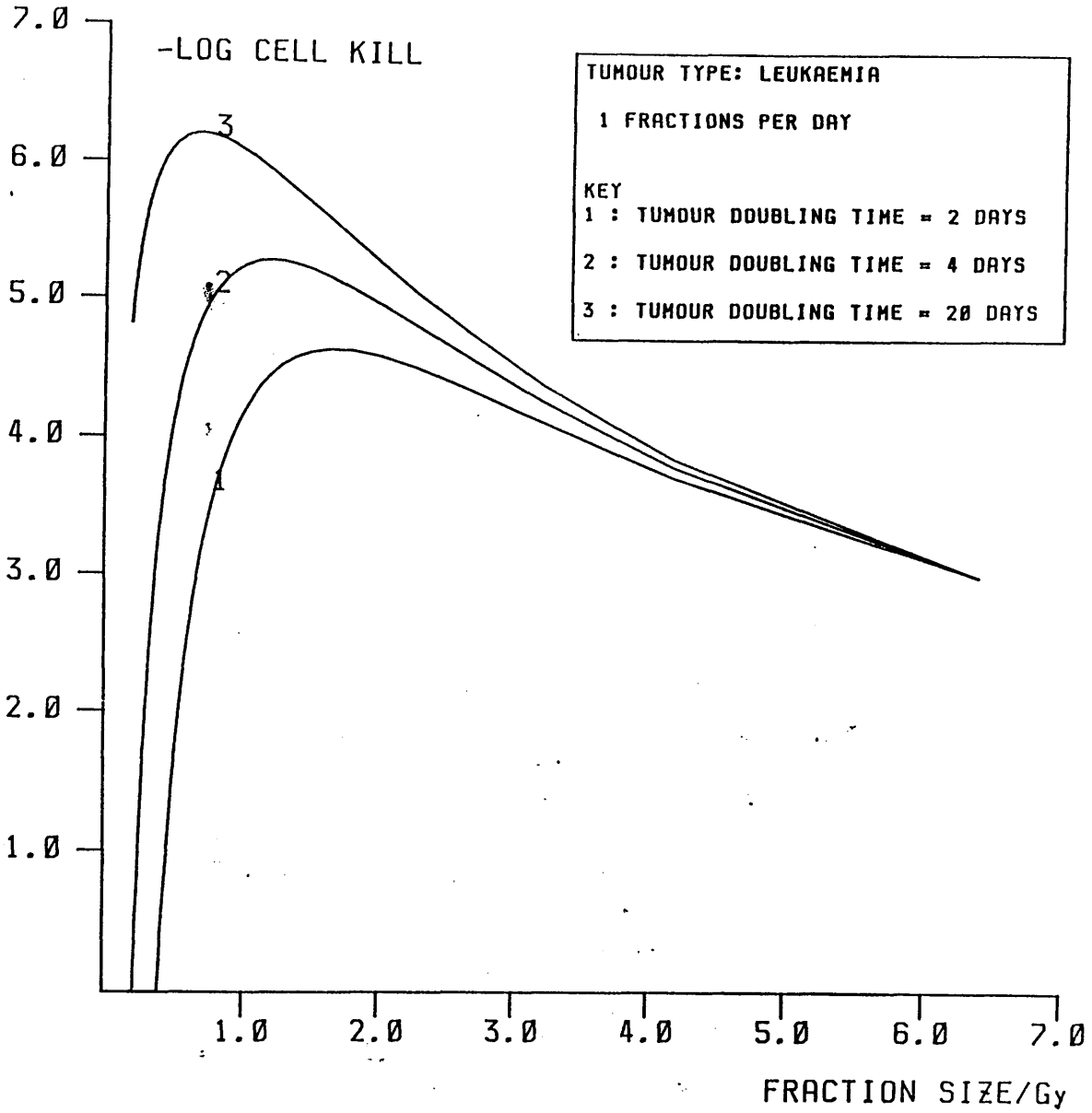

Fig 7.2 Leukaemic log cell kill as a function of dose per fraction for TBI schedules of one fraction per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The leukaemic radiosensitivity parameters are the median values for 40 cell lines as described in the text.

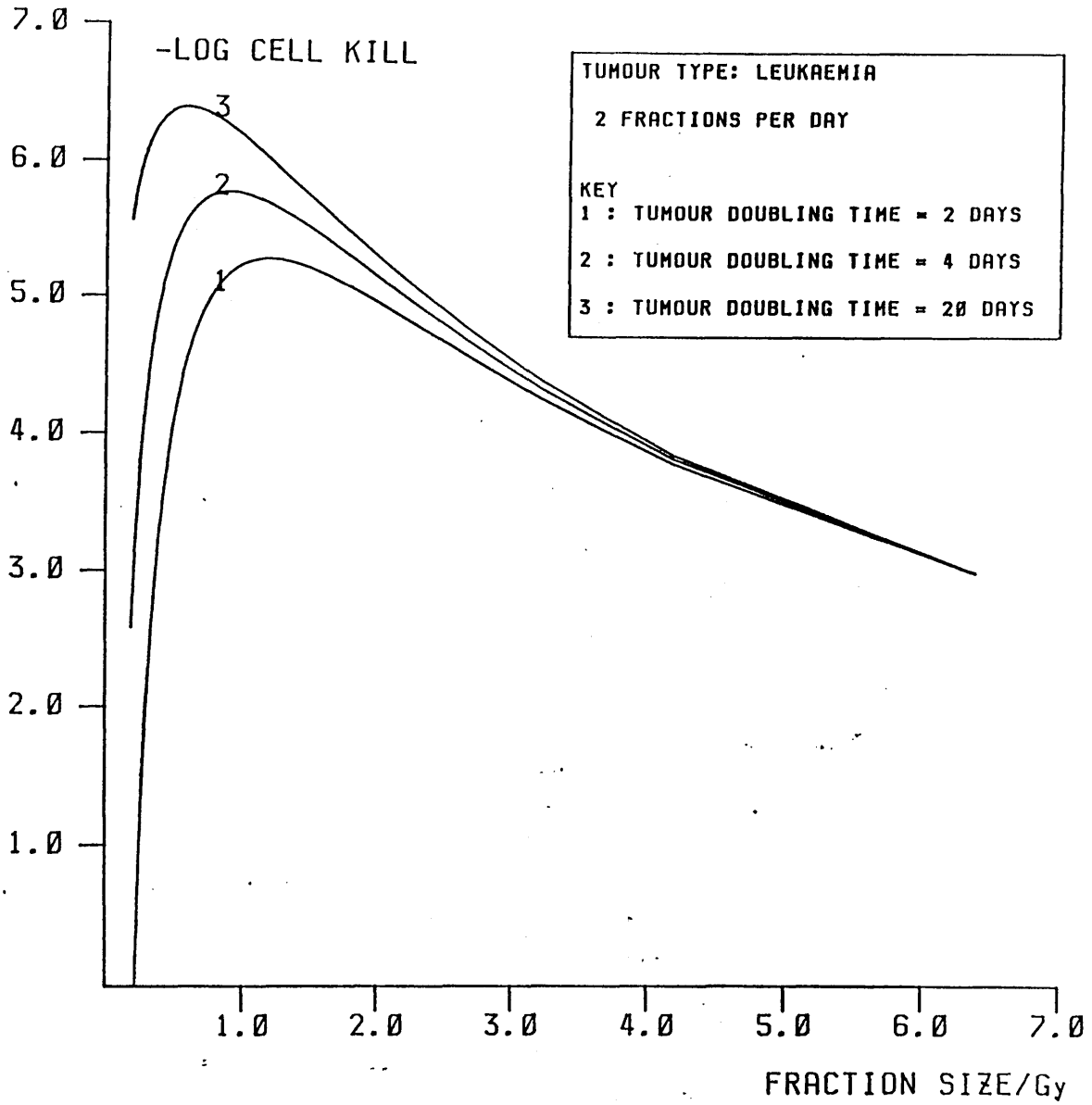

Fig 7.3 Leukaemic log cell kill as a function of dose per fraction for TBI schedules of two fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The leukaemic radiosensitivity parameters are the median values for 40 cell lines as described in the text.

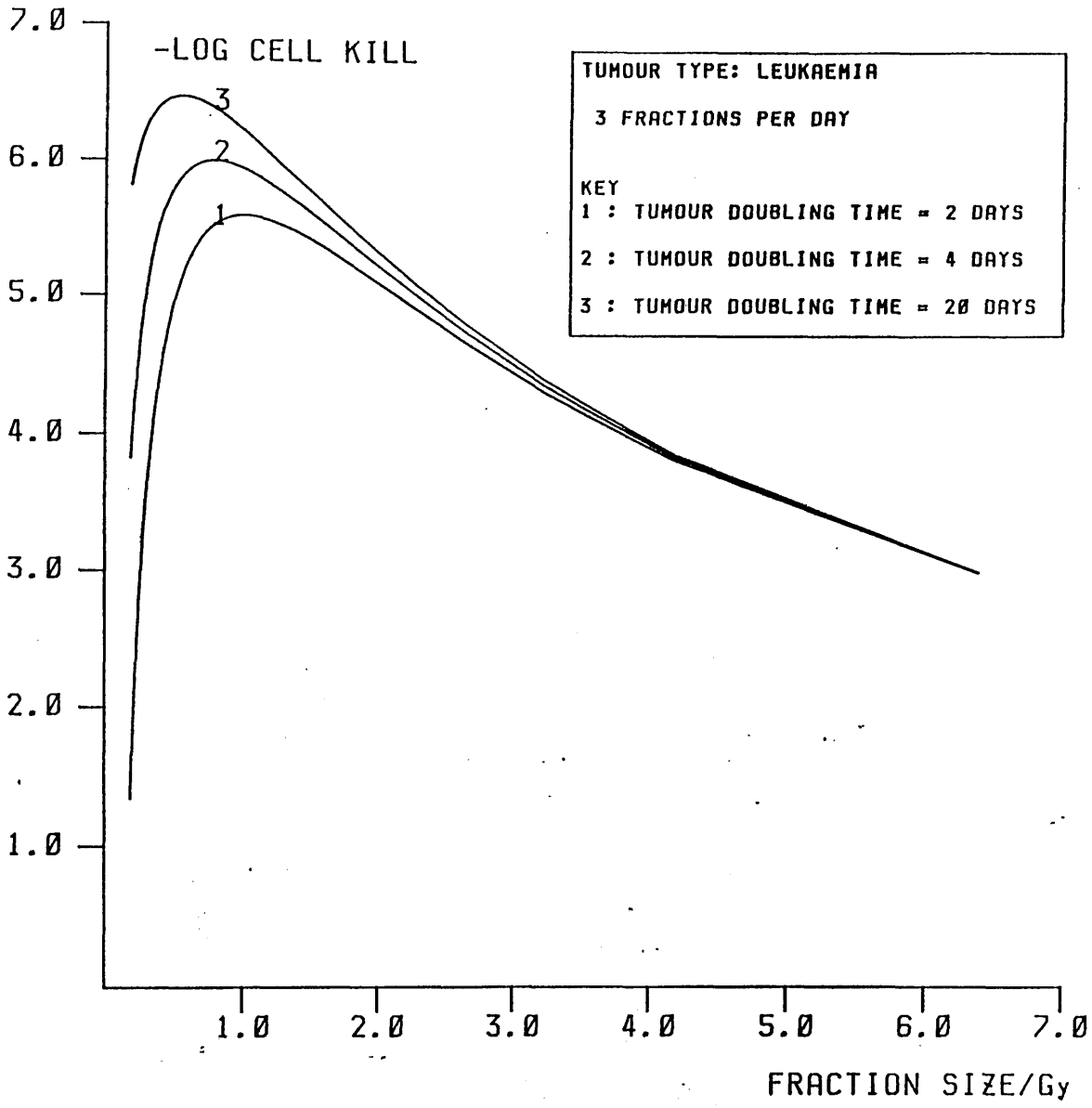

Fig 7.4 Leukaemic log cell kill as a function of dose per fraction for TBI schedules of three fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The leukaemic radiosensitivity parameters are the median values for 40 cell lines as described in the text.

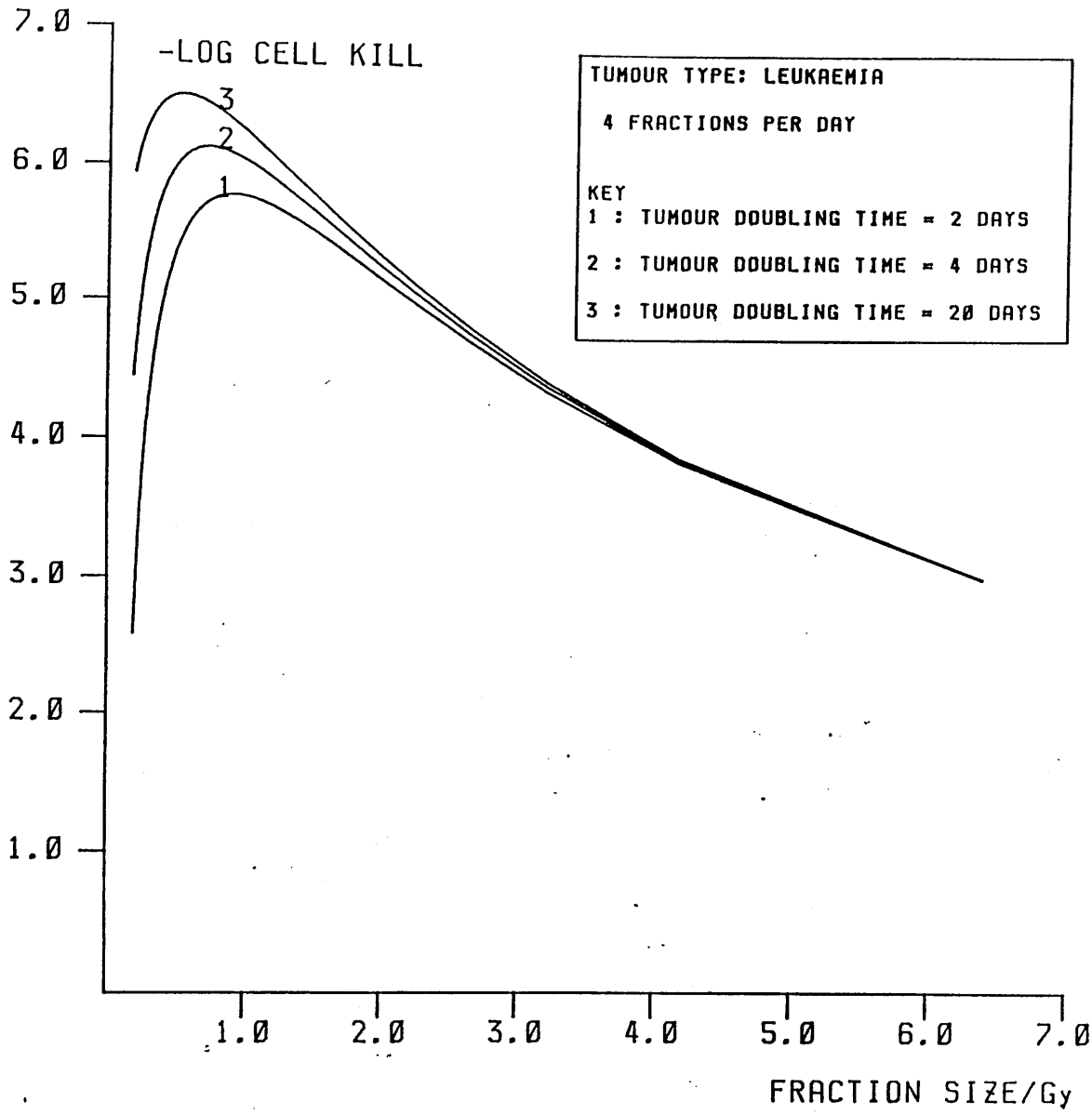

Fig 7.5 Leukaemic log cell kill as a function of dose per fraction for TBI schedules of four fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The leukaemic radiosensitivity parameters are the median values for 40 cell lines as described in the text.

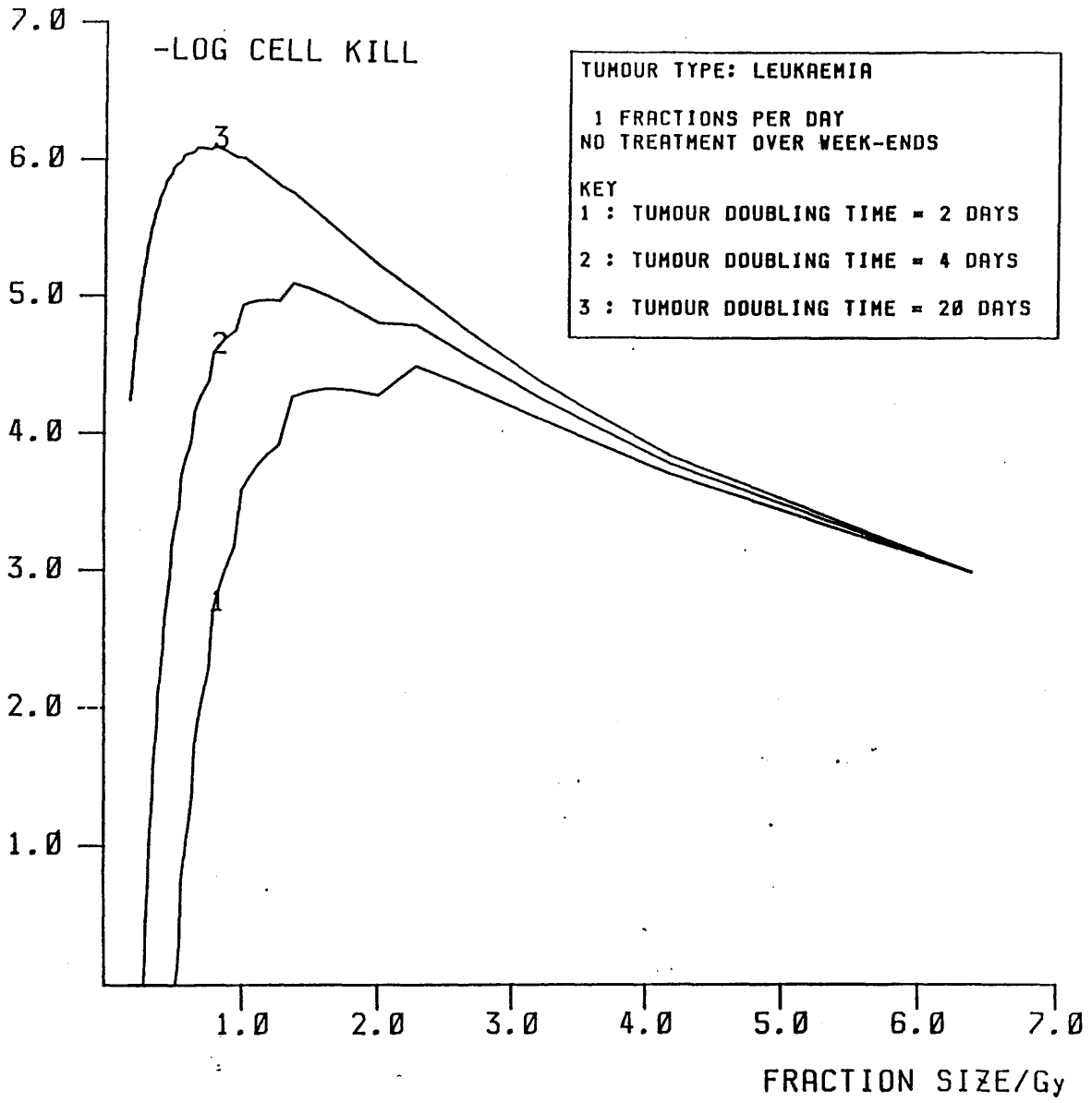

Fig 7.6 Leukaemic log cell kill as a function of dose per fraction for "socially constrained" TBI schedules of one fraction per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The leukaemic radiosensitivity parameters are the median values for 40 cell lines as described in the text.

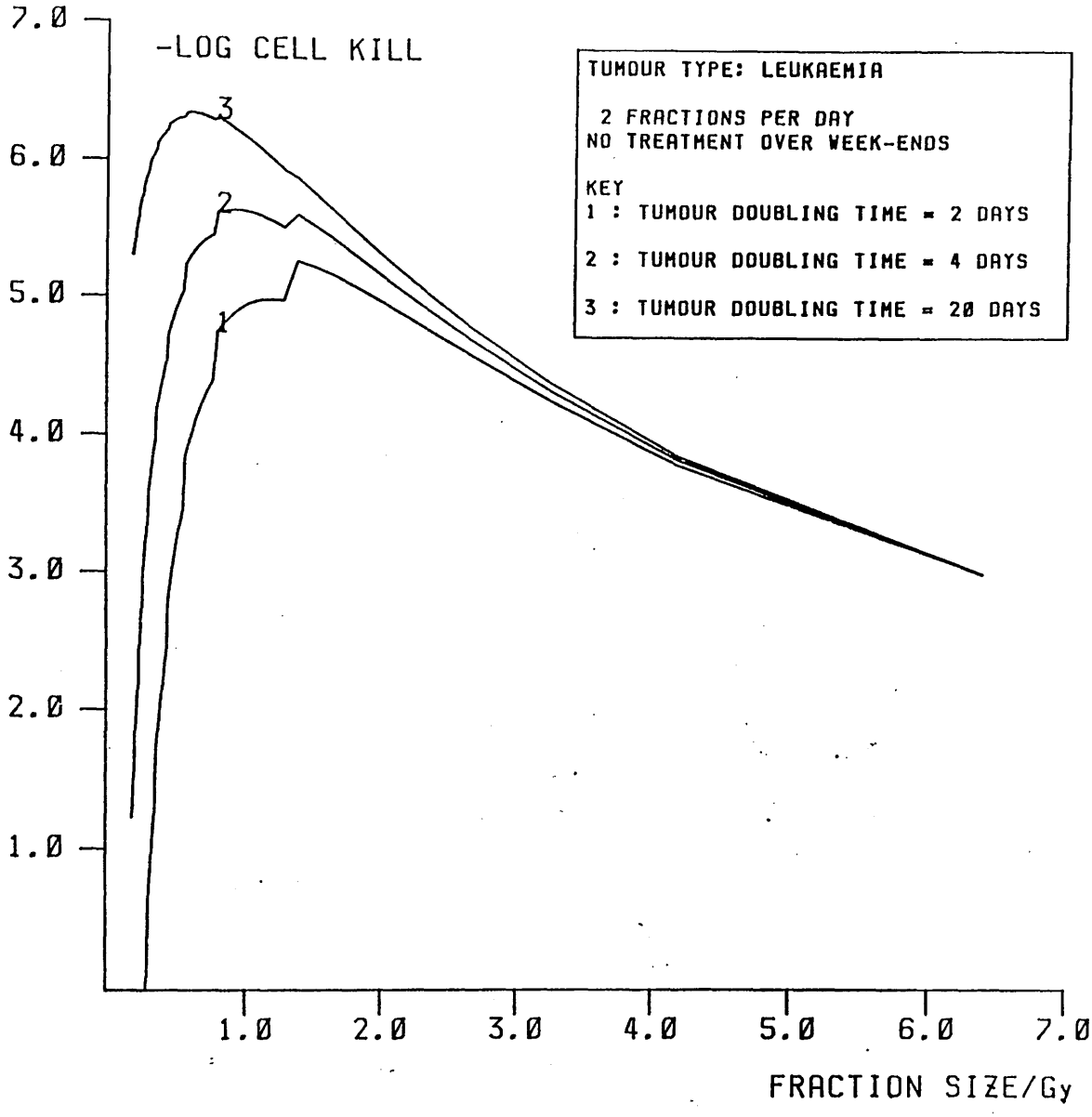

Fig 7.7 Leukaemic log cell kill as a function of dose per fraction for "socially constrained" TBI schedules of two fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The leukaemic radiosensitivity parameters are the median values for 40 cell lines as described in the text.

temporal factors become increasingly important as the doubling time decreases and there is an advantage in having schedules with shorter durations. In no case does the optimal schedule consist of a large single fraction. This provides the justification for the assertion in chapter 6 that multifractionated (or single low dose-rate fractions) are most appropriate for the treatment of radiosensitive neoplasms with TBI followed by marrow rescue. For the leukaemic cell radiosensitivity parameters used here the optimal dose size in the absence of proliferation was found to be 0.46 Gy when the normal tissue constraint was provided by a LQ model tissue with an α/β ratio of 3 Gy. This value provides a lower limit on the optimal fraction size. One would expect that the optimal fraction sizes would all be larger than this to greater or lesser extents. The more important proliferation effects are (i.e. for low fractionation frequencies and fast doubling times) the greater would be the expected deviation from the no-proliferation value. Figure 7.8 shows the relationship between the optimal fraction sizes, the doubling time of the leukaemic cell population and the number of daily fractions in cases where treatment is given every day. On examination of this graph three observations can be made:

1/ The faster the tumour cell proliferation the larger the optimal fraction size.

