M.D. THESIS

# A QUANTITATIVE STUDY OF HYPERTHERMIA IN HUMAN TUMOURS

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CONTENTS

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Abstract

A review of published clinical data shows that there is a substantial increase in complete response rate, from an average of 30% to 70%, when heat is added to radiation. In the present study, the interaction of heat and radiation has been investigated quantitatively in measurable superficial human tumour metastases. Ninety-six nodules, in 10 patients, have provided evidence of enhancement of radiation response by the addition of heat 3-4 hours after a single dose of radiation.

Growth delay, tumour regression rate, maximum volume reduction and time to maximum response have been calculated from growth curves obtained from ultrasonic and caliper tumour volume measurements. Thermal enhancement ratios (TER) have been derived from growth delay measurements. The greater effect of heat plus radiation, compared with radiation alone, was reflected in thermal enhancement ratio (TER) values ranging from > 1.6 - 4.2. Nodules subjected to the combined treatment regressed more rapidly than those receiving radiation alone with median tumour volume halving times of 15.5 and 70 days respectively. The total volume reduction was also greater in heated nodules and the nadir values were observed later compared with nodules receiving radiation alone.

Xenon clearance and thermal wash-out techniques were used to examine the effect of a standard hyperthermia treatment (43°C minimum temperature for 60 minutes) on small, superficial tumours. There was no evidence of vascular destruction and in most instances tumour and normal tissue behaved similarly with a transient increase in blood flow immediately after heating.

With the radiofrequency system employed, 54% (30/56) of nodules were successfully heated to the prescribed level (43°C for 30 or 60 minutes). The median volume of successfully heated nodules was significantly smaller than that of the failures ($p = 0.002$). Temperature distribution was homogeneous in 73% of nodules, but non-uniform in 27%. The potential relevance of these observations to the clinical situation is discussed.

The observations on tumour response are encouraging and suggest that prospective clinical studies of sequential radiation - heat therapy should be undertaken, although there are no quantitative data of the type described in the present study available for fractionated treatment schedules.

# PART I

# LITERATURE REVIEW

Chapter 1

# HYPERTHERMIA AS AN ANTI-TUMOUR AGENT

# CHAPTER 1

## 1.1 Introduction

Interest in hyperthermia as an antitumour agent stems from a clinical observation made in 1866 when a histologically diagnosed sarcoma regressed following an episode of erysipelas (Busch, 1866). Subsequently, 10 out of 23 patients with malignant tumours were reported to have a complete tumour regression after intentional induction of erysipelas (Coley, 1893). The development of a bacterial extract to stimulate fever in cancer patients however was less successful, both in terms of efficacy and toxicity. Despite this, the possibility of utilising anti-cancer properties of heat has been pursued for more than a century. Activity has intensified over the past 25 years as the biological potential of heat has been elucidated by the application of radiobiological techniques, in vivo and in vitro. However, concurrent technical advances in heat delivery and thermometry have not been able to overcome the anatomical and physiological constraints of tumour heating and have severely limited clinical application.

The inadequacies of tumour heating, in terms of severity and uniformity of temperature distribution, have directed interest towards an adjuvant role for hyperthermia. Present clinical exploitation of hyperthermia relies on its combined application with radiotherapy or chemotherapeutic agents. Although published clinical data is still insufficiently precise to delineate the contribution of heat in this

context, there is growing evidence to support the possibility of a therapeutic gain.

## 1.2 The Biological Basis for Clinical Hyperthermia

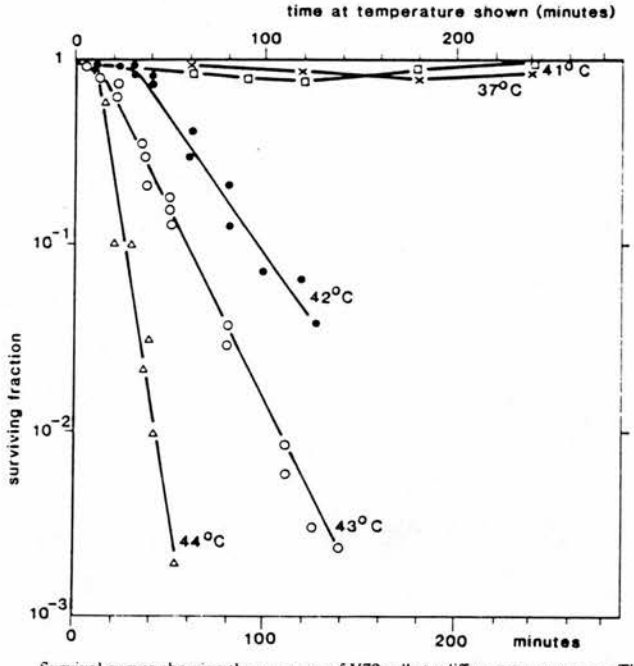
### 1.2.i Hyperthermia as a Single Agent

Heat kills mammalian cells in a predictable, repeatable manner that is both temperature and time-dependant (Henle & Dethlefsen, 1980, Westra & Dewey, 1971). For this reason, the concept of a "thermal dose" requires consideration of both parameters. Of the several attempts to define thermal dose, minutes equivalent at 43°C is the most generally accepted, referring exposures at different temperature levels to a standard reference temperature (43°C) (Sapareto & Dewy, 1984). In theory, such a system allows comparison between heat applications, an important maneouvre in clinical hyperthermia where delivery of a standard, reproducible heat dose is impossible at present. Numerous in vitro and in vivo animal experiments have demonstrated that with increasing temperature, the time required to produce a given level of cell kill is reduced and, at least for the approximate temperature range 42-46°C, this can be expressed as a simple relationship; for every degree centigrade rise in temperature treatment time can be halved to produce the appropriate iso-effect (Field & Morris, 1983). However, the validity of this relationship has not been confirmed for the conditions encountered in clinical hyperthermia.

Heat damage can be quantified by clonagenic cell assay and the resultant survival curves are remarkably similar to those obtained following irradiation with an initial shoulder followed by an exponential decrease in survival ( Fig. 1).

Figure 1

Survival Curves Showing the Effect of Time and Temperature on Response of Cells In Vitro




Survival curves showing the response of V79 cells to different temperatures. Time at temperature is plotted against surviving fraction. × 37°C, □ – 41°C, ● 42°C, ○ 43°C, △ 44°C.

Time at temperature is plotted against surviving fraction

Although individual cell types vary widely in their sensitivity to heat (Dewey et al, 1977), there is no consistent difference in response between normal and

malignant cells (Hahn, 1982). Despite this, a clinically useful tumour : normal tissue differential can be expected because micro-environmental conditions and tumour kinetics strongly influence the heat effect. Thus, cells' sensitivity to heat is greatest in areas of nutritional deprivation and low PH, conditions which arise more commonly within tumour than in normal tissue probably as a result of the aberrant, vulnerable tumour-neovasculature (Gerweck, 1977, Overgaard & Bichel, 1977). Also, enhanced sensitivity of S phase cells (Westra & Dewey, 1971) may allow selective killing of faster cycling tumour cells, with the relative protection of slowly proliferating normal cells responsible for late tissue damage.

The possibility of selectively heating tumour, compared with surrounding normal tissue, may also contribute to a differential effect. In animal and clinical studies intra-tumoral temperatures, higher by several degrees compared with adjacent normal tissue, have been described (Kim et al, 1982). Less efficient heat dissipation resulting from poor tumour perfusion may be responsible (Jain et al, 1979), but the limited published tumour blood flow data, especially from clinical studies, do not allow the generalisation that tumour perfusion is less than that in normal tissue. More important may be the ability of normal tissues to vasodilate in response to heat, with consequent increased blood flow, whereas the tumour vasculature can be

considered to be in a state of maximum vasodilation, and therefore unable to adapt to stimuli (Mattsson et al, 1980).

The precise mechanism of heat cell kill remains unknown. Nevertheless, cell damage is often histologically evident immediately after heating and is usually expressed within a matter of hours (Overgaard & Overgaard, 1975, Hill & Denekamp, 1982, Coss & Dewey, 1983). Diverse functional, morphological and biochemical changes have been described in cells following exposure to heat, but the biological basis for cell death is poorly understood. For example, heat interferes with membrane function (Kwock et al, 1978, Yatvin, 1977), inhibits metabolism (Streffer, 1982) and macromolecular synthesis (Henle & Leeper, 1979) and alters cytoskeletal conformation (Lin et al, 1985), and the possible implications of these lesions have been reviewed (Leeper, 1984).

Heat cytotoxicity may rely on more than one lethal cellular mechanism. Cells heated in S-phase for example, show marked thermal sensitivity which is not expressed until after the subsequent mitosis, whereas $G_1$ cells mostly die before mitosis (Coss & Dewey, 1983). The correlation of chromosome aberrations with S-phase cell killing supports the implication of a DNA-related mechanism, whereas this is probably not applicable with $G_1$ cells (Dewey et al, 1980). Wong & Dewey (1982) have described an increase in single stranded regions of DNA as a result of heat induced inhibition of replicon initiation and retardation of DNA

chain elongation. This could permit exchange between DNA molecules and produce chromosome aberrations (Warters & Stone, 1983). The enzymes believed to be responsible for these DNA abnormalities may be made relatively deficient by the accumulation of non-histone protein on the nuclear matrix/nuclear membrane. This accumulation seems to be a constant dose related effect of heat (Roti Roti & Winward, 1978, Warters & Roti Roti, 1982).

Cells exposed to heat develop a transient resistance to further temperature elevation known as thermotolerance (for review see Field & Anderson 1982). This phenomenon is also described in murine normal and tumour tissues (Law, 1981, Neilson & Overgaard, 1982), but it has not yet been demonstrated in a clinical setting. However, the recent demonstration of thermotolerance in human tumour cell lines and xenografts indicates the likelihood that the process is universal and must be considered in clinical application (Rofstad et al, 1984, Rofstad & Brustad, 1984). At temperatures below 43°C, thermotolerance develops during the heating process, but at higher temperatures it tends to appear shortly after completion of heating. In both cases, decay of tolerance is slow, taking 17-14 days in most normal tissues, but there is some evidence that it may occur more rapidly in tumours, a property which could enhance tumour sensitivity relative to normal tissues (for review see Urano, 1986).

### 1.2.ii Hyperthermia Combined with Irradiation

Heat and radiation are effective in combination because of preferential toxicity on different cell populations. Thus, those cells most resistant to radiation, such as hypoxic, S-phase and low PH cells, are at least as sensitive, if not more sensitive, to heat than the general cell population (Westra & Dewey, 1971, Gerweck & Rottinger, 1976, Hahn, 1974). Therefore, a potentially useful additive effect can be achieved irrespective of the sequencing and scheduling of the two modalities as no true interaction is involved.

Conversely, hyperthermia also has a radiosensitising effect, as the result of a direct interaction between the two modalities (for review see Dewey et al, 1977, Hahn, 1982). Enhanced radiation damage is expressed by an increase in the slope ($D_0$), and decrease in extrapolation number (n) of the radiation cell survival curve (Dewey et al, 1977). As with direct heat toxicity, thermal radiosensitisation is most marked in S-phase cells and consequently this radioresistant population can be made more radiosensitive than $G_1$ cells. After heating, loss of activity of the enzyme DNA polymerase beta correlates well with radiosensitisation but it is not clear whether the decreased activity is due to alteration of chromatin structure, by for example non-histone proteins, or to a direct effect on the enzyme (Jorritsma et al, 1984).

Because thermal radiosensitisation involves a true interactive process it is critically dependent on sequencing, and on the time interval between the two modalities. Maximal

sensitisation is achieved with simultaneous application and any separation of the two components decreases the effect until, with intervals of 3-4 hours, it is reduced virtually to zero (Dewey et al, 1977, Denekamp et al, 1980, Overgaard, 1980). Unfortunately, hyperthermic radiosensitisation may occur similarly in normal tissue and tumour which limits the usefulness of the interaction unless selective tumour heating operates, or normal tissues can be excluded from the heat application.

### 1.2.iii Hyperthermia Combined with Chemotherapy

Hyperthermia and cytotoxic chemotherapy can be applied in combination to exploit different effects; heat modification of the drug effect or drug modification of the heat effect. In either case, the outcome, potentiation or protection, is to some extent dictated by the sequencing of the two modalities. Of these possible interactions, hyperthermic chemopotentiation has been the most investigated and as a result, several mechanisms of action for the process have been described. These include increased free drug level, increased cell uptake, induction of activation enzymes and inhibition of repair (Dahl, 1986).

In clinical practice, whole body hyperthermia provides a means of achieving chemo-potentiation in metastatic disease. The combination of chemotherapy with local or regional hyperthermia allows for possible therapeutic enhancement of tumour effect, relative to normal tissues, thus promoting a therapeutic gain.

## 1.3 Heat Delivery in Clinical Practice

Intense technical activity has generated a versatile range of heating techniques in an attempt to accommodate diverse clinical situations. Obviously, selection of the most appropriate method necessitates clinical assessment of the required treatment volume and physical consideration of the optimal means of heating the region. Each heat producing modality has its own physical properties, and every tumour possesses unique characteristics in terms of size, depth and local anatomy so that individual methods may have specific application or limitations in a given setting (Perez, 1983).

The clinical extent of heating is broadly divided into systemic (whole body), regional or localised hyperthermia. The methodological options and clinical application will be considered for each below, and the results, in terms of tumour and normal tissue effects, will be summarised in the next section.

### 1.3.i Whole Body Hyperthermia (WBH)

Currently, there are three main approaches to systemic heating:

(a) Direct skin contact

(b) Power deposition using externally applied non-ionising radiations.

(c) Extra-corporeal perfusion

Regardless of technique, 42°C is considered the safe upper limit of core temperature elevation and is usually monitored by oesophageal, rectal and bladder thermometers.

Above 42°C the incidence of toxicity increases sharply and, depending on the method of heating, a number of side effects may become evident. Whole body hyperthermia toxicity, along with other aspects of the biology of systemic heating, has recently been reviewed (Robins & Neville, 1984).

The narrow temperature range for therapeutic effectiveness and safety, and the serious side effects associated with overheating, make the ability to control heat deposition a major consideration in selecting a technique for whole body heating. At present, extra corporeal perfusion seems to offer the greatest potential for rapid and accurate temperature control (Parks, et al, 1979). In this system, femoral arterial blood is exteriorised, pumped through a heat exchanger and returned via the femoral vein. High flow through-put and freedom from rate-limiting heat transfer interfaces permit rapid control of temperature.

In contrast, externally applied power-absorption heating methods employ non-ionising radiation to raise core temperature. Microwave antennae (Pomp, 1978) and infra-red lamps (Heckel, 1975) have been used successfully to achieve systemic heating. Recently, a radiant heat device has been described (Robins et al, 1985) which allows target temperatures to be achieved within approximately 60 minutes, and which avoids the need for general anaesthesia and endotracheal intubation. Other authors, however, consider general anaesthesia to be essential to the safety and efficiency of whole body hyperthermia (Cronau et al, 1984).

The simplest approach to whole body hyperthermia is direct contact heating, in which the skin is encased in heated fluid, such as water, melted wax or air, and conductive and convective processes then permit heat transfer into the body. The practical clinical alternatives are hot wax baths as pioneered by Pettigrew et al (1974), heated water blankets (Barlogie et al, 1979, Larkin et al, 1977) and, as an extension of the latter, the space suit (Bull et al, 1978).

Disseminated malignancies are generally refractory to conventional therapy. The necessity for an effective systemic treatment approach in this situation provides a potential therapeutic role for whole body hyperthermia. With a safe upper temperature limit of 42°C, this form of heating is likely to prove more useful when combined with chemotherapy and/or radiation than when used as a sole agent. The potential of chemo-thermotherapy has been outlined in Section 1.2.iii and clinical results will be reviewed in a following Section (1.4.ii).

### 1.3.ii Localised and Regional Hyperthermia

Because localised and regional treatments employ the same heating modalities they will be considered together. In any case, in current clinical practice the distinction between the two is rather arbitrary, with the term "localised" and "regional" usually implying superficial and deep heating respectively. Ideally, systems should be sufficiently adaptable to achieve adequate heating throughout

a treatment volume at any site, regardless of depth within the body. At present the bulk of clinical information concerns superficial tumours and reports of deep heating are sparse.

Current clinical practice relies on two major modalities for tissue heating; electromagnetic radiation [radio-frequency (RF) and microwave (MW)] and ultrasound (US).

(a) Electromagnetic Heating

Radio-frequency and microwave regions of the electromagnetic spectrum cover frequencies between 300KHz and 300GHz but the clinically useful range is limited to 1MHz to 10GHz, corresponding to wave lengths in air of 1,000M to 1mm. Electromagnetic energy in this frequency range can be transmitted through, absorbed by and reflected within the body tissues. Lower frequencies allow better tissue penetration whereas higher frequencies facilitate focusing. In general, the requirements for uniform heating of deeper-seated tissues are best met by the lower RF frequency. On the other hand, the enhanced focusing provided by higher microwave frequency allows better differential heating of tumour compared with normal tissue. Both radiofrequency and microwave are suitable for interstitial, intracavitary and deep heating.

(i) Interstitial Heating

The attraction of delivering heat interstitially lies in the potential to improve 'uniformity', and to selectively deliver high temperatures to tumour. Radiofrequency and microwave have both been adapted to provide safe and effective interstitial heating but the details of application such as material for implantation and thermometry, geometry and probe spacing differ.

Radiofrequency in the range 0.5 - 1MHz generates resistive heating by electric currents driven between pairs of electrodes (Cosset 1984, Cosset et al, 1984, Emani et al, 1984a, Lilly et al, 1983, Manning et al, 1982, Vora et al, 1982, Yabumoto & Suyama, 1984). Acting as electrodes, conventional brachytherapy implant needles can be made more comfortable for the patient by incorporating a plastic terminal portion at either end. Additionally, the "metallic-plastic" probes allow heating to be confined within the metallic portion so that, by selection of 'active' lengths, the surrounding normal tissues can be spared (Cosset et al, 1984).

The alternative is to use radiative diathermy by means of coaxial microwave antennae which can be inserted into the plastic catheters commonly used for brachytherapy implantation (Coughlin et al, 1984, Emami et al, 1984a, Gidman, et al, 1984, Wong et al, 1984.

Interstitial hyperthermia is no longer employed as a sole agent and is mostly used in association with brachytherapy; "interstitial thermo-radiotherapy". However, the combination of interstitial heating with external irradiation (Yabumoto & Suyama, 1984) or with external and interstitial irradiation is being explored.

(ii) Deep Regional Heating

Interstitial techniques have proved effective for small to moderate sized (50-250 mls), accessible volumes but are highly invasive and unsuitable for multifractionated courses or for treatment of inaccessible, large, irregular tumours. Recently, the possibility of overcoming some of the constraints of conventional interstitial techniques has been proposed by magnetic induction heating of implanted ferromagnetic seeds (Lilly et al, 1985, Stauffer et al, 1984). Seeds are surgically implanted within the treatment volume and later heated by the magnetic fields created within a concentric coil positioned around the patient. Once the seeds are in position, heat can be delivered repeatedly. Both constant power, and constant temperature seeds have been evaluated in theoretical and animal studies with the latter providing better temperature distributions (Matloubieh et al, 1984).

Several non-invasive electromagnetic deep heating systems are currently being evaluated, but in general the long wavelengths, large applicator size and broad

surface area coverage necessary for adequate penetration result in unfocused regional heating (Oleson, 1982, Turner, 1984, Song et al, 1986). Clinically, the techniques have mainly been evaluated in thoracic, abdominal and pelvic tumours with, as yet, no convincing evidence of selective tumour heating (Storm et al, 1985, Abe et al, 1986, Sapozink et al, 1986).

(iii) Intracavitary heating

Warm saline irrigation provides the simplest technique for intracavitary heating and lends itself to intravesical application (Hall et al, 1974). The concurrent promotion of intravesical chemotherapy, and the recognition of synergism between heat and radiation, has made thermal chemotherapeutic intravesical irrigation a logical union. Unfortunately, clinical studies have not yet determined whether this is an effective treatment (Kubota et al, 1984).

Intracavitary applicators, generally designed to operate at microwave frequencies, are available for hyperthermia treatment of hollow viscera and, in the light of radiotherapy experience, gynaecological malignancies are an obvious area for their assessment. Consequently, carcinoma of the uterine cervix supplies much of the available data, which reports encouraging tumour responses but proper evaluation of the technique must follow (Tian et al, 1984, Kohno et al, 1984).

Endoscopic introduction of radiofrequency and microwave antennae is an alternative approach for attempted tumour heating in accessible hollow viscera. The feasibility of endotract heating has been assessed in a series of oesophageal tumours and appears to have been well tolerated (Sugimachi et al, 1984). However, as intratumoural temperatures were not measured and as radiation and chemotherapy were given simultaneously with heat, the authors' conclusion that hyperthermia is a highly effective treatment for oesophageal cancer is not justified.

(b) Ultrasound Heating

The capacity of ultrasound to heat tissues is long established, but its comparability with other heating methods for the induction of hyperthermia in superficial animal and human tumours was only demonstrated in the late 1970s (Marmor & Hahn, 1978). At frequencies of 0.3-10MHz the mechanical friction set up by molecular vibrations in tissues is transformed into heat. At frequencies useful from the point of view of penetration into tissue, the corresponding wave lengths permit beam focusing, with the result of greater deposition of energy at tumour depth compared with overlying and surrounding normal tissues. This feature endows ultrasound with a potential advantage over other heating modalities, but its clinical application is limited by high absorption in bone, and by the acoustic mismatch at gas/tissue interfaces (Lele, 1985). The advantages and disadvantages are summarised in Table 1 and contrasted with those of electro-magnetic heating.

TABLE 1

ADVANTAGES AND DISADVANTAGES OF ELECTROMAGNETIC AND ULTRASOUND HEATING TECHNIQUES

| | ELECTROMAGNETIC | ULTRASOUND |
|---|---|---|
| Advantages | 1. Widespread clinical application<br>- Propagates through<br>- not hindered by bone<br>- MW penetrates deeply into low water-containing tissues such as fat | 1. Deep penetration possible. |
| | 2. Large volumes encompassed by multiple applicators or phase-arrayed MW | 2. Focusing possible |
| | 3. Suitable for non-invasive or invasive application. | 3. No significant fat-muscle interface reflection |
| | | 4. Non-interactive with thermometry |
| Disadvantages | 1. Normal tissue overheating<br>- Fat, due to large reflections from fat-muscle interface<br>- Hydrate tissues, due to EM absorption in water-containing tissue | 1. Limited clinical application<br>- no transmission through air.<br>- High bone absorption.<br>- Large reflection bone tissue interface.<br>- Acoustic impedence at air-tissue interface |
| | 2. Limited depth of dose with single applicator | |
| | 3. Focusing difficult | 2. Reflected energy cannot be focused |

## 1.4 Clinical Achievement with Hyperthermia

As yet there is no conclusive evidence that hyperthermia has any impact on cancer cure rates. However, this may not so much reflect the effectiveness of hyperthermia as the nature of the available clinical material, which in most experience involves advanced tumours with a high likelihood of systemic disease. Patient prognosis may also preclude examination of local control rates and late normal tissue effects, and necessitate the introduction of other end points for evaluating hyperthermia. Some, such as symptom palliation, have clinical use, but biological inferences cannot be drawn from parameters such as response rate.

Although the advantage of adding hyperthermia to other treatment modalities has not been established, the unique properties of this agent warrant optimism. As heat is most effectively delivered to poorly vascularised regions of tumour, in which microenvironmental conditions favour enhanced heat cytotoxicity, hyperthermia should provide a means of delivering treatment to those areas of tumour which may harbour radioresistant cells and have poor drug access. Clinical benefits may result from applying hyperthermia as a local treatment, systemic agent, or by combining the two approaches. It is conceivable that tumours resistant to conventional treatment, such as radiation, have a higher likelihood of metastatic disease, so that attempts to improve local control must be accompanied by intensified systemic treatment for any worthwhile survival effect. However, at certain sites, such as cervix, ovary, oropharynx and

colorectum, it has been estimated that improved local treatment is likely to be more effective than the introduction of systemic treatment in terms of potential increase in survival (Suit, 1982).

Survival aside, what has clinical effort established regarding the optimal utilisation of hyperthermia and its role in cancer management? Alone, hyperthermia produces impressive tumour responses, but is an inadequate treatment and is more likely to make a useful contribution to management in combination with radiotherapy or chemotherapy (Meyer, 1984). Further discussion is therefore confined to hyperthermia in an adjuvant setting.

Phase I studies have established that therapeutically useful temperatures can safely be achieved locally or systemically, and in combination with drugs or radiation. Local heating has mostly involved the external application of heat to superficial lesions, and in this situation toxicity has been defined and minimised. Attempts to improve heat treatment in terms of temperature achieved and uniformity of dose have also led to the development of interstitial techniques and intracavitary devices.