2/ The lower the fractionation frequency the larger the optimal fraction size.

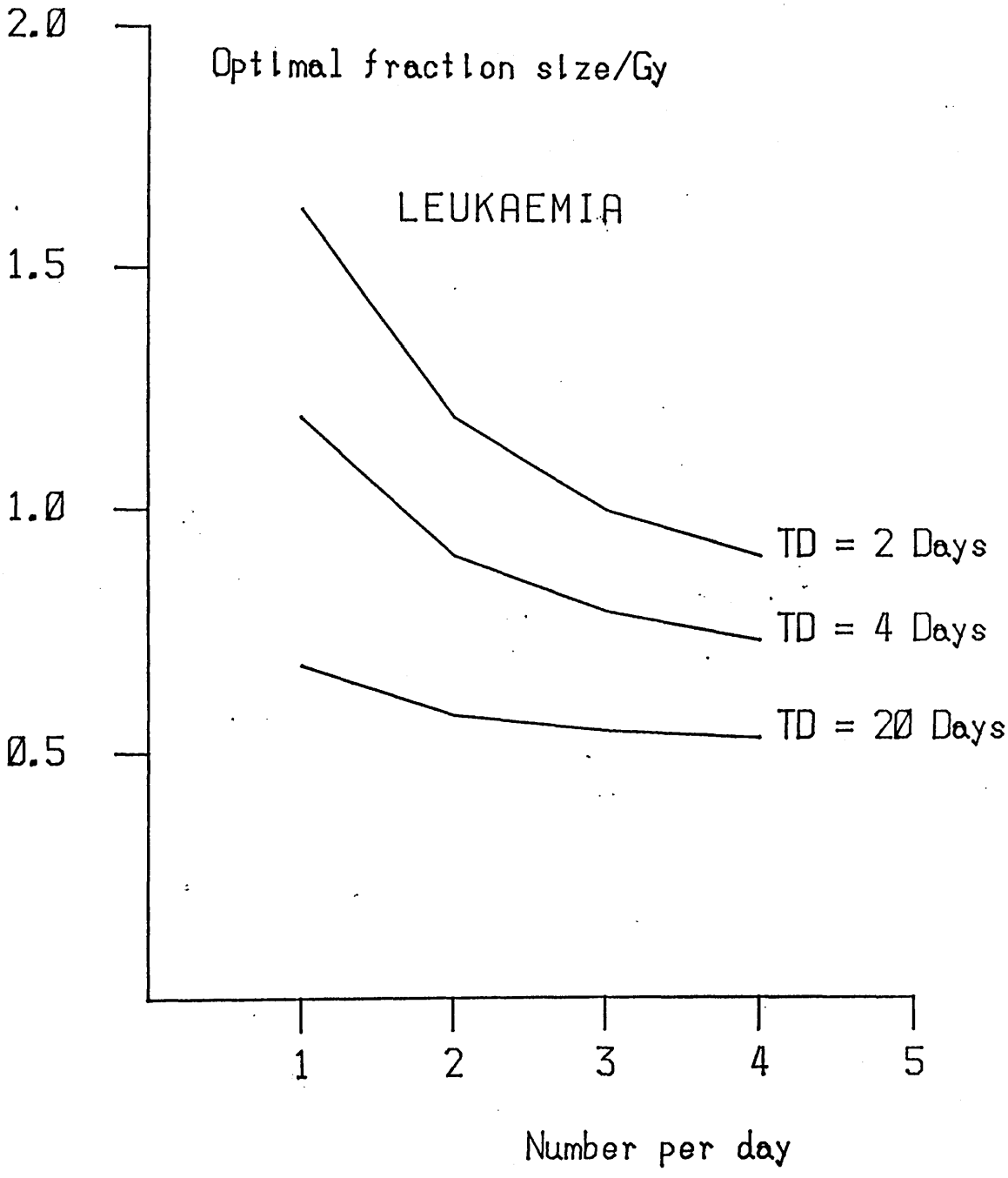

Fig 7.8 The optimal dose per fraction as a function of the number of daily fractions for three different values of leukaemic doubling time.

3/ The faster the tumour cell proliferation the more variation there is between the fraction sizes of optimal schedules with different fractionation frequencies.

These effects are caused solely by proliferation and reflect the fact that the longer a schedule takes to complete, in terms of tumour doublings, the more the differential radiosensitivities of tumour cells and lung are overcome by kinetic factors.

The tumour log cell kills produced by the optimal schedules as a function of the fractionation frequency are shown in figure 7.9. These schedules produce a degree of tumour cell kill of the order of 5-7 logs. This is higher than the corresponding values for neuroblastoma, as will be seen later, and reflects the relative radiosensitivity of human leukaemia/lymphoma cell lines. Two important effects are immediately obvious from figure 7.9 :

1/ The faster the tumour cell proliferation the more advantageous is increasing the fractionation frequency.

2/ There is a greater advantage in going from one fraction per day to two a day than there is in going from two to three a day, which in turn is more advantageous than going from three to four a day i.e. there is a steadily diminishing return as the fractionation frequency is increased.

Now going on to consider those schedules which are subject to "social" constraints (i.e. treatment during the period 9 a.m. to 5 p.m. on weekdays only), figures 7.6 and 7.7 show the tumour log cell kills against fraction size for

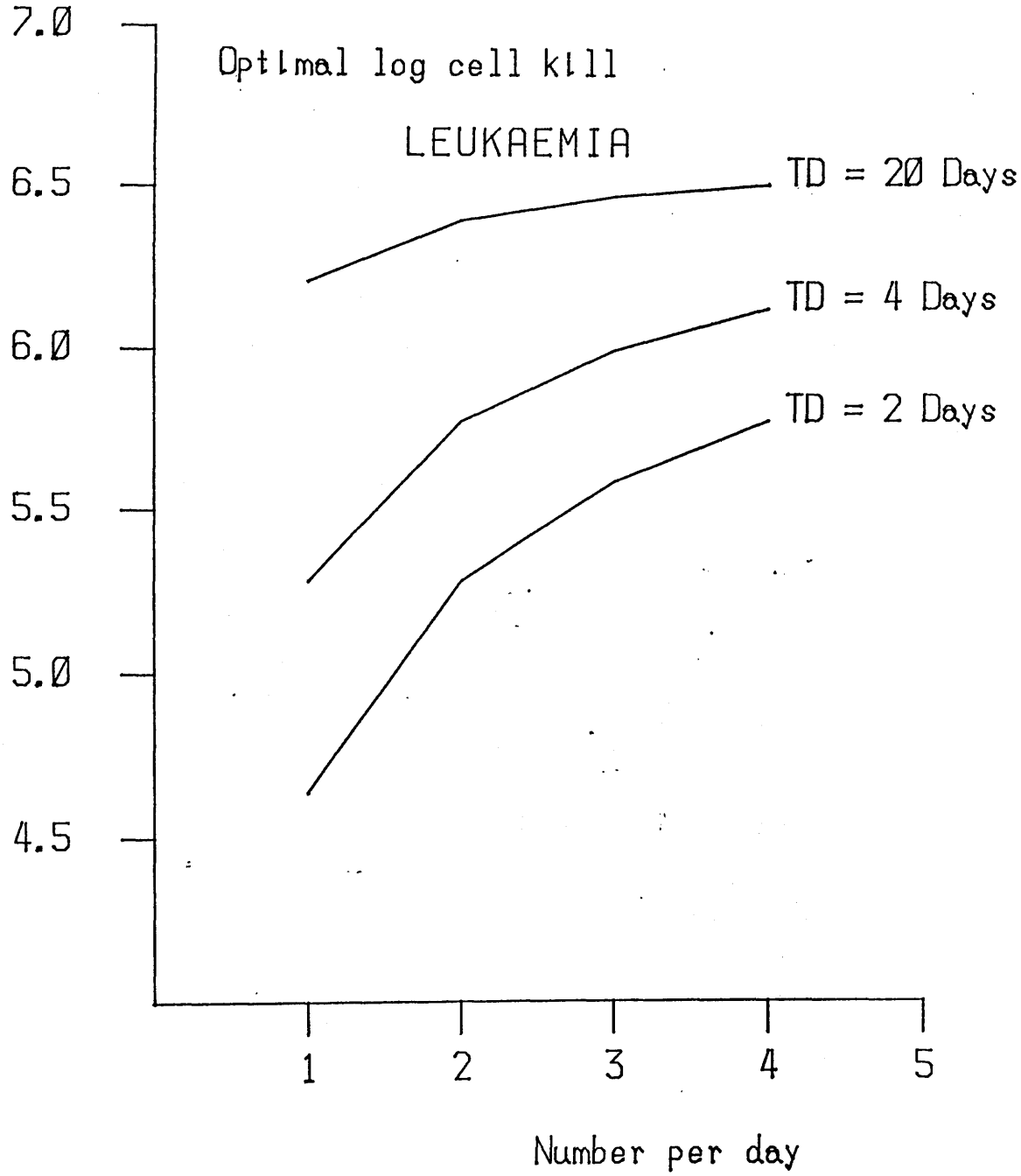

Fig 7.9 The leukaemic log cell kill produced by the optimal schedules as a function of the number of daily fractions for three different leukaemic doubling times.

the lung-isoeffective schedules in this category. The discontinuities in the graphs correspond to weekend breaks in treatment. The number of fractions in the optimal schedules tends to be an integral number multiplied by 5n where n is the number of daily fractions. This is not surprising as schedules on either side of these divides are separated by 2 days of tumour proliferation but no radiation-induced sterilization. It is always true that for schedules with the same numbers of equal dose fractions, those with twice daily fractionation are better than those with one fraction per day. Figure 7.7 shows that the twice daily schedule with a fraction size of 1.37 Gy is close to the optimal for tumour doubling times of both 2 and 4 days. This schedule is, in fact, 10 fractions of 1.37 Gy. As doubling times of 2-4 days are thought to more relevant to human leukaemia/lymphoma, it appears that a fractionated TBI schedule of 10 fractions of 1.37 Gy would be appropriate for the treatment of this disease. In order to test this prediction further the effects of two schedules, 6 fractions of 2 Gy in 2.5 days and 10 fractions of 1.37 Gy in 4.5 days, have been calculated for all 40 independent sets of radiosensitivity parameters. This information is shown in Table XII. This lends further support to the clinical use of the latter schedule. The precise numerical value of 1.37 Gy is dependent on the value chosen for the reference schedule (6 X 2 Gy) and on the fractionation response of lung. Errors in radiation dosimetry and variations in the behaviour of lung from

SCHEDULE COMPARISON FOR LEUKAEMIA/LYMPHOMA

DESIGNATION	TD=2	TD=4	TD=20
HL60	A	A	A
K562	A	B	B
45/K45	B	B	B
KG1/KG+	A	A	A
176	A	A	A
Reh	A	A	A
P3HR-1	B	B	B
U715	B	B	B
U698	A	B	B
SU-DHL-4	B	B	B
JY	A	A	B
Daudi	B	B	B
U937	A	A	A
MOLT-4	B	B	B
JM(Jurkat)	A	A	A
CCRF-CEM	B	B	B
APHID-D2	A	B	B
RCH-ACV	B	B	B
HRIK	B	B	B
FMC-HU-1B	B	B	B
1	A	A	B
2	B	B	B

TABLE XII

3	A	A	B
4	A	B	B
5	A	B	B
6	B	B	B
7	B	B	B
8	B	B	B
9	B	B	B
10	B	B	B
11	B	B	B
12	A	B	B
CS	B	B	B
GH	B	B	B
JR	B	B	B
TC	B	B	B
GS	B	B	B
HF	B	B	B
JL	B	B	B
LJ	B	B	B

This table shows which of the two, twice daily, schedules A or B produce the greatest sterilizing effect on the cells of the specified line, for different values of the tumour cell population doubling time. Schedule A is 6 fractions of 2 Gy in 2.5 days; schedule B is 10 fractions of 1.37 Gy in 4.5 days. The number of times schedule A is better for doubling times of 2 or 4 days is 24. The corresponding number for schedule B is 56.

TABLE XII (CONTD)

patient to patient as well as deviations from a strict LQ response may be expected to introduce some uncertainty to estimates of the optimal schedule. Bearing this in mind the conclusions of the optimal scheduling study for leukaemia/lymphoma may be summarized as follows :

1/ It is clear that accelerated hyperfractionation is the optimal strategy for the treatment of leukaemia/lymphoma by TBI with marrow rescue.

2/ Single high dose-rate treatments are substantially less effective than multifractionated schedules.

3/ A TBI schedule of 10 fractions of between 1.3 and 1.5 Gy, two fractions per day, is practicable, worth considering for the treatment of leukaemia/lymphoma and should be tested clinically.

7.4 NEUROBLASTOMA

The equation arrived at in chapter 5 as a best estimate of the single dose survival curve for human neuroblastoma is

$$S(d) = \left[\left(1 - \left(1 - e^{-d/1.06}\right)^{1.75}\right) \left(e^{-d/2.04}\left(1 - \left(1 - e^{-d/2.21}\right)^{1.75}\right)\right)\right]^{1/2}$$

Exactly the same computer analysis as was carried out for leukaemia/lymphoma is reported here for neuroblastoma.

7.4.1 RESULTS OF COMPUTER SIMULATION FOR NEUROBLASTOMA

Figures 7.10-7.15 show the effects on neuroblastoma cells of the set of lung-isoeffective schedules given at 1-4 fractions per day and once and twice daily subject to conventional "social" constraints. Figures 7.10-7.15 are all of similar shape, both to each other and also to those for leukaemia/lymphoma. Optimal schedules are dependent on

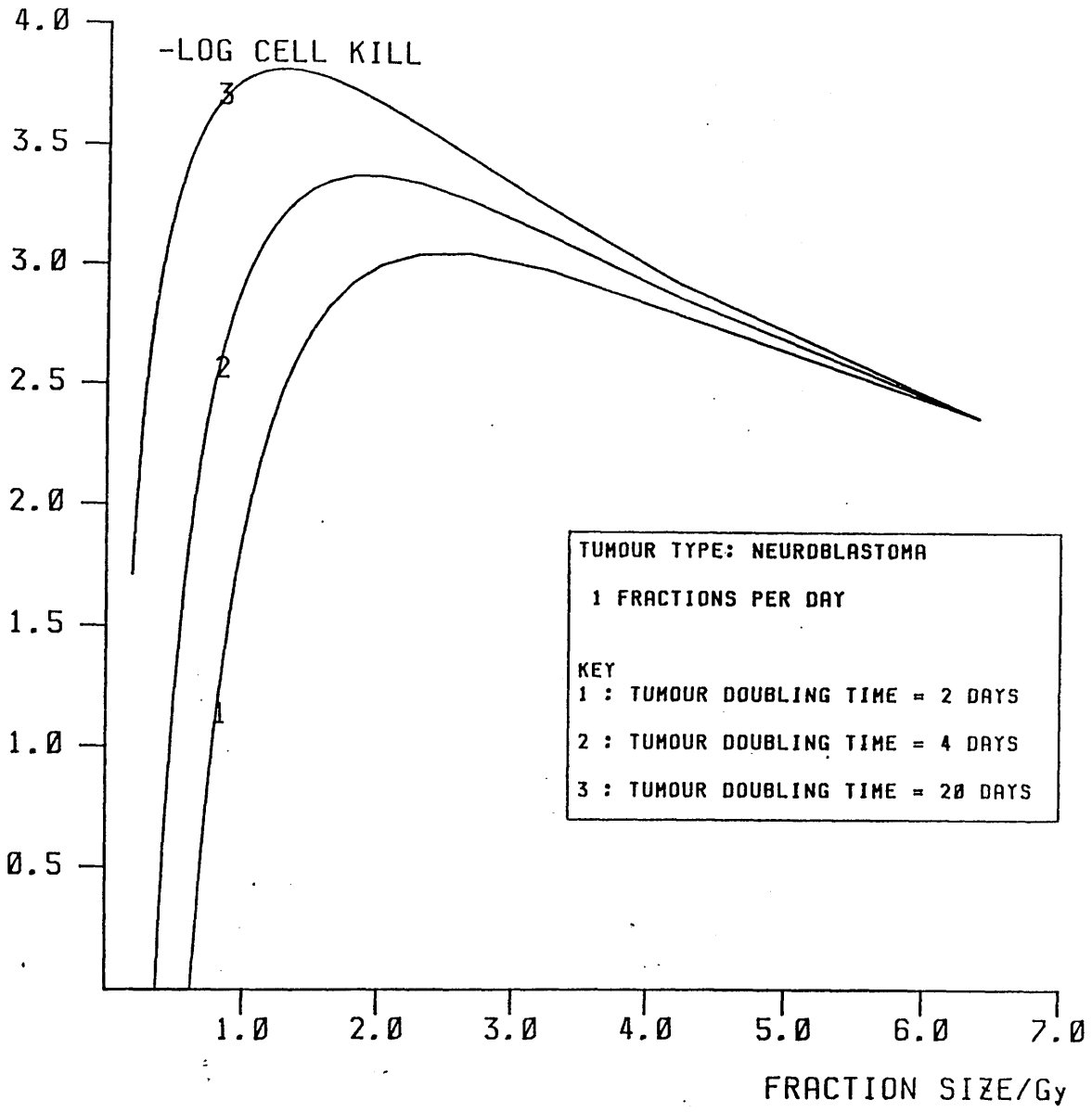

Fig 7.10 Log cell kill for neuroblastoma as a function of dose per fraction for TBI schedules of one fraction per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The neuroblastoma radiosensitivity parameters are the median values for 8 cell lines as described in the text.

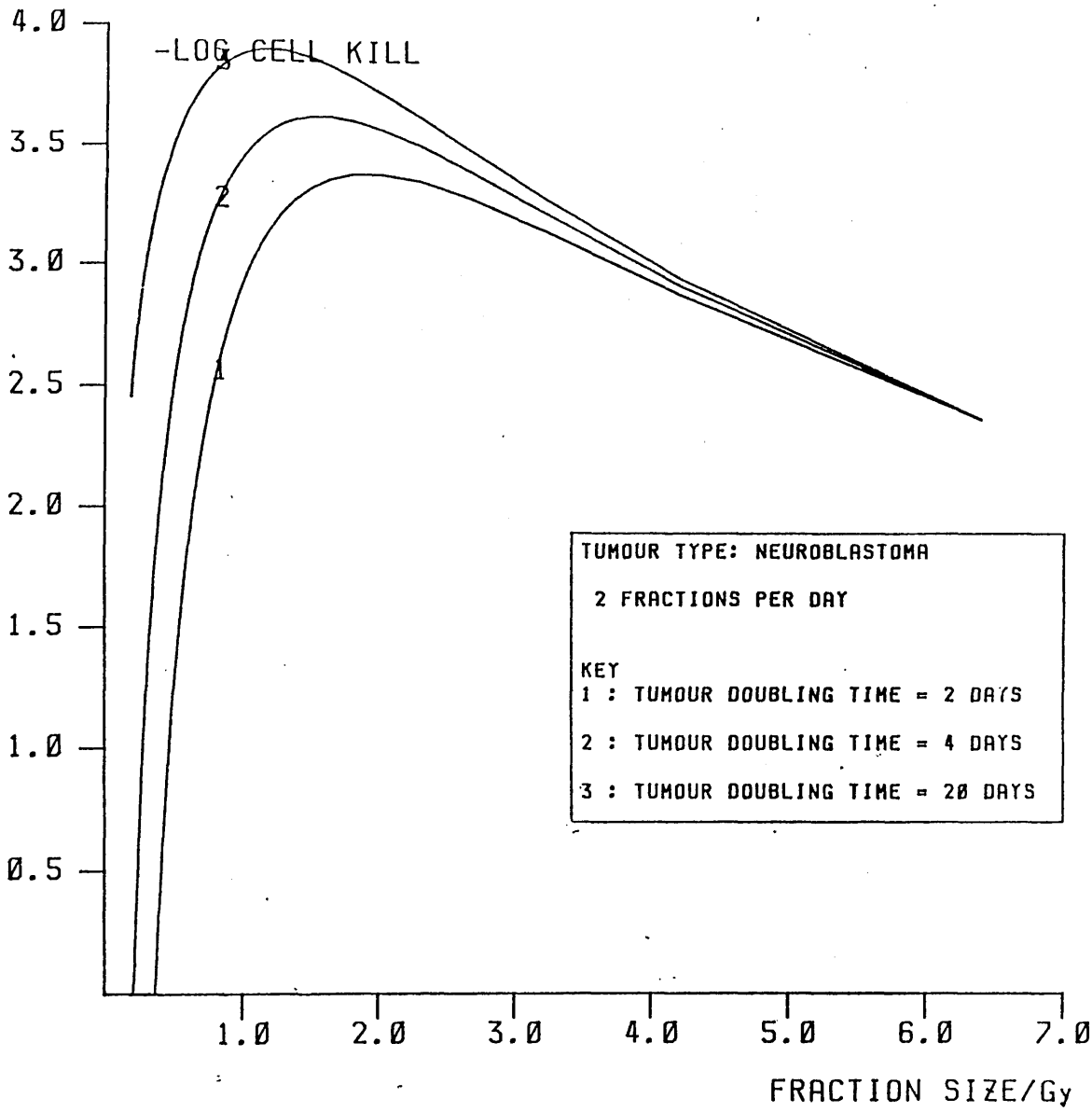

Fig 7.11 Log cell kill for neuroblastoma as a function of dose per fraction for TBI schedules of two fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The neuroblastoma radiosensitivity parameters are the median values for 8 cell lines as described in the text.

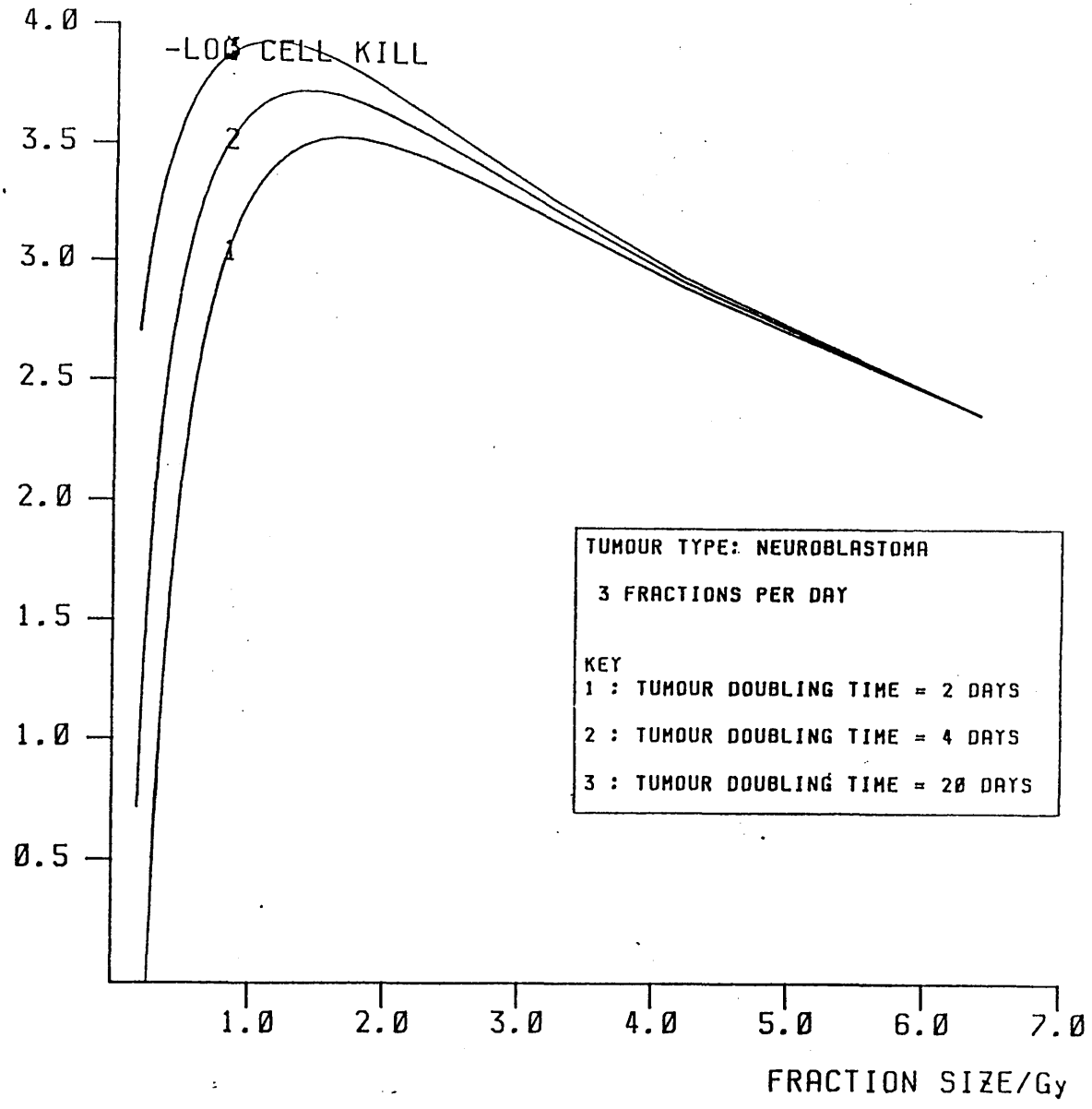

Fig 7.12 Log cell kill for neuroblastoma as a function of dose per fraction for TBI schedules of three fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The neuroblastoma radiosensitivity parameters are the median values for 8 cell lines as described in the text.

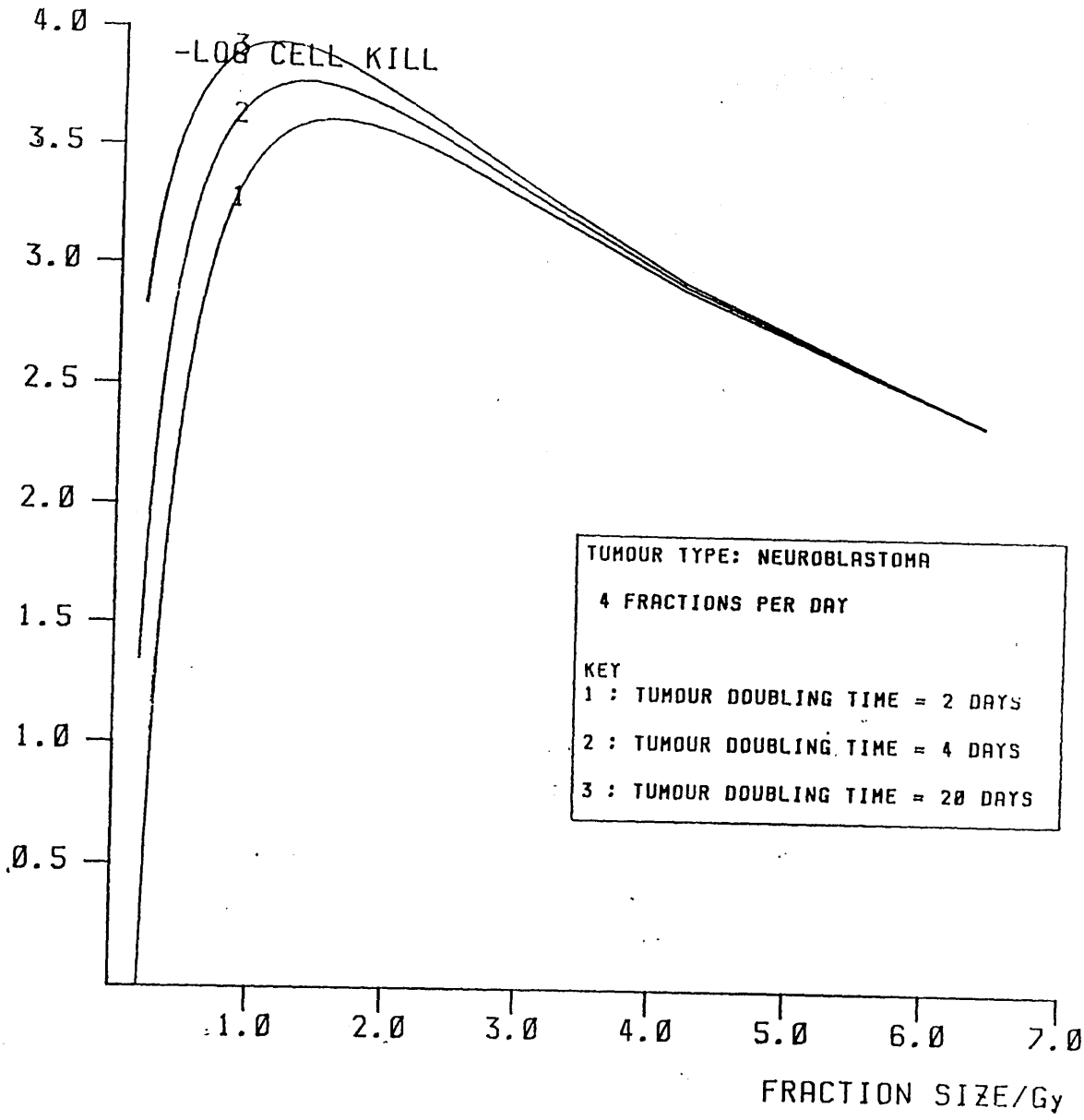

Fig 7.13 Log cell kill for neuroblastoma as a function of dose per fraction for TBI schedules of four fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The neuroblastoma radiosensitivity parameters are the median values for 8 cell lines as described in the text.

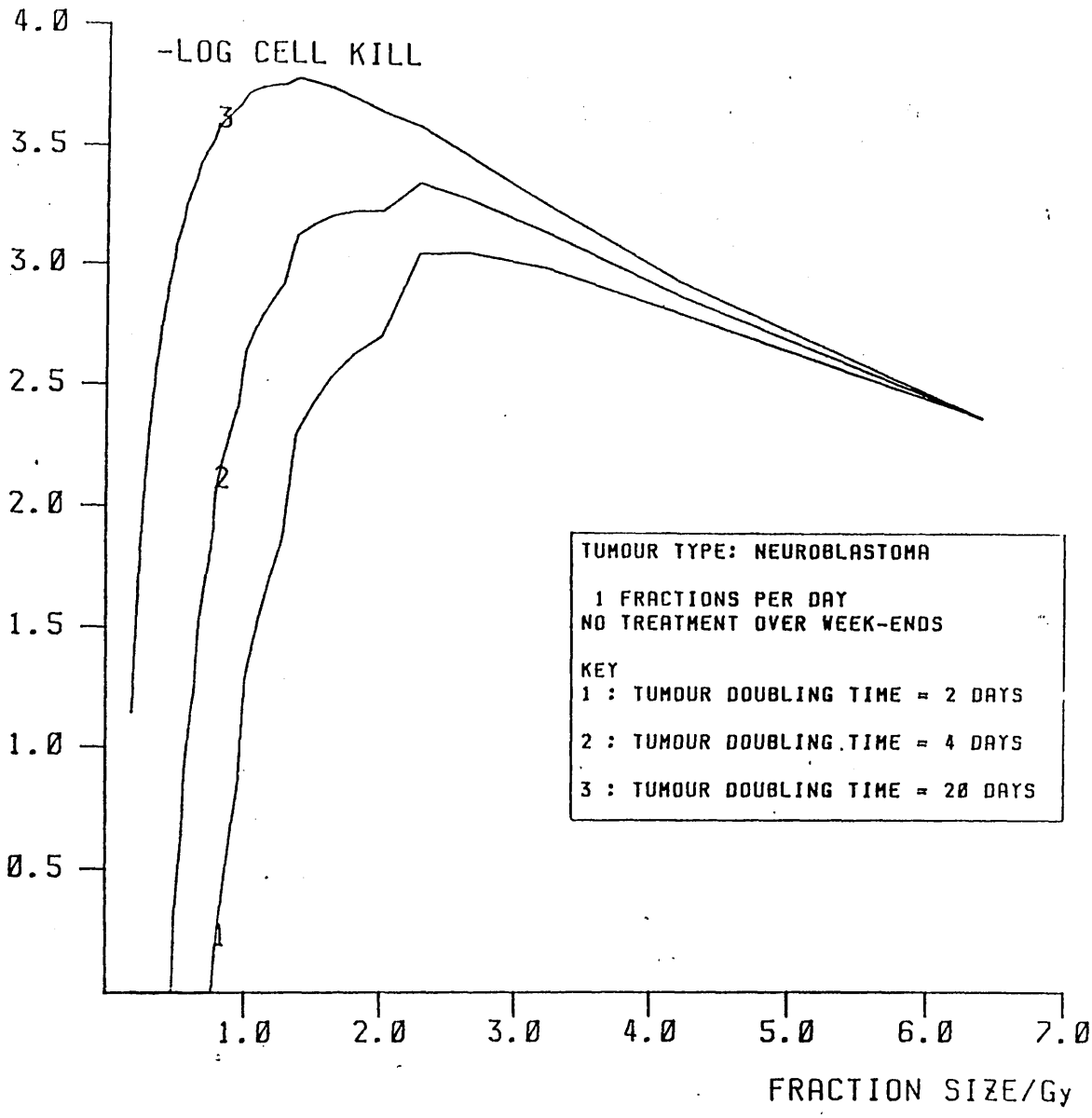

Fig 7.14 Log cell kill for neuroblastoma as a function of dose per fraction for "socially constrained" TBI schedules of one fraction per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The neuroblastoma radiosensitivity parameters are the median values for 8 cell lines as described in the text.

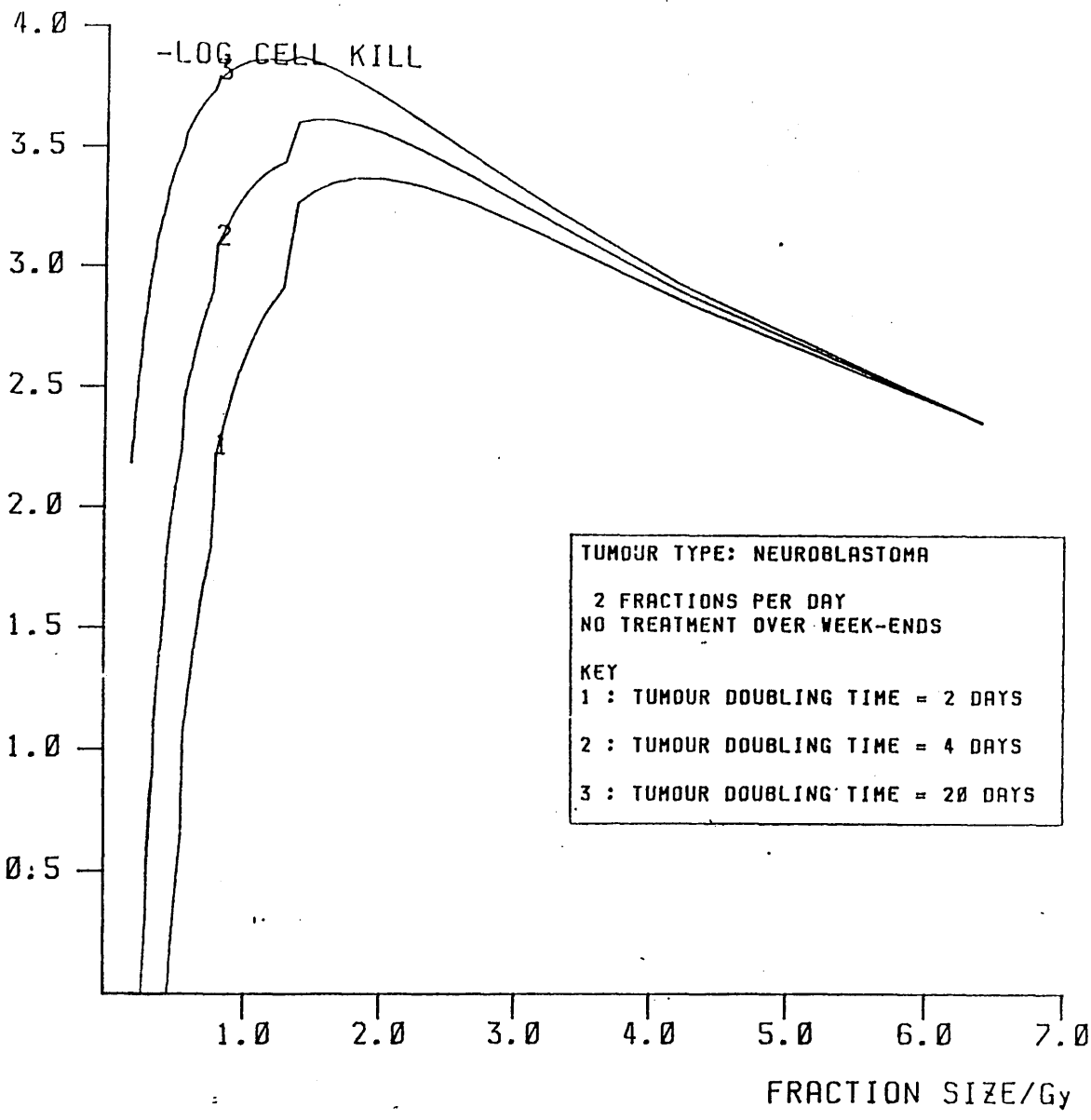

Fig 7.15 Log cell kill for neuroblastoma as a function of dose per fraction for "socially constrained" TBI schedules of two fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy. The neuroblastoma radiosensitivity parameters are the median values for 8 cell lines as described in the text.

the fractionation frequency and the population doubling time. In no case is a single fraction optimal. This further supports the underlying assumption of chapter 6, namely that multifractionated (or single low dose-rate) treatments are most appropriate for the treatment of radiosensitive neoplasms, with small shouldered survival curves, by TBI and marrow rescue. The optimal fraction sizes are shown in figure 7.16 as a function of tumour cell doubling time and fractionation frequency. In figure 7.17 the log cell kills produced by the optimal schedules are shown. It is apparent that the absolute level of tumour sterilization is less than that for leukaemia/lymphoma (by about 2 logs). Apart from this all the observations made on the corresponding leukaemia/lymphoma graphs are equally applicable to neuroblastoma. Treatment schedules subject to "social" constraints are shown in figures 7.14 and 7.15. As can be seen in figure 7.15 twice daily schedules of between 6 fractions of 2 Gy and 10 fractions of 1.37 Gy are in the optimal region for doubling times of 2 and 4 days. The evidence presented in chapter 2 suggests that doubling times of this order are characteristic of neuroblastoma. The effects of two schedules, 6 X 2 Gy in 2.5 days and 10 X 1.37 Gy in 4.5 days, were again calculated for the 8 independent sets of neuroblastoma radiosensitivity parameters. These results are shown in Table XIII and support the relative superiority of the 10 fraction schedule. The conclusions of the optimal scheduling study

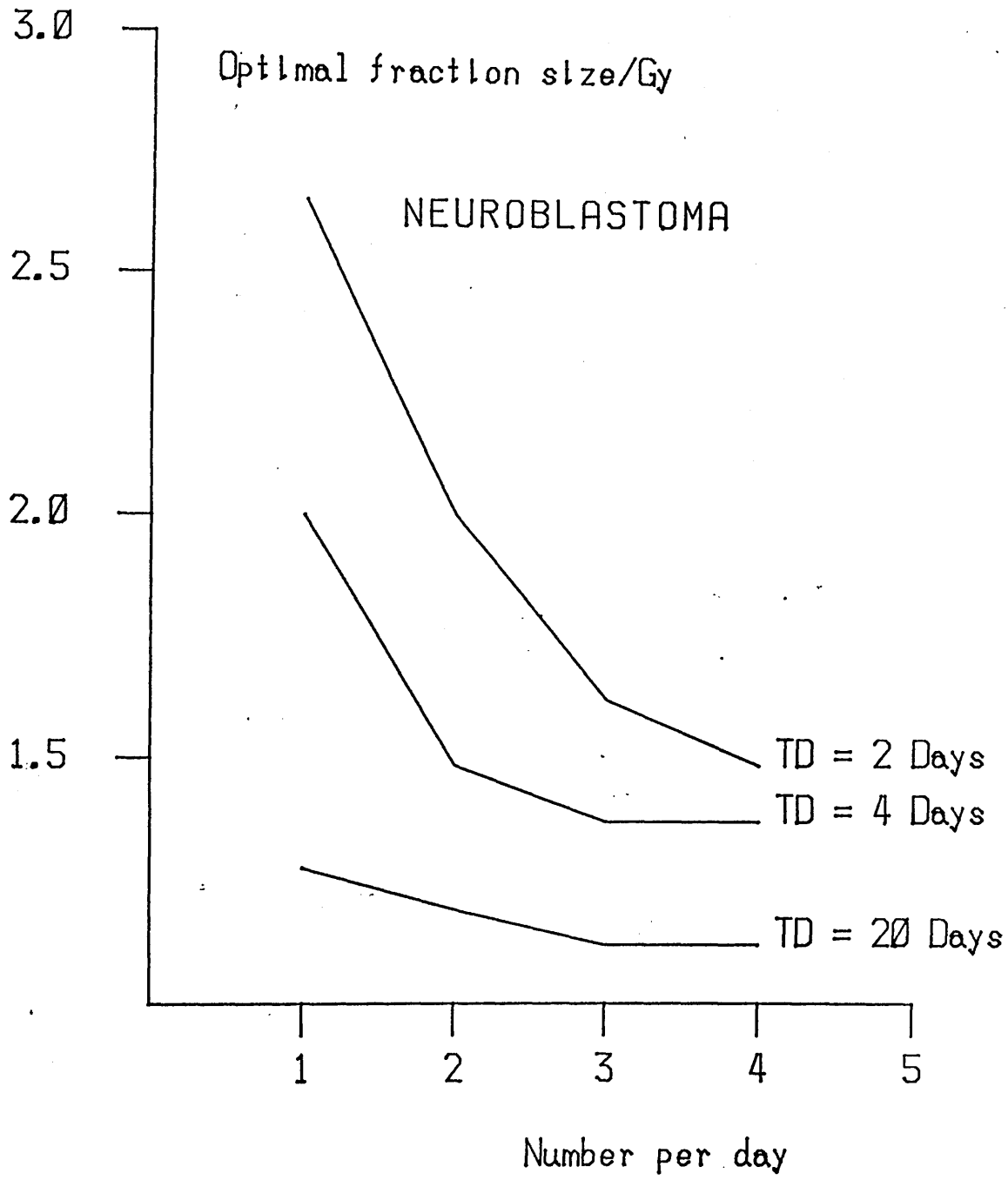

Fig 7.16 The optimal dose per fraction as a function of the number of daily fractions for three different values of tumour doubling time for neuroblastoma.

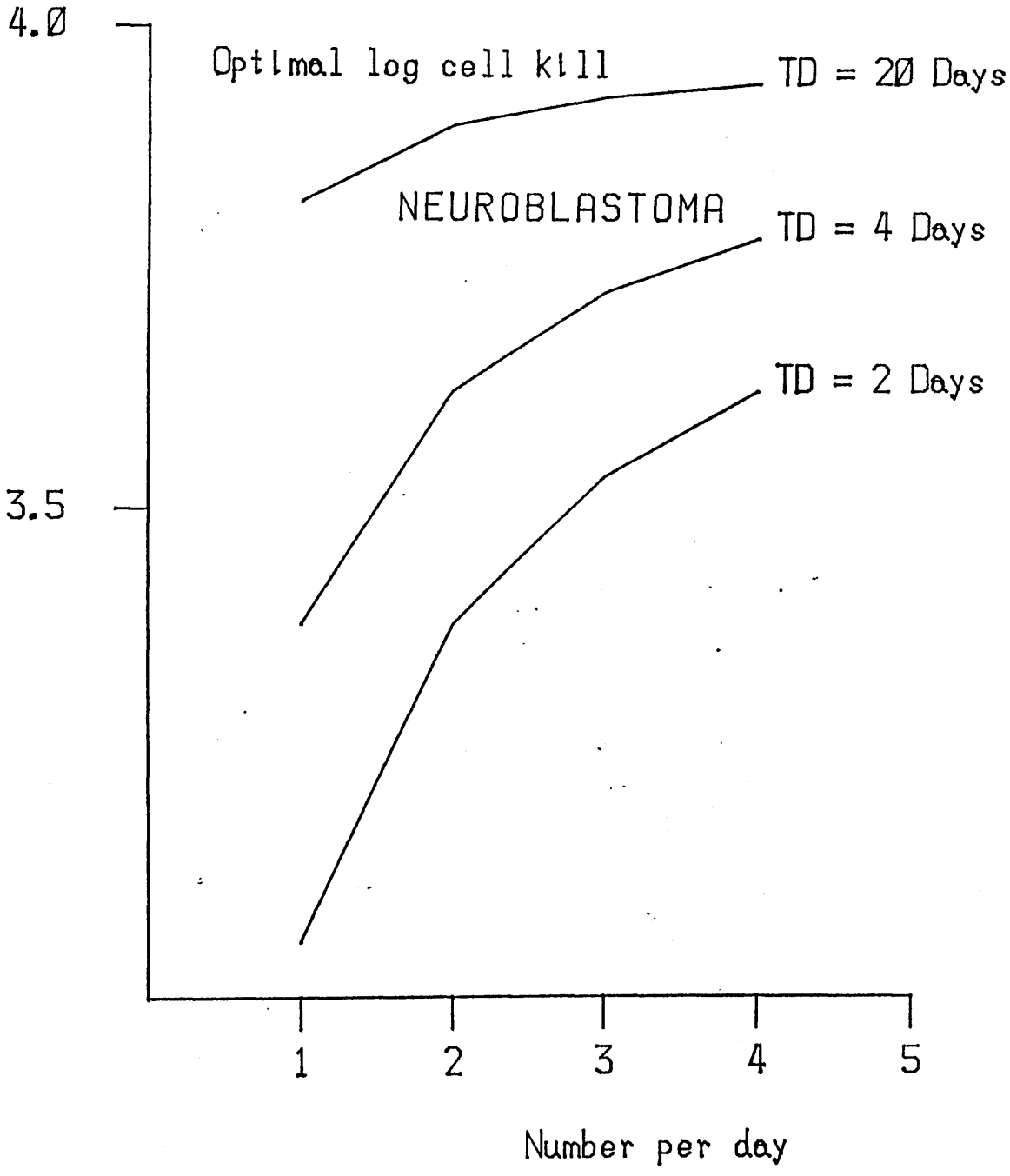

Fig 7.17 The log cell kill produced by the optimal schedules as a function of the number of daily fractions for three different values of tumour doubling time for neuroblastoma.