### 1.4.i Results of Localised Hyperthermia Combined With Radiation

Published results from centres using combined heat and radiation are difficult to interpret collectively. Heterogeneous groups of patients, often heavily pre-treated,

tend to have been given combination treatment in a non-standardised fashion. However, despite the suboptimal clinical setting an impressive and consistent increase in complete response rate, from an average of 30% after radiation alone to an average of about 70% following combined heat and radiation, has been demonstrated (Overgaard, 1982). Although the reported increase in complete response rate is encouraging, this end-point in itself does not provide information about the severity of treatment, nor on the possible advantages of heat and radiation in terms of survival and local control.

Information on long term response in comparable lesions after single or combined modality treatment is available in a limited number of series, and provides an opportunity to establish thermal enhancement ratios (TER). In experimental animal tumours, values for TER are derived from ratios of radiation response giving the same biological effect in the absence or presence of heat (Robinson et al, 1974). Construction of dose response curves from clinical data is difficult, hence TER values have usually been derived from comparison of tumour responses to the same dose of radiation with or without tumour heating (Kim et al, 1982, Archangeli et al, 1983, Scott et al, 1982). Patients with multiple lesions provide an opportunity to assess a range of radiation dosages in the same tumour, to obtain dose response information, and calculate Thermal enhancement ratios along conventional lines (Overgaard & Overgaard, 1984). A TER so

derived may be considerably smaller than those deduced from comparison of responses (Gillette, 1982).

Thermal enhancement of radiation response is, at worst, comparable with the enhancing effect of chemical hypoxic-cell sensitisers and, at best, is considerably greater (Table 2). This effect should be accentuated by defining optimum schedules for combination treatment, in biologically suitable tumours.

The influence of sequencing of the two modalities has been demonstrated in animal tumours, where simultaneous administration achieved a 67-75% cure rate compared with only 20% after sequential treatment (Emami et al, 1984b, Mittal et al, 1984). The clinical relevance of the biological rationale proposed in these studies is supported by the observation of a greater TER in human tumours after "simultaneous" rather than sequential treatment (Overgaard & Overgaard. 1984). The pattern of enhancement was similar in the surrounding skin with a TER of 1.4 after simultaneous application whereas sequential treatment only enhanced the tumour response. The study concluded that although tumour effect was maximal with simultaneous treatment no therapeutic gain was achieved, whereas the lesser tumour effect with sequential treatment resulted in a therapeutic gain factor of 1.3. Clinical advantage of the maximum

TABLE 2

CLINICALLY DERIVED THERAPEUTIC ENHANCEMENT RATIOS FOR CHEMICAL RADIOSENSITISERS AND HYPERTHERMIA

| Reference | Tumour type | Response Endpoint | TER:- Value | TER:- Ratio of |
|---|---|---|---|---|
| A. CHEMICAL RADIOSENSITISERS | | | | |
| Thomlinson et al 1976 | Sq Ca Cervix | Growth delay | 1.2 | R/T dose |
| Dawes et al 1978 | Teratoma | Growth delay | 1.2 | R/T dose |
| Ash et al 1979 | Osteosarcoma ) | Growth delay | 1.2) | R/T dose |
| | Leiomyosarcoma) | | >1.5) | |
| | Sq Ca ) | | 1.1)<br>1.3) | |
| | Ca Prostate ) | | >1.5) | |
| B HYPERTHERMIA | | | | |
| Scott et al 1982 | Carcinoma<br>melanoma | CRR | 2.2<br>1.3 | Response |
| Kim et al 1982 | Melanoma | CRR | 2.3<br>1.4<br>1.5<br>1.4 | Response |
| Overgaard & Overgaard 1982 | Melanomas | CRR | 1.5<br>1.3 | R/T dose |
| Archangeli 1983 | Melanoma & others | CRR | 1.74<br>2.05<br>1.79<br>2.63 | R/T dose |

R/T = Radiotherapy
CRR = Complete response rate

sensitising effect of heat can therefore only be utilised where the tumour is susceptible to selective heating. Any interval between radiation and heat markedly reduces hyperthermic radiosensitisation until, by 3-4 hours, true interaction ceases and direct heat cytotoxicity is solely responsible for the heat effect. The tumour micro-environment provides optimal conditions for hyperthermic cell kill, so that where normal tissue is included in the heated volume sequential application ensures a therapeutic gain.

Arcangeli (1983) has compared four different schedules of combined radiation and hyperthermia, and, on the basis of these results has emphasised the variation in thermal enhancement, in tumour and skin, and the therapeutic gain that can be obtained by modifying the therapeutic protocol. Although the magnitude of enhancement varied, it was consistently greater in tumour than normal tissue irrespective of radiation fraction size or whether hyperthermia immediately followed irradiation or was delayed four hours. Comparative hyperthermia studies of this type are difficult to execute but provide valuable indications of the relative benefits of different treatment regimes. However, the derived information does not necessarily translate to other treatment settings because of the strong dependence on the heating technique employed and lesion characteristics. It is particularly dangerous to extrapolate from results obtained in superficial lesions to the potential benefit of deep tumour heating.

Hyperthermic therapeutic advantage is hindered by inadequate, non-homogenous tumour temperatures, compounded by poor normal tissue sparing. Since the suggestion that interstitial techniques might overcome these problems of heat delivery and localisation, and provide an ideal vehicle for combination with irradiation, several institutions have developed and evaluated systems (Vora et al, 1982, Cossett, 1984, Coughlin et al, 1983). The principles of the technique are discussed in Section 1.3.

Until now, clinical assessment of interstitial thermo-radiotherapy has largely been confined to patients with superficial relapses in previously irradiated areas. Despite this, the procedure appears to be well tolerated. Rapid tumour necrosis is reported with the formation of open wounds, but healing is usually complete (Emami et al, 1984a). Occasionally, however, extensive painful necrosis has occurred (Cosset, 1984).

Evaluation of tumour response from pooled published data is confined to examination of complete and partial response rates and there are no studies comparing effect in comparable lesions treated with brachytherapy alone. A complete response rate of 65%, from a total response rate of 83%, may now be a rather conservative estimate of effect since this encompasses the entire interstitial experience and includes early, less reliable prototypes (Cosset, 1984). The enormous variation in radiation and thermal dose between, and within, series has allowed limited investigation of dose response

relationships, but supports the influence of both minimum tumour temperature and radiation dose on biological effect (Aristizabel & Oleson, 1984).

At present, interstitial hyperthermia seems to offer greater uniformity of heating in non-surface lesions than any other available technique. In combination with interstitial brachytherapy, which as sole or partial primary treatment can achieve local control rates of 70-100%, it may be possible to demonstrate a therapeutic advantage at least in terms of long-term control rate.

Phase I trials have identified prognostic variables which should be incorporated into the design of Phase II and III protocols (Oleson et al, 1984). Comparison of response by histology has not shown any consistent correlation, although irradiation doses have probably been chosen to compensate for histological radiosensitivity. The influence of tumour volume on response to combined treatment, is conflicting. There is, however, emerging evidence that, although large volumes are more difficult to treat, they are more likely to benefit from the addition of heat to radiation (Kim et al, 1982, Dewhirst et al, 1984). With external heating systems, the depth of tumour below the skin surface is an important variable which effects the ability to heat tumours and can be translated into poorer response Perez et al, 1984). Minimum tumour temperature provides the best correlation of temperature with response (Oleson et al, 1984) and confirms earlier or similar

observations in large animal spontaneous tumours (Dewhirst et al, 1984).

### 1.4.ii Results of Whole Body Hyperthermia Combined with Systemic Chemotherapy (Thermo chemotherapy - TCT)

Phase I and II studies combining WBH with systemic chemotherapy agents have demonstrated the feasibility of this approach. However, extracting information regarding tumour response from these studies is hazardous, but has been attempted by Engelhardt (Engelhardt et al, 1983, Engelhardt, 1985). Of 377 patients treated in 11 centres, positive effects were described in 147. Little information is available from the remainder as in many instances the terms used to describe response were too imprecise for collective assessment.

Initial studies indicate that whole body hyperthermia may be particularly promising in tumours with a high growth fraction such as small cell bronchogenic carcinoma and high-grade non-Hodgkin's lymphoma. A pilot study in small cell carcinoma has perpetuated the encouraging climate (Engelhardt, 1983) and has initiated a randomised trial from which results are awaited. Interim data suggests that the addition of WBH has improved response rates and nearly doubled the median duration of response.

The design of future randomised trials should be improved by a better understanding of drug-temperature interaction in individual tumour types. Present information

on scheduling and the pharmaco-dynamic effects of heat are inadequate for logical planning of further treatment.

### 1.4.iii Results of Regional Hyperthermic Chemotherapy Perfusion

Regional isolated perfusion was introduced in 1957 and modified in 1967 to incorporate heat (Creech et al, 1958, Stehlin, 1969). Its main clinical application has been in limb perfusion for malignant melanoma and sarcoma, both as an adjuvant treatment in high-risk patients and in patients with overt disease. Although more than 3,500 patients have undergone this procedure there are no controlled studies to evaluate the technique and the clinical role remains to be defined.

The contribution of heat to the effects seen with thermochemotherapy has been indicated in two studies comparing hyperthermic with normothermic perfusion (Martijn et al, 1981, Cavaliere et al, 1982). Both implied improved results with hyperthermic treatment. No further statement regarding hyperthermic limb perfusion should be made without the benefit of randomised studies and fortunately these are now under way.

## 1.5 Optimisation of Heat Delivery and Effect

After twenty years of clinical effort it is still not clear what contribution, if any, hyperthermia can make to cancer management. Continued allocation of resources and intensification of effort require some degree of confidence

in the likely advantages of combining heat with radiation or chemotherapy, in terms of survival, local control and early and late normal tissue damage. These issues can only be satisfactorily addressed in Phase III studies. Unfortunately, current technical limitations of thermometry and heat delivery make the design and implementation of stringent protocols unattainable, but the alternative of imprecise, non-comparable treatments is unsatisfactory However, while technical developments proceed, it seems valid to assess what the best heating presently available can achieve in the standardised clinical setting of a trial, although it would be inappropriate to attempt to answer biological questions with such a system. The European Society for Hyperthermic Oncology (ESHO) is presently attempting to set up Phase III studies in advanced carcinoma of the breast, advanced neck nodes and malignant melanoma.

Radiation dose is a critical consideration in the design of combined modality trials. Anticipating heat potentiation of normal tissue effects, a reduction in conventional radiation dose might be considered appropriate. However, this assumes a uniform biological heat effect throughout the treated volume, but this is unlikely in the face of inhomogeneous clinical heating. For example, with most localised heating devices, temperatures in adjacent normal tissues, where micrometastases are likely to occur, infrequently reach temperatures adequate for potentiation of the radiation effect. Therefore, it is vital that the inadequacy of the hyperthermic contribution to treatment of

micrometastatic disease is taken into account when determining the radiation schedule. In many Phase I trials of combined hyperthermia and radiation, not only has the radiation dose been sub-optimal, but, in addition, unconventional radiation dose/fractionation schemes, with unproven adjuvant efficacy, have been employed. Future trials should be designed to combine hyperthermia with the best conventional radiotherapy.

However it may be that, in combination with heat, alternative radiation dose/fractionation schedules would be more effective, at least in certain tumour types. Therefore, concurrent with Phase III studies employing conventional radiation practices, these possibilities should be explored in carefully designed Phase II studies.

There are two major approaches to optimising the heat effect; technical development and biological manipulation.

### 1.5.i Technical Developments

An ideal thermometry system would provide continuous temperature measurement to the nearest 0.1°C, have spacial resolution of < 1cm. and be well tolerated by patients. A non-invasive system is the only means of encompassing these criteria and a number of techniques are currently being explored for their potential to provide images of thermal distribution. There are two possible approaches to this; the first involves the detection of thermal radiation images from tissue, but is limited to superficial situations, and

the second concerns the measurement of heat-sensitive tissue properties. At present, the latter is the main focus of investigation in deep tumour thermometry. Ultrasound (Husson et al, 1982), nuclear magnetic resonance (Parker et al, 1983, Parker, 1984) and computerised transaxial tomography (Fallone et al, 1982, Cetas, 1984) are all interesting possibilities which require further evaluation to ensure that they can provide the necessary accuracy and spacial resolution and that they are compatible, in terms of synchronous function, with the commonly used heating methods. The practical problem of simultaneously employing the bulky equipment required both for heat delivery and temperature measurement can only really be overcome in specially designed dual-function machinery.

The invasive nature of current thermometry systems severely limits the number of measurement points available during heat delivery. However, description of temperature distribution throughout a region of interest has been attempted by interpretation of information available from implanted thermometry probes. Thus, thermal modelling relies on applicator power deposition pattern and tissue characteristics to infer an entire treatment volume temperature based on measurement at only a few points. Present models make many assumptions, and the availability of an accurate technique for measuring tissue perfusion would make a major contribution to improving the validity of these analytical methods.

The ability to measure tumour blood flow would offer additional advantages. Firstly, it would allow the selection of tumours according to their potential 'heatability', and secondly it would provode a means of assessing the effect of vasoactive drugs aimed at decreasing tumour blood flow in an attempt to further facilitate selective tumour heating.

### 1.5.ii Biological Manipulation

The sequence of critical cellular events involved in heat killing, heat radiosensitisation, heat chemopotentiation and thermal tolerance need to be determined, and points of reversibility and irreversibility defined. This would allow identification of targets most likely to enhance the heat effect in tumour or to diminish normal tissue response. The possibility of selective modification of events within tumour promises a potential therapeutic gain.

The strong influence of microenvironmental conditions on heat cell toxicity offers an opportunity for altering the effectiveness of hyperthermia by manipulation of the internal milieu. Accordingly, attempts to lower intratumoural PH have been pursued by, for example, the infusion of glucose (Jahde & Rajewsky, 1982a). The relationship between serum glucose concentration and intratumoural PH was carefully examined with the finding that intratumoural PH could be reduced from about 6.9 to 6.2 by maintaining the serum glucose concentration at 50 mM. More intriguing was the finding of a reduced cell survival in tumours heated to 42.2°C for one hour after attaining a serum

glucose concentration of 50 mM (Jahde & Rajewsky, 1982b). The greatly enhanced thermotoxicity of some chemotherapeutic agents, such as BCNU, in the presence of low PH further supports the notion that efforts to reduce PH in human tumours may be a worthwhile manoeuvre.

Selective reduction of tumour blood flow should provide improved tumour heating and induce those micro-environmental conditions most favourable to cytotoxicity. Experimental work in animal tumours suggests that a significant fall in tumour blood flow can be achieved by a number of pharmacological manoeuvres, and this is discussed in Chapter 2. As yet there is no clinical data supporting the feasibility or usefulness of this approach.

The biological implications of thermotolerance in clinical heating have not yet been determined. Although thermotolerance in tumour cells may diminish the anticipated cytotoxic effect of subsequent heat treatments, its development in normal tissue may be protective and provide a means of augmenting the therapeutic efficacy of localised hyperthermia. So far it has not been possible to identify exploitable differences in the process of thermotolerance in tumour and normal tissue, and until the mechanism is better understood it is probably best to avoid thermotolerance altogether by allowing sufficient time between heat fractions for its complete decay.

Chapter 2

# TUMOUR BLOOD FLOW

## 2.1. Introduction - The Clinical Implication of Tumour Blood Flow

Sustained growth of a solid tumour requires provision of a tumour microcirculation. The necessity for this renders a tumour potentially vulnerable and has raised the possibility of targeting treatment towards the tumour neovasculature (Denekamp, 1982). Alternatively, there are sound theoretical reasons why tumour blood flow may be responsible for decreased effectiveness of established treatments. For example, with radiotherapy, chemotherapy and hyperthermia, the disorganised aberrant tumour vasculature, with its consequent metabolic heterogeneity, probably plays a central role in determining treatment failure. Thus, hypoxic tumour regions harbour cells which enjoy a degree of protection from radiation injury (Thomlinson & Gray, 1955) and similarly, nutritionally deprived cells exhibit resistance to cycle specific chemotherapy agents (Twentyman, 1976, Valeriote & van Putten, 1975). The distribution of systemic agents between tumour and normal tissue depends on the relative vascular perfusions. Variable blood flow within a tumour makes drug access unreliable in necrotic, poorly nourished, areas (Goldacre & Sylven, 1962). With hyperthermia the inhomogeneity of tumour blood flow makes it difficult to achieve uniform heating within a treatment volume (Gullino et al, 1982).

It would therefore seem reasonable to suggest that harnessing tumour blood flow might either increase the efficacy of existing treatments or provide a novel means of

destroying tumours. Thus, selective manipulation of tumour blood flow should enable optimal conditions to be provided for application of different treatment modalities. Additionally, tumour:normal tissue differentials could be exaggerated to exploit a therapeutic gain. Alternatively, inhibiting angiogenesis or destroying existing tumour vessels is an intriguing approach to tumour therapy.

## 2.2 Angiogenesis

The provision of optimal conditions for tumour cell proliferation requires an intimate relationship between individual tumour cells and their nutritive capillaries. Correlation between tumour cell proliferation rate and capillary proximity has been demonstrated using tritiated thymidine to calculate a labelling index (Tannock, 1968). At increasing distance from open capillaries, a decrease in the labelling index of malignant cells was demonstrated. Initiating and sustaining angiogenesis is therefore a critical tumour process, but is not unique to malignant tissue. Neovascularisation accompanies wound healing, chronic inflammatory conditions (Fromer & Klintworth, 1975) and certain immune reactions (Sidky & Auerbach, 1975). Inflammatory cells, such as activated macrophages and lymphocytes, have strong angiogenic activity and presumably play a prominent role in these conditions. From study of experimental systems, the difference between angiogenesis induced by tumour and non-tumour tissues seems to be quantitative rather than qualitative.

Neoplastic cells acquire, or reveal, angiogenic activity early in the process of transformation from the normal cell state, before the morphological stigmata of neoplasia become evident (Gimbrone & Gullino, 1976a & b). In mouse and rat mammary gland, the frequency with which hyperplastic lesions undergo neoplastic transformation can be predicted from their angiogenic capacity (Maiorana & Gullino, 1978). Similarly, hyperplastic lesions of human breast display angiogenic activity before the onset of histological malignancy (Brem et al, 1978; Chodak et al, 1980).

Humoral mediators of angiogenesis have long been suspected (Ide et al, 1939), but detection of soluble angiogenic factors in animal and human tumour homogenates is more recent (Folkman et al, 1971). The active agent in these crude angiogenic tumour extracts has been termed "tumour angiogenesis factor" or TAF, although the precise structure had not been identified. A chondrosarcoma-derived growth factor which stimulates capillary endothelial cell migration and proliferation has now been purified and its activity in vivo confirmed (Shing et al, 1984 and 1985).

The sequence of events involved in angiogenesis has been reconstructed from light microscopic observations (Clark & Clark, 1939; Warren & Shubik, 1966) and confirmed by electron microscopic studies (Warren et al, 1972). The literature is rich in detailed descriptions of the minutiae of the process (Schor & Schor, 1983; Folkman 1974). Briefly, new vessels arise from pre-existing, but altered, "host"

vessels in which endothelial proliferation produces buds which emerge as capillary sprouts. These generally advance along the course of least resistance, although tumour type has some influence on the architectural arrangement of the new vascular network (Goodall et al, 1965; Hirano & Zimmerman, 1972). Anastomoses between adjacent capillary sprouts create capillary loops.

Once neovascularisation has been established it must be maintained and updated so that in fact the capillary network is a dynamic system constantly undergoing remodelling, retraction and degeneration. To achieve this, endothelial turnover must be rapid and proliferation rates of 30-40 times those seen in normal vessels have been described in experimental tumours by Denekamp & Hobson (1982).

## 2.3 Morphology and Architecture of Tumour Neovasculature

A morphological classification of tumour vessel types is given in Table 3. The capillary is of fundamental interest being the nutritive structure which dictates the availability and exchange of nutrients, metabolites and chemical substances. Because of their rapid construction, tumour vessels often represent immature vascular channels, devoid of an external basement membrane and lined by a single layer of epithelial cells. In parts, cords of tumour cells may entirely constitute the vessel wall (Willis, 1960). Random fusion between capillary sprouts produces a tortuous capillary network conducive to sluggish blood flow which is further impeded by dilatations and sacculations within the

vessel walls. Episodes of complete stasis have been observed in an experimental tumour system, making it likely that flow, at least in some regions of tumour, is not only inefficient but also intermittent (Reinhold, 1971, Suzuki et al, 1982).

TABLE 3

MORPHOLOGICAL CLASSIFICATION OF BLOOD VESSELS FOUND WITHIN TUMOUR

| |
|---|
| Arteries and arterioles |
| Capillaries with basement membrane |
| Capillary sprouts |
| Sinusoidal vessels - interrupted endothelial lining |
| Blood channels without endothelial lining |
| Giant capillaries |
| Capillaries with fenestrated endothelium |
| Venules and veins |
| Arterio - venous anastomoses |

Expansion of the neovasculature occurs concurrently, but not necessarily equally, with tumour growth so that the delicate vessels become distorted, elongated and compressed by rising extravascular pressure. If this process leads to complete cessation of flow, necrosis follows in the area of

supply. Simultaneous construction and obliteration of tumour vessels enforces inhomogeneity of tumour blood flow.

Newly formed tumour vessels appear to be defective in both adrenergic innervation and contractile smooth muscle elements (Hafstrom et al, 1980; Mattsson et al, 1979). Gullino and Grantham (1961a) demonstrated vascular reactivity after intratumoural injection of adrenalin and suggested that normal host vessels incorporated within the mass might be responsible. Certainly, normal arteries and arterioles seem to be highly resistant to neoplastic invasion and can be traced through densely infiltrating tumour in which the normal architecture has been completely destroyed (Willis, 1973). Some tumours, such as lymphoma, rely on co-opting existing vessels to supply much of their nutritional demands (Denekamp & Hobson, 1982). Persistence of these vessels may provide some tumour vasomotor activity, but this will be compromised by stretching and elongation of the incorporated vessels as the tumour mass expands.

Vascular morphology varies between tumours of different histology, the same histology and between different regions of the same tumour. Progressive tumour size, or age, enhances this diversity. In small tumours a haphazardly organised fine capillary bed intermingles with unchanged incorporated normal vessels with intact vessel innervation. Subsequent tumour growth causes distortion and disruption, with occlusion of fine wall capillaries and functional alteration of the more robust normal vasculature. The inhomogeneity

seen in the early stages of tumour formation becomes exaggerated with tumour progression.

## 2.4 Functional Properties of Tumour Neovasculature

Vascular reactivity relies on two parameters; vessel innervation and the presence of contractile elements, and both are defective in tumour vessels. Pharmacoangiography, using vasoconstrictive drugs to visualise tumour vessels against a background of constricted normal vasculature, proffers functional evidence of contractile inadequacy (Ekelund, 1979). However, the inclusion of innervated normal vessels may provide some vascular reactivity, and a number of experimental animal studies suggest even an increased sensitivity to vasoconstricting drugs in the vascular bed of the tumour compared to normal tissue. The hypoxic tumour environment could be responsible for this.

If incorporated normal vessels are the main contributors to tumour reactivity, then the characteristics of the host tissue vasculature should determine the qualitative and quantitative response observed. However, this is probably not a static contribution because of the effect of tumour expansion on incorporated vessels (see 2.3). Consequently it is impossible to predict the overall influence that this will exert on tumour blood flow. These features may help to explain some of the conflicting reports regarding the effect of vasoactive, and other, stimuli on tumour blood flow (see section 5).

## 2.5 Measurement Of Tumour Blood Flow

### 2.5.i Concepts of Flow

Morphological assessment of tumour vascularity is a poor reflection of functional blood flow. An extravagant production of capillaries in the stroma of carcinomata, resulting in an increased capillary density per unit volume of tumour compared with normal tissue, does not necessarily equate with a greater tumour blood flow. Functional, rather than morphological, techniques are necessary to derive blood flow measurement per unit volume of tissue, and such methods have only become available relatively recently (Gullino & Grantham, 1961b). There are now a number of different approaches to tumour blood flow measurement in animals but it is important to recognise that direct comparison between techniques may be inappropriate. For example, the precise aspect of flow being measured by a given technique must be established. Venous outflow techniques measure total volume blood flow, a term which describes total blood throughput irrespective of the contribution of different types of transmitting vessels. Concealed within this parameter is capillary flow or perfusion, but the technique cannot detect what proportion of total tumour flow is available for this vital function. In addition, regional inhomogeneity of flow within a tumour mass cannot be appreciated by this 'blanket' approach. More relevant to tumour biology and therapy is nutritional flow, or tissue perfusion, which describes accessibility of capillary blood and constituents to tumour cells. A number of techniques

claim to measure this parameter but there are pitfalls which must be recognised and will be outlined in the section below.

### 2.5.ii Flow Measurement Techniques

Tumour blood flow can now be measured in animals by a variety of techniques (Table 4). The published data is difficult to interpret collectively because direct comparison requires a common absolute unit of blood flow, usually mls./min./100g. of tissue, and results in the literature are frequently expressed as ratios of tumour to normal tissue flow or as fractional proportion of cardiac output per/gm. of tissue. Technical, tumour and host variables further deter unification and have prevented the emergence of recognised patterns of tumour flow, or of predictable changes in flow induced by chemical and physical agents.