SCHEDULE COMPARISON FOR NEUROBLASTOMA

DESIGNATION	TD=2	TD=4	TD=20
NB1	B	B	B
HX138	B	B	B
LAN-1	B	B	B
CHP-100/NB-100	A	A	A
HX142	A	A	A
NB1-G	B	B	B
NB2-G	B	B	B
SK-N-SH	A	B	B

This table shows which of the two, twice daily, schedules A or B produce the greatest sterilizing effect on the cells of the specified line, for different values of the tumour cell population doubling time. Schedule A is 6 fractions of 2 Gy in 2.5 days; schedule B is 10 fractions of 1.37 Gy in 4.5 days. The number of times schedule A is better for doubling times of 2 or 4 days is 5. The corresponding number for schedule B is 11.

TABLE XIII

for human neuroblastoma are:

1/ Accelerated fractionation is the optimal strategy for the treatment of neuroblastoma by TBI with marrow rescue. The value of hyperfractionation (i.e. < 2 Gy per fraction) seems less clear cut than for leukaemia/lymphoma, although table XIII does show some advantages of 10 X 1.37 Gy in comparison with 6 X 2 Gy.

2/ Single high dose-rate treatments are substantially less effective than multifractionated schedules.

3/ TBI schedules of between 6 fractions of 2 Gy and 10 fractions of between 1.3 and 1.5 Gy, two fractions per day, are recommended for the treatment of neuroblastoma.

7.5 EWING'S SARCOMA

As the only two sets of survival curve measurements on this tumour type are dissimilar, each set of radiosensitivity parameters are taken separately. It is interesting to see how the differences in radiosensitivity parameters are expressed in terms of the implied optimal schedules. In this case only radiotherapy schedules of twice daily fractions are considered. Tumour cell population doubling times of 2, 4 and 20 days are allowed.

7.5.1 RESULTS OF THE COMPUTER SIMULATION FOR EWING'S SARCOMA

Figures 7.18 and 7.19 show the effects on the two Ewing's sarcoma lines of the lung-isoeffective schedules. In contrast to the leukaemia/lymphoma and neuroblastoma lines both Ewing's sarcoma lines are relatively radioresistant.

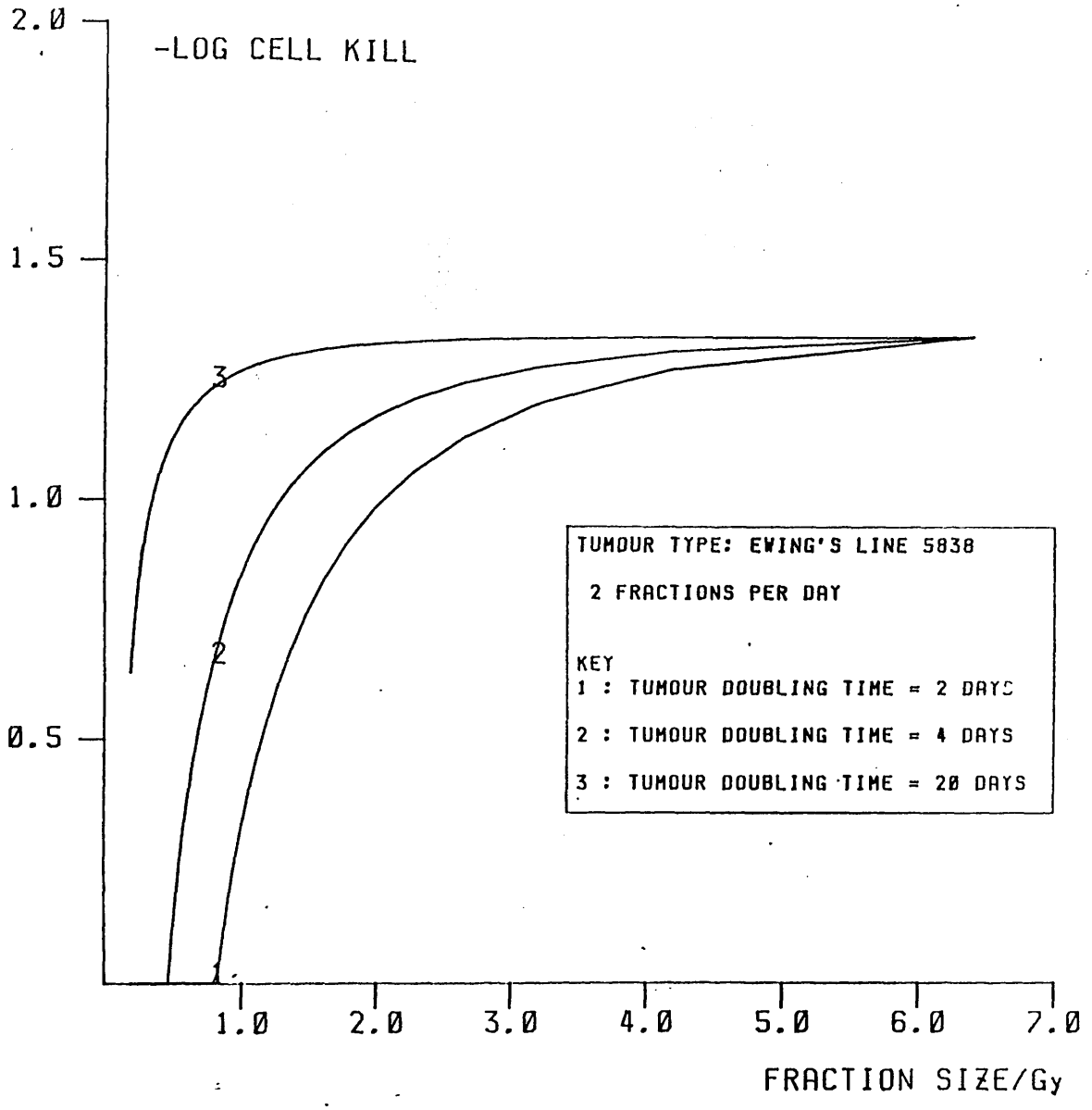

Fig 7.18 Log cell kill for Ewing's sarcoma line 5838 as a function of dose per fraction for TBI schedules of two fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy.

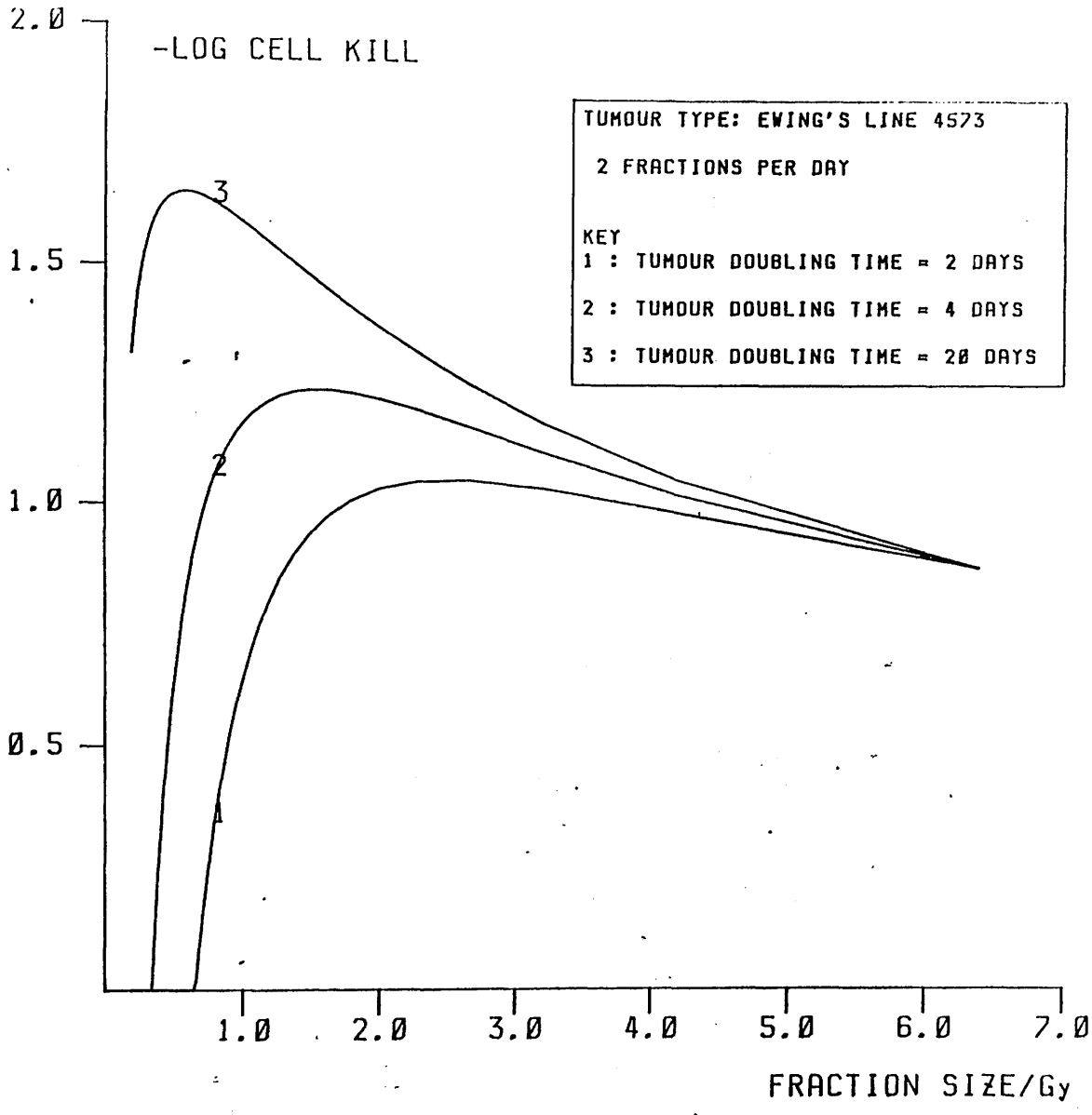

Fig 7.19 Log cell kill for Ewing's sarcoma line 4573 as a function of dose per fraction for TBI schedules of two fractions per day, isoeffective to 6 fractions of 2 Gy on the basis of the LQ model with α/β = 3 Gy.

On the basis of the survival curve parameters no more than 2 logs of tumour cell reduction are possible in stark comparison to the 5-7 logs for leukaemia/lymphoma and 3-4 logs for neuroblastoma. One line (5838) displays a high degree of quadratic curvature (α/β = 3 Gy). The calculations show that, for this line, there is no advantage to be gained by fractionation. The optimal schedule is a large single fraction treatment. There are possible advantages achievable by fractionation from the point of view of the other line (4573 : α/β = 25 Gy) but these are not significant if fast proliferation is assumed. No prediction of a suitable "optimal schedule" can be made for Ewing's sarcoma due to the inadequacy of the present experimental database.

7.6 DISCUSSION

In this chapter a restricted form of optimal scheduling for TBI with bone marrow rescue has been attempted. It was seen that the optimal schedules were determined by the interaction between the numbers and sizes of dose fractions, the kinetics of the tumour cell population and the temporal distribution of radiation dose as well as the intrinsic radiosensitivity of the tumour cells. For the radiosensitive neoplasms of leukaemia/lymphoma and, to a lesser extent, neuroblastoma optimal treatment schedules have an accelerated hyperfractionation form. But it is important to note that accelerated hyperfractionation is not a sufficient condition for a schedule to be therapeutically advantageous. Qualitative statements about

what sort of schedule is optimal are of little use. It is necessary to derive quantitative predictions of what actual schedule can be used as the basis for clinical testing. Fairly robust predictions can be made by introducing additional constraints on the possible schedules due to "social" factors (9 to 5 working, 5 days per week treatment). It was found that a fractionation schedule of 10 fractions of 1.3 - 1.5 Gy, two fractions per day, starting Monday, ending Friday was a suitable candidate for clinical evaluation and embodies the available evidence on the radiation responses of the tumour cells and lung tissue.

For Ewing's sarcoma the findings of this work were that no optimal schedule predictions can be made at present due to the scarcity of experimental data. However the study did produce some useful insights. Only one line (5838) displayed a high degree of quadratic curvature with an α/β ratio of 3 Gy. The α/β ratio of the other line (4573) was 25 Gy, which following conventional wisdom (Williams et al, 1985) is sufficient proof of the advantages of hyperfractionation. This analysis shows that whether or not hyperfractionation is worthwhile depends on the interaction between cell sterilisation by radiation and population growth by mitotic division and the α/β ratio is not, by itself, sufficient information on which to base optimal schedule predictions. Absolute radiosensitivity parameters are necessary to enable this interaction to be assessed.

7.7 EXPERIMENTAL TEST OF THE ADVANTAGES OF HYPERFRACTIONATION

An experiment was designed and has been performed to test the advantages of an accelerated hyperfractionation schedule on a tumour line, NB1-G grown as multicellular tumour spheroids (MTS). The radiosensitivity parameters for this line were estimated as n = 1.18; D0(initial) = 1.47 Gy; D0(final) = 1.04 Gy. This experiment has been reported (Wheldon et al, 1987). Briefly the LQ model was used to calculate a set of isoeffective schedules consisting of from 1 to 8 fractions, using an α/β ratio of 3 Gy. Multifractionated schedules were given twice daily. Two experimental endpoints were used to quantify tumour response, regrowth delay and proportion cured. The results were as follows :

1/ The lung-isoeffective schedules did not have equal effects on the neuroblastoma MTS.

2/ By either endpoint radiation damage to the MTS increased with total dose however it was delivered. This meant that schedules consisting of smaller fractions were more effective.

This experiment does no more than generally support the superiority of moderate hyperfractionation when treating radiosensitive cells with a low capacity to repair sub-lethal damage. However it does constitute the first experimental study of a normal tissue / tumour optimisation and may be a forerunner of more precise testing of theoretical predictions.

8.0 BIOLOGICALLY TARGETED RADIOTHERAPY

In the chapter on total body irradiation it was stated that the aim of radical treatment of malignant disease is to sterilize every tumour cell. This principle is equally valid for biologically targeted radiotherapy. TBI has the advantage that all tumour cells in the body, whether they are detectable or not, are treated. It suffers from the disadvantage that it lacks biological specificity, all cells in the body, whether tumour or not being subject to irradiation.

Generally, the larger the fractional body volume irradiated the less total dose can be given. The possible dose which can be given by TBI is, therefore, relatively low. Conversely, local radiotherapy has the advantage that, when the tumour cells are restricted to a particular limited volume, the radiation dose to other volumes which do not contain tumour cells can be reduced or eliminated. In this way much larger doses can usually be given. It has the disadvantage that collections of tumour cells which are too small to be detected will not be treated at all.

Biologically targeted radiotherapy (BTR) is an attempt to combine the good aspects of both systemic and local radiotherapy. Ideally, all tumour cells in the body will be irradiated by radionuclides carried by some biological molecule with an ability to target tumour cells. The selectivity of tumour treatment will be achieved both by the effects of biological targeting and also the limited range of the radiation from the radionuclide. Needless to

say the real situation is considerably more complicated than this idealized one.

8.1 VEHICLES AND WARHEADS

For successful BTR two distinct requirements are neccessary. The first is the ability of the exogenous substance to discriminate between target and non-target cells and become intimately associated with the target cells in some way. The second is the ability to deposit a sterilizing dose of ionizing radiation in the DNA of the target cell nucleus. This duality of function can be extended to consider a targeting modality as being composed of two distinct parts; a vehicle part, and a warhead part. The vehicle consists of a biological molecule with some specificity for the target cell. The warhead consists of a radionuclide atom or atoms which produce suitable ionizing radiation. For any one cell there are two foreseeable ways in which BTR could be achieved :

1/ attachment of the radionuclide/vehicle complex to the cell surface.

2/ incorporation of the radionuclide/vehicle complex into the interior of the cell.

When the vehicle molecule has affinity for some cell surface feature, any targeting would be of the first type. If the targeting vehicle is a "building block" precursor used, perhaps, in the synthesis of a protein or in the replication of DNA, it would lead to targeting of the

second type. Before going on to discuss suitable vehicles and warheads it is useful to consider how molecules can be described as "tumour associated".

8.3 TUMOUR ASSOCIATED MOLECULES

Molecules can be associated with tumour cells in a variety of ways. In order to classify these ways and their relevance for biological targeting it is useful to consider what it is that distinguishes tumour from non-tumour cells at the molecular scale. True tumour-specific targeting can only be possible where there are true tumour-specific molecules. This applies in only a limited set of circumstances. Where there are no true tumour-specific molecles, quantitatively specific biological targeting may still be possible.

8.3.1 ONCOGENE ASSOCIATED MOLECULES

Recent advances in the molecular biology of oncogenes have revealed some of the relationships between the genetic lesions ultimately responsible for the induction and promotion of neoplasia and their products via the transcription of DNA to RNA and translation of RNA to proteins. At present, it is mainly through the processes of protein expression that biological targeting may be envisaged.

It appears that genetic defects responsible for cancer may be due to three main pathways either singly or in combination:

1/ Point mutations in cellular proto-oncogenes giving rise

to aberrant proteins. This has been observed in oncogenes of the ras family (Reddy et al, 1982; Tabin et al, 1982)
2/ Oncogene amplification giving rise to overexpression of (possibly normal) proteins e.g. c-myc in lung cancer (Little et al, 1983), N-myc in neuroblastoma (e.g. Brodeur et al, 1984)
3/ Oncogene translocations possibly involving chromosomal abnormalities. Oncogenes may be translocated to another part of the genome e.g. in Burkitt's lymphoma about 90% of cases involve the translocation of the c-myc oncogene to the regions of the genome coding for immunoglobulin heavy chain (Klein, 1983). In these cases proteins may be overexpressed by the coding DNA being read too often.
The protein products of activated oncogenes seem to have a role in cellular control mechanisms. A variety of oncogene products have been identified as cellular growth factors, e.g. c-sis (Waterfield et al, 1983; Doolittle et al, 1983), and growth factor receptors, e.g. c-erb-B (Downward et al, 1984). The products of several oncogenes, e.g. src, abl, yes appear to have protein kinase activity, especially tyrosine kinase activity (see Hall, 1986). The genes of the ras family code for GTP-binding proteins (McGrath et al, 1984) involved in the control of intracellular levels of the secondary messenger cyclic AMP which can lead to the activation of intracellular kinases (Hall, 1986). Some oncogenes (e.g. c-myc) code for nuclear proteins which may be involved in DNA transcription and cell cycle progression (Rabbitts et al,

1985; Campisi et al, 1984). It can be conjectured that overexpression of these proteins or defects in their structure may result in an increase in cellular proliferation or a dissociation from homeostatic growth control.

Biological targeting of oncogene products may be possible. In order to achieve this they would have to be accessible to the targeting agent, suggesting that oncogene products on the cell surface would offer the best bet, e.g. growth factor receptors. The recent study by Chan et al (1986) on the possibility of tumour localization by targeting the c-myc oncogene product seems to contradict this, as c-myc codes for a nuclear protein. However this is "the exception which proves the rule" as it is only through cell death and the consequent release of nuclear contents that targeting of the c-myc product is possible. True tumour-specificity would only be realistic in cases where the oncogene product was an aberrant protein, although it is unlikely that proteins differing by only one amino-acid could be satisfactorily distinguished. In other cases quantitative specificity may be possible where there are enhanced levels of expression compared with normal cells. The oncogene-associated molecules described above constitute one extended set of possible targets for therapeutic approaches.

8.3.2 FUNCTION ASSOCIATED MOLECULES

A different type of targeting may be possible with another set of tumour associated molecules. Tumour cells may have

greater or lesser degrees of differentiation depending on the status of the initial transformed cell and any further evolutionary changes in the descendants. They may still retain vestiges of the function which the initial cell used to perform. These cells may synthesise molecules (possibly aberrant) which can be used as "tumour markers". The ability of some neuroblastomas and phaeochromocytomas to synthesize the catecholamines epinephrine and nor-epinephrine, and also the synthesis of melanin by some malignant melanomas are examples. Biological targeting of the process of synthesis may be possible. A radioiodinated catecholamine precursor analogue meta-131I-iodo benzylguanidine (mIBG) has been used for both scintigraphic imaging and therapy of neuroblastoma and phaeochromocytoma (Horne et al, 1985; Gerrard et al, 1987). The basis of this approach is that cells, behaving as "assembly lines" for the synthesis of catecholamines will preferentially take up mIBG, storing it in neurosecretory granules for later use.

Other sorts of "functional" molecules may be expressed on the cell surface. Tumour cell surface markers may correspond to those of the original transformed cell, but they may be expressed to a greater extent or they may be altered in some way. A situation where the latter seems to be the case is in the expression of a 400 kD mucin molecule associated with the human milk fat globule (HMFG) membrane. It has been suggested that the mucin produced by breast carcinoma cells may be aberrantly glycosylated (

Burchell et al, 1987). A monoclonal antibody SM-3 directed against the protein core of this mucin has been reported to react with 91% of breast carcinomas but shows little or no reactivity with pregnant or lactating breast, normal resting breast or benign breast tumours. However monoclonal antibodies (HMFG1, HMFG2) directed against different epitopes of the complete mucin molecule also cross-react with normal breast, Fallopian tube and ovarian epithelium (Ward et al, 1987). It may be possible to target "functional" cell surface molecules by radionuclide-conjugated antibodies, in particular monoclonal antibodies. Several clinical studies have used this method for both diagnosis and therapy (e.g. Order et al, 1980; Carrasquillo et al, 1984; Hammersmith Oncology Group and ICRF, 1984)

To summarize this section, molecules associated with tumour cells may be of two main types :

1/ Oncogenes and their products. These may be true tumour specific molecules, related to the transcription and translation of mutated DNA. This will result in aberrant proteins, not found in normal cells. Alternatively, there may be quantitative overproduction of certain molecules as a result of gene amplification or translocation processes. Enhanced levels of expression may therefore occur in tumour cells of proteins which also exist in normal cells.

2/ Molecules related to the differentiation status of the original transformed cell and the subsequent evolution of the clone. These may be "assembly line" molecules such as

catecholamines or "functional" cell surface markers such as mucins.

Both types of tumour associated molecules give rise to the possibility of biological targeting.

8.4 PROSPECTS FOR BIOLOGICALLY TARGETED THERAPY

Over the course of evolution vertebrates have developed methods of coping with invasion by potentially harmful microorganisms. These methods go under the umbrella term of the immune system. The feature they all have in common is the ability to distinguish foreign molecules from self molecules. The functions of the immune system are conventionally sub-divided into two general types of mechanism:

1/ Humoral antibody responses

2/ Cell-mediated immune responses

Both types of response are mediated by lymphocytes, immunoglobulin secreting B-cells for the first type and T-cells for the second type. Immunology is a vast subject and no attempt will be made here to review it; suffice it to say that it is through understanding and modifying both components of the immune system that nearly all present approaches to biologically targeted therapy are based. The main types of biologically specific molecules used so far have been whole antibodies or their Fab and F(ab)2 fragments. This is especially so since 1975 when Kohler and Milstein first obtained monoclonal antibodies. Both polyclonal and monoclonal intact antibodies may be directly cytotoxic through complement lysis. Alternatively

they may be modified by the addition of a cytotoxic "warhead". To date these have included the A chain of the plant toxin ricin (Thorpe et al, 1982; Uhr, 1984), conventional cytotoxic drugs such as methotrexate (Garnett et al, 1983) and vindesine (Rowland et al, 1985) and also radionuclides. Only radionuclide-conjugated antibodies will be considered further. Together with precursor-like molecules such as mIBG they are likely to be the main mediators of biologically targeted radiotherapy for the immediate future.

8.5 RADIONUCLIDE IMAGING WITH TARGETED MODALITIES

The requirements for imaging and therapy with biologically targeted radionuclides are different. Before discussing therapeutic approaches further, it will be useful to consider the requirements for imaging. The basic mechanism of radionuclide imaging is the detection, by a gamma camera, of γ- ray photons emitted from inside the body. From this perspective it is desirable to use a γ-emitting radionuclide with a suitable energy for imaging. This contrasts with the requirements for therapy, as will be discussed later. It has been found when using antibody-radionuclide conjugates for imaging that the tumour to background discrimination improves as the time since administration increases, although the absolute level of activity in the tumour steadily diminishes (Begent and Bagshawe, 1985). This is believed to be caused by the prolonged retention of antibody by the tumour compared with the faster loss of activity from the

bloodstream. The optimal times for scanning depend on the characteristics of the radionuclide used but, for 131-I, are from two to six days following administration (Leichner et al, 1983; Carrasquillo et al, 1984; Riggs et al, 1988). This phenomenon has no counterpart in therapy - since this starts immediately after administration. Consequently the initial high blood pool concentrations of radionuclide seem unavoidable in systemic BTR. A possible exception to this may be the use of the streptavidin-biotin system (Paganelli et al, 1987). Another technique used in scanning is computer assisted background subtraction (e.g. Begent and Bagshawe, 1985; Chan et al, 1986). The blood pool activity can be electronically removed from the image, thereby improving the picture quality. Obviously there is no counterpart to this in therapeutic applications. These differences indicate that success in biologically targeted radionuclide diagnosis is not a guarantee of success for therapy.

8.6 TUMOUR TARGETS FOR BTR

Tumour targets for BTR may be conveniently divided into three categories:

1/ Bulk disease, essentially a primary tumour or a large secondary deposit having a diameter greater than 1 cm and probably greater than 5 cm.

2/ Disease confined to a specific body region such as the peritoneal cavity.

3/ Disease which is widely disseminated and, in general,

not clinically locatable.

Although these categories can be distinguished it is possible for more than one type to be present in a patient, possibly all three. The corresponding requirements for therapy, however, are likely to differ.

8.6.1 BULK TUMOURS

Bulk disease consists of a mass of viable clonogenic and non-clonogenic tumour cells, non-viable and necrotic tumour cells, stromal cells, infiltrating lymphocytes and macrophages, non-cellular material and blood vessels including capillaries. The access of targeted radionuclides to bulk disease is provided by diffusion through capillary walls. In order to give a high uniform dose to a bulk tumour there are, at least, four requirements:

1/ Vehicle-warhead specificity is necessary to provide a differential between tumour and normal tissues. It should be noted that this broad prerequisite will include not only biochemical specificity for tumour associated antigens or products but also:

a) firm binding of radionuclide to vehicle to prevent free radionuclide from circulating

b) minimizing reticulo-endothelial uptake of non-self proteins

c) favourable pharmacokinetics of vehicle-warhead distribution.

2/ Vehicle-warhead sensitivity or the ability to target all tumour cells is also neccessary. The potential

problems in this instance can be related to two main co-factors as follows:

a) The biological heterogeneity of human tumours by the time of diagnosis (Owens et al, 1982). This is partly due to cellular differentiation in less anaplastic tumours and also the result of the "genetic instability" of tumour cells leading to the continual emergence of mutant clones as tumour growth proceedes. Not all tumour cells will express a particular antigen (Edwards, 1985) or synthesize a particular molecule (Buck et al, 1985) even if the majority do. The phenomenon of "antigen modulation" (Old et al, 1968) i.e. suppression of a previously expressed antigen, will also contribute to the heterogeneity of tumour cells.

b/ Another important problem area is likely to be access of the vehicle to the tumour cells. Large molecules like antibodies are likely to diffuse only a short distance (Pimm and Baldwin, 1985; Hagan et al, 1986). Problems of accessibility may not only be confined to large tumours. Restricted access of even small molecules such as adriamycin (Durand, 1981; Kerr et al, 1986) and methotrexate (West et al, 1980) has been observed in sub-millimeter diameter tumour spheroids in vitro and may be inferred for micrometastases in vivo.

These potential problems highlight another requirement.

3/ The choice of a suitable range of ionizing radiation, either

a/ long-range emissions to exploit "cross-fire"

irradiation of tumour cells from radionuclides not directly associated with them or

b/ short-range emissions to exploit intimate localisation if and when it occurs.

4/ A high level of radionuclide concentration throughout the tumour.

The prospects of antibody-targeted radiotherapy for bulk disease have been considered by Vaughan and co-workers (Vaughan et al, 1986; 1987). These authors used a compartmental model to evaluate doses to a bulk tumour and the whole body, based on current estimates of tumour uptake of antibody-radionuclide conjugates administered systemically (about 0.005% of injected activity per gram of tumour) and their effective half-lives in tumours and other body compartments. They calculated that, in order to give a "sterilizing" dose of 60 Gy to the tumour, the whole body would receive a dose of 17 Gy. Vaughan et al pointed out that bone marrow toxicity is the dose-limiting effect for BTR, as has been reported by others (Ettinger et al, 1982), and that doses of this magnitude are far in excess of what would enable marrow recovery. They concluded that it was inappropriate to plan therapeutic studies using this technique.

This led to a correspondence (Wheldon and O'Donoghue, 1986; Epenetos et al, 1986; Begent et al, 1986; Ward et al, 1986) in which several alternative interpretations and therapeutic approaches were described. Vaughan et al took a dose of 60 Gy as representing a sterilizing dose.

This may be open to criticism on the grounds that the dose required for sterilization depends on tumour size. To a first approximation it is directly proportional to the logarithm of the number of clonogenic tumour cells. This means that smaller tumours will be eradicated at lower doses than are required for larger ones. The other objection to the figure of 60 Gy is that it is set, not because it is the dose required for tumour sterilization, but because it corresponds to the tolerance of connective tissues for fractionated radiotherapy. Having said this it may be acknowledged that 60 Gy is a reasonable figure of merit for BTR and doses of this order, or even higher will be neccessary for the eradication of bulk tumours. The suggestion of bone marrow rescue (Wheldon and O'Donoghue) and its likely consequences will be described in detail in the next chapter, suffice it to say here that one would anticipate a possible dose escalation. Begent et al pointed out that the limited diffusion of antibody-radionuclide conjugates from capillaries may be similar to that of nutrients and oxygen and that the distribution of antibody-radionuclide may correspond to the distribution of viable tumour cells. For this reason, BTR may be more effective than would be suggested by estimates of the mean dose throughout a tumour volume. This possibility is supported, to some extent, by the appearance of cords of viable tumour cells surrounding capillaries with intervening necrotic areas in some human tumours (e.g. Thomlinson and Gray, 1955).

Epenetos et al and Ward et al pointed out that there may be therapeutic potential in regional administration of radiolabelled antibodies. This will be described in the next section.

8.6.2 REGIONAL DISEASE

Several common cancers, such as cancer of the ovary, primary brain neoplasms and malignant serous effusions have a propensity for confinement to specific body cavities. The advantages of this situation for therapy are twofold:

1/ A higher proportion of the administered activity is available for binding to tumour.

2/ The accessibility of distant normal organs and tissues to radionuclide is diminished. Absence of a "first pass" encounter with the liver is especially important.

Apart from the limitation to a bounded region of the body, regional malignancy may be essentially bulk tumour (in which case most of the discussion of the last section will be equally relevant) or composed of small disease foci - micrometastases or single cell suspensions. In the latter case the considerations for disseminated malignancy, discussed in the next section, will be applicable. Candidate neoplasms for regional therapy include ovarian carcinoma treated by intraperitoneal infusion (Epenetos et al, 1987); liver metastases treated by intrahepatically administered antibody-radionuclide with biodegradable starch microspheres which temporarily block the flow of blood creating an artificial regional

environment; primary malignant melanoma in limbs treated by isolated limb perfusion possibly with antibody-radionuclides; leptomeningeal malignancy deriving from medulloblastoma, acute lymphocytic leukaemia, lymphoma, melanoma and others, treated by intrathecal or intraventricular administration of antibody-radionuclide (Lashford et al, 1988). These examples have little in common apart from the regional aspect. Any other clinical conditions where regional confinement, both natural or artificial, may be a factor will also be worth considering for BTR.

8.6.3 DISSEMINATED MALIGNANT DISEASE

It is in the treatment of disseminated but undetectable micrometastases that BTR holds the greatest theoretical promise. The reason for this is quite simple. Malignant disease less than 1cm in diameter is unlikely to be visualized by imaging procedures. Its existence may be inferred by other clinical investigations e.g. serum markers, or by experience of the natural course of a particular disease. In theory, if disease is locatable, it may be treated by local means - surgery or radiotherapy. If disease is not localized it must be treated systemically by chemotherapeutic drugs and possibly TBI. Either way, in this case, it is not so much the disease which is being treated as the whole patient. This severely restricts the allowable doses. With BTR, as was described at the beginning of this chapter, there is the possibility of the all-encompassing aspect of systemic treatment

coupled with the spatial specificity of local treatment. This is, of course, in the ideal case. In reality, for the foreseeable future, BTR will be more like non-uniform TBI and, like other forms of cancer therapy, will rely on the accumulation of small advantages and differentials in order to be a success. Some discussion of the strategic aspects of treatment by BTR have been reported (Wheldon et al, 1988) and this will be expanded in the next chapter. For the moment, some comparisons will be drawn between disseminated neoplastic disease and bulk tumours. Access to small avascular metastatic conglomerations of tumour cells is provided by diffusion from the outside. To this extent the situation is similar to bulk disease where diffusion is from the tumour vasculature. The levels of uptake of targeted radionuclide in microtumours are not known. The studies of Moshakis et al, 1981; Menard et al, 1983 and Hagan et al, 1986 demonstrated that, for specific antibody, the radionuclide uptake per gram of tumour was inversely proportional to tumour mass. Epenetos et al (1982) reported that non-specific antibody uptake varied directly with tumour size but was diminished by necrosis. Baldwin and Pimm (1983) also reported tumour growth to be accompanied by linear uptake. The latter two studies imply a constant or only slowly increasing uptake per gram as tumour size decreases. None of these experimental systems measured radionuclide uptake in microtumours. The balance of the evidence appears to suggest that smaller tumours concentrate specific antibody more efficiently.

Estimates of tumour uptake made for bulk disease may be misleadingly low for micrometastases.

Micrometastases are probably in an exponential growth phase and therefore less likely to harbour quiescent (G0) cells. Consequently, they may be more radiosensitive than bulk tumours, although there is no direct clinical evidence for this. The requirements for vehicle-warhead specificity and sensitivity are the same as for bulk tumours.

The ideal range of ionizing radiation will depend on the dimensions of the collection of tumour cells. Sizeable microtumours (1 mm diameter or greater) will have different range requirements from single freely circulating cells. Targeting of single cells calls for a radionuclide with a very short range emission, of the order of the cell radius. If a long range emitter is used, virtually no ionization will occur within the target cell. Conversely, given a situation of limited diffusion into a sizeable microtumour, a longer range emitter is called for. In this case a short range radionuclide could not hope to satisfactorily treat tumour cells in the interior. In the case where both single circulating tumour cells and microtumours may be present, no one radionuclide is likely to be satisfactory. This may be a reason for using several radionuclides, having different emissions, simultaneously. However, it is difficult to envisage all tumour cells being adequately treated by BTR in isolation. For curative therapy it may be necessary to combine BTR

with some other cytotoxic therapy. The combination of BTR with external beam TBI is a possibility considered in the next chapter.

There have not been any clinical studies of BTR for disseminated disease other than in situations where large primary tumours were also present. This is mainly due to the pattern of development of any new method of cancer treatment, where it is first tried out on patients unlucky enough to have advanced bulky disease with little hope of cure. Although this is unavoidable it should be borne in mind that it is in treatment of widely disseminated disease where BTR should be most beneficial.

8.7 RADIONUCLIDE WARHEADS FOR BTR

In the previous section it was seen that the requirements for a radionuclide warhead are highly dependent on the type of tumour target. In this section radionuclides which may be candidates for BTR will be examined. Following Humm (1986) radionuclides may be split into five groups, three of which are only quantitatively independent. These groups are:

1/ Alpha sources, e.g. 211 At, 212 Bi

2/ Short range beta sources (mean range < 200 um), e.g. 33 P, 199 Au

3/ Medium range beta sources (200 um < mean range < 1 mm), e.g. 131 I, 67 Cu, 109 Pd

4/ Long range beta sources (mean range > 1 mm), e.g. 32 P, 90 Y

5/ Electron capture and internal conversion decaying

sources, e.g. 77 Br, 125 I, 131 Cs

Alpha radiation is a very efficient agent of cell sterilisation. The range is short, typically of the order of cellular dimensions, 50 - 90 um. This means that one would expect only cells directly targeted to be sterilised. This implies that α - emitters may be suitable for therapy of single cells. Their potential for therapy of substantial microtumours or bulk disease is less obvious. Uniform radionuclide distribution throughout the tumour mass would be nec essary, otherwise there will be a problem relating to tumour cells which escape effective targeting.

Astatine - 211 is a halogen. It therefore will be likely to mimic the chemistry of iodine to some extent. In particular it may be possible to replace iodine by astatine in mIBG, which would become mAtBG. Astatination of antibodies should also be possible. Some investigations into the use of astatine labelled monoclonals have been reported (Vaughan et al, 1982; Harrison and Royle, 1984). Radiobiologically one would not expect any dose-rate effect for α - emitters, either for tumour cell sterilisation or also for damage to normal tissues.

Short range beta sources again are likely to be of most use in the therapy of single cells or smaller micrometastases. Treatment of larger deposits may suffer from the heterogeneity of vehicle distribution.

Medium range beta sources include 131 I, the only radionuclide used so far in clinical BTR. 131 I also

produces high energy photons. This has advantages and disadvantages. The distribution of radionuclide may be visualized by scanning, which enables attempts at dosimetry to be made. Conversely, the photon component increases the dose throughout the whole body. Medium range beta sources like 131 I are of limited use for the therapy of single cells as most of the disintegration energy will escape. This may increase the cross-fire effect in therapy of larger tumours but, in this case, the range is rather too short to compensate for the heterogeneity of distribution.

Long range beta sources are best suited for BTR of bulk tumours and large metastatic deposits. They are not appropriate for single cells or small microtumours. 90 Y is under investigation as a therapy radionuclide (Hnatowich, 1985; Leichner et al, 1988).

Radionuclides such as 77 Br, 125 I and 131 Cs decay by electron capture or internal conversion causing an inner shell vacancy. The subsequent cascade of electronic transi tions produce a large amount of X-rays and Auger electrons. The Auger electron range is very short (~ 1 um) and therefore close proximity to the genomic DNA is required for cell sterilisation. The compounds 77 Br - bromodeoxyuridine (BUDR) and 125 I - iododeoxyuridine (IUDR) are pyrimidine analogues and are taken up and used in DNA synthesis. They are extremely effective as sterilizing agents once incorporated into DNA. In the future one may speculate on the use of Auger emitters in

targeting specific oncogenes, perhaps incorporated in a specific gene probe in a genetically engineered virus. These approaches are, of course, a long way off.

The spectrum of radionuclides presented above illustrate two important points:

1/ No one radionuclide is suitable for all tumour targets. Long range beta sources e.g. 90 Y are probably best for bulk disease. Medium range beta sources e.g. 131 I may be best for sizeable microtumours (~ 1 mm diameter). Short range beta sources and alpha emitters may be best for single cells and small microtumours (< 1 mm diameter). The time for Auger electron emitters may not be right yet, although these may be useful in the future.

2/ It is likely that there will be a proportion of tumour cells which are effectively untargeted. They may pose a serious obstacle to cure and it will be important to examine alternative methods of ensuring that they are sterilised.

8.8 BTR BIODISTRIBUTION AND DOSIMETRY

The subjects of radionuclide biodistribution and dosimetry are intimately connected, one could even say that they are aspects of the same thing. For this reason they are considered together here.

If monoclonal antibodies and other BTR modalities were, truly, "magic bullets", studies of biodistribution and dosimetry would be unnecessary. One would simply administer enough targeted radionuclide to sterilize all tumour cells. However, there are no magic bullets, at

least not yet, and the biodistribution and dosimetry of BTR are of vital importance. For the treatment of disseminated malignant disease, intravenous or intraarterial administration of targeted radionuclide seems unavoidable. This means that initially all the radionuclide is contained in the blood pool. The removal of radionuclide from the blood pool is brought about by several mechanisms, including:

1/ Uptake of targeted radionuclide by tumour cells

2/ Uptake by cross-reacting normal cells

3/ Reticulo-endothelial uptake of non-self protein, leading to sequestration of radionuclide in the liver and spleen.

4/ Excretion of targeted radionuclide, e.g. via the urinary tract (kidney, ureter, bladder).

Radionuclides in the blood pool will irradiate tissues through which blood passes i.e. all tissues. Photon emission from radionuclides such as 131 I will also irradiate the whole body by virtue of its long range. Several remedial measures have been proposed to reduce the problems of biodistribution. These include:

1/ Improved physical chemistry and purification techniques (Gansow et al, 1986; Meares et al, 1988). The ease of iodination of antibodies is compromised by the ease of deiodination. This is especially important in vivo, where it seems to occur primarily in the liver and kidneys (Carrasquillo et al, 1984), but the minimization of radiolytic products and aggregated proteins before

administration is very important. Other radionuclides e.g. 90 Y may be chelated to antibodies (Hnatowich et al, 1985). This is a more secure binding and should improve the situation with respect to in-vivo breakdown.

2/ The identification of more specific tumour markers or antigens is a worthwhile goal e.g. SM-3 antigen in breast cancer (Burchell et al, 1987). This may reduce the cross-targeting affinities. However, as has been described, true tumour-specific molecules may not be present in all cases.

3/ The use of chimeric (Sahagan et al, 1986; Reichman et al, 1988) or human (Carrasquillo et al, 1987; Yiu et al, 1987) antibodies may help to reduce the immune response to foreign proteins.

4/ The use of antibody fragments may help in three respects:

a) Reticulo-endothelial uptake of antibody is probably facilitated by Fc receptors (Buraggi et al, 1985). The use of Fab or F(ab')2 fragments may reduce this.

b) Antibody fragments may diffuse further than intact antibodies and may therefore improve the intratumour distribution of radionuclide (Matzku et al, 1986).

c) Monovalent antibody fragments (Fab) will help to reduce the problems of antigenic modulation (Cobbald and Waldmann, 1984).

5/ Second antibody techniques may help to accelerate the clearance of targeted radionuclide from the blood pool (Begent et al, 1982; Goodwin et al, 1984; Sharkey et al,

1984).

It is difficult to see how these measures will radically change the nature of BTR, but they are part of the accumulation of small differentials which is the hallmark of cancer therapy. For the foreseeable future BTR will be equivalent to non-uniform TBI. By analogy with TBI one would expect that the introduction of marrow rescue would enable an increase in the tumouricidal effect. This will be discussed in the next chapter. Meanwhile it would seem sensible to adopt the treatment philosophy used in radiotherapy. This may be described as "treat to tolerance". Adherence to this principle requires that the amount of BTR which is given be determined solely by consideration of the dose-limiting constraints which are imposed by normal organs and tissues. For sytemic treatment of minimal residual disease i.e. micrometastases and single cells, it is unlikely that tumour doses will be directly evaluable. For this case the principle of treating to tolerance would appear particularly useful. The primary purpose of biodistribution and dosimetric studies is the evaluation of doses to normal tissues.

8.8.1 THE MIRD APPROACH TO DOSIMETRY

The quantitative basis for calculation of doses from internally distributed radionuclides is the set of equations and recommendations of the Medical Internal Radiation Dosimetry committee of the Society of Nuclear Medicine. These rules have been published as a series of pamphlets in the years since 1968 (Loevinger and Berman,

1976 refer to most of the other pamphlets). The basis of the approach is the separation of the relevant variables into two main groups:

1/ Processes which involve the temporal distribution of radionuclide i.e. biodistribution kinetics

2/ Other processes, namely physical (i.e. related to radionuclide disintegration) and spatial (i.e. related to the anatomical distribution of radionuclide)

The MIRD approach amounts to calculating the dose or dose-rate produced in a "target organ" due to radioactivity in a "source organ", including the special case where the target and source organs are the same i.e. self-irradiation. Strictly only normal tissue doses can be calculated, as the calculations are based on an anatomical model of a normal body. The effects of processes independent of biodistribution kinetics i.e. spatial anatomy and nuclear transformations are summarized in tables of "S" values of absorbed dose in a target organ per unit cumulated activity of a particular radionuclide in a source organ. The S values are arrived at by Monte Carlo simulations of the paths of individual ionizing particles from radioactive decays. They are, therefore, average values over the whole of the target organ based on a uniform distribution of radionuclide throughout the source organ and do not account for local variation. It seems clear that where this approach is likely to break down is in self-irradiation processes. This will be especially pronounced for short range emitters. For these

cases microdosimetric models will probably be required (Myers, 1987; Humm 1987)

9.0 ISOEFFECT AND DOSIMETRIC MODEL STUDIES OF BIOLOGICALLY TARGETED RADIOTHERAPY

In this chapter the subject of biologically targeted radiotherapy (BTR) is considered from the perspectives of the LQ isoeffect model and the medical internal radiation dosimetry (MIRD) approach. Using the LQ model the relationships between predicted allowable dose to bone marrow and a "late responding" organ as functions of the effective radionuclide half-life are investigated. The doses to micrometastases from antibody targeted 131-I and 90-Y are estimated using the MIRD equations and the models of Humm (1986) and Vaughan et al (1987).