Whether or not animal data makes a useful contribution to our understanding of tumour blood flow in a clinical setting is not clear. A tumour arising spontaneously, within the appropriate normal tissue, is a different biological system from that produced by the implantation of experimental tumour into accessible normal tissue. Furthermore, the two tumour systems have very different cellular kinetics with animal tumours often doubling in volume over hours or days, whereas for human tumours doubling over a period of months is not uncommon. Expansion of the vascular component of a

TABLE 4

TECHNIQUES COMMONLY USED FOR BLOOD FLOW MEASUREMENT IN ANIMAL STUDIES AND THEIR CLINICAL APPLICATION

| Technique | Measured parameter | Animal experience: Advantages | Animal experience: Disadvantages | Clinical Application | Reference |
|---|---|---|---|---|---|
| 1. ISOLATED ORGAN-VENOUS OUTFLOW | Total blood flow | Isolated controllable system | Limited anatomical application<br>No discrimination between shunted & nutritive blood | No | - |
| 2. UPTAKE TECHNIQUES | | Serial measurements | Left ventricular injection | Possible application | Crean et al 1986 |
| (i) Radiolabelled microspheres | Capillary perfusion | | Inaccuracies due to:<br>-variable tumour vessel diameter<br>-variable microsphere size<br>-poor counting statistics in low flow areas | | |
| | | | + | | |
| (ii) Radiactive tracers ) $^{86}Rb$ ) $^{42}K$ ) | Capillary perfusion (active uptake) | | Cardiac out put required<br>Single observation | Potential usefulness of $^{81}Rb$ | |
| $^{131}I$ Antipyrene ) $H_2{}^{15}O$ ) | (passive diffusion) - | See $^{81}Rb$ | - | With PET | -<br>Beaney et al 1984 |
| 3. CLEARANCE TECHNIQUES | | Relatively non invasive | Difficult clearance curve analysis | Superficial tumours | Mantyla 1979 |
| (i) $^{133}Xe$ - Interstitial | Capillary perfusion (passive diffusion) | | Interstitial 133Xe<br>-Capillary trauma<br>-Poor reproducibility | Intra-arterial | Olch et al 1983a<br>Wheeler et al 1986 |
| - Intra-arterial | | | -Superficial tumour only | | |
| (ii) Heat | Capillary perfusion | No recirculation | Thermally induced vascular perterbation<br>Precise temperature control and thermometry | Utilising power deposition for temperature maintenance | Carnochan & Tait 1986 |

tumour must emulate parenchymal growth. The preponderance of primitive vessels in experimental tumours is in keeping with rapid vascularisation. A high endothelial labelling index in the order of 30-40 times of that of normal endothelium, in experimental animal tumours confirms the intense vascular effort necessary to maintain tumour growth. Human tumour vasculature often expresses features of maturation, but endothelium labelling index is unknown with mention of only one report in the literature (Denekamp & Hobson, 1982). Observations on the vasculature of human tumours xenografts are not necessarily applicable to tumours in man as the stroma is of host origin (Giovanella & Fogh, 1978).

Table 4 describes the most commonly used flow measuring techniques with respect to method, parameter measured and clinical application. The relative advantages and disadvantages, are outlined below.

(a) Venous Outflow

Quantification of tumour circulation was engineered by Gullino & Grantham using "tissue-isolated" tumours (Gullino & Grantham, 1961b). Organs, such as ovary and kidney which can be isolated with respect to blood supply are implanted with tumour which, if growth is successful, eventually replaces normal tissue providing a completely encapsulated tumour preparation for circulatory studies. By catheterisation of the exiting vein, venous outflow can be recorded and total volume blood flow measured. It is not possible from the

derived value to discriminate between nutritional and shunted blood.

(b) Tumour Uptake Techniques

The proportional distribution of cardiac output to different tissues reflects local tissue blood flow. This principle forms the basis of a variety of techniques in which a tracer substance is introduced into the systemic circulation and is distributed to tumour and normal tissues. Detection of proportional tracer uptake provides a measure of tumour blood flow.

Radioactive microspheres were first used to measure tumour blood flow by Rudolph & Heymann (1967) and have now been extensively applied to the problem. The method involves injection of microspheres into the left ventricle of the heart to ensure good mixing. Peripheral trapping must be complete and irreversible otherwise recirculation distorts distribution. In normal capillaries 15 Mm microspheres are virtually 100% trapped (Archie et al, 1973) and those that bypass the microcirculation, by way of arteriovenous shunts, become lodged in the primary capillary bed prohibiting recirculation (Kaihara et al, 1968). Aberrant tumour vessels with a greater variability of size are likely to lead to errors unless a combination of different size spheres is used. Another drawback to the method is that in areas of low flow the number of microspheres trapped may be too few for statistical significance (Buckberg et

al, 1971). Further, the method requires sacrifice of the animal, and excision of the tissues of interest, making it unsuitable for human application. It has, however, proved a useful technique in animal studies allowing repeated measurement in the same tissue by using microspheres labelled with different isotopes, and providing an absolute value for flow provided the cardiac output can be calculated (Peterson, 1979). Adaptation of the technique for clinical tumour blood flow measurement now seems a possibility as a technique using bio-degradable human albumen microspheres, with external quantification of activity, has recently been devised for estimating the fractional distribution of cardiac output to organs in non cancer patients.

Intravenously injected radioactive tracers such as Rubidium 86, Carbon 14, Iodine 131-anti pyrene are similarly allocated to tumour and normal tissues according to the proportional distribution of cardiac output (Sapirstein, 1948). In this case the tracer substances are effectively trapped by peripheral cellular extraction, and following sacrifice of the animal radioactivity can be measured in the appropriate tissues. Similar in many ways to the microsphere technique, it is simpler in practice because good mixing of the tracer in the right and left ventricle after intravenous injection eliminates the need for left ventricular catheterisation. This makes it a suitable technique for use in small animals such as the mouse.

Although Rubidium and potassium do not cross the blood brain barrier, Iodine 131-anti pyrene, being lipophilic, does and can therefore be used to measure brain blood flow.

(c) Tumour Clearance Techniques

An alternative approach is to deliver a chemical or physical agent to tumour and use the rate of its clearance as a measure of blood flow. A number of radioactive isotopes such as Kryton $^{85}$ and Xenon $^{133}$, have been employed in this way (Kety, 1951). For example, Xenon $^{133}$ is an extremely lipophilic gamma emitting (0.081 MeV) radioactive gas, which readily disperses throughout tissue compartments and passes through the alveolar membrane of the lungs. Rapid excretion of Xenon$^{133}$ via the lungs results in negligible activity in the recirculating blood which is redistributed according to cardiac output. Following intra-tumoural injection, Xenon$^{133}$ diffuses rapidly and is considered to be in diffusion equilibrium with the tissue and therefore with the blood passing through it. The rate of clearance of the isotope will depend both on the rate of blood flow, and on the partition coefficient (Lambda) of the isotope between blood and tissue. As the partition coefficient is influenced by the proportional lipid content of the tissue, biopsy is required for accurate measurement in any individual tissue (Kallman et al, 1972). However, the

impracticality of this in clinical studies usually results in the use of a standard coefficient. The technique is attractive because Xenon$^{133}$ washouts from the tumour can be monitored externally making this a technique appropriate for clinical studies (Mantyla et al, 1982, Olch et al, 1983a).

Measurement of blood flow by thermal clearance relies on the principle that temperature decay after a transient or prolonged thermal perturbation is a function of convected heat transfer by blood flow within the tissue. The technique was originally developed by Gibbs (1933) and has been widely applied to physiological studies (for review see Eberhart et al, 1980). The technique usually involves implantation of a probe with both heating and thermometry functions, but a number of variations have been described. For example, Johnson (1976) applied external heat sinks and sources to superficial tumours in order to document thermal flux as a representation of blood flow change in underlying tissue. Although flow changes were detected with this method following 4 Gy irradiation, the sensitivity and reproducibility of the technique was not described.

The physical parameters associated with localised hyperthermia have also been considered to give an estimate of perfusion in the heated tissue (Roemer et al, 1985). The interpretive difficulties inherent in applying simple mathematical models, such as the

"bio-heat" transfer equation (Eberhart et al, 1980), to derive absolute flow values are encountered in either approach. Heat-induced vasoactive changes are a further problem with this technique and it is therefore essential to minimise thermal perturbations. This imposes exacting constraints on the temperature resolution and stability of the thermometry and heating systems.

d. Other Flow Measuring Techniques

In theory, both uptake and clearance techniques provide a measure of tumour perfusion, but neither has adequate resolution to examine events at the level of individual capillaries. The heterogeneity of tumour vasculature makes it important to have this information available when investigating the distribution of drugs, oxygen and heat in neoplastic tissues. In experimental animals, transparent chambers allow direct, continuous observations of individual tumour capillaries in vivo, but tumour growth is restricted to a sheet-like, 2-dimensional, structure which imposes limitations on the system when examining physiological responses (Algire 1952, Clark, 1952). Nevertheless, the setup has provided quantitative and qualitative information regarding vascular development (Reinhold, 1979). Flow calculation using this system involves measurement of red cell velocity, by a dual slit photomatic technique, and calculation of vessel diameter, using an image shearing monitor. Serial measurement can be made over a

period of days and the effects of pharmacological agents or heat monitored (Dudar & Jain, 1984).

Doppler ultrasound provides an alternative approach to estimating flow in individual vessels. Provided the vessel diameter is known, flow can be calculated from the ultrasound measurement of red cell velocity profiles. A criticism of any method incorporating vessel diameter is that this is not a static parameter and a single observation is not representative.

## 2.6 Intrinsic Factors Affecting Tumour Blood Flow

### 2.6.i Tumour Type

The variation in human tumour growth rate can broadly be categorised according to histological type. For example, Burkitt's lymphoma is a rapidly growing tumour which can double in volume in less than 40 hours (Iversen et al, 1974), whereas primary colo-rectal lesions are often slow growing with doubling times in the order of 632d. In order to sustain the energetic growth of Burkitt's lymphoma the tumour vascular kinetics, morphology and architecture are likely to differ from that seen in the slower growing tumour. It is, therefore, not surprising that the measured blood flow from different tumour types is correspondingly diverse. Raczka et al, (1983) observed tumour growth and blood flow in two experimental tumours, the Lewis lung carcinoma and the JW sarcoma, over a two week period. Initially, the flow was greater in the Lewis lung carcinoma than in the sarcoma, but

by 11-14 days post transplantation the situation had reversed, with the sarcoma flow unchanged, but that in the carcinoma having decreased by 50%. Histological examination of the tumours showed necrosis in the Lewis lung carcinoma whereas none was observed in the sarcoma. Raczka inferred that the tendency to develop necrosis may influence blood flow.

Techniques such as Rubidium 86 and radiolabelled microspheres, which can estimate blood flow in different regions of tumour, allow for separate identification of flow within central and peripheral areas. In most tumours necrosis occur centrally whereas peripheral tissue is composed of viable, proliferating cells. Using these techniques, perfusion appears to be greater at the periphery, compared with the centre (Young et al, 1979; Groothuis et al, 1983). Techniques which sum these component areas give a flow value which to some extent reflects the degree of necrosis present. When comparison of vascular volume in different tumours is confined to viable areas, there seems to be remarkable consistency (Jirtle & Clifton, 1971). Despite this report, Vaupel (1973) observed significant inhomogeneity of blood flow when sampling at a number of peripheral sites within the same tumour. This inconsistency may be a reflection of the tumour type studied and of the technique employed.

The degree of tumour differentiation, as judged histologically, is an indicator of cell kinetics and has been

used by Allen et al (1975) to look at blood flow in relation to cellular activity. In this study ENU induced rat tumours, of well differentiated and anaplastic histologies, were grown to an equivalent size. Blood flow in the well-differentiated tumours was greater than that in their anaplastic counterparts. Mantyla (1979a) supports this finding in his study of superficial human metastasis using Xenon 133 to measure blood flow. Well differentiated tumours had higher flow values compared with anaplastic carcinomas, although the highest flows of all were seen in a group of lymphomas.

In summary, it seems most likely that tumour perfusion is dependent on tumour type. The exact tumour characteristics which dictate this spread of values have been studied in animal tumours, and to a much more limited extent in a clinical setting. The intimate relationship of tumour features such as cell-proliferation rate, tumour doubling time, cell loss and tumour necrosis makes it difficult to implicate any one component, or to quantitate its influence. The following sections (2.6.ii and 2.6.iii) emphasise the interdependence of these gross tumour features.

### 2.6.ii Primary and Metastatic Tumour

In experimental and clinical studies metastases respond better to chemotherapy than do the primary tumours from which they are derived (Steel & Adams, 1975; Laster et al, 1969). Differential drug access may account for this disparity as Houghton suggested by demonstrating a significantly greater uptake of cyclophosphamide in pulmonary metastases compared

with primary tumour (Houghton et al, 1976). A greater proportion of necrotic tissue in large primaries could account for this difference, but Donelli found a higher concentration of adriamycin in metastatic tumours compared with primary growths when only viable regions were compared (Donelli et al, 1977). Possibly the microvasculature of metastases is 'superior' to that of primary tumours, allowing not only better drug delivery but also greater tumour cell proliferation thus providing a favourable environment for enhanced chemosensitivity (Hill & Stanley, 1975). These studies only indirectly reflect blood flow, through information on drug delivery and distribution. Direct measurement however confirms higher blood flow values in metastatic lesions compared with primary disease (Raczka et al, 1983).

2.6.iii Tumour Size

As tumours increase in size their relative vascular volume and blood flow decreases (Gullino & Grantham, 1961a, Vaupel, 1977). Necrosis has been implicated in this trend because of its close correlation both with tumour size and blood flow (Edlich et al, 1969, Cataland et al, 1962, Jirtle et al, 1978). Vascular surface area also decreases with increasing tumour size, but this precedes the fall in vascular volume and is in keeping with the observation that it is the smallest vessels that are compromised first (Yamaura & Sato, 1974). Increasing intratumoural pressure, with increasing tumour mass, could be responsible for compression of these thin walled fragile vessels.

Additionally, endothelial cell proliferation may become retarded as tumours enlarge (Tannock, 1970). The outcome is the development of necrotic areas transected by vascular spaces containing blood which does not exchange with general circulation and is effectively stagnant (Tannock & Steel, 1969). Alternatively, transmitting vessels may shunt blood through tumour substance without allowing it to contribute to tumour nutrition. Increased shunting may be a feature of enlarging tumours; Weiss has estimated that at least 40% of blood flow into rats tumours was delivered to shunting vessels (Weiss et al, 1979).

2.6.iv Tumour Site

Alteration of tumour perfusion by manipulation of the surrounding normal vasculature, suggests that the tissue in which a tumour arises, or is implanted, and its vascular characteristics will influence tumour perfusion. However, the majority of studies investigating the effect of tumour site on blood flow do not confirm that this is the case. Edlich et al, (1969), Tveit et al, (1984) and Suzuki et al, (1979) examined a number of anatomical locations including intrahepatic, sub-renal capsule, intramuscular, renal and gastrointestinal and were unable to demonstrate dependence on tumour site. To the contrary, Young has reported variation in blood flow according to tumour site. Using a microsphere technique and comparing only viable tumour areas, it was observed that high blood flow organs bore high blood flow tumours (Young et al, 1979).

### 2.6.v Systemic Blood Pressure

In experimental tumours, blood flow can be very sensitive to changes in systemic blood pressure (Algire et al, 1954; Vaupel, 1975). Suzuki has induced escalating hypertension in rats given Angiotensin II (Suzuki et al, 1981). Thermoelectric and hydrogen clearance techniques showed that while blood flow remained unchanged in normal tissue up to a mean arteriole pressure of 150mm of mercury, an average 6.5 fold increase in flow was seen in tumours. The findings were reproducible in tumour transplanted to a variety of organs, and also in induced primary tumours. The important inference is that by manipulating systemic blood pressure, selective and specific enhancement of tumour blood flow is possible. The therapeutic potential of this is discussed later.

## 2.7 Extrinsic Factors Influencing Tumour Blood Flow

### 2.7.i Pharmacological Manipulation of Tumour Blood Flow

#### a. The Potential

Conventional anti-cancer treatment could theoretically be improved by coincidental manipulation of tumour blood flow. Increased tumour perfusion should permit better drug access and distribution, and provide an improved tumour micro-environment, particularly with respect to oxygenation, with consequent sensitisation to radiation and chemotherapy. With hyperthermia, higher temperatures are achieved in regions of poor tumour

perfusion which also provide an environment which enhances heat cytotoxicity. Selective manipulation of tumour blood flow, with flow to normal tissues remaining unaffected or altered in the opposite direction, allows the possibility of exploiting a tumour/normal tissue differential. Augmented anti-tumour activity coincident with diminished normal tissue toxicity constitutes a therapeutic gain and an increased likelihood of treatment success.

b. The Basis of a Therapeutic Gain

Structural and functional differences between normal tissue and tumour vasculature make selective alteration of tumour blood flow feasible. Vessel reactivity relies on two parameters; the presence of tumour vessel innervation and the presence of contractile elements. Morphologically tumour vessels appear to be defective in both (Hafstrom et al, 1980, Mattsson et al, 1979) and pharmacoangiography, in which vasoconstrictive drugs aid the visualisation of tumour vessels against a background of constricted normal vasculative (Ekelund, 1979) proffers functional evidence of contractile inadequacy. That some tumours do show reactivity, however, has been demonstrated and attributed to the incorporation of preformed normal tissue vessels (Gullino & Grantham, 1961a).

Vasoactive drugs influence local normal tissue capillary flow by constricting or dilating feeder arteries and

arterioles. The tumour vascular bed, effectively lying in parallel with the normal tissue capillary network, will necessarily be influenced by events in the latter. For example a vasodilator causing smooth muscle relaxation in the host vasculature, and consequent increased blood flow, may diminish flow in the less reactive tumour compartment on the basis of a "steel phenomenon" (Kruuv, 1967). Indirect means of adjusting tumour flow by vasomotor agents are being investigated in animal and clinical studies.

An alternative approach is to directly target drugs on tumour vessels by exploiting any deviant characteristics. For example, an unusual sensitivity to circulating amines has been suggested by measurement of oxygen tension in tumour and muscles following administration of 5-Hydroxytryptamine (Cater et al, 1966). On the basis of this data Knapp et al, (1985) measured blood flow changes in tumour and normal tissues following intraperitoneal injection of 5-Hydroxytryptamine in rats. Tumour blood flow decreased by 79±29% accompanied by a decreased muscle blood flow of only 14±4.8%. The decreased likelihood of systemic effects make agents acting primarily at the tumour site an attractive therapeutic proposition.

c. Experimental Studies

Most experimental studies have attempted to manipulate tumour blood flow indirectly, by affecting the surrounding normal tissue vasculature. Less commonly, drugs directed at the intratumoural vessels have been employed to induce tumour flow changes, on the basis that tumours possess some vasoactivity (Cater et al, 1962, Rankin & Phernetton, 1976, Jirtle et al, 1978, Young et al, 1979, Tveit et al, 1981). On the other hand Mattsson, using xenon clearance to measure blood flow, combined a vasoactive agent with intratumorally injected xenon and demonstrated less reactivity in tumour vessels compared with their normal tissue counterparts (Mattsson et al, 1982). Other observers agree with these findings (Tvete et al, 1981, Wickersham et al, 1977).

In essence, tumour vessel reactivity greater than, equal to and less than that of normal vessels has been reported. Since the microvasculature of the tumour and that of the surrounding normal tissue are critical determinants of the outcome of vasoactive stimulation these conflicting results in experimental systems encompassing technical, as well as biological and pharmacological, variables are not surprising.

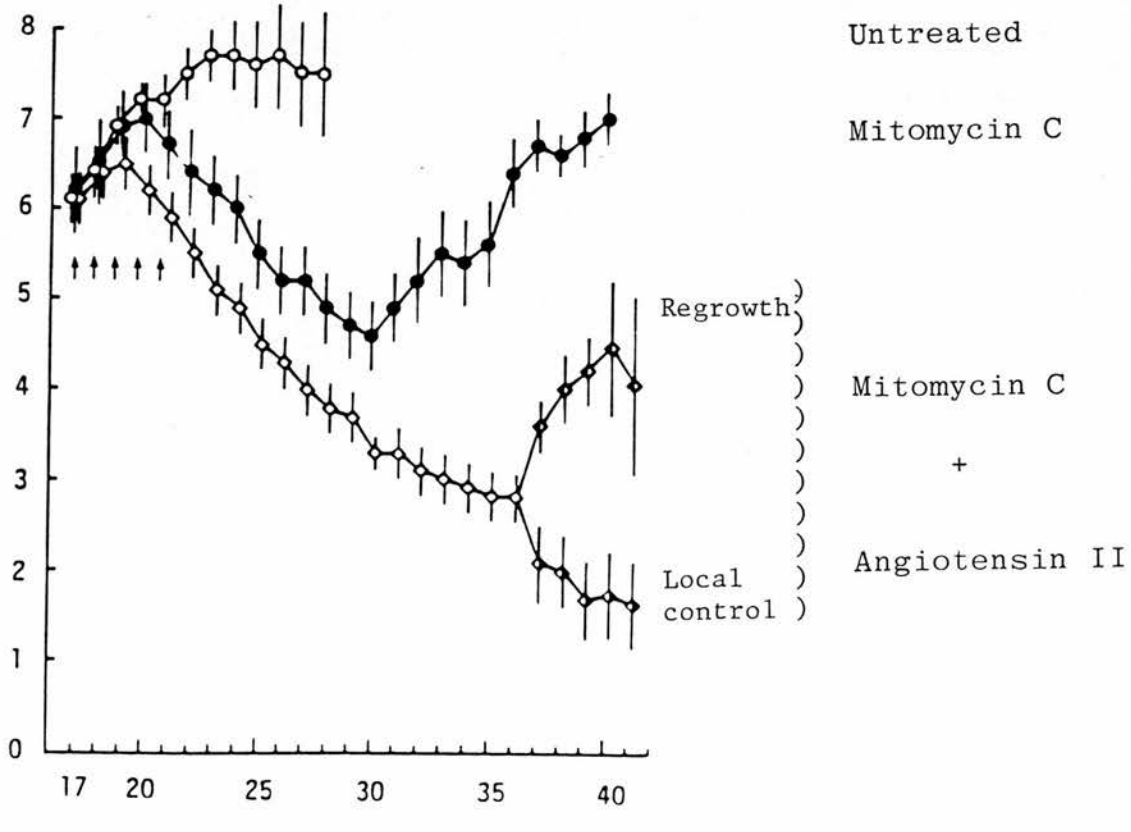
The importance of histological tumour type in determining vascular reactivity has been proposed (Rockoff, 1966) but is counterbalanced by observations

of flow changes of similar direction and magnitude in tumours of different morphology and growth kinetics (Knapp et al, 1985). The influence of tumour vasculature on tumour reactivity has been elegantly demonstrated using two experimental tumours derived from the same squamous cell carcinoma which diversified with respect to microscopic vascular pattern (Chan et al, 1984). In this study tumours were designated as either "hard" or "soft", the former tumour showing a distribution of vessel size in keeping with normal tissue and containing a proportion of large vessels with wall thickness greater than 7 microns, whereas "soft" tumours displayed an abnormal distribution of vessels and a striking paucity of large channels. Two drugs, phenylephrine, a vasoconstrictor, and hydralazine, a vasodilator, were given intravenously and changes in tumour and normal tissue flow monitored using a radioactive microsphere technique. In both tumours phenylephrine decreased and hydralazine increased normal tissue perfusion, but tumour blood flow was only significantly altered in "hard" tumours exposed to phenylephrine where a fall in flow was produced. Chan concluded that the virtual absence of smooth muscle in "soft" tumour, due to a predominance of small vessels, produced a quantitatively different response compared with "hard" tumours. The presence of vascular smooth muscle in "hard" tumour was supported by their ability to respond to phenylephrine.

Since tumour blood flow is influenced by systemic arterial pressure, manipulation of the former could be achieved by alteration of the latter. Suzuki's work with angiotensin II in rats suggests this may be a realistic approach (Suzuki et al, 1981). The potential of this system has been further explored by combining angiotensin with chemotherapy in solid transplanted tumours in rats. Fig. 2 shows the effect of Mitomycin C alone or in combination with angiotensin II on tumour diameter. The conclusion drawn was that angiotensin-induced increase in tumour blood flow could be usefully employed in combination with chemotherapy to enhance the biological effect (Suzuki et al, 1981).

Figure 2

Enhancement of the Effect of Mitomycin C by Angiotensin II



The potent vasodilator activity of calcium antagonists has been directed at tumour blood flow. The results obtained to date are as confusing and conflicting as any others concerned with tumour perfusion, but this may not be surprising (Knapp et al, 1985). The use of different calcium antagonists with different pharmacological properties makes it likely that in different systems, using different dosages, different effects will predominate. However, with the described effects ranging from an approximate 50% increase in tumour blood flow with Verapamil (Kaelin et al, 1982) to a 63±8% decrease in flow following Nisoldipine, it is difficult to make any predictions as to their usefulness in a clinical setting.