9.1 ISOEFFECT MODEL STUDIES OF BTR

There is evidence which suggests that haemopoietic toxicity is currently the dose-limiting effect for BTR (Ettinger et al, 1982). It is, therefore, important to know what dose may be given to the bone marrow. Other organs which may be particularly vulnerable are the liver (due to sequestration of antibody) and kidney (due to renal excretion of radiolabel). As with conventional TBI, radiation damage to lung may also be of importance. For BTR the dose-rates in these organs may be complex functions of time, related to the biodistribution kinetics of the vehicle-warhead. The simplest possible case is an exponentially decaying dose-rate. This is the only situation considered in what follows.

In order to calculate acceptable doses to normal organs

the extension of the LQ model to exponentially decaying dose-rates (Dale, 1985) will be used.

As described in chapter 3 the biological effect of an exponentially decaying radiation exposure of initial dose-rate, r_0 , and effective decay constant, λ ($= \ln 2/T_{1/2}$; where $T_{1/2}$ = effective half-life) can be expressed as an ERD where

$$ERD = RE \times D$$

$$RE = 1 + \frac{r_0}{(\mu+\lambda)\alpha/\beta}$$

$$D = r_0/\lambda$$

$$\therefore ERD = \frac{r_0^2}{\lambda(\mu+\lambda)\alpha/\beta} + \frac{r_0}{\lambda} \qquad (9.1)$$

For this biological effect to be equivalent to that produced by a fractionated schedule of n fractions of size d

$$ERD = ERD_f = nd\left(1 + \frac{d}{(\alpha/\beta)}\right) \qquad (9.2)$$

Equations 9.1 and 9.2 may be solved for $D = r_0/\lambda$ to yield

$$D = \frac{\sqrt{(\mu+\lambda)\alpha/\beta)^2 + 4\lambda(\mu+\lambda)nd(\alpha/\beta + d)} - (\mu+\lambda)\alpha/\beta}{2\lambda} \qquad (9.3)$$

The first situation to be considered is for haemopoietic toxicity. Here the dose-rate of importance is that produced in the bone marrow. The single fraction tolerance dose taken for the whole body (without marrow rescue of some sort) is 1 fraction of 2 Gy. The LQ model parameters

for bone marrow are taken from Thames et al, 1984 as

α/β = 10 Gy : $T_{1/2}$(repair) = 0.5 hr (μ = 1.4 hr^{-1}).

Equation (9.3) may now be solved to give the predicted allowable doses for BTR as a function of radionuclide effective half-life. These are plotted as the lower curve in figure 9.1

The other organs to be considered fall into the radiobiological category of "late-responders". These are likely to assume greater importance when haemopoietic toxicity is by-passed by the use of marrow rescue. A conventionally fractionated schedule which is close to tolerance is taken to be 6 fractions of 2 Gy, with the assumption of complete repair between fractions. This schedule is currently used clinically for TBI when marrow rescue is performed (Clift et al, 1982; Goolden et al, 1983). In the TBI situation the dose limit is set by lung tolerance rather than by liver or kidney, which may be able to withstand higher doses. The LQ model parameters taken to represent these organs which impose dose limitations are taken to be those of a "late responding organ" (Dale, 1985) and are

α/β = 3 Gy : $T_{1/2}$(repair) = 1.5 hr (μ = 0.46 hr^{-1})

Equation (9.3) may now be solved to give the predicted allowable doses as a function of radionuclide effective half-life. These are plotted as the upper curve in figure 9.1.

9.2 DISCUSSION OF THE ISOEFFECT MODEL STUDY

It can be seen from figure 9.1 that there is apparently

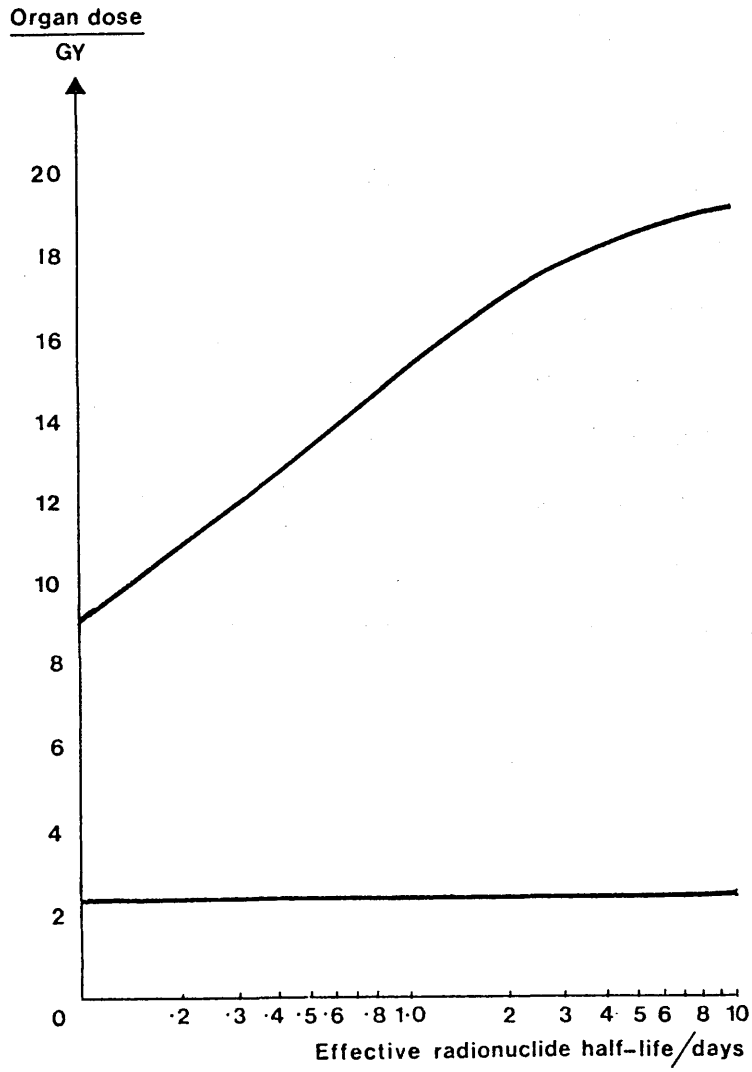

Figure 9.1

The predicted allowable doses as a function of effective radionuclide half-life. The logarithmic scale of the half-life axis should be noted. The upper curve is for a "late responding" organ with an α/β of 3 Gy and a repair half-time of 1.5 hours. "Tolerance" for this organ is taken to be equivalent to six fractions of 2 Gy. The lower curve is for bone marrow with an α/β of 10 Gy and a repair half-time of 0.5 hours. "Tolerance" for bone marrow is taken to be equivalent to a single fraction of 2 Gy.

little dose-rate effect for haemopoietic toxicity. This is due to a combination of the low repair capacity of haemopoietic cells, the low value of tolerance dose and also the assumed fast repair kinetic half-time of 30 minutes. In practice the result of this is that, from the point of view of repair, one would not expect any significant sparing of bone marrow cells by using a radionuclide-carrier combination with an effective half-life longer than a few hours.

The dose value represented by the lower curve of figure 9.1 is 2.4 Gy. This corresponds to the ERD i.e. the dose assuming all possible repair has been completed. For effective radionuclide half-lives tending towards zero time, the isoeffective dose tends towards 2.0 Gy, equal to the isoeffective single fraction dose. The equivalent dose becomes 2.2 Gy (i.e. the half-way value) for an effective half-life of 0.7 hours.

There is no allowance for repopulation in the LQ equations. For this reason the real curve for haemopoietic toxicity will diverge from the lower trace in figure 9.1 having an upward curvature at the long half-life end.

The findings for haemopoietic toxicity contrast with those for "late responding" organs. Here there is a marked dose-rate effect; 100% in the range of effective half-lives shown in figure 9.1. This is due to a combination of the higher repair capacities of late responding tissues, the higher dose equivalent of the tolerance level for these and the slower repair kinetic

half-time of 1.5 hours which is assumed. Figure 9.1 shows that the isoeffective dose for the exponentially decaying exposure can be lower than the reference fractionated dose of 12 Gy. This is because the biological effect of the fractionated schedule itself embodies a significant degree of sparing (complete repair being assumed to take place between fractions). Similarly to the case for haemopoietic toxicity, the isoeffective dose for late responders will tend towards the single fraction equivalent of 6 fractions of 2 Gy (6.4 Gy) as the effective half-life tends to zero. The isoeffective dose as the half-life tends to infinity tends to the ERD value of 20 Gy. This means that the theoretical maximum sparing factor is 20/6.4 = 3.125. The consequences for BTR are that late responders (kidney, lung, etc) will be increasingly spared by using radionuclide-vehicle combinations with longer effective half-lives. They will be spared preferentially with respect to bone marrow cells and tumours, if tumours behave more like acute than late responders. This argument neglects tumour proliferation effects, which may remove the relative benefits of longer half-lives. For BTR which is limited by marrow tolerance it is possible that the doses to organs such as liver, kidney and lung will be below the levels of clinical toxicity. If however bone marrow rescue is incorporated these organs will probably become dose-limiting and their tolerances will determine the amount of activity which can be administered.

9.3 DOSIMETRIC MODEL STUDIES OF BTR

In this section the prospects for treatment of micrometastatic disease by BTR are considered. The conclusions reached are based on purely dosimetric reasoning. Use is made of the work of Humm (1986) and Vaughan et al (1987) as well as the standard MIRD radionuclide dosimetry works (Berger, 1971; Dillman and von der Lage, 1975; Snyder et al, 1975; Loevinger and Berman, 1976). The strategies of treatment of micrometastases by antibody-targeted 131-I and 90-Y will be considered.

9.4 ANTIBODY UPTAKE IN MICROMETASTASES

In the course of their analysis, Vaughan et al (1987) surveyed published data on antibody uptake by human tumours. They concluded that uptake values were mostly very low, typically 0.005% of injected activity per gram of tumour. These data were mainly on uptake by bulk tumours, which are often composed of viable and non-viable material. Autoradiographic studies show higher uptake in viable regions. It has been suggested (Begent et al, 1986) that viable tumour cells have higher levels of antibody uptake than would be surmised from the figure of 5E-5/g which is a gross average for the whole tumour.

This viewpoint is supported by experimental studies on uptake of radiolabelled antibody by murine tumours (Moshakis et al, 1981; Menard et al, 1983; Hagan et al, 1986). These studies suggest an increase by about a

factor of 2 in specific uptake per unit mass as tumour size decreases. In the following sections the doses to micrometastases will be estimated based on both the 5E-5/g suggested by the survey of Vaughan et al (1987) and also on a figure of 1E-4/g obtained by incorporating a factor of 2 for improved uptake in micrometastases.

9.5 INJECTED ACTIVITIES OF ANTIBODY-TARGETED 131-I

The amount of radionuclide injected will be limited by the radiation dose to the bone marrow. The assumption is made that the radionuclide distribution is uniform throughout the body except in the collections of tumour cells. For present purposes the activity in the whole body is assumed to clear exponentially according to the formula

$$W = A_0 \exp\{-(m+c)t\} \qquad (9.4)$$

where W = whole body activity

A_0 = initial injected activity

c = physical decay constant for 131-I

and m = biological loss rate of activity from the body. Vaughan et al (1987) in their review of the literature suggested that a suitable value of m for antibody-targeted 131-I would be 0.23 day-1, corresponding to a biological half-life of 3 days.

The whole body radiation dose to a standard man may now be calculated using the MIRD scheme. The cumulated whole body activity

$$\tilde{W} = \frac{A_0}{(m+c)} = 2.73 \times 10^5 A_0 \quad (Bq\,s) \qquad (9.5)$$

and the whole body dose

$$D = \tilde{w} S \qquad (9.6)$$

where S is the standard value (Snyder et al, 1975) for 131-I.

$$S = 7.43 \times 10^{-16} \ Gy / Bq\,s$$

$$\therefore \quad D = 2.03 \times 10^{-10} A_0$$

The predicted allowable dose for bone marrow for an effective half-life of 2.19 days is 2.4 Gy (corresponding to a 2 Gy single fraction equivalent dose) from section 9.2. If the whole body dose must not exceed 2.4 Gy then the maximum permissable injected activity may be calculated

$$A_0 = \frac{2.4}{2.03 \times 10^{-10}} = 11.81 \ GBq$$

Therefore in order to give a whole body dose of 2.4 Gy assuming uniform radionuclide distribution 11.81 GBq (319 mCi) of antibody-targeted 131I may be given.

9.6 DOSES TO MICROMETASTASES FROM ANTIBODY-TARGETED 131-I

The dose to tumour cells is due not only to radionuclide directly bound but also to irradiation from radionuclides elsewhere in the body. This can be expressed as

$$D_{tumour} = D_{bound} + D_{non\text{-}bound} \qquad (9.7)$$

For 131-I, disintegration energy is released in the form of β-particles and γ-ray photons. The tumour dose may thus be further divided

$$D_{tumour} = D^{\beta}_{bound} + D^{\gamma}_{bound} + D^{\beta}_{non\text{-}bound} + D^{\gamma}_{non\text{-}bound} \qquad (9.8)$$

Each of these four components will be evaluated individually.

9.6.1 DOSE FROM BOUND BETA ACTIVITY

The model of Vaughan et al (1987) provides an expression for the proportion of activity per unit mass of tumour at time t after administration as follows

$$C(t) = \frac{B_0 k \left[\exp(-(k+m)t) - \exp(-lt)\right]}{(l-k-m)} \qquad (9.9)$$

where B_0 = the maximum value C(t) would reach if there was no loss from the tumour or the whole body

k = biological uptake rate of radiolabelled antibody into tumour

l = biological rate of loss of activity from the tumour and m = biological rate of loss of activity from the whole body, as previously.

From a survey of published data Vaughan et al estimated the rate constants for antibody targeted 131-I as follows:

k = 2 day-1 : l = m = 0.23 day-1

It can be shown that the time for maximal uptake

$$t_m = \frac{1}{k} \ln\left(\frac{k+m}{l}\right) \qquad (9.10)$$

B_0 is found from equation (9.9) by setting the maximum proportional activity per gram of tumour to be (a) 5 E-5, or (b) 1E-4 for an improved uptake factor of 2. The maximum proportional activity per gram Cm is given by

$$C_m = 0.69\, B_0$$

$$\therefore B_0 = \frac{C_m}{0.69} = 7.25 \times 10^{-5} \text{ (a)} \text{ or } 1.45 \times 10^{-4} \text{ (b)}$$

The activity per gram of tumour is given by

$$Y(t) = A_0 \, C(t) \, e^{-ct} \qquad (9.11)$$

where c = physical radionuclide decay constant.

The total cumulated activity is given by

$$\tilde{Y} = \int_0^\infty Y(t)\,dt = \frac{A_0 B_0 k}{(\ell + c)(k + m + c)}$$

$$= 202 \; GBq\,s \quad (a)$$

$$\text{or} \quad 404 \; GBq\,s \quad (b)$$

In order to calculate the dose the cumulated activity must be multiplied by the equilibrium dose constant. These have been tabulated for a large number of radionuclides (Dillman and von der Lage, 1975). The total equilibrium dose constant for all β-particles and Auger electrons for 131-I is

$$\Delta_\beta = 3.07 \times 10^{-11} \; gm\,Gy/Bq\,s$$

The product of the cumulated activity and the equilibrium dose constant is the absorbed dose for conditions of electronic equilibrium. This is very far from the real situation for micrometastases. Humm (1986) has published values of the absorbed fraction, ϕ for small volumes. These are shown in Table XIV

TUMOUR DIAMETER	ABSORBED FRACTION (ϕ)
200 um	0.17
1 mm	0.54
1 cm	~1.0

Diameters of micrometastases and associated absorbed fractions of equilibrium dose for 131-I

TABLE XIV

The tumour doses from β-activity bound to the tumour can now be calculated and are shown in the first line of table XV. The figures in brackets are the doses assuming an enhanced uptake by a factor of 2 in the two smaller sizes of tumour.

9.6.2 DOSE FROM BOUND GAMMA ACTIVITY

This component is considered to be negligible for micrometastases of this size.

9.6.3 DOSE FROM UNBOUND BETA ACTIVITY

This component is taken to be the dose to a spherical "cold" volume in a continuum of β-activity at an initial concentration of 0.169 MBq gm-1, calculated by assuming 100% uptake of the administered activity throughout a 70 kg total body mass. The effective radionuclide decay constant in the whole body, λ, is .316 day-1 and the cumulated activity per gram

$$\tilde{A} = \frac{A_0}{\lambda} = 46.06 \ GBq\,s$$

The contribution to the "cold" region is found by multiplying the equilibrium dose by a factor ($1-\phi$) where ϕ is the absorption factor given in Table XIV (Rossi and

Ellis, 1950)

$$D^{\beta}_{non\text{-}bound} = \frac{A_0}{\lambda} \Delta_{\beta} (1 - \phi) \qquad (9.12)$$

The doses from unbound β-activity are shown in the third line of Table XV

9.6.4 DOSE FROM UNBOUND GAMMA ACTIVITY

This is the γ-component of the whole body radiation dose. The effective photon dose constant can be calculated by multiplying the "S" value by the mass of the reference phantom (70 kg) and subtracting the equilibrium beta dose constant.

$$\Delta^{\gamma}_{eff} = 2.14 \times 10^{-11} \text{ gm Gy / Bq s}$$

The photon dose is the product of this dose constant and the cumulated activity per unit mass. This is shown in the fourth line of Table XV

Contribution to dose (Gy)	Tumour diameter 200um	1mm	1cm
D^{β}bound	1.05 (2.11)	3.35 (6.69)	6.20
D^{γ}bound	0	0	0
D^{β}non-bound	1.17	0.65	~0
D^{γ}non-bound	0.98	0.98	0.98
Dtumour	3.20 (4.26)	4.98 (8.32)	7.18

Estimated radiation dose (Gy) to micrometastases of varying diameters from 11.81 GBq 131-I labelled antibody assuming a uniform distribution of radionuclide throughout the body. The fractional uptakes in the micrometastases are 5 x 10-5/gm and 1 x 10-4/gm in brackets

TABLE XV

The results show that the doses to micrometastases lie within the range 3.2 - 8.3 Gy depending on the uptake of antibody in the smaller tumours. These doses are low, even in comparison with TBI schedules, and it would not be expected that they would be curative, even for micrometastases. To this extent the analysis supports the conclusions of Vaughan et al that significantly improved antibody uptake is necessary for tumour sterilization when antibody-targeted radiotherapy is used as a treatment modality in isolation. Vaughan et al also concluded that for BTR of bulk disease 90-Y is a superior warhead to 131-I. The use of this radionuclide will now be examined for treatment of micrometastases.

9.7 INJECTED ACTIVITIES OF ANTIBODY TARGETED 90 Y

Again assuming the radionuclide distribution is uniform throughout the body except in the collections of tumour cells, bone marrow will be the dose limiting organ. The activity in the body clears exponentially according to the formula

$$W = A_0 \exp\{-(m+c)t\} \qquad (9.4)$$

where W = whole body activity

A_0 = initial injected activity

c = physical decay constant for 90-Y = .256 day-1 (half-life = 2.71 days)

and m = biological loss rate of activity from the body.

Vaughan et al (1987) suggest that there is no biological

loss of 90 Y from the body, or effectively that the physical decay is the dominant mechanism of removal of activity (i.e. m=0 in equation (9.4)).

The whole body dose is again calculated using the standard MIRD method. The cumulated whole body activity

$$\hat{W} = \frac{A_0}{c} = 3.375 \times 10^5 \; A_0 \qquad (9.13)$$

The whole body dose

$$D = \hat{W} S$$

The "S" factor for 90-Y

$$S = 2.1 \times 10^{-15} \; Gy/Bq.s$$

$$\therefore \; D = 7.09 \times 10^{-10} \; A_0$$

Again the predicted allowable dose for the low LET emissions of 90-Y is 2.4 Gy for an effective half-life of 2.7 days.

$$\therefore \; A_0 = 3.38 \; GBq$$

Therefore in order to give a whole body dose of 2.4 Gy assuming uniform radionuclide distribution 3.38 GBq (91.4 mCi) of antibody targeted 90 Y may be given.

9.8 DOSES TO MICROMETASTASES FROM ANTIBODY-TARGETED 90-Y

The situation here differs from that for 131-I as there is no photon component in the disintegration of 90 Y. The dose to tumour cells may be expressed as

$$D_{tumour} = D^{\beta}_{bound} + D^{\beta}_{non\text{-}bound} \qquad (9.14)$$

Both components will be evaluated individually

The equation for proportional activity per gram is taken from the model of Vaughan et al (1987)

$$C(t) = \frac{B_0 k \left[\exp\{-(k+m)t\} - \exp(-lt) \right]}{(l-k-m)} \qquad (9.9)$$

where the symbols have the same meaning as for 131-I. The estimates of the rate constants for 90 Y are

$$k = 2 \text{ day}^{-1} \; : \; l = m = 0$$

Equation (9.9) can thus be reduced to

$$C(t) = B_0 (1 - e^{-kt}) \qquad (9.15)$$

The maximal value of C(t) is simply given by

$$C_m = B_0$$

$$\therefore \; B_0 = 5 \times 10^{-5} \quad (a)$$

$$1 \times 10^{-4} \quad (b)$$

It should be noted that this value of B is not the same as that used for antibody targeted 131-I. This is because Vaughan et al estimated Cm rather than B .

Actvity per gram of tumour is given by

$$Y(t) = A_0 \, C(t) \, e^{-ct} \qquad (9.11)$$

The cumulated activity is given by

$$\hat{Y} = \int_0^\infty Y(t) \, dt = \frac{A_0 B_0 k}{c(c+k)} = 50.6 \text{ GBqs (a)}$$

$$\text{or } 101.2 \text{ GBqs (b)}$$

The dose to micrometastases from bound activity is product of the cumulated activity, the equilibrium dose constant and the absorbed fraction, ϕ . From standard tables (Dillman and von der Lage, 1975) the equilibrium dose constant may be expressed as

$\Delta = 1.49 \times 10^{-10}$ gm Gy / Bqs

Humm has published estimates of ϕ for tumour diameters of 200 um and 1 mm. The estimate of ϕ for a tumour diameter of 1cm was derived from Berger, 1971 by the following method. This MIRD pamphlet deals with absorbed doses from point sources of beta activity in small volumes. The percentile absorption corresponding to a radius value of 0.5 cm was scaled by the corresponding ratio of percentile absorption to Humms' factor, ϕ for the radius value of 0.5 mm. This gives an estimate of 0.73 for ϕ for a 1cm diameter tumour. The values of ϕ for these three volume sizes are shown in Table XVI.

TUMOUR DIAMETER	ABSORBED FRACTION (ϕ)
200 um	0.015
1 mm	0.097
1 cm	0.73

Diameters of micrometastases and associated absorbed fraction of equilibrium dose for 90-Y

TABLE XVI

The doses to micrometastases from radionuclides bound to the tumour cells may now be calculated and are shown in the first line of Table XVII The figures in brackets are doses from bound activity assuming an enhanced uptake by a factor of 2.

9.8.2 DOSES FROM UNBOUND ACTIVITY

Again this is taken to be the dose to a spherical "cold" volume in a continuum of 90-Y activity at an initial concentration of 0.048 MBq gm-1 with an effective half-life equal to the physical half-life i.e. 2.7 days. The cumulated activity

$$\tilde{A} = \frac{A_0}{\lambda} = 16.31 \text{ GBq s}$$

The dose to the cold region is found from

$$D^{\beta}_{\text{non-bound}} = \frac{A_0}{\lambda} \Delta (1 - \phi) \qquad (9.12)$$

These values are shown in the second line in Table XVII.

Contribution to dose	Tumour diameter 200um	1mm	1cm
D^{β}_{bound}	0.11	0.73	5.50
	(0.23)	(1.46)	
$D^{\beta}_{\text{non-bound}}$	2.39	2.19	0.66
Dtotal	2.50	2.92	6.16
	(2.62)	(3.65)	

Estimated radiation dose (Gy) to micrometastases of varying diameters from 3.38 GBq 90-Y labelled antibody assuming a uniform distribution of radionuclide throughout the body. The fractional uptakes in micrometastases are 5 x 10-5/gm and 1 x 10-4/gm in brackets

TABLE XVII

The results of this analysis of the predicted doses to micrometastases using antibody-targeted 90-Y show that they lie within the range 2.5 - 6.2 Gy depending on the antibody uptake. For the two smaller tumour sizes the

majority of the dose is produced by a "cross-fire" effect, very little actually coming from bound radionuclide. For the 1 cm diameter tumour the estimate of ϕ was 0.73. This means that 73% of the available energy is absorbed. The tumour dose for 100% absorption would be ~ 7.5 Gy. Doses of this order are unlikely to be curative. For the largest tumour size considered the dosimetric analysis supports the contention of Vaughan et al (1987) as regards the necessary improvement in antibody uptake for the clinical use of this modality in isolation. However for the two smaller tumour sizes an increase in antibody uptake would have a small, possibly negligible effect. This is because of the highly inefficient absorption of 90-Y energy in small volumes. It must be concluded that for tumours of this size 90-Y is not an appropriate radionuclide for BTR.

9.9 DISCUSSION

The limitations of this analysis should be borne in mind. The compartmental model of Vaughan et al (1987) is simplistic, although this reflects the limited available data. In particular the assumption of uniform distribution of radionuclide throughout the body is unrealistic. This point will be returned to later.

Other radionuclides, apart from iodine and yttrium may have been considered, but these two are the most relevant at the present stage of development of BTR. The α-emitting radionuclide 211-At has several theoretical advantages, namely extreme localisation of energy deposition and

highly effective ionizing radiation. However there is no information on the effects of antibody targeted 211-At on normal tissues and consequently no guide to acceptable tolerance dose levels. The above analysis is in appropriate for 211-At for another reason which has not yet been addressed. This is to do with the problem of tumour cells which have escaped targeting, a matter which will be discussed further in chapter 10.

For the treatment of micrometastases it is apparent that 131-I is better than 90-Y. This finding is in contrast to the conclusion of Vaughan et al that 90-Y was superior to 131-I as a cytotoxic label for therapy of bulk tumours. The reason for this difference is the relatively poorer absorption of the β-energy from 90-Y. Although the energy from 131-I is not efficiently absorbed in the smallest volume considered (200 um diameter) it is much better than 90-Y. Consequently, in the next sections on strategies incorporating bone marrow rescue, only the use of 131-I as a cytotoxic label will be considered further.

9.10 THE USE OF BONE MARROW RESCUE

The tumour doses calculated to be achievable by administration of antibody-targeted 131-I are determined by the amount of injected activity which is allowed. This, in turn, is limited by the predicted allowable whole body dose. The whole body dose from BTR is important since above 2 Gy equivalent there is a sharply increasing incidence of bone marrow toxicity. The conclusions drawn from the previous section are that BTR of micrometastases

does not seem curative, for the currently achievable levels of antibody uptake, so long as the injected activity is limited by haematological tolerance. The use of bone marrow rescue would remove this obstacle. In this section the implications of using this procedure are examined for 131-I.

If bone marrow were replaced following BTR, much higher activities of radionuclide could be given. Limitation of dose would no longer depend on the risk of marrow ablation but on damage to other critical organs. The next organ to become dose-limiting will depend on the cross-targeting affinities of the delivery vehicle and the excretion route of the radionuclide. The most vulnerable organs would probably be lung (as for external beam TBI), liver, urinary tract and gastro-intestinal tract. In section 9.1 the predicted allowable doses to late responding organs equivalent to 6 fractions of 2 Gy were calculated using the LQ model and are shown in figure 9.1. Assuming the effective whole body half-life for antibody targeted 131-I remains at 2.19 days, the predicted allowable dose is 17.25 Gy to the whole body assuming uniform distribution of radionuclide. By the same reasoning as previously the injected radionuclide activity to give 17.25 Gy may be calculated. This activity is a factor of 7.2 (i.e. 17.25/2.4) higher than the activity required for a 2.4 Gy whole body dose. For a 70 kg body weight and assuming uniform distribution of radionuclide this proves to be 85 GBq (2.3 Ci). The doses to micrometastases can also be

estimated by scaling up the previous doses by a factor of 7.2. These dose values are shown in Table XVIII

	Tumour diameter		
	200 um	1 mm	1 cm
Dtotal	23.0-30.7	35.9-59.9	51.7

Estimated radiation doses (Gy) to micrometastases of varying diameters from an administered activity of 85 GBq of 131-I labelled antibody. These values are arrived at by scaling up those in table XV by a factor of 7.2

TABLE XVIII

This analysis suggests that very substantial radiation doses could be delivered to micrometastases if BTR were combined with marrow rescue. Bearing in mind that the smaller a tumour the less dose will be required for sterilisation, the achievable doses seem very encouraging. However, there would be major obstacles to this treatment strategy. The most serious of these are:

1/ The change to BTR plus marrow rescue is potentially a large one. As yet there is no clinical experience of such a procedure. For this reason caution is to be recommended. In the UKCCSG protocol for the mIBG trial for disseminated neuroblastoma, storage of autologous marrow is suggested as a precautionary measure in case of severe haemopoietic depression. This may be the route by which some clinical experience of BTR and marrow rescue may be obtained.

2/ So far in the analysis the assumption of uniform

distribution of radionuclide throughout the body has been made. This is an unrealistic assumption. Particular organs (e.g. liver, kidney, bladder) may receive considerably higher doses than suggested by the whole body average. The effect of this will be to limit the allowable injected activity (and hence tumour dose) to levels less than those of the forgoing analysis. The relative benefits of marrow rescue may therefore be rather less than average dose values would suggest.

3/ As with chemotherapy there may be "sanctuary sites" which are inaccessible to the delivery vehicle. This may be a special problem for antibodies which are of higher molecular weight than cytotoxic drugs.

4/ The quantities of radionuclide which could theoretically be given (85 GBq of 131-I) would pose very serious problems of radiation protection.

5/ It is possible that reinfusion of marrow would have to be delayed until dose-rate levels from the radionuclide fell to a low level. The time for this to occur would obviously depend on the amount of radionuclide injected. The longer the interval the greater the risk to the patient from immune suppression and prolonged aplasia. These points illustrate the potential problems likely to exist with single-agent BTR and marrow rescue. They suggest that a sudden change from a strategy of BTR without marrow rescue (2.4 Gy whole body dose) to BTR with marrow rescue (17.25 Gy whole body dose) may be too drastic. A more practicable policy may be to combine lower

levels of BTR with external beam TBI and marrow rescue. It will be suggested in the next chapter that this strategy is actually the optimal one for the treatment of systemic malignant disease. However, for the moment, only the dosimetric aspects will be considered.

9.11 COMBINED BTR WITH TBI AND MARROW RESCUE

One way of looking at the rationale for this therapeutic strategy is to consider the following: TBI consists of the delivery of a uniform dose (so far as possible) throughout the body. Any non-uniformities are caused by physical rather than biological factors (e.g. the reduced density of lung tissue). Biologically targeted radiotherapy entails biological non-uniformity of the dose distribution with, hopefully, tumour cells receiving higher doses than normal tissues. Without marrow rescue the dose from BTR is unable to sterilize even microscopic tumours. With marrow rescue we have the problems listed in the previous section 9.10. Combination of the uniform distribution and high dose of TBI with the non-uniformities introduced by BTR could lead to higher probabilities of tumour sterilization than could be achieved by TBI alone. Normal tissues which receive inappropriately high doses from BTR (e.g. liver, kidney) could be preferentially shielded during TBI.

9.11.1 ISOEFFECT RELATIONSHIPS FOR COMBINATION BTR AND TBI

The amount of radiation damage sustained by an organ or tissue can be described using the LQ model as an ERD

value. When two independent treatments are given the combined damage can be expressed by the sum of the ERDs of each individual treatment. The following considerations are valid only when the fractionated TBI and the BTR are sequential (i.e. non-simultaneous). To describe simultaneous treatments modified versions of these equations would be necessary. These are, however, presently unavailable and their derivation is beyond the scope of the present work.

The requirement for a sequential combination BTR and TBI schedule to be equivalent to a specified "reference" toxicity level is

$$ERD_{BTR} + ERD_{TBI} = ERD_{REF} \qquad (9.16)$$

this condition can be written as

$$\frac{r_0}{\lambda}\left(1 + \frac{r_0}{(\mu+\lambda)\alpha/\beta}\right) + nd\left(1 + \frac{d}{\alpha/\beta}\right) = n_r d_r \left(1 + \frac{d_r}{\alpha/\beta}\right)$$

If d = dr this may be rewritten as

$$\frac{r_0^2}{\lambda(\mu+\lambda)\alpha/\beta} + \frac{r_0}{\lambda} - (n_r - n)\, d\left(1 + \frac{d}{\alpha/\beta}\right) = 0 \qquad (9.17)$$

which is identical to equation 9.1 with n being replaced by (nr-n). The solution for D is

$$D = \frac{\sqrt{((\mu+\lambda)\alpha/\beta)^2 + 4\lambda(\mu+\lambda)(n_r - n)d(\alpha/\beta + d)} - (\mu+\lambda)\alpha/\beta}{2\lambda} \qquad (9.18)$$

The injected activity corresponding to the allowable BTR doses may now be calculated from equation 9.18. The doses to micrometastases of different sizes may also be calculated following the methods outlined earlier. This has been done as one-by-one the TBI fractions are replaced by an "equivalent" amount of BTR. All this information is shown in Table XIX

Treatment strategy		Tumour diameter		
TBI	BTR (GBq)	200um	1mm	1cm
6 X 2	-	12.0	12.0	12.0
5 X 2	15.93	14.3-15.7	16.7-21.2	19.7
4 X 2	31.00	15.1-19.2	21.1-29.8	26.9
3 X 2	45.34	18.3-22.4	25.1-36.9	33.6
2 X 2	59.05	20.0-25.3	28.9-45.6	39.9
1 X 2	72.21	21.6-28.1	32.5-52.9	45.9
-	84.87	23.0-30.7	35.9-59.9	51.7

Estimated radiation doses (Gy) to micrometastases of varying diameters from combination strategies (TBI + targeted 131-I BTR) incorporating marrow rescue.

TABLE XIX

9.12 DISCUSSION

In the present stage of development of biologically targeted radiotherapy for systemic malignant disease doses are limited by bone marrow toxicity. Analysis of the situation using the LQ model suggests that there will be little or no dose-rate effect on haemopoietic cells. Allowable whole body doses are therefore likely to be similar to those for external photon irradiation (i.e. around 2 Gy). With the present reported levels of antibody uptake in tumours BTR is unlikely to result in curative doses for both bulk disease, as reported by Vaughan et al (1987) and micrometastatic disease, as described in this chapter. This is true for both 131-I and 90-Y as cytotoxic labels. For micrometastatic disease

131-I is a superior therapy radionuclide to 90-Y. This is because of better absorption of the disintegration energy. Higher levels of BTR may be possible if bone marrow rescue is incorporated into treatment procedures. This is reasonable by analogy with external beam TBI. LQ model studies indicate that dose-rate effects due to the exponentially decaying low dose-rates from radionuclides will be significant in this case. If a uniform distribution of radionuclide is assumed, simple calculations suggest that single-agent BTR may be curative in these circumstances. However, the real situation will be rather different and there are good reasons for using a combination approach to treatment involving a proportion of BTR, a proportion of external beam TBI and bone marrow rescue. This has the potential advantage that organs receiving high doses from BTR may be shielded during TBI treatment. It appears from this analysis that the advantages of the combination BTR/TBI treatments are greatest for tumours in the 1mm - 1cm diameter range. While containing large numbers of cancer cells, these tumours are below or close to the threshold of clinical detectability, so not treatable by local modalities, and may therefore be important obstacles to cure. If this is true the combination approach may have a significant impact on patient survival in particular those for whom bone marrow rescue is feasible.

9.13 RADIATION PATHOLOGY AND MICRODOSIMETRY

In this chapter doses delivered by BTR and also by

external beam radiation were assumed to be equally effective at the microdosimetric level. The pathological damage to normal organs is related to the pattern of distribution of ionization events. Assays of normal tissue damage up till the present have been based on the patterns of ionization produced by external beam radiation. For biologically targeted radiotherapy (BTR) these patterns are quite likely to be different. Myers (1987) has pointed out the need for special microdosimetric approaches to radiation deposition in the bone marrow. Other organs like the kidney may also require a dosimetry protocol based on the to-be-observed micropathology of BTR toxicity. The detailed study of the effects of BTR on individual organ systems will be a task for the future.

10.0 MODEL STUDIES OF OPTIMAL SCHEDULING OF BTR

10.0.1 THE PROBLEM OF UNTARGETED TUMOUR CELLS

The previous work has been based on largely dosimetric reasoning. Combination of BTR with external beam TBI was introduced as a method of reducing the problems of radionuclide biodistribution and radiation protection. The calculated doses to micrometastases are average values. This neglects the dosimetric non-uniformities which exist at the microscopic level. These will assume greater or lesser degrees of importance depending on the range of ionization of the radionuclide. For instance, with a long range emitter like 90-Y there will be a large amount of cross-fire. So much so that, as was seen in the last chapter, most of the dose to micrometastases comes from cross-fire from non-bound radionuclide. On the other hand for an extremely short range emitter such as 211-At there will be little or no cross-fire. Microdosimetric fluctuations will ensure that all tumour cells will not receive the same dose. In addition to microdosimetry there will be other mechanisms in operation which will further increase the heterogeneity of absorbed dose in tumour cells and consequently the probabilities of sterilisation. Several of these mechanisms were discussed in section 8.6.1. They include:

1/ Differences in protein synthesis between tumour cells (this will be important for delivery vehicles like mIBG)

2/ Differences in antigenic expression between tumour cells

3/ Antigenic modulation

The cumulative result of the mechanisms described above will be a wide heterogeneity in the probabilities of sterilization from one tumour cell to another. The possibility exists of some tumour cells receiving virtually no radiation dose at all and being effectively untargeted. As a preliminary "thought experiment" it is easy to see that in the best case situation (i.e. where all targeted tumour cells are sterilised) the tumour cell population will regrow from those cells which escaped targeting. Using a combination of radionuclides may alleviate this to some extent but will not eliminate the problem. From this perspective the introduction of an element of TBI is an obvious step. It should be noted that the rationale presented here is different to that of chapter 9. In this case it is to sterilize those tumour cells which have escaped targeting. However, this option would only be feasible in the context of bone marrow rescue. For BTR limited by haematological toxicity the allowable doses are so small that sub-dividing them further would be unrealistic. In order to assess the consequences of untargeted tumour cells for BTR and investigate the question of optimization of combination BTR/TBI treatment a mathematical model description of the tumour cell population is required.

10.1 A MODEL FOR TUMOUR EFFECTS

To calculate the effects of BTR, possibly in combination

with TBI, on tumour cells five assumptions are made:

1/ The radiation survival curve for the tumour cells is "shoulderless". The surviving fraction of cells is an exponential function of dose and the relationship can be characterized by one single parameter DO. The value of DO used in the numerical examples is 1 Gy. This assumption is reasonable for leukaemia/lymphoma and for neuroblastoma (see chapter 5) but will be less good for some other tumours.

2/ Tumour cells proliferate exponentially with growth constant, γ .

3/ A proportion, δ, of tumour cells completely escapes targeting and receives no dose from BTR. This is a gross simplification of the real situation where there will be a spectrum of dose-rates applicable to tumour cells in different localities. The validity of the assumption will also depend on the emission range of the radionuclide used.

4/ The dose-rates in the whole body, and by implication the dose limiting organ, and also at the tumour cells can be described by simple exponential decays, although not necessarily at the same rate. This neglects the initial "accumulation" phase in tumours described by the analysis of Vaughan et al. As a measure of the specificity of the BTR the tumour to non-tumour dose-rate ratio (R) is taken; defined for the purposes of the model as the ratio of initial dose-rate at the tumour cells to that in the dose-limiting organ. If the allowable dose-rate in the

dose-limiting organ is given by

$$r_d = r_0 e^{-\lambda_N t} \qquad (10.1)$$

The dose-rate at the targeted tumour cells is given by

$$r_t = R r_0 e^{-\lambda_T t} \qquad (10.2)$$

5/ In combination treatments TBI and BTR are administered sequentially. This is due to the requirements of the isoeffect model for normal tissue damage as described in section 9.9.1. For reasons of mathematical simplicity the TBI is assumed to be given first. It will be seen that from the point of view of the tumour effects (though not damage to normal tissues) it is unimportant what order these treatments are given in, or even if they are given simultaneously.

With these assumptions a general equation for the effects of combination schedules can be constructed.

10.1.1 EFFECTS OF BTR

For convenience the tumour cell population may be split into 2 sub-populations. If the original number of viable tumour cells is N_0 then there is a number $\sigma N_0 = N_1$ which are untargeted by BTR, effectively receiving zero dose. The remaining number of tumour cells $(1 - \sigma)N_0 = N_2$ receive a uniform dose at a dose-rate of $r(t) = Rr_0 \exp(-\lambda_T t)$. The total dose delivered in a time, t for these cells is

$$D(t) = \int_0^t R r_0 e^{-\lambda_T t} dt = \frac{R r_0}{\lambda_T} (1 - e^{-\lambda_T t})$$

In the absence of repopulation effects, for the moment, the surviving numbers for the 2 sub-populations are

$$N_1(t) = N_1(0)$$

$$N_2(t) = N_2(0) \exp\left[-\frac{R r_0}{\lambda_T D_0} (1 - e^{-\lambda_T t})\right]$$

The total number of viable cells at a time t from the start of BTR is

$$N(t) = N_0 \left\{ \alpha + (1-\alpha) \exp\left[-\frac{R r_0}{\lambda_T D_0} (1 - e^{-\lambda_T t}) \right] \right\} \quad (10.3)$$

10.1.2 THE EFFECTS OF CELLULAR PROLIFERATION

Proliferation is assumed exponential with a growth constant, η . When this is included equation 10.3 becomes

$$N(t) = N_0 \exp(\eta t) \left\{ \alpha + (1-\alpha) \exp\left[-\frac{R r_0}{\lambda_T D_0} (1 - e^{-\lambda_T t}) \right] \right\} \quad (10.4)$$

It can be noted that if all the targeted cells are sterilized the overall population will regrow according to

$$N(t) = \alpha N_0 \exp(\eta t) \quad (10.5)$$

10.4 is the final equation for the effects on tumour cells of single-agent BTR.

10.1.3 THE EFFECTS OF EXTERNAL BEAM TBI

For mathematical convenience it is assumed that the TBI is given first. For times longer than the duration of the TBI schedule the surviving number of tumour cells is given by

$$N(t) = N_0 \exp\left(\eta t - \frac{n d}{D_0}\right) \left\{ \alpha + (1-\alpha) \exp\left[-\frac{R r_0}{\lambda_T D_0} (1 - e^{-\lambda_T (t-\tau)}) \right] \right\} \quad (10.6)$$

Where d = fraction size of the TBI schedule

n = number of TBI fractions

τ = duration of the TBI schedule = (n-1) x IT

Where IT = inter-fraction interval

For times shorter than the duration of the TBI schedule

$$N(t) = N_0 \exp\left(\eta t - \frac{n' d}{D_0}\right) \quad (10.7)$$

Where n' = INT (t/IT) + 1

INT denoting the integer part of the expression in

brackets.

The pair of equations 10.6 and 10.7 describe the time course of sterilization of tumour cells for a combination BTR/TBI schedule. However, it is more convenient to use the log cell kill function

$$T(t) = -\log_{10}\left(\frac{N(t)}{N_0}\right)$$

10.2 PARAMETER VALUES USED IN THE CALCULATIONS

A value of 1 Gy was taken for D0, as has been stated already. This is appropriate for radiosensitive tumour cells such as leukaemia/lymphoma or neuroblastoma. A value of 4 days was taken for the tumour cell population doubling time. The effective half-times for loss and decay of radionuclide were taken to be 2.4 days for both the dose limiting organ and tumour respectively. This is intermediate between the values for antibody targeted 131-I and 90-Y values, based on the review of Vaughan et al, 1987. In reality these values may be expected to vary, depending on factors such as tumour morphology, the identity of the dose-limiting organ and whether antibody fragments or second antibody clearance are used. In this model the intensity of BTR is determined solely by the tumour to non-tumour ratio, R. For the computer simulation this parameter was allowed to vary from 1.0 to 10.0 in steps of 1.0. It may be noted that the ratio of effective half-lives plays a similar role to the tumour to non-tumour ratio. This is because the tumour to non-tumour dose ratio is directly proportional to the ratio of

half-times. Variations in the latter ratio may be simulated approximately by varying the tumour to non-tumour dose-rate ratio, R. For example the following situation; tumour:non-tumour = 3, $t_{1/2}$(tumour) = 4.8 days, $t_{1/2}$ (organ) = 2.4 days: is approximately equivalent to; tumour:non-tumour = 6, $t_{1/2}$(tumour) = 2.4 days, $t_{1/2}$(organ) = 2.4 days. The proportion of untargeted tumour cells, σ, was allowed to vary by factors of 10 from 1 to 1 E-10.

10.2.1 SINGLE-AGENT BTR LIMITED BY HAEMOPOIETIC TOXICITY

The allowable whole body dose calculated in section 9.1 to be equivalent to a single fraction of 2 Gy (LQ model: α/β = 10 Gy; $T_{1/2}$ (repair) = 0.5 hour) was 2.4 Gy. The initial dose-rate corresponding to this dose and an effective radionuclide half-life of 2.4 days is 0.69 Gy/day. As uniform distribution of radionuclide is assumed this will also be the initial dose-rate in the dose-limiting organ.