## 2.7.ii Physical Agents Affecting Tumour Blood Flow

### a. Ionising Radiation

In experimental solid tumours the radioresistant hypoxic cell population varies but is commonly in the order of 10-20% (Denekamp, 1983). Following irradiation improved tumour oxygenation is reflected by a fall in the proportion of hypoxic cells, which may be a consequence of increased blood flow (Thomlinson 1968, Van Putten & Kallman, 1968, Du Sault 1969). There is no comparable data on hypoxic cells in human tumours but evidence of reoxygenation following irradiation, for example in cervical and breast tumours, supports the parallel (Bergsjo & Evans 1968, Evans & Naylor 1963). However,

in addition to improved oxygen supply diminished tissue metabolism may contribute to post irradiation reoxygenation (Thomlinson, 1968).

Radiation-induced vascular changes in mouse mammary tumours have been observed using a modified algire chamber system (Reinhold, 1971). A course of fractionated radiotherapy, giving 576 rads daily, produced an improvement in tumour microcirculation, in terms of vascular density and flow velocity. The peak of these changes coincided with tumour regression, consistent with the idea that the vascular effect is partly attributable to tumour cell decay and elimination.

The published data on vascular changes during and following single dose and fractionated radiotherapy has been reviewed (Mattsson & Peterson, 1979). The effect of single fraction radiation appears to be dose-dependent with low dosage generally increasing, and high dosage decreasing, tumour blood flow. Fractionated irradiation does not seem to damage tumour vascular endothelium to the same extent (Gillette, 1975).

The effect of radiation on human tumour blood flow is virtually unexplored. However, measurement of xenon clearance during and following a course of radiation showed significant changes in flow, with an increase

after the first week of treatment and a progressive decrease thereafter (Mantyla et al, 1982).

b. Hyperthermia

Although it is not infrequently stated that blood flow in tumours is less than that in the normal tissue of origin (Vaupel, 1977, Gullino & Grantham, 1961a, Cataland et al, 1962, Peterson, 1979), a generalisation to this effect cannot be made from the limited available data. However, the application of heat seems to accentuate any disparity between the two by a differential action on tumour and normal tissue vasculature. This has important consequences for heat delivery during hyperthermia treatment and also in promoting a selective tumour effect.

## 2.8 Heat Effect on Blood Flow

### 2.8.i Heat and Normal Tissue Vasculature

Normal vasculature responds to heat by vasodilation with consequent increase in blood flow. In skin, this is apparent in the development of a transient erythema. The area immediately adjacent to tumour may not be representative of normal tissue having higher than usual resting flow values, perhaps as a result of inflammation (Song et al, 1980a). Temperatures within the therapeutic range induce significant increases in normal tissue flow which are rapidly and totally reversible. Vascular volume and permeability generally increase following heating at 43°, with total recovery within

hours after completion (Song et al, 1980a & b; Ackerman & Heckner, 1978). Vascular induced changes in different normal tissues are qualitatively similar but quantitatively different.

2.8.ii Heat and Tumour Vasculature

The effect of heat on the tumour circulation depends on the temperature delivered. In general, modest temperatures of 40-41°C either exert no effect (Gullino et al, 1978; Song et al, 1980b) or cause a reversible increase in tumour blood flow (Bicher et al, 1980; Emami et al, 1980; Vaupel et al, 1980). However, temperatures in the therapeutic range of 42-44°C have produced a significant decrease in tumour blood flow in a number of experimental studies (Bicher et al, 1980; Vaupel et al, 1980; Eddy, 1980; Endrich et al, 1979). The damage induced by these higher temperatures is irreversible (Emami et al, 1980; Song et al, 1980a & b). The development of thermotolerance to heat vascular damage has been suggested by Eddy & Chmielewski (1982) who observed a decreased vascular effect when an interval of only 5-24 hours separated two sequential heating sessions.

Histological examination of the vascular damage induced in tumours heated to different temperatures shows a dose response which correlates well with the physiological changes described above (Emami et al, 1981). Up to 40.5°C there seems to be no specific change, but heating to 43°C produces dilatation and congestion within the tumour vessels. When

the temperature is increased further, hemorrhage, necrosis and blood vessel disruption occur.

### 2.8.iii The Anti-tumour Effect of Hyperthermic Vascular Damage

The vascular component of heat damage may contribute to tumour cell death in a number of ways.

a. Ischaemic Tumour Cell Damage

A continuous decrease in the number of surviving clonogenic cells in tumours left in situ post hyperthermia implicates a delayed cell death process (Song, 1980c). Similarly tumour growth delay after a given treatment is considerably longer than that expected from cell survival measured immediately after treatment (Rofstad et al, 1984).

This progressive hyperthermic cell death is thought to be due to protracted hypoxia, increasing acidity and nutrient deficiency caused by heat-induced vascular damage. This suggestion is supported by a measured reduction in tumour vascular volume prior to the onset of delayed cell killing (Kang et al, 1980).

b. Enhanced Selective Heating of Tumours

The opposed responses of tumour and normal tissue vasculature to therapeutic temperatures probably facilitates selective tumour heating (Kim et al, 1982).

Thus, as local perfusion rate is an important determinant of temperature rise (Jain et al, 1979), increased normal tissue and decreased tumour flow, consequent on heating, provides a useful tumour:normal tissue differential.

c. Promotion of a Favourable Micro-environment

Collapse of the tumour capillary network could procure local environmental conditions favourable for direct heat toxicity. The importance of pH has been repeatedly observed and is probably the most critical factor in the thermosensitivity of mammalian cells (Gerweck & Rottinger, 1976; Overgaard & Bichel, 1977).

## 2.8.iv Pharmacological Manipulation of Blood Flow to Enhance the Heat Effect

Pharmacological exaggeration of naturally occurring selective tumour heating should provide a therapeutic advantage. Approached by either vasoconstriction of the tumour vascular bed, or by vasodilation of the surrounding normal tissue vasculature animal studies suggest that this may be a feasible manoeuvre. Improved selective heating was achieved in an experimental study involving three species, three tumour types and three different vasodilators (Babbs et al, 1982). Normal tissue blood flow, measured with thermal clearance and radioactive microspheres, increased to several times that in tumour allowing steady state intratumoural temperatures of over 45°C to coexist with normal tissue temperatures of 40°C. The most pronounced

effect was seen with large masses. This manoeuvre has not been attempted in clinical hyperthermia but intra-arterial prostaglandin $E_1$, a potent vasodilator, increased blood flow in normal tissue relative to tumour in an angiographic study in patients (Jonsson et al, 1978).

Many human tumours appear to be heat resistant in that they fail to reach therapeutic temperatures. Preservation of some heat/stress response within tumours may increase blood flow during hyperthermia and dissipate the delivered heat. Retained reactivity may predict for tumour heating capacity and subsequent therapeutic response (Olch, 1983a). In heat resistant tumours a locally infiltrated vasoconstricting drug could sufficiently inhibit blood flow to allow an adequate temperature rise (Olch, 1983b) but this has not yet been investigated in the clinic.

### 2.8.v Mechanisms of Heat Vascular Damage

The reason for increased heat susceptibility in tumour vessels remains speculative. The possible contributing mechanisms are outlined below, but the responsibility may not be singular:

a) Decreased Red Cell Deformability

Red blood cell membranes lose their deformability at the pH levels commonly detected in tumours after hyperthermia and become rigid spherical corpuscles, which tend to stick in tumour microvessels and cause vascular occlusion (Vaupel et al, 1980).

b. Increased Leukocyte and Platelet Adhesiveness

During hyperthermia leukocytes become adherent to the postcapillary wall with the result that there may be up to a 300-fold increase in postcapillary resistance (Ferguson et al, 1982). Alteration in the leukocyte membrane, or changes in the endothelial surface, may be responsible for this effect.

c. Direct Endothelial Damage

Normal vascular endothelium is generally considered to be very heat resistant but marked morphological changes have been reported in tumour endothelium. A short exposure of only 12 minutes to 43°C produced deeply staining endothelial cells which became vacuolated and protruded into the lumen (Fallin et al, 1972). Heating to 66.3° for only 40 seconds caused total endothelial destruction. Emami has demonstrated the temperature dependence of tumour endothelial damage, with obvious rupture and destruction of the capillary wall at therapeutic temperatures (Emami et al, 1981). The disrupted vascular endothelium may impede blood flow and promote hemorrhage in heated tumours.

d) Normal Tissue - "Steal" Phenomenon

Heat-inducted increase in adjacent normal tissue blood flow may effectively shunt blood from the tumour vascular bed producing a "Steal" phenomenon. As a result, decreased pressure within the tumour vasculature

allows collapse of the fragile walled, rapidly constructed vessels.

## 2.9 The Potential Implication of Tumour Blood Flow in Clinical Oncology

Published tumour blood flow data overwhelmingly concerns experimental animal tumours. Extrapolation from this to human tumours may be inappropriate because of fundamental biological differences between the two systems.

In order to exploit tumour blood flow in patient management, a means of assessing individual tumour perfusion is essential. This requires availability of a non-invasive, reproducible, quantitative technique for measuring flow, with the ability to detect intra-tumoral heterogeneity. At present, PET scanning is the only technique which approaches this ideal but its application is confined to centres possessing a Cyclotron.

An appropriate human tumour flow measuring technique could provide answers to important biological and clinical questions. For example, the assumption that tumour perfusion influences the effectiveness of radiotherapy, chemotherapy and hyperthermia remains unproven and, in fact, there is experimental evidence to suggest that blood flow cannot be used as a prognostic indicator of the radiation or drug response of individual animal tumours (Pallavicini & Hill, 1979). Tumour characterisation with respect to blood flow, and subsequent correlation with response to treatment

and local control, could elucidate this point. If perfusion is a critical factor then flow characteristics might predict the likelihood of response and help to determine treatment policy for a group of tumours in general, or for an individual tumour in particular.

Quantitative and qualitative changes in perfusion are likely to accompany therapeutic intervention and may have important consequences particularly for combined modality management. The radiation-induced alteration in flow described by Mantyla (Mantyla et al, 1982) could be construed either as a therapeutic benefit or hindrance. The described early increase in flow should improve oxygen and drug delivery to the tumour, enhancing the effect of further radiation or concomitant drug administration. The later fall in flow, however, would have the opposite effect, but might indicate a more appropriate time to attempt hyperthermia. Serial flow measurements throughout the course of active management could permit optimal utilisation of the most appropriate treatment.

Compromise of the tumour vasculature may be a useful therapeutic tool but its development relies on a means of assessing human tumour perfusion. The possibility of harnessing this vulnerable component of the tumour has been discussed, but properties of the tumour microcirculation can be exploited in other ways. Tumour capillary permeability for example, can be exaggerated by heat or calcium blocking drugs to allow better cellular access of cytotoxic agents. The necessity for continued endothelial proliferation in growing

tumours makes inhibition of angiogenesis a potentially useful mechanism.

Detection and control of angiogenesis has other intriguing clinical implications. Angiogenic activity emerges early in the transformation of normal cells to a neoplastic state, making its detection a valuable preneoplastic marker. This could provide a pioneering approach to screening programmes for the early detection of cancer, which now begin to appear fruitful in their aims to reduce cancer mortality (Tabar et al, 1985). Similarly, monitoring high risk tissue for angiogenic activity might provide a sensitive indicator of early metastatic growth and allow treatment to be instigated at a time of minimal tumour burden.

Tumour progression, in terms of metastatic potential, seems to require vascularisation of the primary mass. The number of cells shed into the circulation correlates with vascular density and with the number of lung metastases later observed (Liotta et al, 1976). Prevention of neovascularisation or destruction of the network at an early stage might influence the metastatic potential of cancers.

Chapter 3

# CLINICAL MEASUREMENTS AND ENDPOINTS

## 3.1 Clinical Endpoints

Objective evidence of tumour response is vital to establish antitumour activity but is only useful if it correlates with clinical benefit to the patient. This is particularly so in palliative treatment settings where alleviation of a symptom, such as pain, may be a more relevant endpoint than tumour shrinkage. Table 5 gives a summary of the clinical endpoints commonly used in the evaluation of radical and palliative cancer treatment.

TABLE 5

CLINICAL ENDPOINTS USED TO EVALUATE CANCER TREATMENTS

| |
|---|
| Survival |
| Disease free survival |
| Metastases free survival |
| Local control |
| Objective tumours response - complete<br>partial |
| Symptom alleviation |
| Performance status |

Although survival is the ultimate endpoint it usually involves a long follow-up period, and may be inappropriate in experimental treatments which are generally evaluated in advanced disease. Alternative endpoints providing more immediate information generally require precise tumour assessment, usually in terms of direct tumour measurement

although indirect measurement, for example by monitoring tumour markers, is appropriate in certain malignancies.

3.2 Tumour Volume Measurement

Direct tumour measurement is achieved either by calipers in accessible superficial lesions, or by imaging techniques in deeper seated tumours. Although the bulk of measurement data in humans is derived from radiological assessment of lung lesions, the availability of ultrasound, computerised tomography and magnetic resonance imaging will expand the range of tumours suitable for study and allow more accurate volume measurement of irregular masses (Husband et al 1982). Ideally, the three dimensions of a tumour should be measured and used to calculate the tumour volume. However, in practice this is rarely achieved and it is usually considered acceptable to confine measurement to length and width. The adequacy of this compromise will depend on the configuration of the tumour and is obviously more appropriate in spherical lesions such as is commonly the case in lung metastases where growth is generally spatially unrestrained. Gurland & Johnson (1966) have shown that with such lesions a simple measurement of greatest diameter will suffice. At other sites, and particularly in superficial lesions, tumours often assume an eccentric shape during growth, and may show eccentric regression in response to treatment. In such circumstances a single measurement is obviously inadequate and at least the tumour "area" should be calculated from the product of the longest perpendicular diameters. The most accurate expression of change in tumour extent will be obtained from a three-dimensional measurement which may

require a combination of techniques. For example, in this study, superficial lesions were measured by calipers in the longitudinal and transverse lateral dimensions, and by ultrasound in depth. It was felt that this combined the advantages, and minimised the drawbacks, of both methods (Yarnold et al 1986). Computer reconstruction of tumours imaged by ultrasound, computerised tomography, and magnetic resonance imaging provide accurate volume assessment in irregularly shaped and inaccessible masses (Bamber et al, 1987, Husband et al, 1982) which are a more realistic clinical proposition.

## 3.3 Reliability of Tumour Volume Measurement and its Influence on Clinical Endpoints

Consideration of measurement error will be confined mainly to superficial tumours in keeping with the interests of the present study. In these accessible lesions, caliper evaluation is the commonest approach to tumour measurement and has been investigated for source and magnitude of variation in measurement simulation experiments (Moertel & Hanley, 1976, Lavin & Flowerdew, 1980, Warr et al 1985). Moertel invited 16 experienced oncologists to apply their usual clinical measurement technique to 12 simulated lesions constituting a range of sizes which included two identical pairs. With the same investigator, there was a 7.8% chance that two sequential measurements, on a single nodule, would demonstrate an objective response, as defined by a 50% reduction in the product of perpendicular diameters. This increased to a 19% chance when the criterion for scoring

response was taken as a 25% reduction in the same parameters. The degree of error between different investigators was of similar magnitude with the chance of an objective response rate of 6.8% and 25% respectively for a 50% and 25% reduction. The authors therefore recommended that only the 50% reduction criterion be employed, with the anticipation of a 5-10% objective response rate occurring by error alone. This study encouraged investigator precision and offered ideal conditions for measurement making it likely that the error incurred in a less diligent clinical setting could be greater than these estimates. On the other hand, Moertel's results were derived from only two sequential simulated measurements and in the clinical setting measurement reliability increases considerably with the frequency of observation.

In a similarly constructed simulation study, Lavin and Flowerdew (1980) demonstrated a log-normal distribution for tumour area measurements and proposed the use of the ratio of tumour area at each evaluation as the most appropriate parameter for determining, not only tumour response, but also tumour progression. Applying power curves to determine the probability of declaring tumour response or progression, according to the criteria commonly used by the Eastern Cooperative Oncology Group, they demonstrated that when tumour volume was unchanged between consecutive measurements there was a greater likelihood of scoring progression than response. They calculated that in 100 patients given treatment which stopped tumour growth, 64 would be falsely

classified as having progressive disease on the basis of 4 follow-up evaluations conducted by different investigators. New criteria of 50% decrease in tumour area for designation of response and 100% increase in tumour area for declaration of progression were suggested. Applied to the above example false progression fell from 68% to 6%. It was felt that similar simulation studies would be of value in identifying appropriate statistical analysis for different approaches to tumour measurement. The study also addressed the influence of tumour texture and shape on measurement error with a negative outcome.

More recently Warr et al (1985) designed a simulation study which included clinically relevant lesions such as lymphadenopathy, hepatomegaly and pulmonary metastases. Although introducing some of the variables encountered in clinical practice, only clearly defined lesions were assessed by a group of consenting investigators, making it likely that the derived error still underestimates clinical reality. The calculated influence of measurement error on response rate concurred with previous studies, showing a false partial response rate of approximately 10% for most lesion types with a greater frequency of false progression, in the order of 30%. More stringent criteria of response and disease progression decreased the percentage of false categorisations.

The reliability of caliper measurement in human tumours has been evaluated by different approaches (Thomlinson, 1982,

Yarnold et al, 1986). In Thomlinson's large measurement study of breast cancer the variation in perpendicular tumour diameter obtained by two different observers was investigated. Thirteen thousand five hundred measurements were compared and the differences obtained were found to follow an almost normal distribution with a mean difference of 2.5mm for lesions ranging in diameter from 16 - 161mm. Sixty per cent of the compared measurements were within 2mm of one another. Assuming a spherical configuration for the lesions, tumour volumes were derived from the mean of the measured dimensions and, from the standard deviation of these means, the 95% confidence limits of the volumes were calculated and expressed as error bars on the growth curves.

In a small series of superficial excisable malignant lesions, Yarnold (1986) compared tumour volumes derived from preoperative caliper measurement with those directly obtained by immersion of the excised specimen in a displacement chamber. Caliper measurements tended to overestimate tumour diameter, partly because of inclusion of overlying tissues, but the significance of this error is minimised in serial measurements which detect a change in relative tumour volume rather than describing absolute values. In the same study comparison of preoperative ultrasound measurement with excised tumour dimensions indicated that ultrasound measured tumour depth accurately but tended to underestimate the lateral extent of the tumour. As a result of this, ultrasound was used to provide a depth measurement in superficial lesions measured in the present study.

Satisfactory incorporation of tumour measurements into response criteria in multicentre clinical trials requires evaluation of the reliability of tumour measurement. Henderson et al, (1984) attempted to assess the consistency of tumour measurements, from chest X-ray and radionucleotide scans, made by investigators at participating hospitals compared with a central radiologist. Overall, differences in mean tumour dimension, and products of dimension, between all local investigators and the central radiologist were small, although individually there were large differences in three of the eight participating hospitals when compared with the central radiologist. Classifying disease status on the basis of these measurements as either progressive, stable or remission, there was only 75% agreement between local and central examiners, with particularly poor (41.7%) agreement for the subset of patients regarded as having remission by one of those examiners.

## 3.4 Human Tumour Doubling Time Data Derived from Clinical Measurements

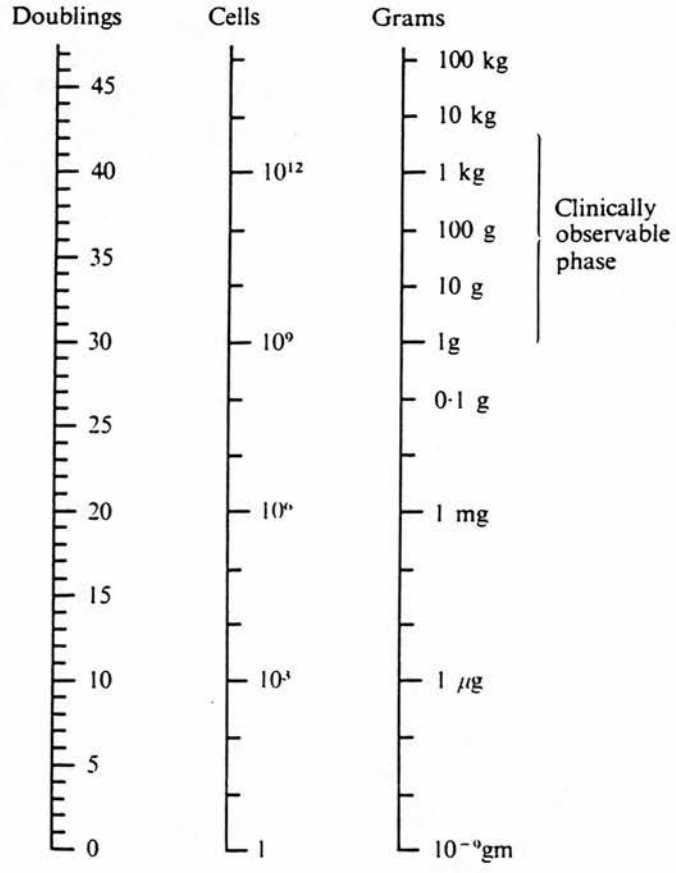
A single histological tumour type can include neoplasms varying widely in biological behaviour, as demonstrated by doubling times ranging from 23-280 days for adenocarcinoma of breast (Batterman et al, 1981), and 83-547 days in adenocarcinoma of corpus uteri (Breur, 1966a). If tumour growth rates could be determined readily, and without delaying treatment, this information could be incorporated into the design of clinical trials by stratifying according to doubling time. Comparison of biologically similar groups

of tumours could be advantageous in certain situations; for example, tumour growth rate has been shown to correlate with the relative biological effectiveness of neutrons (Batterman et al, 1981).

However, information regarding doubling time is generally not available at the start of treatment and knowledge of tumour growth rate is generally confined to a few carefully selected tumours. Thus, most of the available doubling time data has been accrued from tumours which are regular, discrete and accessible for measurement, by calipers or radiological techniques, and those that can be monitored over a period of weeks or months without therapeutic intervention. The latter stipulation implies that tumours are either indolent, incurable or metastatic and precludes collection of information on early disease which is potentially curable. Unless a serum tumour marker is available which is stoichiometrically related to tumour volume as in female choriocarcinoma (Bagshawe 1973), growth rate data are limited to clinically detectable disease and hence to a range of approximately 6 - 10 volume doublings. (Fig. 3). The major component of tumour expansion is therefore excluded from observation although earlier detection of metastatic lesions by C.T. scanning permits some insight into the "preclinical" phase of growth (Husband et al 1982).

Figure 3

Relationship Between Cell Number, Tumour Weight and the Number of Doublings of Cell Population




Human data generally demonstrate exponential growth rates (Collins et al, 1956, Schwartz 1961, Breur, 1966a, Brenner et al, 1967, Weiss et al, 1966, Van Peperzeel, 1972). However, where frequent observations are made over an extended period of time, examples of both growth retardation (Breur 1966a) and abrupt acceleration are available (Spratt & Spratt, 1964). In a study of growth rate in soft

tissue metastases, Lee and Spratt (1972) were unable to differentiate between linear and exponential growth, taking into account frequency of observation and measurement error. On the assumption that human tumours grow in a continuous exponential fashion, attempts have been made to construct the natural history of a tumour from the moment of its inception by extrapolating backwards (Collins et al, 1956).

Despite the problems of accruing and interpreting human data the number of published estimates of volume doubling time runs into several hundreds and a summary of the data on 780 human tumours has been presented (Steel, 1977). Consolidation of this data demonstrated the very considerable range of growth rates found with any particular tumour type. Adenocarcinomas, for example, had a geometric mean doubling time of 77.8 days but in 36% of tumours the doubling time fell outside the range 37-163 days, and 4% lay outside the range 18-343 days. However, individual series report a log-normal distribution for tumour doubling time, considering either a specific tumour type or observations on a mixed population of tumours (Spratt and Spratt 1964, Charbit et al 1971 and Lee and Spratt 1972).

## 3.5 Tumour Growth Delay and Other Endpoints

Growth delay is an established experimental endpoint which has been successfully used to evaluate a wide variety of treatments, particularly those involving radiation. This approach was used by Thomlinson (1960) to investigate the potential of hyperbaric oxygen and subsequently has been

widely employed in a range of radiobiological studies including the investigation of fractionation, (Denekamp & Harris, 1976), comparison of photons with high L.E.T. radiations (Field et al, 1968, Barendsen & Broerse, 1969) and evaluation of hypoxic cell radiosensitisers (Denekamp & Harris, 1975, 1976). Growth delay has also been successfully applied in the assessment of other treatments including cytotoxic drugs (Steel et al, 1983) and hyperthermia (Rofstad & Brustad, 1986). In the laboratory, growth delay has the advantage of being applicable to almost all solid tumour systems and of being sensitive over a wide range of responses. However, because of the complex relationship between cell kill and growth delay, it is not possible to quantify the surviving cell fraction using this technique. The proliferation kinetics of those cells surviving treatment, from which repopulation will occur, critically influence growth delay. This effect is amplified when treatment modalities involving different mechanisms of cell damage are compared. For example, Stephens & Peacock (1977) investigated cell survival and growth delay in B16 melanoma treated with cyclophosphamide or 1-(2-Chloroethyl) - 3-Cyclohexly-1-nitrosourea (CCNU). Doses of the two drugs that produced the same level of cell kill, resulted in different growth delays due to slower clonogenic cell repopulation after cyclophosphamide compared with CCNU. Experiments involving comparison between radiation and drugs (Rowley et al, 1979) and radiation and hyperthermia (Walker et al, 1983) have demonstrated similar discrepancies. In the latter experiment, at the treatment level producing 37% cure

with each of the two modalities, the corresponding growth delays were 22.9 days for hyperthermia and 69.5 days for radiation.

Characteristics of the regrowth phase may also be influenced by damage to the stroma, or bed, of the tumour. For example, in animal experiments, prior irradiation to the site of implantation results in slower tumour growth compared with tumour sited in unirradiated host tissue (Stenstrom et al, 1955, Hewitt & Blake, 1968, Urano & Suit, 1971). This effect, termed the tumour bed effect (TBE), demonstrates that tumour growth is not simply a reflection of the inherent proliferation potential of clonogenic cells. This has important consequences for growth delay; firstly, if the growth curves for treated and untreated tumours are no longer parallel, then the endpoint size selected influences the estimated delay. Secondly, the non-linear variation in magnitude of TBE with radiation dose (Urano, 1966) complicates the use of growth delay in dose response experiments (Begg & Denekamp, 1983).

A reduction in growth rate has also been demonstrated after cytoxic treatment although the magnitude of the effect is usually less than that induced by radiation. In this situation TBE is an unlikely explanation and, as general host health has been shown to influence tumour growth rate (Brown, 1979), a systemic effect is more probable.

Although localised hyperthermia can result in severe normal tissue damage, a tumour bed effect is probably not significant for clinical application. In animal experiments, pre-transplantation hyperthermia taken to tolerance dose, failed to demonstrate retardation of tumour growth (Yerushalmi & Weinstein, 1979, Urano & Cunningham, 1980). However, the addition of heat to radiation doses which alone failed to produce a TBE, resulted in significant enhancement of radiation effect, and consequent retardation of tumour growth (Yerushalmi & Weinstein, 1979).

Clearly, a greater growth delay does not necessarily imply greater cell kill but it does indicate a greater effect which may in itself confer clinical benefit. Thus despite the mechanistic problems of interpreting growth delay, it is a valid clinical endpoint in its own right providing a means of comparing severity of treatments in patients.

The clinical application of growth delay is now well established (Ash et al, 1979, Dawes et al, 1978, Thomlinson et al, 1976). Yarnold (1986) investigated the reproducibility and sensitivity of radiation-induced growth delay and was able to detect 20% differences in radiation dose thus supporting the validity of the technique for identifying enhancement ratios as low as 1.2. However, the method was limited as a clinical endpoint because of the scarcity of patients with measurable metastases and an adequate prognosis, who were not receiving active antitumour therapy. Urtason et al (1980) have also emphasised the

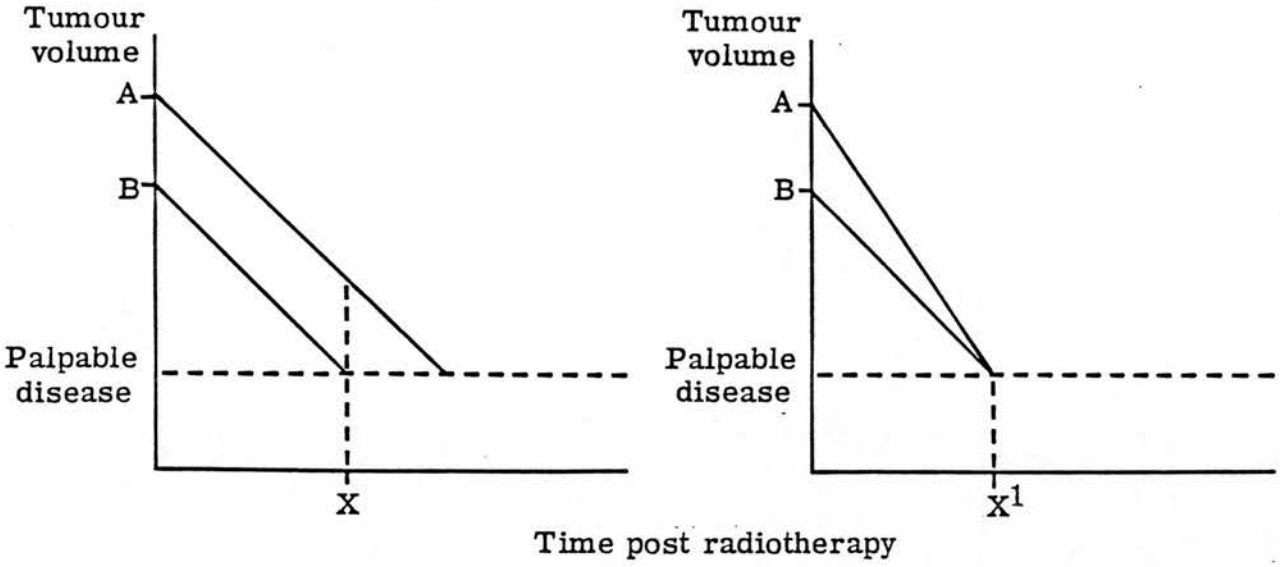
difficulty of applying the growth delay assay to the investigation of clinical problems.

### 3.6 Tumour Shrinkage

Two parameters, rate of regression and extent of volume reduction, can be used to describe tumour shrinkage and need to be clearly defined, although in many reports this distinction is not made. Rate of regression, expressed as tumour volume halving time in days, is an absolute measure which can be used to compare treatment response in different tumours. Calculation of regression rate requires construction of a response curve derived from serial tumour measurements following treatment. On the other hand, extent of volume reduction is a less precise endpoint, often employed in clinical studies and estimated variously by assessment of complete response, tumour clearance and percentage volume reduction. Extent of volume reduction is usually based on comparison between the clinical assessment prior to treatment with an assessment made at an arbitrary time following treatment. This usually relies on clinical judgement rather than precise measurement and is therefore a crude endpoint. Figure 4 demonstrates the necessity for serial tumour measurement and the inadequacy of tumour clearance or complete response, as an expression of shrinkage.

Figure 4

Schematic Representation of Initial Tumour Volume, Rate of Regression and Extent of Volume Reduction




Two tumours of unequal volume, A and B, which regress at the same rate appear to vary in extent of volume reduction when assessed at time X, at which time A is designated complete response, whereas B remains palpable. On the other hand, if the two tumours regress at different rates, they may both be impalpable at time $X_1$. Introduction of pre-treatment

tumour assessment adjusts for initial volume variation and allows expression of percentage volume reduction. However, this endpoint is assessed at a specified time so that, in the example above, tumours A and B at time X would be classified as 100% and 75% volume reduction respectively. Only serial tumour measurements permit meaningful comment on tumour regression and complete response is an inadequate endpoint for measurement of either extent, or rate, of tumour shrinkage.

Human tumour data suitable for construction of response curves are sparse and confined to lesions accessible for clinical measurement or repeated radiological assessment (Friedman and Pearlman, 1955, Wambersie and Dutriex, 1980, Thomlinson, 1982, Bartelink, 1983). Following irradiation, tumour volume decreases in an exponential manner, at least over the period of maximum change in tumour dimension. However, considering the entire response curve, a sigmoid shape may become evident (Friedman and Pearlman, 1955, Thomlinson, 1982). In a series of 168 patients with irradiated breast cancer, Thomlinson was frequently able to fit a second exponential to the terminal part of the response curve. This second exponential represented a phase of slower regression, compared with the first exponential, and commonly became established when tumour shrinkage was of the order of a quarter of the original volumes. Resorption of stromal elements may be responsible for this terminal phase. Careful observation, in the first few days after conventionally fractionated radiation, has demonstrated that the initial

portion of the response curve is flat (Friedman and Pearlman, 1955, Wambersie and Dutriex, 1980, Thomlinson, 1982). Because of this, it has been suggested that a critical level of cellular damage is necessary to trigger the mechanisms involved in tumour shrinkage, a hypothesis supported by the more rapid onset of regression after large single doses of radiation.

Tumour shrinkage is a complex mechanism reflecting the multiple cellular processes, occurring in response to treatment, in both malignant and stromal elements. These include tumour cell kill, proliferation kinetics of surviving and non-surviving tumour cells, kinetics of cell lysis, mechanisms of debris clearance, proportion of stroma and its response to irradiation and host reaction against residual disease.

Animal experiments have demonstrated that tumour volume regression does not provide a measure of clonogenic cell kill. For example, Thomlinson and Craddock (1967) demonstrated that rat tumours given single doses ranging from 5 to 60 Gray, regressed at the same rate regardless of whether there was subsequent tumour regrowth or cure. Similarly, Suit (Suit et al, 1965) reported an absence of correlation between tumour regression rate and probability of tumour control after single dose irradiation in spontaneous mammary tumours in $C_3H$ mice. Over a range of doses, corresponding to local tumour control rates of 0.3 to 99.7%, the median tumour regression rate of 21 days did not differ

between treatments. In a review of experimental studies investigating the significance of tumour regression on local control, Suit and Walker (1980) calculated that, at least for tumour systems with no effective host resistance, virtually all tumour failures represented regrowth from less than 10 surviving cells. The ensuing argument hinged on the unlikelihood that the proliferation characteristics of these 1-10 surviving cells would be reflected in tumour regression during irradiation. The same authors substantiated this hypothesis with regression and regrowth data from Barendsen and Broerse (1969). However, Denekamp, (1977) has challenged the significance of these studies, which employed single, large dose irradiation and investigated tumour regression during fractionated irradiation, more representative of clinical scheduling. In tumours of similar volume, tumour size at the end of treatment was significantly correlated with local control for three of the four fractionation schedules, whereas, there was only a weak correlation in those lesions given single doses. Although it was conceded that tumour shrinkage is unlikely to reflect the number of cells killed, it was postulated that it could influence re-oxygenation and thus, sensitivity to subsequent irradiation.

Human tumour regression rate, and extent, have been investigated as indicators of radiocurability and as early predictors of the outcome of treatment. The attraction of an early determinant of radiocurability is that treatment could be tailored accordingly and, for example, the radiation dose

boosted or surgical salvage considered. To the clinician, rapid tumour regression is an encouraging sign which it is tempting to equate with radiosensitivity and, extrapolating even further, with radiocurability. However, radiotherapy experience refutes the general validity of this supposition with, for example, prompt, and usually complete, regression of small cell lung cancers after only moderate radiation doses which rarely lead to tumour control. Similarly rapid response in irradiated Hodgkin's disease led to the practice of employing inadequate dose levels with consequent local relapse and the eventual realisation that 40Gy. in four weeks was required to achieve local control in more than 90% of patients. On the other hand, very slow regression, or even static disease following irradiation does not necessarily indicate treatment failure (Choi et al 1979).

Accepting that there is variation in regression pattern between different histological tumour types, and a lack of clear connection with treatment outcome, it is nevertheless still possible that tumour shrinkage is of prognostic significance in a group of like tumours. For example, the 38 fold range in the slope of regression in 60 patients with primary breast cancer, essentially treated in the same way, might be expected to detect differences in treatment outcome (Thomlinson 1979). A number of clinical studies have investigated the influence of either regression rate during treatment, or extent of regression shortly after completion of treatment (Table 6).

As shown, data on regression rate are scanty and most observations relate to the extent of tumour regression scored either at the end of radiotherapy or within the first six months after treatment. From these studies documenting extent of regression, timing of assessment is obviously a critical determinant of correlation with subsequent outcome. For example, Sobel (Sobel et al, 1976) in a series of head and neck cancers, documented response by scoring for tumour clearance or persistence at specific time intervals during, at the completion of, and following a course of fractionated radiotherapy. The use of the term "rate of regression" by these authors is imprecise as tumour volumes were not calculated. However, the allocation of tumours into early and late responders is valid and demonstrates that the time of tumour clearance is less important than the completeness of tumour clearance as a predictor of local control. In the majority of sites studied, the third assessment interval, 30-90 days after the completion of treatment, most accurately predicted outcome although this information obviously fails to provide an early indication of treatment outcome and therefore has less impact on individualising treatment. The conclusions of the study challenge the practice of

TABLE 6

CORRELATION OF PROGNOSIS WITH PARAMETERS OF TUMOUR REGRESSION

| Tumour | No. of patients | Regression parameter, assessed at | Clinical endpoint | Correlation | Reference |
|---|---|---|---|---|---|
| Head & Neck Cancer | | | | | |
| Oropharynx $T_3$ $T_4$ | 72 | TC** - completion R/T | LC, 2y | No | Suit et al 1965 |
| Oropharynx<br>Oral cavity | 75 | | LC | No | Fazekas et al, 1972 |
| Oropharynx<br>Oral cavity | 144 | TC - during R/T )<br>- completion RT) | LC | No | Sobel et al 1976 |
| | | - 30-90 d post R/T)<br>- 120-180 d " " ) | | Yes | |
| Squamous carcinoma Head & Neck (Split course R/T) | 110 | %VR* - during R/T<br>- completion R/T | LC, 2y | Yes (early tumours)<br>Yes (advanced " ) | Mantyla et al, 1979b |
| Squamous carcinoma Head & Neck | 534 | TC - 30d post R/T<br>- 90d post R/T | LC<br>S | Yes<br>Yes | Dawes 1980 |
| Squamous carcinoma Head & Neck lymph node metastases | 47 | $T\frac{1}{2}$° - during) R/T<br>- after ) | LC, 3y | Yes | Bartlink, 1983 |
| Uterine Cervix | | | | | |
| Advanced disease | 116 | %VR - 7-14/7 post R/T | LC, 3y | Yes | Dische et al, 1980 |
| | | | MFS | Yes | |
| Invasive disease | 200 | TC - 30d post R/T | LC | Yes | Hardt et al, 1982 |
| Bronchus | | | | | |
| Locally advanced | | %VR - 2-3/12 post R/T | S | No | Dische & Saunders, 1980 |
| Breast | | | | | |
| Primary | 60 | $T\frac{1}{2}$ - during) RT<br>after ) | ET | No | Thomlinson, 1979 |

R/T = Radiotherapy

**TC = Tumour clearance
*%VR = Volume reduction
°$T\frac{1}{2}$ = Volume halving time

LC = Local control
S = Survival
MFS = Metastases free survival
ET = effectiveness of treatment'

evaluating tumour persistence during treatment as the criterion for surgical intervention (Marcial & Bosch, 1970, Lederman, 1972). In addition, Sobel's data suggests that the apparent lack of concordance of results as shown in Table 6, may arise from selection of an inappropriate time interval for observation in different tumour types.

Suit & Walker (1980) re-examined those clinical series in which tumour extent was evaluated at the completion of treatment and could therefore contribute to management policy. They calculated true and false positive rates with regard to expected and actual local relapse on the basis of residual tumour. False positive rates ranged from 2-86%, demonstrating that the presence of statistically significant correlation does not necessarily imply practical application.

Only one study addresses the prognostic value of regression rate during a course of radiotherapy, by calculating tumour volume halving time from serial tumour measurements (Bartelink, 1983). The results suggest that following a dose of 70 Gy to neck nodes involved with metastatic squamous carcinoma, recurrence is significantly ($p = 0.044$) more common in slowly regressing tumours with a halving time of > 20 days. As the prediction could be made as early as 28 days after the start of radiotherapy, regression rate may provide an early indicator of response and thus be of value in determining treatment policy, at least in this particular clinical setting.

In human tumours, both regression rate (Thomlinson, 1982) and the extent of volume reduction (Breur, 1966b, Van Peperzeel, 1972 and Bartelink, 1980) seem to be related to pre-irradiation growth rate. However, the influence of other factors has been defined with, for example, more rapid regression occurring after large (8-10 Gy) compared with small (2 Gy) fraction size (Holsti et al, 1978, Wambersie & Dutreix, 1980).

## CONCLUSIONS OF REVIEW AND PROPOSALS OF STUDY

Hyperthermia now requires proper clinical evaluation and definition of its role in improving local tumour control when used in combination with radiotherapy. Concerns regarding the adequacy of current technology, in terms of heat delivery and thermometry, have, until very recently, discouraged the introduction of a prospective randomised clinical trial to test these questions. Although this has now been undertaken by the European Society for Hyperthermic Oncology, it is likely to be several years before clinical trials in man can confirm or refute the value of hyperthermia in clinical practice. For this reason, attempts to derive quantitative clinical data provide more immediate information which can subsequently be used to direct the design of future clinical trials.

The aim of the present study was to provide quantitative data regarding heat modification of the radiation response, using multiple metastatic nodules in individual patients. Because of interpretative difficulties with fractionated studies and the possibility of invoking thermotolerance or inducing vascular change, the therapeutic schedule was confined to a single dose of radiation followed three to four hours later by a single hyperthermia treatment. Further, it was hoped to use the clinical measurements obtained during the study to investigate the biological comparability, in terms of growth rate and response to radiation, of these multiple nodules and thus determine the most effective use of clinical material of this sort.

Tumour blood flow is an interesting aspect of tumour physiology which may have important implications for treatment modalities such as radiotherapy and chemotherapy. Acquisition of means of manipulating this parameter may provide mechanisms for enhancing the effectiveness of conventional treatment, or for establishing an alternative approach to tumour management. In hyperthermia, blood flow has an even more crucial impact. Not only is heat delivery, qualitatively and quantitatively, dependent on blood flow, but also vascular changes induced by heat may contribute to hyperthermic cell death and influence the effectiveness of other treatment modalities. Because of this critical relationship between heat and tumour blood flow, one of the aims of the present work was to evaluate flow measuring techniques which might be incorporated into hyperthermic studies. In particular, it was hoped that a reproducible technique could be used to demonstrate whether flow changes do occur after a moderate heat delivery (43°C minimum for 30 or 60 minutes) and, if so, to assess roughly their direction, magnitude and duration.

The studies included in this thesis employed the same radiofrequency system to deliver heat to a series of small superficial metastatic tumours. Standardisation of thermometry was attempted by ultrasound localisation of the thermometry cannula at the tumour base and minimum tumour temperature used to control delivery of the treatment. It was hoped that this setup would permit performance analysis of the technique.

# PART II

## PATIENTS AND METHODS

Chapter 4

# INVESTIGATION OF THE MODIFYING EFFECT OF HEAT ON RADIATION RESPONSE

## 4.1 Patient Accrual and Selection

### (a) Tumour Selection

Patients with multiple, discrete metastases were referred by physicians, radiotherapists and surgeons, providing access to a wide spectrum of disease. Fifty-six of the total referred patient population had suitable lesions and were considered eligible for initial tumour measurement and eventual entry into the treatment study (see Eligibility Criteria - Table 7).

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TABLE 7

HEAT AND RADIATION IN SUPERFICIAL METASTASES:
ELIGIBILITY CRITERIA FOR ENTRY INTO STUDY

| | | |
|---|---|---|
| (1) | Tumours considered incurable by conventional therapy | Tumour criteria |
| (2) | Presence of multiple, discrete superficial metastatic nodules | |
| | (i) minimum nodule size 5 mm linear dimensions: limited by spatial resolution of the measurement technique | |
| | (ii) maximum nodule size 3 cm linear dimensions: limited by heating capability of the system | |
| (3) | Patients with a life expectancy of at least 3 months | Patient criteria |
| (4) | Patients in whom the introduction of systemic treatment was not anticipated during the course of study | |
| (5) | Patients fit and willing to attend hospital regularly for clinical measurement | |
| (6) | Patients giving informed consent | |

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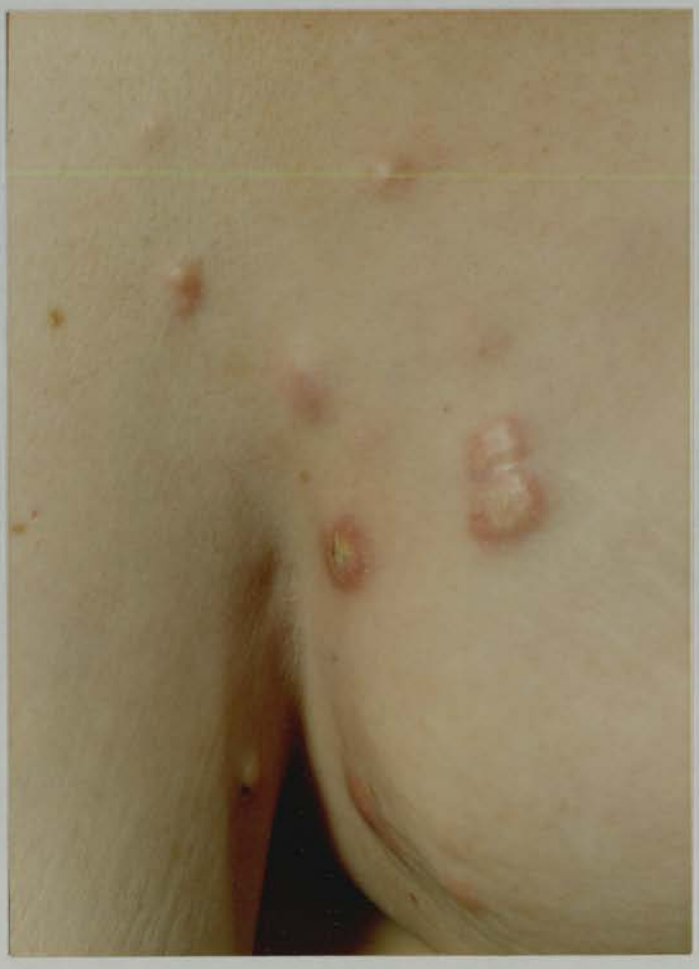
The commonest tumour type was locally recurrent adenocarcinoma of the breast, which proved particularly suitable in view of its tendency to develop accessible metastases compatible with a relatively prolonged survival. Breast cancer nodules from three patients entered into the measurement study are shown in Figs. 5, 6 & 7. Other histological types included uncommon tumours such as chordoma and carcinoid, which rarely present with superficial metastases (Fig. 8).

Figure 5

Cutaneous Metastases Over the Chest Wall in a Patient with Breast Cancer



Figures 6 & 7

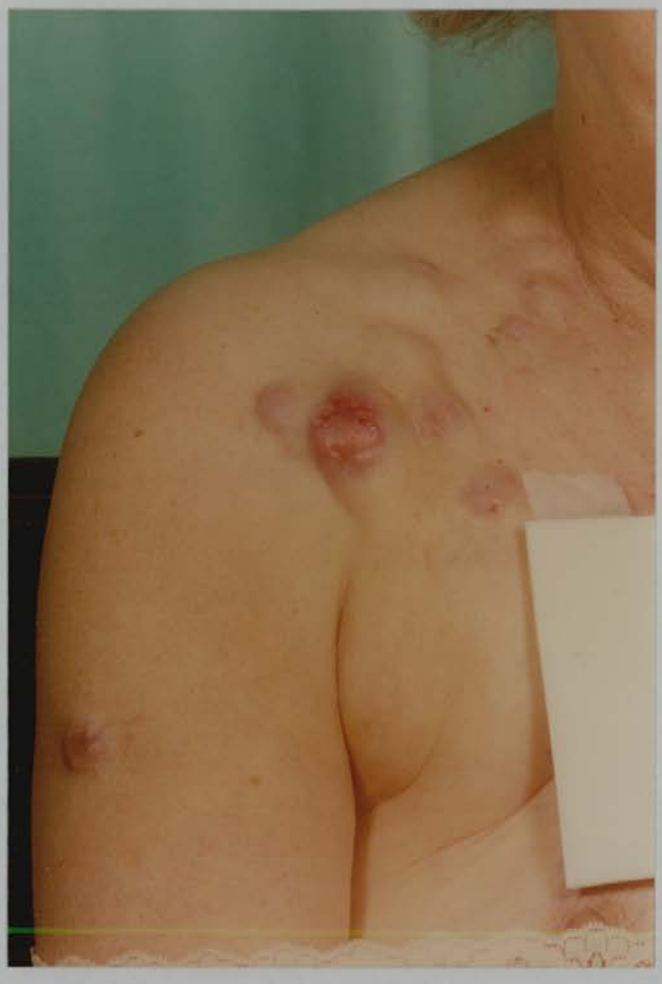


Fig. 6
Cutaneous and subcutaneous metastases of varying size.