10.2.2 ALLOWABLE BTR-TBI COMBINATION SCHEDULES

In Section 9.9.1 allowable combination schedules of antibody targeted 131-I BTR and external beam TBI were calculated. The criterion was that they were all isoeffective to a fractionated schedule of 6 X 2 Gy on the basis of the LQ model (α/β = 3 Gy; $T_{1/2}$ (repair) = 1.5 hrs). The BTR component was expressed as the injected activity in GBq. These values were arrived at by first calculating the allowable whole body dose assuming uniform distribution of radionuclide. The whole body doses are not only applicable to 131-I but also to any low LET emitting

radionuclide with an effective half-life of 2.19 days (the value used in the calculation). They would not be applicable to a high LET emitter like 211-At for which tolerance doses and isoeffect models are not available. The effective half-lives for different combinations of carrier molecule and radionuclide would be expected to vary. As described in 10.2 rather than carry out a separate calculation for every value an approximation is made. If a value of 2.4 days is chosen this is intermediate between the effective whole body half-lives of antibody targeted 131-I and 90-Y according to the review of Vaughan et al. The allowable BTR/TBI doses calculated for this half-life would therefore be reasonable approximations for both radionuclides. This information is shown in table XX along with the corresponding initial whole body dose-rates.

TBI COMPONENT	BTR DOSE (Gy)	r_o (Gy/day)
6 X 2	-	-
5 X 2	3.24	0.94
4 X 2	6.33	1.83
3 X 2	9.27	2.68
2 X 2	12.10	3.49
1 X 2	14.81	4.28
-	17.43	5.03

Allowed schedules for combined TBI and BTR when bone marrow rescue is used. Doses and dose-rates to the whole body are shown. The effective radionuclide half-time is taken as 2.4 days as described in the text.

TABLE XX

The optimization principle is as follows: a clinically used external beam TBI schedule (6 X 2 Gy) is taken as representing a certain acceptable level of toxicity. This toxicity level can be achieved in the various ways described above depending on the proportions of BTR and TBI. Some of these combinations will produce a greater tumour effect than others. The combination schedule parameters which maximize the tumour effect, while not exceeding the toxicity level of the "reference" TBI schedule identify the optimal schedule.

10.3 RESULTS OF COMPUTER SIMULATIONS

10.3.1 SINGLE-AGENT BTR LIMITED BY HAEMOPOIETIC TOXICITY

The maximum tumour log cell kills, Tmax, as a function of (R, δ) are plotted in figure 10.1. It can be seen that

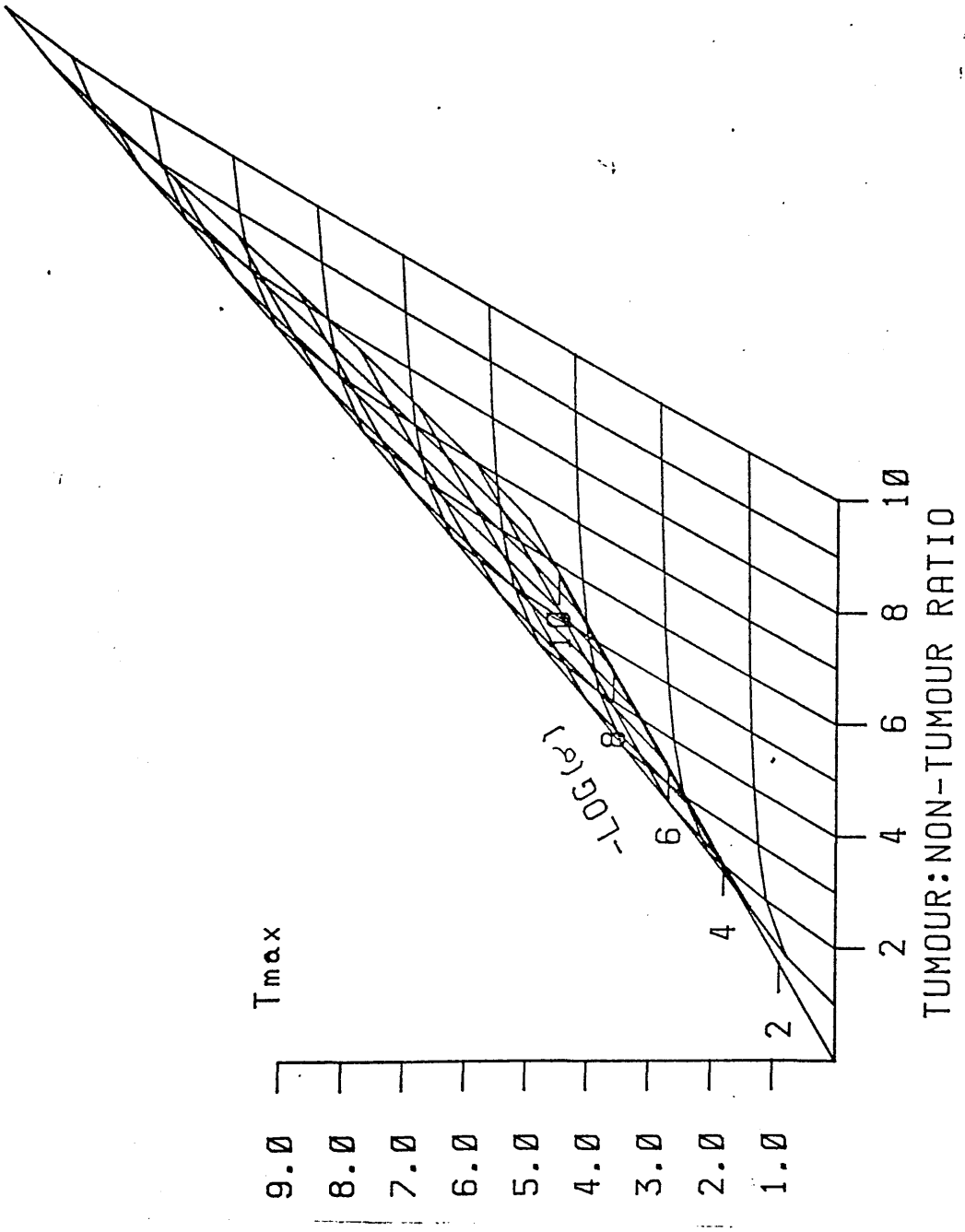

Figure 10.1

Maximum log cell kill values produced by single agent BTR as a function of the tumour:non-tumour ratio and $-\log(\sigma')$, where σ' is the proportion of effectively untargeted tumour cells.

this quantity increases as the tumour:non-tumour ratio increases and as the proportion δ decreases. Tmax reaches a value of around 9 at the limiting values of R = 10 and δ = 1E-10.

10.3.2 COMBINED BTR/TBI AND MARROW RESCUE

Figure 10.2 is an illustrative example of the time course of the tumour log cell kill function T(t). This is for (R, δ) = (3,0.01), meaning that the initial tumour to dose-limiting normal tissue dose-rate ratio is 3 and the proportion of untargeted tumour cells is 0.01. The graph shows 7 curves, corresponding to the 7 combination schedules isoeffective to a fractionated schedule of 6 X 2 Gy. The combination schedules contain from 0 to 6 fractions of external beam TBI (fraction size 2 Gy). It may be seen that the schedule with 5 fractions of TBI produces a higher peak T value than all the others. This schedule is therefore optimal.

This approach may be used to calculate peak T values and identify the optimal combination schedules for different values of R and δ . The consequence of this is that the optimal schedules can be mapped as a function of (R, δ). Figure 10.3 shows the optimal combination schedules of BTR and TBI as a function of R and δ. The optimal schedules are indexed by the number of TBI fractions (out of a possible 6) which are included in the combination. The corresponding quantities of BTR may be derived from table XX. In moving from small values of R and -log(δ) to larger values, progressively more BTR and progressively

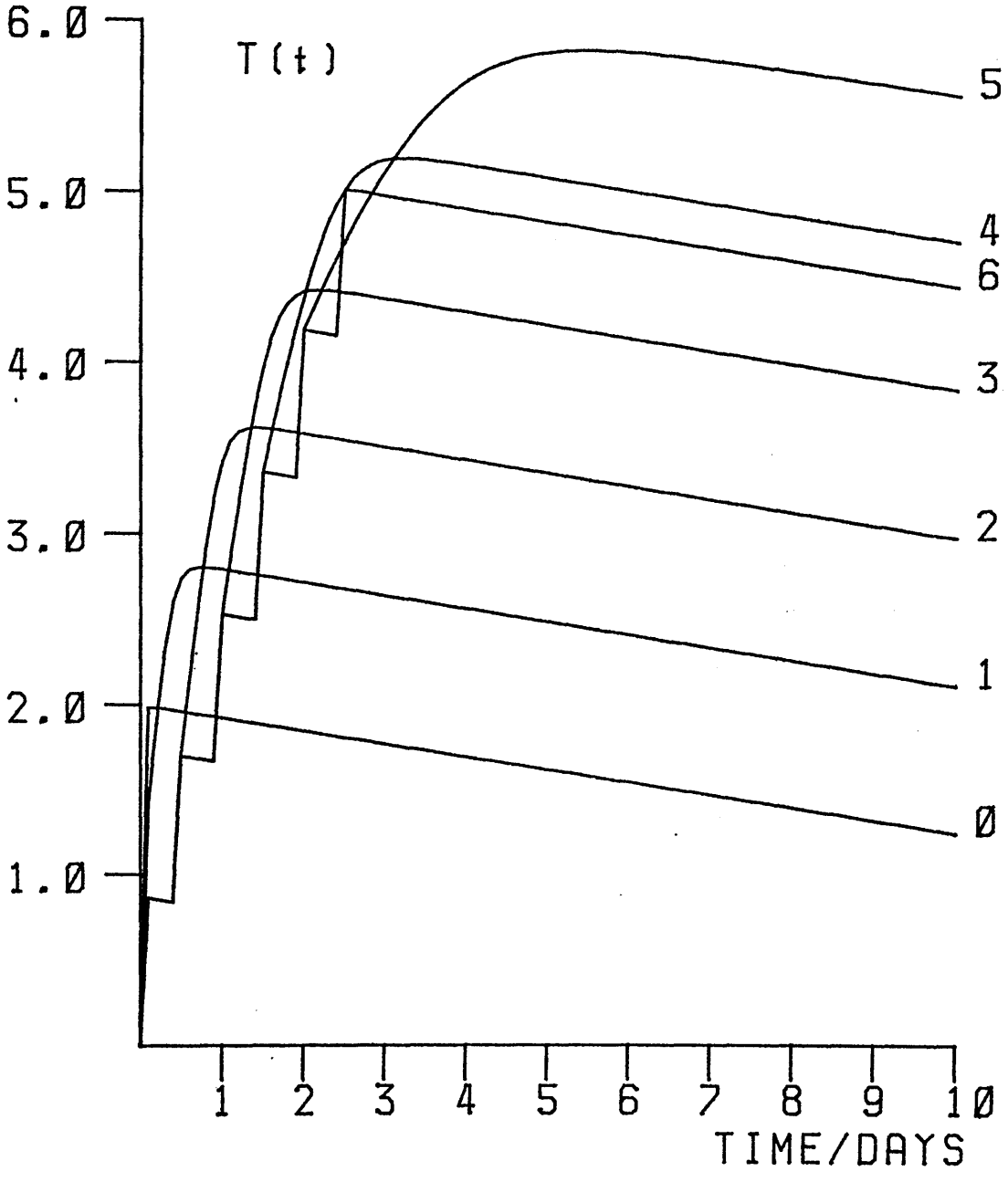

Figure 10.2

The time course of sterilization of tumour cells for allowable combinations of TBI and BTR for the case of bone marrow rescue. Schedules are indexed by the number of fractions of TBI included. This graph is for a tumour:non-tumour ratio of 3 and $\log(\sigma) = -2$. The schedule which contains 5 fractions of TBI is optimal in this case.

TUMOUR:NON-TUMOUR RATIO

-LOG(σ)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
0	6	6	6	6	6	6	6	6	6	6
1	6	6	6	5	5	5	5	5	5	5
2	6	5	5	5	5	5	5	5	5	5
3	4	5	5	5	5	5	5	5	5	5
4	3	4	5	5	5	5	5	5	5	5
5	2	4	4	5	5	5	5	5	5	5
6	2	3	4	4	5	5	5	5	5	5
7	1	3	4	4	4	5	5	5	5	5
8	0	3	4	4	4	5	5	5	5	5
9	0	2	3	4	4	4	5	5	5	5
10	0	2	3	4	4	4	4	5	5	5

Figure 10.3

Optimal combinations of BTR and TBI in the case of marrow rescue indexed by the number of TBI fractions.

less TBI make up the optimal schedules. Figure 10.4 shows the predicted effects of the optimal schedules on the tumour cell population. It is apparent that the Tmax values are substantially higher than those shown in figure 9.2 for BTR alone limited by bone marrow toxicity.

10.4 DISCUSSION

The two main parameters which determine the effects of the combination schedules on tumour cells are:

R, the tumour to non-tumour ratio, defined as the ratio of the initial dose-rate at the tumour cells to that in the dose-limiting normal tissue. This parameter can be thought of as representative of the specificity of the biological targeting.

δ, the proportion of untargeted tumour cells. This may be thought of as representative of the sensitivity of the biological targeting. In the model the factor δ determines the proportion of tumour cells which will never be sterilised by BTR no matter how intense. In the situation where $\delta = 1$, i.e. 100% of tumour cells are unaffected by BTR it is obvious that external beam TBI alone will be optimal. If $\delta = 0$ i.e. 100% of tumour cells are effectively targeted it follows that BTR alone will be optimal, assuming the specificity is high enough. For intermediate values of δ it seems reasonable that some combination of BTR and TBI will be optimal. The results of figure 10.3 are consequently not too surprising. The factor δ ultimately limits the curative potential of BTR. Reduction of the surviving fraction of targeted cells to below the

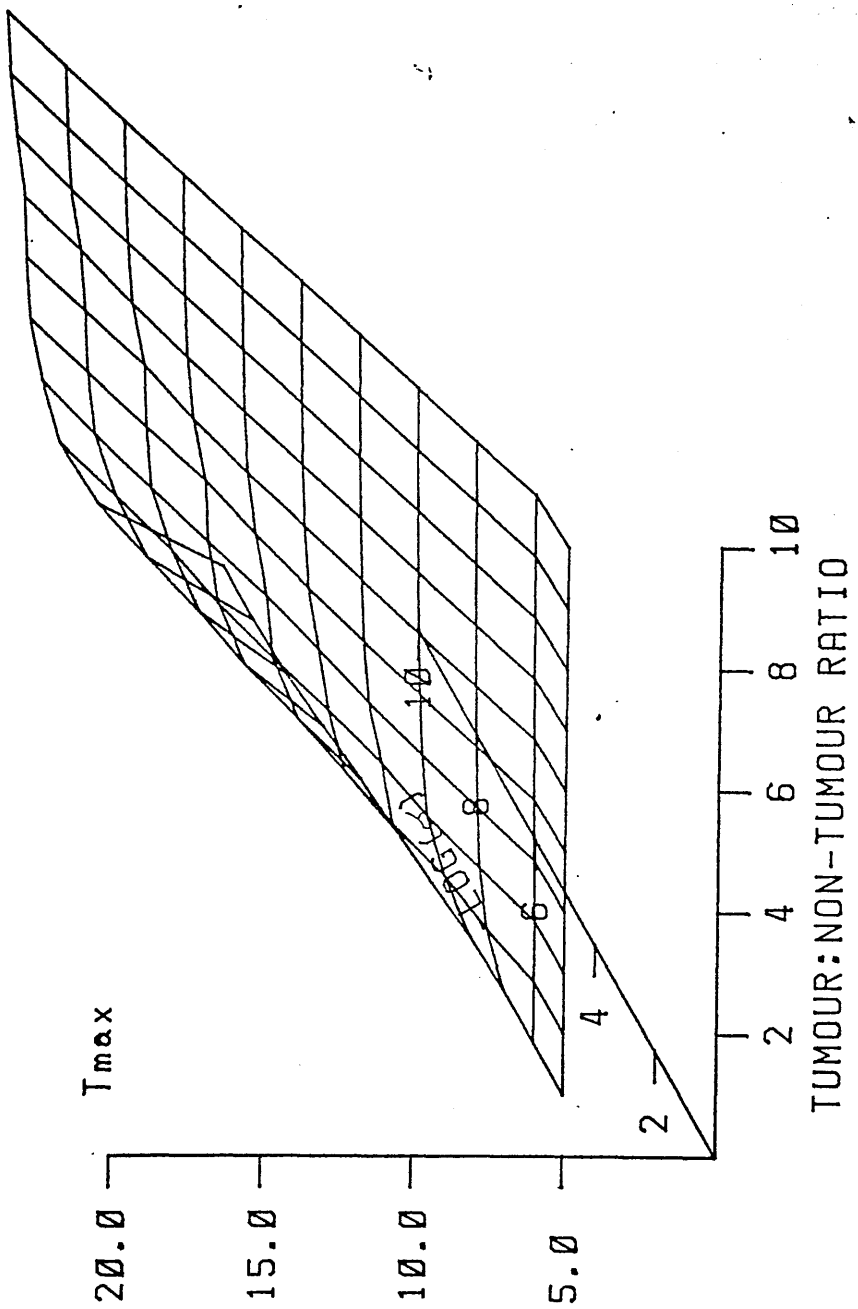

Figure 10.4

Maximum log cell kill values produced by the optimal BTR/TBI combination schedules as a function of the tumour:non-tumour ratio and $-\log(\sigma)$, where σ is the proportion of effectively untargeted tumour cells.

overall level of surviving fraction of untargeted cells is wasted therapy or "overkill". In such a case it is better to exchange a proportion of BTR for some TBI. The "overkill" effect is the main mechanism in determining optimal combination schedules. It was seen that for BTR limited by bone marrow toxicity potentially curative effects were obtained by treatments having not only high specificities, as predicted by several authors, but also high sensitivities, a necessity which has not received much attention to date. When the model was used to examine combination strategies with BTR, TBI and marrow rescue it was found that treatments incorporating elements of both BTR and TBI were usually optimal. The actual amounts of BTR and TBI depended on the values of the specificity and sensitivity of the BTR.

It is important to recognize that the model studies reported here incorporate a high level of approximations and simplistic assumptions. In particular uniform distributions of radionuclides throughout the body are not realistic. For the tumour cells a simple two state dose-rate function is a gross simplification. One would expect a far more complex probability distribution of dose-rates for the population of tumour cells throughout the body; however the possibility of biologically untargeted tumour cells seems highly likely. For this reason it is anticipated that the tumour model will be of most direct relevance to radionuclides with short range emissions where the problem of tumour cells escaping

effective targeting is likely to be most severe. This would be for short range beta emitters like 199-Au and 67-Cu and alpha emitters such as 211-At. However the normal tissue isoeffect model will not be directly applicable to radionuclides which emit α particles. In order to accomodate these further assumptions about RBE values would be necessary. At present the information is not available to address this question. However one may speculate that optimal schedules for BTR with α-emitters will also be mixed. For long range beta emitters like 90-Y and photon emitters like 131-I it is unlikely that there will be any completely untargeted tumour cells, although there may be some with low levels of effective targeting. It seems likely that mixed schedules will be optimal in this case too. It may also be borne in mind that the analysis of chapter 9, albeit from a different perspective, indicates that combination TBI/BTR treatments with marrow rescue would be of benefit here.

11.0 AREAS FOR FURTHER WORK

The work reported in this thesis draws together several strands of radiobiological and clinical study. There are many areas where further work is required.

In normal tissue radiobiology it is still not clear what are the underlying biological processes responsible for distinguishing the response of different organs and tissues to radiation. Work is required to elucidate the general principles of what is happening. The hypotheses of Michalowski (1981), Zeman and Bedford (1984) and Schultheiss et al (1987) are starting points. Mathematical studies need to rigorously determine the predictions of the different models and design experimental tests of these. The radiation responses of individual organs will have to be studied on an individual basis. As yet, theoretical normal tissue radiobiology remains confined to an underlying philosophy where cells are considered as independent, interactionless, self-contained entities. This is essentially unrealistic, the intercommunication and interrelationships of cells of the same and different lineages via a variety of messengers are at the heart of organised tissue function. A wider expansion of normal tissue radiobiology from a cellular to a more integrated approach is warranted.

In tumour radiobiology there is a gulf between models like the multitarget and the biological processes involved in cellular sterilisation. Newer repair models like the LPL are more realistic. As in the case of normal tissues,

current models are unicellular. Experimental work to investigate the role of intercellular factors and mathematically to fully integrate these and the processes covered by the "4 Rs" is required.

The LQ model suffers from the deficiency that there is, as yet, no time factor to take account of proliferation. The kinetics of repair need clarification as to whether a monoexponential, biexponential or some other sort of functional description is most appropriate. The studies reported in this work which use the LQ model yield conclusions which may have to be revised in the light of modifications to the model. Experimental tests of Dale's extension of the LQ model to exponentially decaying dose-rates may be possible. Animal experiments could be designed to compare isoeffect doses for late toxicity given as high dose-rate fractions with those given with exponentially declining dose-rates.

In Clinical TBI the question of the value of T-cell depletion in allogeneic marrow tranplantation must be resolved. Are the cell populations responsible for GvHD and the antileukaemic effect the same? If not, the role of TBI may have to be reconsidered in this context. There is still no proof of the benefits of tumour cell purging in autologous transplants. Likewise, the role of high dose TBI with marrow rescue for disseminated diseases like neuroblastoma, Ewing's Sarcoma, small cell carcinoma of lung, carcinoma of breast and ovarian carcinoma has not been established. Methods of creation and use of

non-uniform dose distributions may be worth investigating. The database of in-vitro radiosensitivities of human tumour cells needs expansion. This will determine whether extrapolation from the laboratory to the clinic is justified. It will also set the scene for the use of individual assays of radiation response as a basis for clinical decision making. The predicted optimal schedules deduced in chapter 7 provide candidates for clinical investigation. These investigations may determine whether the approach is a useful one.

There is enormous scope for further work on the subject of biologically targeted radiotherapy. Improvements in the specificity and sensitivity of treatment, design of alternative vehicle molecules, use of more appropriate radionuclide warheads and targeting of c-onc products, all call for a multidisciplinary programme of research. Individualised microdosimetric models of organ systems based on patterns of radiation damage may well be required. The work reported here suggests that animal experiments and clinical investigation of the use of marrow rescue in conjunction with BTR should be performed. In the future the long term consequences of BTR including induction of second maligancy must be evaluated. Single photon emission computed tomography (SPECT) may be required for clinical dosimetry.

Modification of the LQ model for use in BTR should allow the calculation of the biological effects of exposure when the dose-rate is an arbitrary function of time. This will

also enable situations involving simultaneous fractionated and continuous treatments to be considered. Multicellular tumour spheroids provide a realistic in-vitro model for the test of dosimetric model predictions in the case of micrometastases.

On the subject of optimal scheduling of combined BTR and TBI, more realistic model descriptions of the distribution of dose-rates to tumour cells are required.

The role of mathematical modelling can only be to supplement clinical and experimental investigation. However the use of mathematical model studies may lead to otherwise unforeseen benefits or suggest new directions for clinical investigation. Some of these may prove to be false trails; however if a proportion lead to significant clinical progress the process may be judged a success.

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