Fig. 7
Three discrete cutaneous metastases tattooed for identification

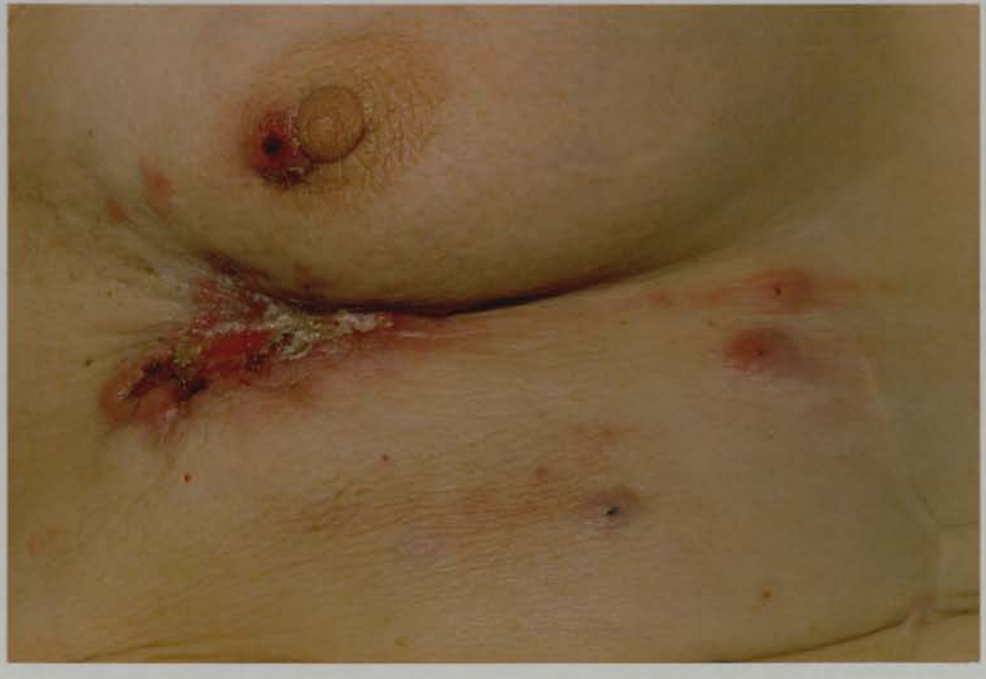
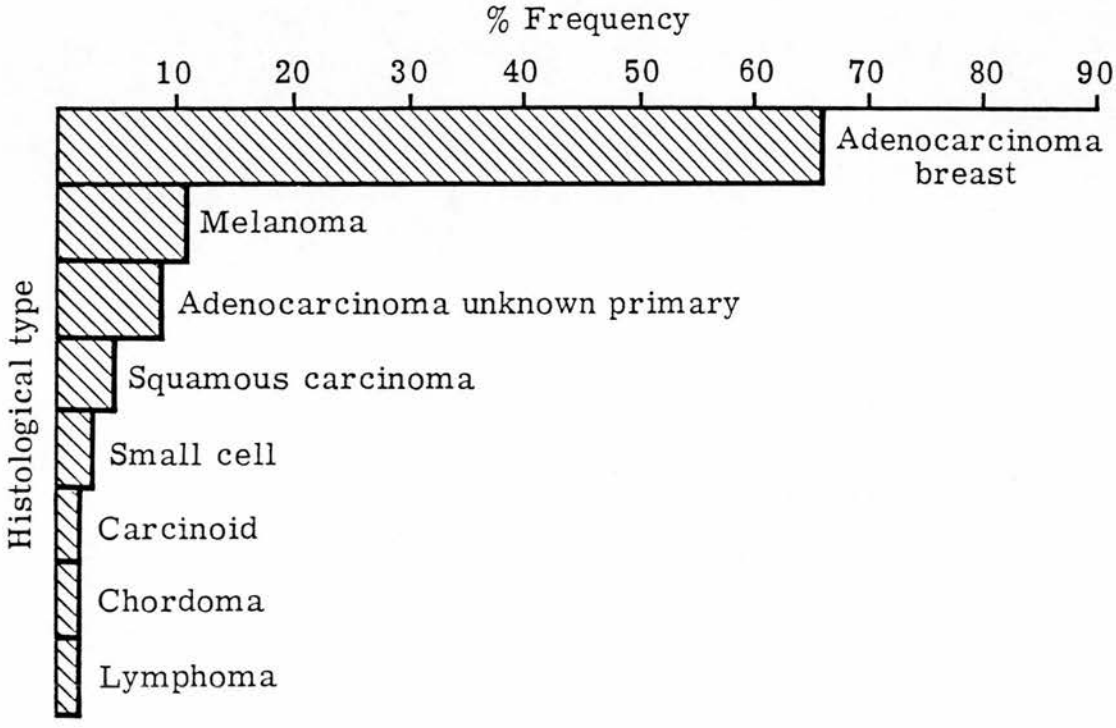


Figure 8

Histological Tumour Type in 56 Patients with Multiple, Discrete Superficial Metastases Entering the Measurement Study




(b) Patient Selection

Patient Elibibility Criteria for entry to the study are outlined in Table 7. The use of growth delay as a clinical endpoint was only feasible in patients with a life expectancy of at least three months. Introduction of systemic treatment during this period would have made interpretation of the role of hyperthermia impossible and was therefore avoided. However, some patients were already established on endocrine therapy and they, or their

physicians, were reluctant to discontinue treatment. Therefore, these patients were only included in the study if initial serial clinical measurements, prior to radiation/hyperthermia, demonstrated continued tumour growth, thus indicating lack of response to endocrine manipulation. A number of patients, particularly those with breast cancer, were referred for tumour measurement after failing to respond to cytotoxic chemotherapy. In those patients, a period of at least three weeks elapsed between final administration of chemotherapy and initiation of measurement observations, and treatment was only subsequently implemented after demonstrating a period of sustained growth.

Informed consent was obtained from all patients participating in the study. Regular clinical measurements, ideally on a weekly basis, required considerable patient cooperation and commitment, but the scheduling of measurements was adjusted in accordance with the physical fitness of individual patients at any time during the study.

After initial tumour measurement, only 18 of the 56 patients with suitable lesions proceeded to treatment according to the protocol. Of the remaining 38, the commonest reason for exclusion was rapid clinical deterioration which either

necessitated the initiation of systemic treatment for symptomatic metastases, prevented adequate hospital attendance, or shortened life expectancy. Ten of the 18 treated patients are evaluable; of the eight non-evaluable patients three died after inadequate follow-up, two required systemic chemotherapy, and two had inadequate tumour measurements.

## 4.2 Clinical Measurement

### 4.2.i Tumour Measurement

#### (a) Frequency of Observation

Serial tumour measurements were obtained pre and post-radiation/hyperthermia, and in control nodules throughout the observation period. Ideally, a minimum of 3-4 weeks prior to treatment was documented to establish tumour doubling time, but this was adjusted depending on the predicted life expectancy, to allow sufficient follow-up time during tumour regrowth. The time interval between measurements depended upon the patient's fitness and willingness to visit hospital. However, where possible, weekly measurements were obtained pretreatment and in the early post-treatment phase. But in the later course of the disease, when regrowth was established, measurement at two-weekly intervals was deemed adequate and appropriate in the face of general clinical deterioration. All

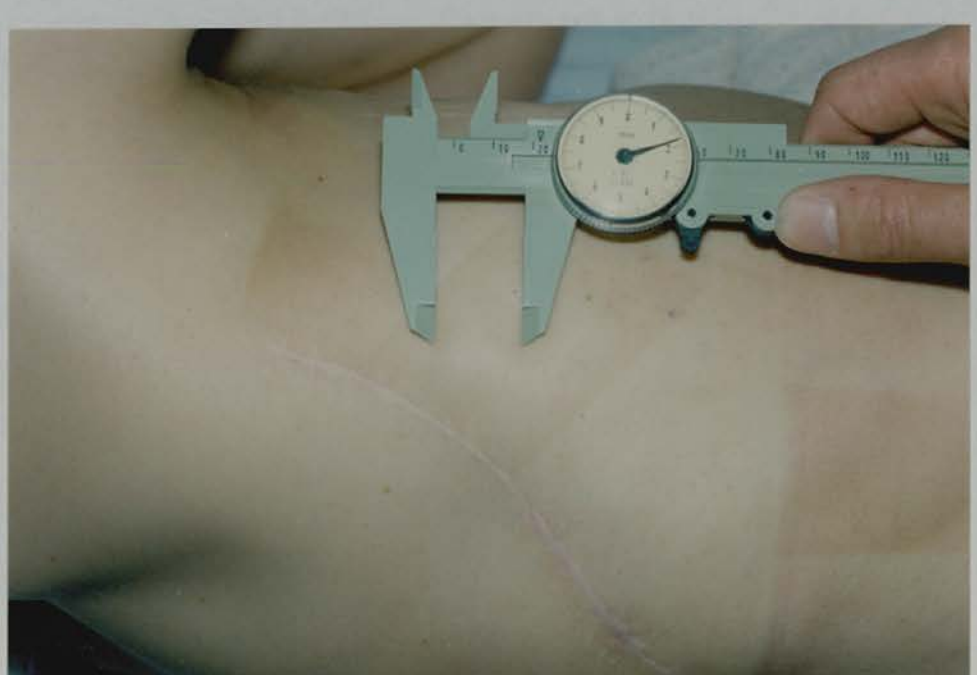
measurements were carried out by the same observer (D.T.). The tendency for further lesions to develop in the vicinity of the treated nodule during the observation period necessitated a means of identifying the study nodule during subsequent shrinkage and regrowth. This was achieved by tattooing the skin over the centre of the nodule at the time of irradiation (Fig. 7).

(b) Caliper Measurements

Engineers' calipers were used to measure each nodule in two planes at right angles to one another (Fig. 9 ). Tumour margins were located by palpation to ensure inclusion of the maximum lateral extension of disease.

Figure 9

Measurement of a subcutaneous nodule (breast adenocarcinoma primary) using engineers' calipers

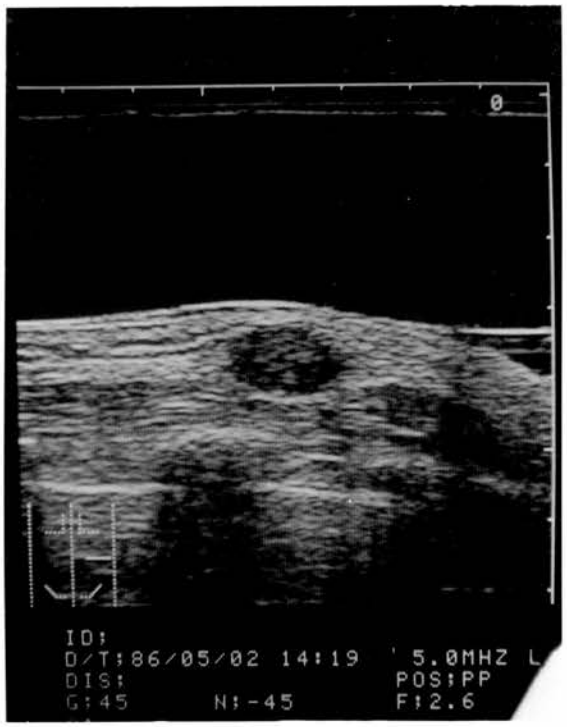


(c) Ultrasound Measurements

Real time ultrasound was available for calculating maximum tumour depth, assessed in the same two coordinates as the caliper measurements, at one of the two hospitals taking part in the study. The ultrasound equipment comprised a Hitachi EUB 25 metre real-time machine linked to a 5 megahertz linear array with 3 centimetre stand/off. Ultrasound images were recorded on Polaroid film, from which tumour depth could be calculated by applying the appropriate magnification factor to the direct measurement (Fig. 10).

Figure 10

Ultrasound Image of a Subcutaneous Nodule



4.2.ii Tumour Volume Calculation and Construction of Growth Curves

(a) Formulae for Volume Calculation Where caliper and ultrasound measurements were available, tumour volumes were calculated using the formula:-

$$V = \frac{\pi (a.b.c.)}{6}$$

where a, b and c represents maximum transverse, longitudinal and depth diameters respectively. Where ultrasound assessment of depth was not available volumes were calculated from the formula:-

$$V = \frac{\pi a^2 b}{6}$$

where "a" represents the minimum lateral diameter.

(b) Computer Constructions of Growth Curves

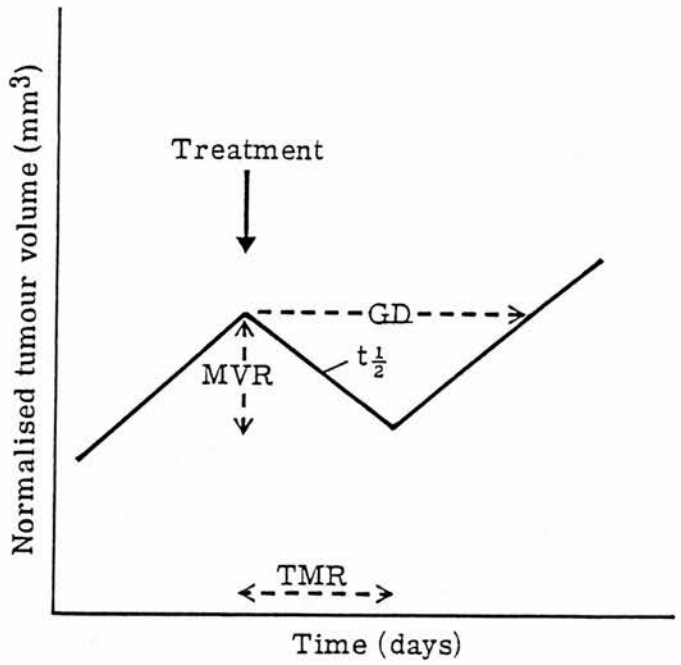
Growth curves were obtained by plotting tumour volume ($mm^3$) against time (days) on a semi-logarithmic scale. In each patient, tumour volumes were normalised to the date of treatment. All patients had at least one control, one radiation and one heat/radiation nodule.

4.2.iii Data Derived from Growth Curves

Fig 11 shows the idealised growth curve of a tumour responding to treatment. The endpoints used in the present study are indicated and defined below:-

Figure 11

Idealised Growth Curve Showing Clinical Endpoints in a Tumour Responding to Treatment




GD = Growth delay MVR = Maximum volume reduction

$t_{\frac{1}{2}}$ = Tumour halving time TMR = Time to maximum response

(a) Tumour Halving Time ($t_{\frac{1}{2}}$)

Defined as the time interval necessary for nodules to shrink to half their treatment-day volume, $t_{\frac{1}{2}}$ has been used to quantify the rate of tumour shrinkage in responding nodules. A line was drawn by eye through data points obtained post-treatment, during the phase of tumour volume reduction. A

statistical regression technique was considered inappropriate because of the variable number of data points, within this phase of the curve, for different nodules in the same patient.

(b) Maximum Volume Reduction (MVR)

MVR was defined as the ratio of tumour volume at the time of treatment to tumour volume at the time of maximum response. In nodules that became impalpable, the last measurement obtained during shrinkage was taken to indicate maximum response for purposes of comparison.

(c) Time to Maximum Response (TMR)

TMR, from the treatment date, was measured in all responding nodules.

(d) Growth Delay (GD)

Growth delay (GD), defined as the time required for regrowth of responding nodules to pretreatment volume, has been used as a semi-quantitative endpoint in clinical studies with radiosensitisers (Thomlinson, 1976, Dawes, 1978, Ash, 1979). In the present study, although all evaluable radiation-only nodules regrew to pretreatment volume, in 2 patients regrowing heated nodules failed to reach this value within the follow-up period available. For these nodules, growth delay was estimated by extrapolating from the regrowth

phase of the curve. This was considered valid as, in the group as a whole, heated nodules regrew at a constant rate, equivalent to the rate of growth of control nodules and to the pretreatment growth rate.

(e) Tumour Doubling Time (TDT) and Regrowth Rate (RR)

Defined as the time interval necessary for untreated nodules to double in volume, TDT was estimated in control nodules and, where available, from pretreatment measurements in treated nodules. A line was drawn by eye through the data points as application of a statistical regression technique was considered inappropriate [see 4.2.3 (a)]. Rate of tumour regrowth after treatment (RR) was calculated in a similar way.

(f) Thermal Enhancement Ratio (TER)

A thermal enhancement ratio for tumour was derived from the ratio of response to a given radiation dose alone and to the same dose of radiation in combination with heat.

The validity of this expression in clinical work and its deviation from thermal enhancement derived from dose response studies in animals will be addressed in the discussion.

## 4.3 The Heating Technique

### 4.3.i The Heating System

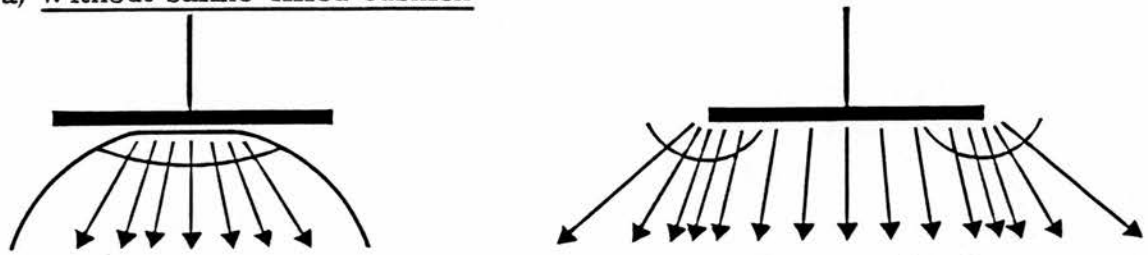
#### (a) Tissue Heating

The heating system used was developed specifically for treating superficial lesions and is based on a physiotherapy short wave diathermy unit operating at a frequency of 27 MHz (Curapuls 418). The equipment has been modified to incorporate an impedance matching unit, which allows more efficient transfer of electromagnetic energy to the patient, and a balanced transmission line connection to the applicator electrodes so that stray electromagnetic fields are reduced in the treatment room. The maximum power output of the unit was rated at 450 W. Localised tumour heating was achieved by electric field divergence beneath the smaller of the two applicator electrodes (Fig.12)

Figure 12

Localised Tumour Heating Achieved By Electric Field Divergence Beneath the Smaller Applicator Electrode

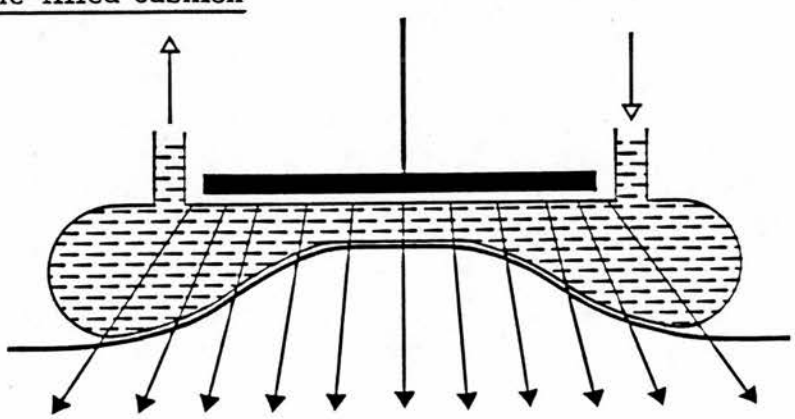
a) Without saline-filled cushion



Unpredictable field size resulting from poor surface contact

Superficial overheating may result from high fringing field intensities

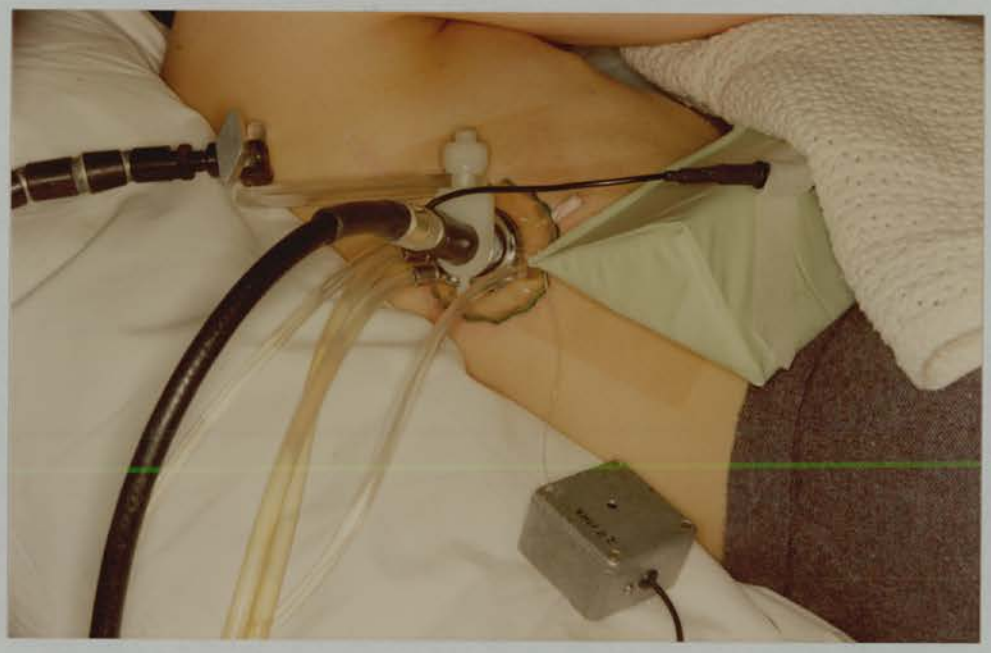
b) With saline-filled cushion



Good surface contact and temperature control is achieved using a perfused saline cushion

Figure 13

The Heating System in Operation Showing the Saline-filled Cushion Beneath the Smaller of the Two Electrodes



The saline-filled cushion is shown beneath the smaller of the two electrodes with the thermometry cannula visible at the end of the cushion.

A saline-filled cushion, positioned between the electrodes and skin, fulfilled a number of functions (Figs.12 and 13):-

(i) The provision of good electrode contact;

(ii) The avoidance of superficial overheating from high fringing field intensity by allowing the fringing field to diverge above skin surface;

(iii) Control of surface temperature by perfusion of the cushion with temperature regulated saline; and

(iv) Definition of treatment field, shape and size, by adjustment of cushion dimensions and degree of inflation.

Clincial experience has demonstrated the system's ability to achieve temperatures > 43°C, at a depth of 3-4 cm in lesions with linear dimensions of up to 10 cm.

(b) Control of Skin Temperature

Accurate control of skin surface temperatures at 43°C throughout the prescribed treatment time was necessary for treatment of cutaneous nodules and for investigation of normal tissue effects, and this was achieved by perfusing the cushions with a temperature-regulated saline solution. The temperature of the flowing saline, measured at the entry and exit points, was taken as a reliable index of skin temperatures provided that a sufficiently high and turbulent flow rate was maintained. A further requirement was that the

contacting membrane of the cushion was of appreciably lower thermal resistance compared with skin.

### 4.3.ii Thermometry and Temperature Control

#### (a) The Reference Temperature - Minimum Tumour Temperature (MTT)

The non-uniform temperature distributions seen in clinically heated tumours are complex in origin. Considering physical parameters first, all heating methods presently employed induce non-uniform power absorption in tumours and adjacent normal tissues. This is further modified by unpredictable physiological characteristics of individual tumours and their surroundings. The lack of a non-invasive thermal measuring technique restricts temperature sampling to a limited number of points which are inadequate for describing treatment of the whole tumour and unlikely to provide a reliable index of biological effect. Recent experimental studies indicate that minimum tumour temperature is the best predictor of long-term biological response when heat is added to radiation

(Dewhirst et al, 1984). Minimum tumour temperature has therefore been used as the reference temperature in the present study.

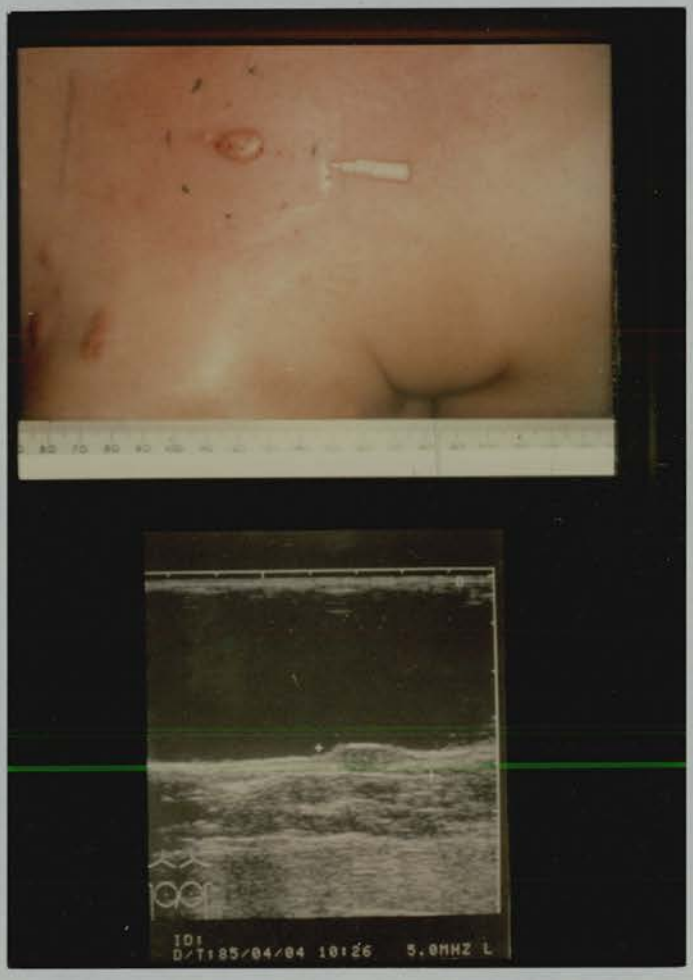
(b) Thermometry Localisation

Ultrasound was used to identify and position a 20 gauge cannula at the macroscopic junction between tumour and normal tissue at the tumour base before all heat treatments. Details of the ultrasound equipment are given in Section 4.2.i (c). Local anaesthetic (1% lignocaine) was used to infiltrate the skin at the entry point and care was taken to ensure that this lay outside the area of skin in contact with the saline cushion (Fig.14).

A thermocouple probe was threaded into the cannula, allowing temperature measurements to be made at between two and four tumour points and four and two adjacent normal tissue points depending on the size of the lesion (Fig. 15).

Figure 14

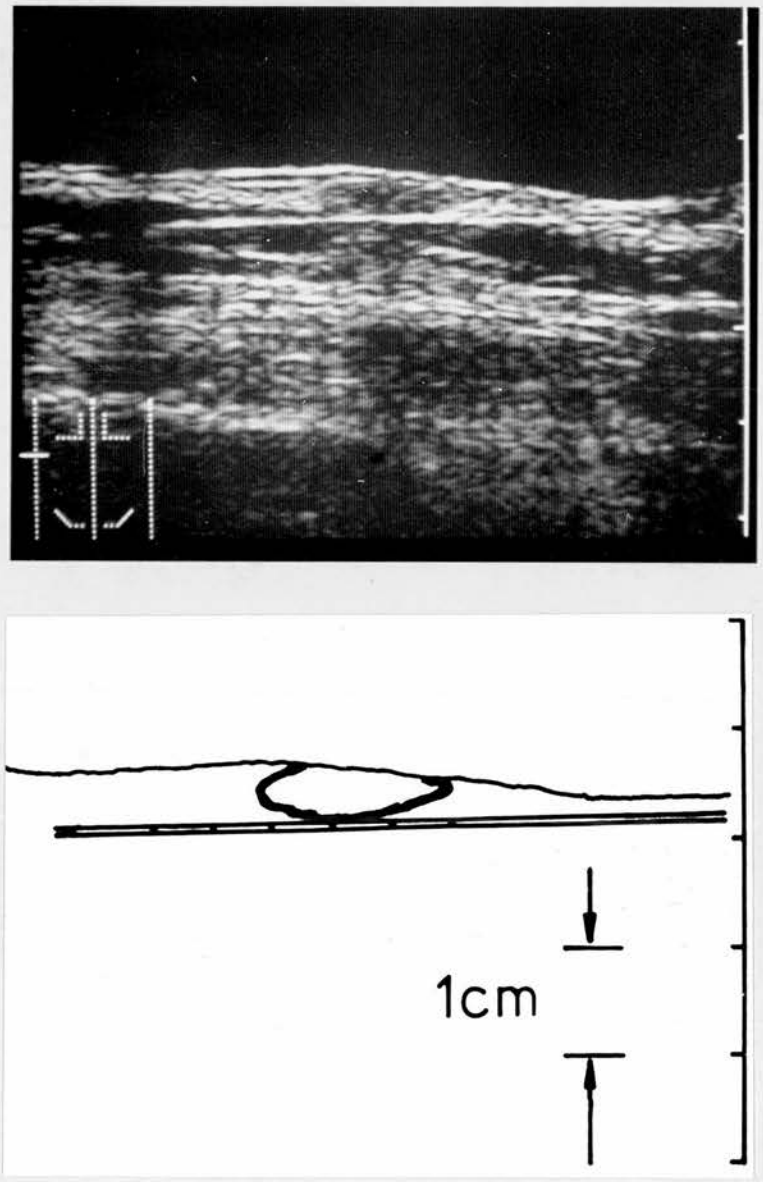
Photograph and Ultrasound Image Demonstrating the Position of the Thermometry Cannula




The black skin marks correspond to the position of saline cushion and the red points demonstrate the thermocouple spacing.

Figure 15

Ultrasound Image and Schematic Representation of the Thermometry Cannula Lying at the Macroscopic Junction Between Tumour Base and Normal Tissue




The position of the six thermocouples is shown with reference to tumour and normal tissue.

(c) Thermometry

An implanted linear array of 6 manganin/constantin thermocouples, used under microcomputer control, provided rapid information about linear temperature distributions. The multijunction thermocouple sensors were developed using the design principles discussed by Carnochan et al (1986).

4.3.iii Heat Treatment

(a) The Prescribed Heat Dose

The treatment aim was to give a minimum tumour temperature of 43°C for the prescribed time of 60 or 30 minutes. Wide lateral field margins were chosen to minimise lateral temperature gradients within tumour tissue, thus increasing the likelihood that the minimum tumour temperature would be located at the tumour base. To increase this probability further the skin surface was maintained at the specified minimum tumour temperature.

(b) Scheduling of Heat with Radiotherapy

Hyperthermia was given 3-4 hours after radiotherapy.

## 4.4. Radiation

### (a) Selection of Radiation Employed

Forty-six nodules were irradiated with single doses of electrons. Electron energies were selected to give as uniform coverage of the lesion as possible, tumour depth having been calculated from ultrasound images. Where overlying skin was infiltrated with tumour, the appropriate depth of perspex buildup was added to ensure adequate dose throughout the nodule.

### (b) Selection of Field Size and Radiation Dose

Field size was chosen to give good clinical coverage of the lesion. Radiation dose ranged from 6-12 Gy and the number of nodules given each dose level, with or without hyperthermia, is shown in Table 8.

TABLE 8

RANGE OF RADIATION DOSE ± HYPERTHERMIA, IN MEASUREABLE SUPERFICIAL METASTASES

| Radiation dose (Gray) | Number of nodules | |
|---|---|---|
| | Radiation only | Radiation & heat |
| 6 | 1 | 1 |
| 8 | 23 | 14 |
| 10 | 4 | 0 |
| 12 | 3 | 0 |

The dose selected was that considered necessary to induce tumour regression while permitting subsequent regrowth within a feasible observation period. For each

patient, this was judged on tumour type, previous radiation response and tumour doubling time. Where possible, a range of radiation doses was given to different nodules in the same patient, but if only two treatment nodules were available the same dose was given to each.

Chapter 5

# INVESTIGATION OF TUMOUR BLOOD FLOW

## 5.1 Xenon Clearance

### 5.1.i Reproducibility of Xenon Clearance as a Technique for Measuring Blood Flow in Small Superficial Tumours

$Xe^{133}$ clearance has been evaluated for its suitability as a technique for measuring vascular change induced by hyperthermia in human tumours. The reproducibility of the technique was assessed first in a group of small, superficial metastases (< 10ml) and later in a group of larger superficial tumours ($\geq$ 10ml) in which lymph node metastases were included. Patients were referred from, and investigated at, two hospitals but the same procedure was followed at each centre and carried out by the same operator (DT).

External conditions were carefully standardised with respect to room temperature and patient positioning. A bolus of about 10 MBq of $Xe^{133}$ in 0.1 ml saline was injected directly into the tumour using a 25 gauge (16 mm) needle. To ensure accurate repositioning of the needle the site of injection was localised by skin tattooing. At one of the centres Ultrasound was available to measure the depth of the tumour centre beneath the skin surface and a marker placed on the needle shaft at the appropriate height.

The barrel of the syringe was held perpendicular to the skin surface during slow injection of xenon. On removal of the needle the skin surface was wiped to remove any

extravasated xenon and the detector rapidly positioned. The equipment used to detect the gamma emissions was either:-

(i) A 5" diameter x 2" thick sodium iodide detector with a focussing collimator connected to a Canberra series 40 multi-channel analyser in multiscaling mode (Royal Marsden Hospital, Sutton)

OR

(ii) a large field of view Siemens ZLC750 gamma camera with a x 3.75 magnification, fitted with a low-energy all purpose collimator (Royal Marsden Hospital, London).

A scheme of the setup is shown in Fig. 16.

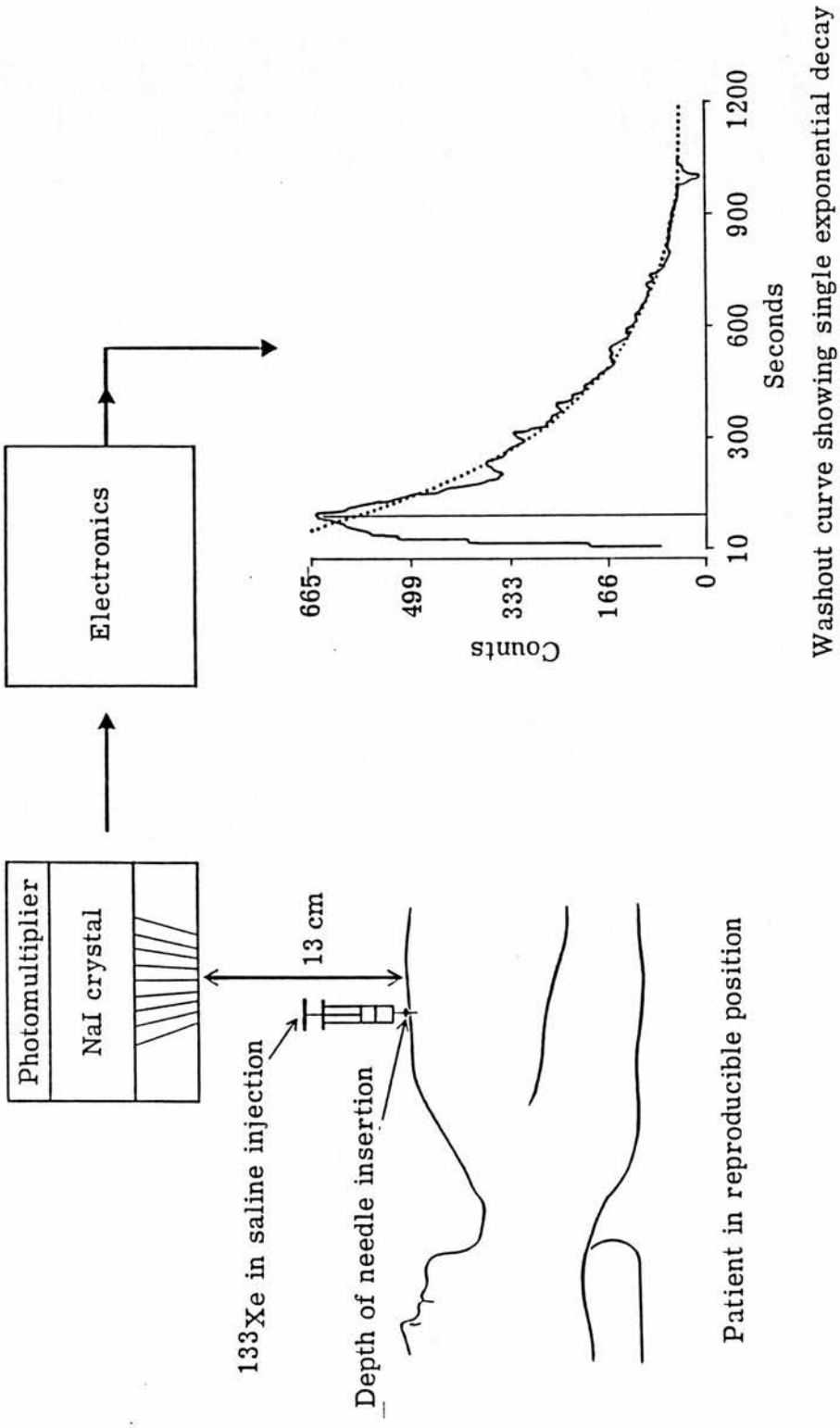
Data was collected at 10 second intervals over a 20 minute period. Washout curves consisted of background plus either a single exponential or the sum of two exponentials. A Gauss-Newton curve fitting programme was applied to the data to extract the exponential functions from the equation shown below:-

$$y\ (t) = Ae^{-K1t} + Be^{-K2t} + C$$

where K1 and K2 are the exponential functions and C represents background activity.

Figure 16

Scheme of the Setup for Xenon 133 Clearance Measurement of Tumour Blood Flow



Blood flow was then calculated from the standard formulae below and expressed in mls/min/100g:

(i) Bi-exponential decay $\quad \text{Flow} = \dfrac{AK_1 + BK_2}{A + B}$

(ii) Mono-exponential decay $\quad \text{Flow} = K$

Delta, the partition coefficient between tumour and blood, was taken to have a value of 1.0 ml/g.

For each tumour the procedure was repeated after a 2-4 hour interval during which time there was no therapeutic intervention. This time interval allowed for adequate fall in xenon activity within the tumour and would be an appropriate schedule for use with hyperthermia.

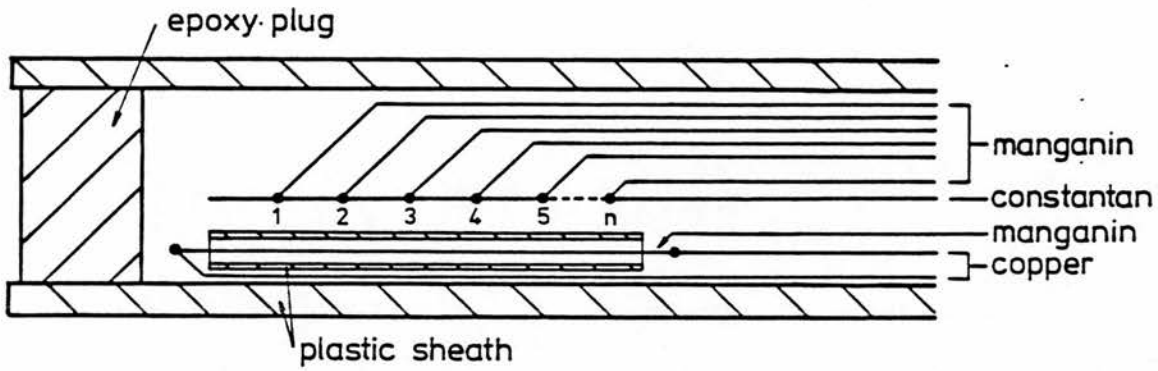
## 5.2 Thermal Clearance

### 5.2.i Thermal Clearance Technique

A flexible multiple-sensor thermal clearance probe has been used to study blood flow in human tumours and surrounding normal tissues. The probe was based upon a multiple-junction thermocouple array of the type commonly employed to monitor localised hyperthermia treatments and the essential features are illustrated in Fig. 17

Figure 17

Illustration of the Essential Features of the Multiple-sensor Thermal Clearance Probe



Initially a nylon sheathed probe (0.5 x 0.63 mm., i.d. x o.d.) housing two thermocouple junctions, with a separation of 10 mm, was used (Design 1). Later, this was replaced by a polytetrafluoroethane (PTFE) sheathed probe (0.4 x 0.6 mm) encasing six thermocouple junctions 5 mm. apart (Design 2). The latter probe was considered superior, despite the better thermal characteristics of the thinner walled nylon sheathing, because of a decreased propensity to kinking. The heating element was formed from a short length of 25 Mm diameter enamelled manganin wire, prevented from making close contact with the thermocouple wires by a PTFE sleeve. Enamelled copper connecting leads (40 Mm diameter) were soldered to the heating element for connection with a constant current supply. The thermocouple junctions were positioned symmetrically about the mid point of the heating element, which was always at least 3 cm longer than the thermocouple array. The design of the probe allowed it to be fixed in position within the tissues so that serial measurements could be taken at several points without the need to mechanically disturb the local environment. The probe was considered to reflect flow changes over a radius of approximately 2-3 mm from each sensing point but the precise distance is dependent upon the thermal characteristics of the surrounding median (Perl, 1962).

Probe heating, using a current of 60mA, was maintained for a period of 5 minutes and the subsequent cooling phase recorded at 5 second intervals until a steady state was achieved (5-15 mins.). Temperature recording was computer

controlled (K3000E, Kemitron Ltd.) and measurements made to a resolution of 0.025°C with an accuracy of ± 0.15°C. A linear fitting procedure was used to characterise the cooling phase, in terms of a single exponential decay constant, for each set of data. The analysis was based upon the assumption that changes in the cooling rate constant, beyond those expected from experimental error, correspond to changes in blood flow.

### 5.2.ii Initial Probe Characterisation and Assessment of the Reproducibility of the Technique

Preliminary physical characterisation of the thermal clearance probe was performed in a polyacrilamide gel phantom considered to be thermally representative of non-perfused tissue of moderate water content, such as necrotic tumour. Thermal characteristics of the nylon sheathed probe, following a 5 minute heating period with a 60mA current are shown in Fig 18, and the subsequent cooling data demonstrated in Fig 19.

Figures 18

Thermal Characteristics of the Nylon Sheathed Probe in Tumour and Phantom, Following a 5 Minute Heating Period

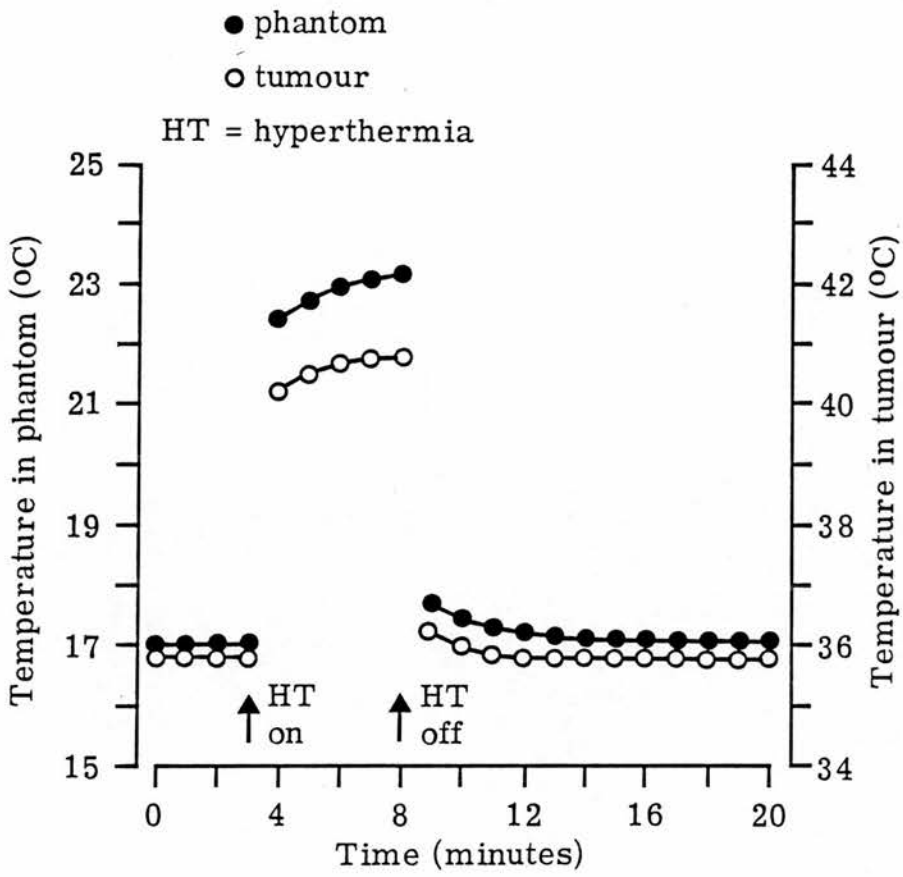
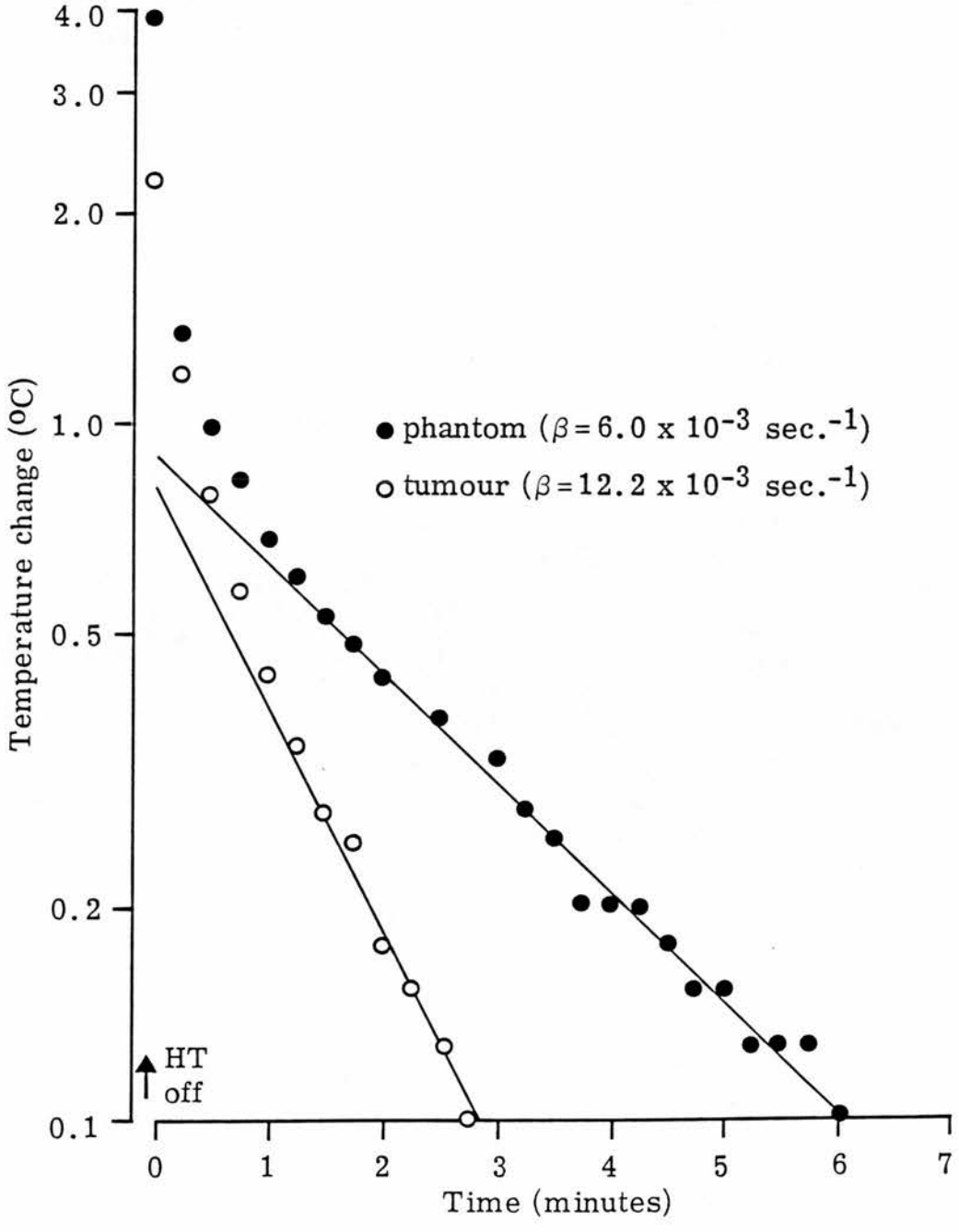


Figure 19

Cooling Curves, in Phantom and Tumour, Following a 5 Minute Heating Period



Inspection of cooling data revealed two distinct components; a fast initial phase and a slower, single exponential component described by the decay constant (beta) derived from the expression:

$$\log_{10} (T - Tf) = \log_{10} (To - Tf) - \frac{Bt}{In(10)}$$

where T is the probe temperature at time t, Tf the final steady state probe temperature and To the "apparent" probe temperature at the start of cooling. Reproducibility of the system was initially assessed by ten consecutive measurements within the gel phantom. Mean values of beta, and respective standard deviations were found to be 6.6 (0.47) x $10^{-3}$ $sec^{-1}$ for position 1 and 5.9 (0.28) x $10^{-3}$ $sec^{-2}$ for position 2.

Thermal clearance characteristics were compared for the gel phantom material and a cutaneous tumour nodule. Two interesting differences are demonstrated in Figs. 18 and 19; firstly, the temperature rise during heating is lower for the tumour and secondly the value of beta is higher. Although dissimilar thermal conduction parameters may account for some of this difference, it seems reasonable to suggest that the presence of blood flow in the tumour is influencing the observations.

The reproducibility of the technique was assessed in a group of 10 patient volunteers with previously untreated superficial tumours. Tumours were selected to reflect the

type of lesion commonly considered suitable for hyperthermia studies. The thermal clearance probe (Design 1) was introduced into tissue such that point 1 lay within the central part of the tumour and point 2 in the surrounding subcutaneous normal tissue. This was achieved in six patients, while the remaining four experienced discomfort which prohibited insertion of the probe to the desired position. In these patients temperature measuring points lay within normal tissue, so that in total six tumour and 13 normal tissue points were assessed. Thermal clearance measurements were repeated at least three times over a 60-90 minute period, resulting in a total of 74 measurements.

5.2.iii Thermal Clearance - Hyperthermia Study

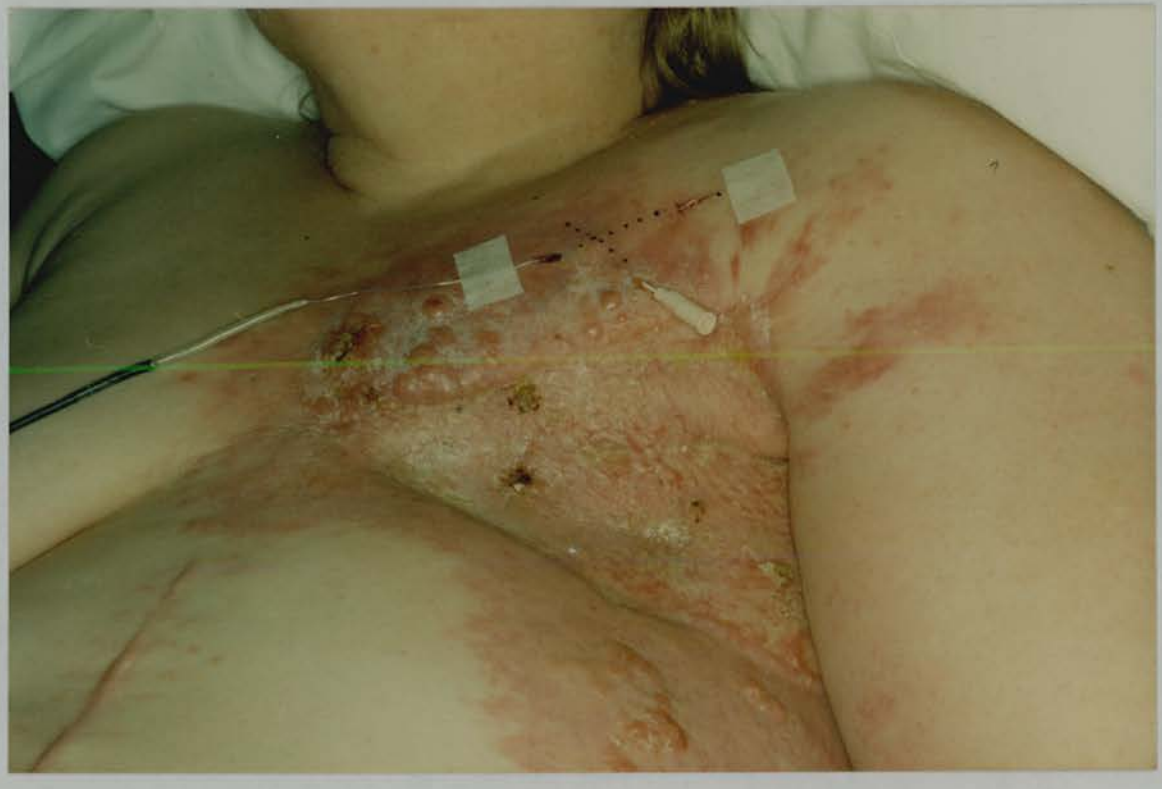
A multiple junction thermocouple probe (six junctions, 5mm spacing) positioned at the base of the lesion, as described in Chapter 4, provided thermal dosimetry and treatment control. In a similar way, the insertion of the thermal clearance probe was guided by placement of 20 gauge PTFE cannulae after infiltration of skin entry and exit sites with local anaesthetic (lignocaine hydrochloride 1%). This second cannula was introduced in a direction perpendicular to the thermometry probe and advanced in a more superficial plane such that the central part of the tumour was traversed.

The distal portion of the cannula was exteriorised and, after inserting the probe, the guide cannula was removed. Both proximal and distal parts of the probe were securely taped to the patients during thermal clearance measurements

and layers of gauze placed over the area to ensure good thermal insulation. Fig. 20 shows the thermal clearance probe secured by tape and, at right angles to it, the cannula which houses the thermal dosimetry probe. the positions of the thermocouple junctions for both probes are marked on the skin.

Figure 20

Thermal Clearance Probe and Thermometry Cannula Positioned at Right Angles to One Another



The position of the thermocouples, in tumour and normal tissue is indicated on the skin surface.

Single doses of hyperthermia, with the aim of achieving a minimum tumour temperature of 43°C for 60 minutes, were delivered using the capacitively coupled electromagnetic field heating system described in Chapter 4. The prescribed dose of heat was considered, in our experience, to be the highest consistent with a reasonable probability of technical success and avoidance of significant normal tissue damage. It was also felt to be within the range where a substantial difference between the vascular response of tumour and normal tissue might be expected.

Thermal clearance measurements were only made during periods of tissue thermal stability better than ± 0.2°C in any 10 minute period and this required a delay after initial probe insertion or following hyperthermia of typically 20 minutes. The thermal clearance probe was disconnected from the recording equipment during hyperthermia sessions to minimise the probability of electromagnetic interference. Care was taken to avoid placing thermocouple sensing points within a centimetre of areas infiltrated with local anaesthetic. Where possible, thermal clearance measurements were made before hyperthermia, immediately following hyperthermia, 4 hours and 24 hours after hyperthermia.

Chapter 6

# PHYSICAL CHARACTERISATION OF HYPERTHERMIA TREATMENTS

## 6.1 Quality of Heat Treatments.

The ability of the heating system to deliver a prescribed treatment has been tested by examining the physical characteristics of 56 single hyperthermia treatments in small superficial circumscribed tumours. Clinically, these formed a fairly homogeneous group with some patients contributing different lesions to more than one study included in this thesis. The 56 treatments have been designated as successes or failures according to the criteria in Table 9. In the present analysis, the term success is used to describe the physical quality of the heat delivery and has no biological implication.

TABLE 9

CRITERIA USED TO ESTABLISH SUCCESS OR FAILURE. OF HEAT DELIVERY

| | | |
|---|---|---|
| Successful heat delivery | | MTT of 43°c for the prescribed time of either 60 or 30 mins. |
| Failed heat delivery | $F_1$ | Failure of MTT to reach 43°c. |
| | $F_2$ | Failure to maintain MTT at 43°c for the prescribed time. |

(MTT = Minimum tumour temperature)

Fifty-four percent (30) of heat deliveries achieved the prescribed aim. The remainder (26) were classified as failures and allocated to one of the two groups according to the criteria in Table 9. F1 failures were more common than F2 failures, with 16 and 10 treatments in each respectively.

The physical characteristics which have been analysed are temperature distribution along the multijunction thermocouple probe, power deposition requirement and time taken to reach treatment temperature. In addition, tumour characteristics, such as volume, site and histology, have been examined to identify factors influencing heat delivery. Patient variables, such as pain tolerance and ability comfortably to maintain the treatment position have also been considered in this study.

## 6.2 Analysis of Heat Delivery Treatments by Temperature Distribution

A typical arrangement of the six thermocouples used to monitor temperature in tumour and normal tissue is shown in Fig 21 . Depending on tumour size, between 2 and 4 points were considered to lie at the tumour base and this was confirmed by ultrasound imaging with the probe in situ.

responses summarised in Fig. 44 showed no significant difference in each group (single tailed $x^2$ test, $p = 0.05$ level). This result was also supported by a careful inspection of temperature data recorded during hyperthermia, which did not reveal any significant relative changes between tumour and normal tissue temperatures throughout the heating period.

Figure 45

Transient Erythema Following Heating of the Skin to 43°C for 60 Minutes

Prehyperthermia Posthyperthermia

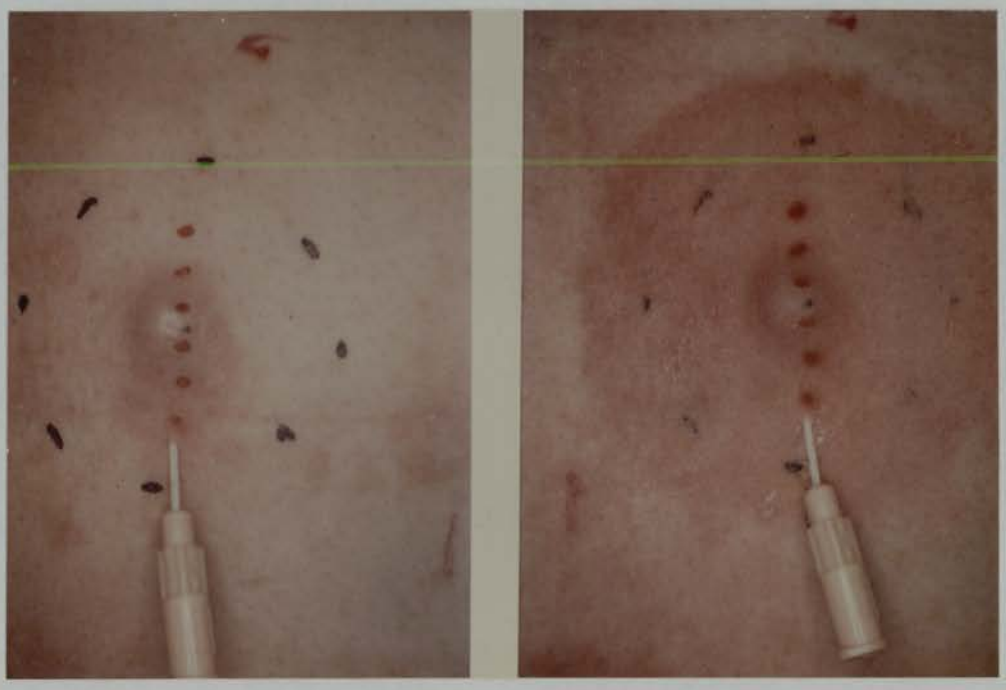
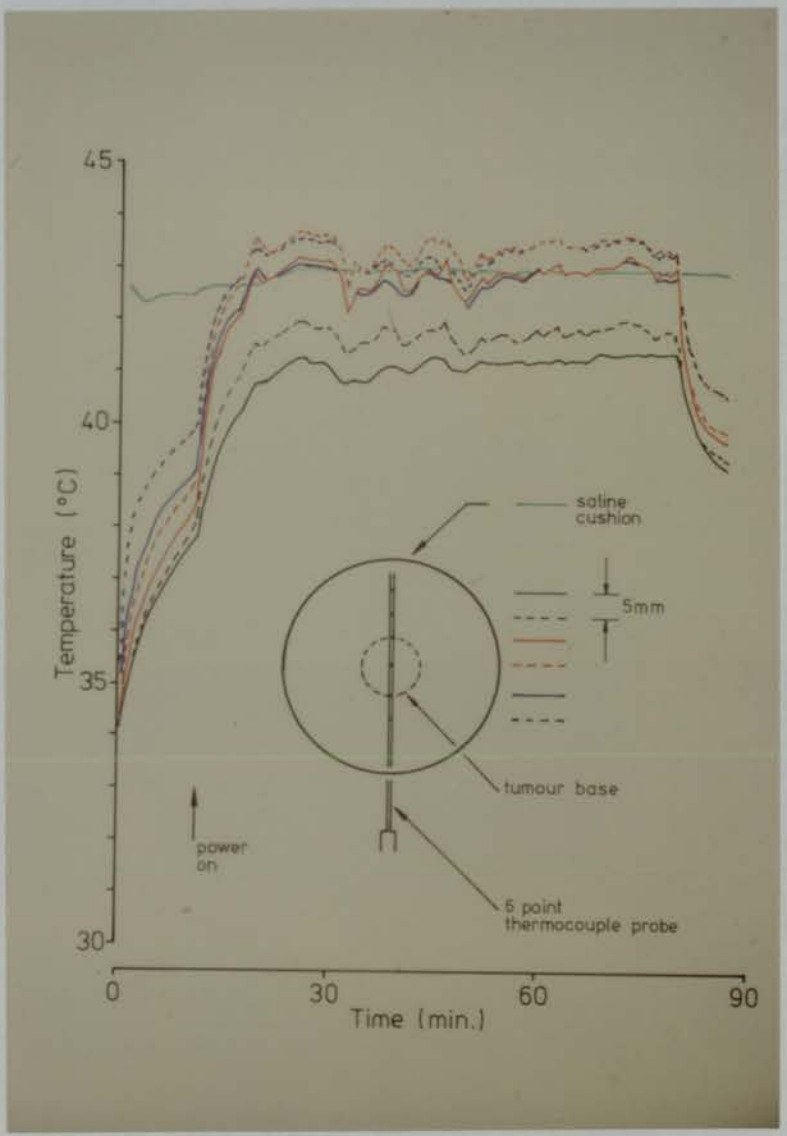


Figure 22

Temperature Profile Obtained from Measurement at Six Thermocouple Junctions, in Tumour and Normal Tissue



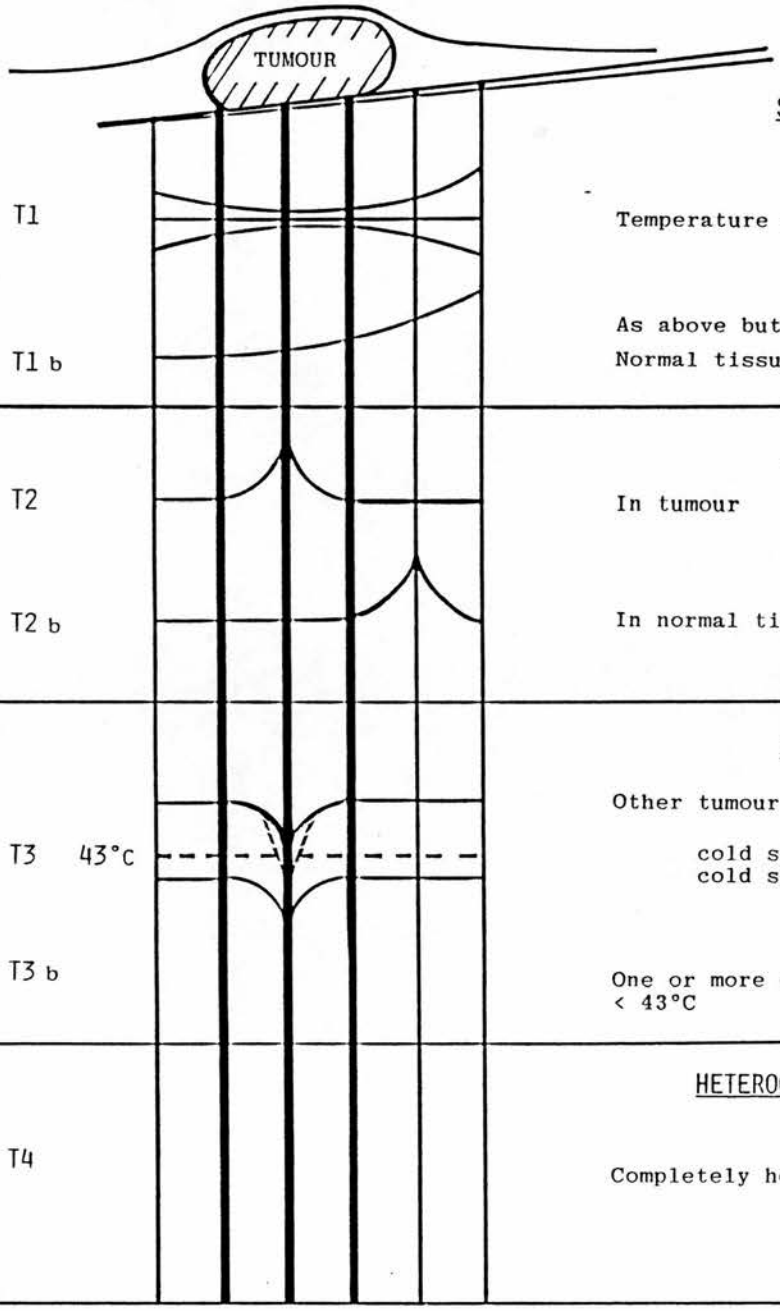
Temperatures measured at this time varied within the range 32-36°C, depending on the depth of the cannula beneath the skin surface. After positioning the saline cushion over the treatment area, temperatures were further observed before turning on the power. During this period, an initial temperature rise was seen and following an interval of 5-10 minutes, the temperature stabilized, usually in the region of 37-40°C.

The initial power input was 25-50 watts, depending on the field size, and was subsequently adjusted manually, with the aim of achieving a temperature rise of 0.5°C per minute. This usually required power adjustment every one to two minutes until the minimum tumour temperature reached the prescribed level. The time of application of non-ionizing radiation is indicated in Fig. 22 by "power on" and, as in this example, was usually followed by a period of linear temperature elevation. The plateau phase indicates the time/temperature specifications of the delivered treatment.

The patterns of temperature distribution observed along the six thermocouples has been classified as shown in Fig. 23. The initial part of the plateau phase of the temperature profile was used to allocate treatments to temperature distribution pattern types, in order to avoid the influences of heat-induced vascular changes likely to occur with progressive heating, particularly in the surrounding normal tissue.

## Figure 23

## A Classification of the Patterns of Temperature Distribution Observed Along the Multi-junction Thermocouple Probe Positioned at the Tumour Base




| CLASS | DESCRIPTION |
|---|---|
| T1 | SMOOTH DISTRIBUTION<br>Temperature gradient < 1°C/cm |
| T1 b | As above but with highest terminal<br>Normal tissue point > 43.5°C |
| T2 | SINGLE HOT SPOT<br>In tumour ] resulting in temperature gradient > 1°C/cm |
| T2 b | In normal tissue ] |
| T3 43°C | SINGLE COLD SPOT<br>Other tumour points ≥ 43°C ] resulting in temperature gradient > 1°C/cm<br>cold spot ≥ 43°C (success)<br>cold spot < 43°C (failure) |
| T3 b | One or more other tumour points < 43°C |
| T4 | HETEROGENEOUS DISTRIBUTION<br>Completely heterogeneous. |

Although temperature fluctuations were seen at individual measurement points during heating, the overall distribution patterns were unaffected during the course of treatment in all but one patient. In this instance a progressive, differential, decline in temperature at the most proximal normal tissue measurement point resulted in a change from T1b to T1. However, T1b was the dominant pattern considering the plateau phase as a whole. Temperature gradients, or change in temperature with distance, were indicated by the slope of the plateau phase, and expressed as degrees centigrade per centimetre.

In temperature profile types T1 and T1b, a smooth distribution was arbitrarily defined as a temperature gradient of < 1°C/cm, or < 0.5°C variation between adjacent thermocouples. The distinction between the two was the high lateral normal tissue temperature in T1b, as a result of progressive small temperature rise in one direction along the probe. With T1 distributions, several profile shapes were possible while maintaining the criteria for a smooth distribution (see Fig. 23). T2b was similar to T1b, with a high normal tissue temperature, but allocation to the former required the presence of a temperature gradient of > 1°C per centimetre. Type T3 temperature profiles displayed a single tumour cold spot which could either be above 43°C (successful treatment) or below (treatment failure).

The aim of this analysis was to examine the homogeneity of heating achieved with the hyperthermia system employed in

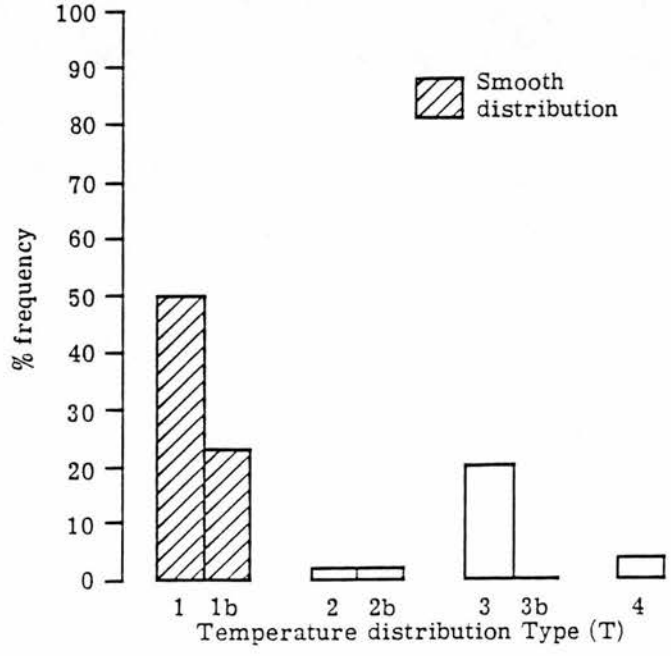
the present study, and to investigate the influence of temperature distribution on successful, and failed, heat delivery.

## 6.3 Homogeneity of Heat Delivery

The frequency of temperature distribution types in the 56 hyperthermia treatments is shown in Fig. 24. A smooth distribution was achieved in 73% (41/56), of which the majority (68%) were included in category T1. A single cold spot in tumour, in an otherwise adequate temperature profile (T3) accounted for a further 20% of heat sessions. There were no T3b distributions and only two completely heterogeneous profiles.

Figure 24

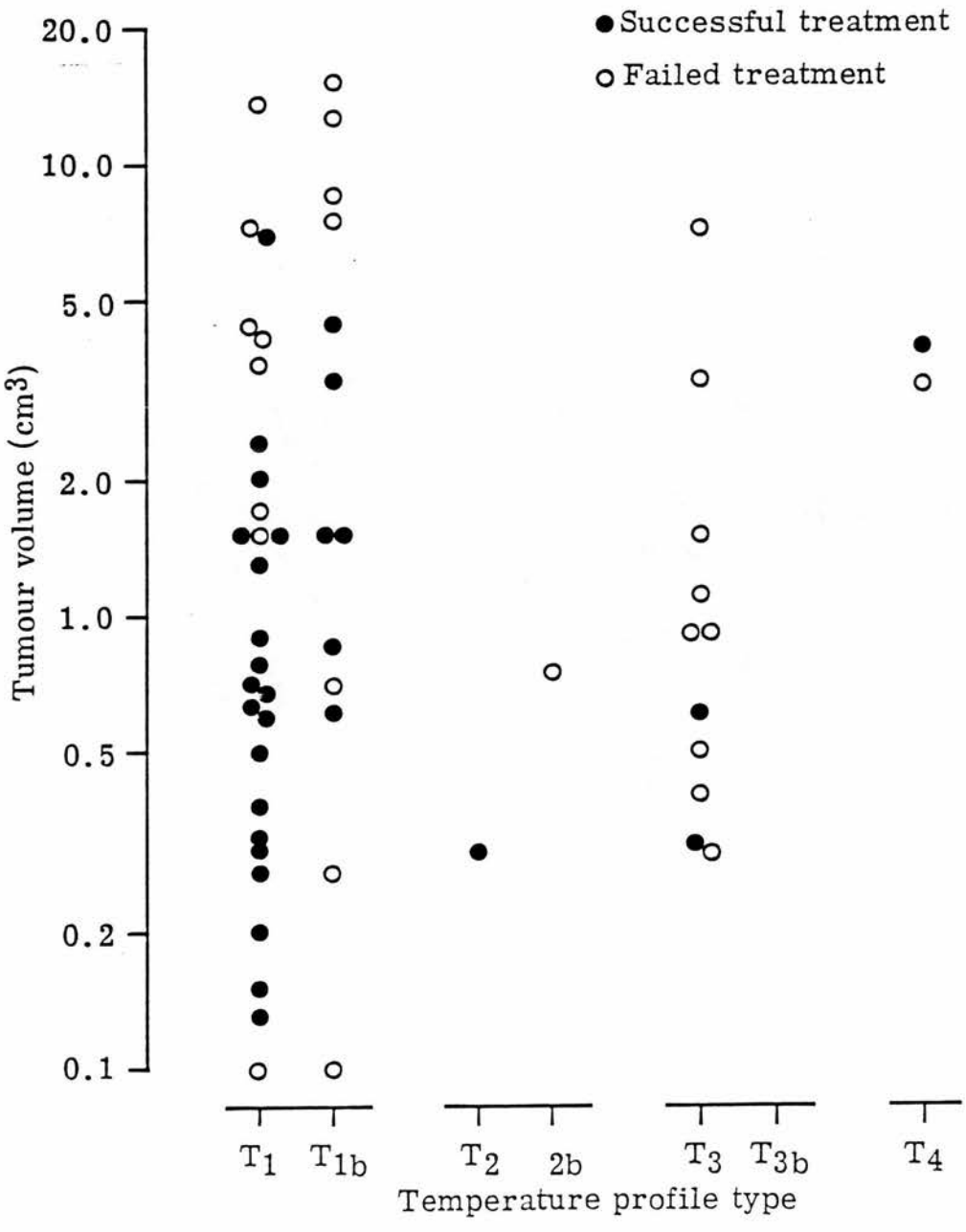
Frequency of Temperature Distribution Patterns (T Type) As An Expression of Homogeneity of Heating in 56 Heat Deliveries



Treated lesions have been ranked according to tumour volume for each of the temperature profile types (Fig 25). Within the range of volumes treated (0.1 - 15 $cm^2$), tumour size did not appear to influence uniformity of heating, although the number of lesions in $T_2$ - $T_4$ is small.

Figure 25

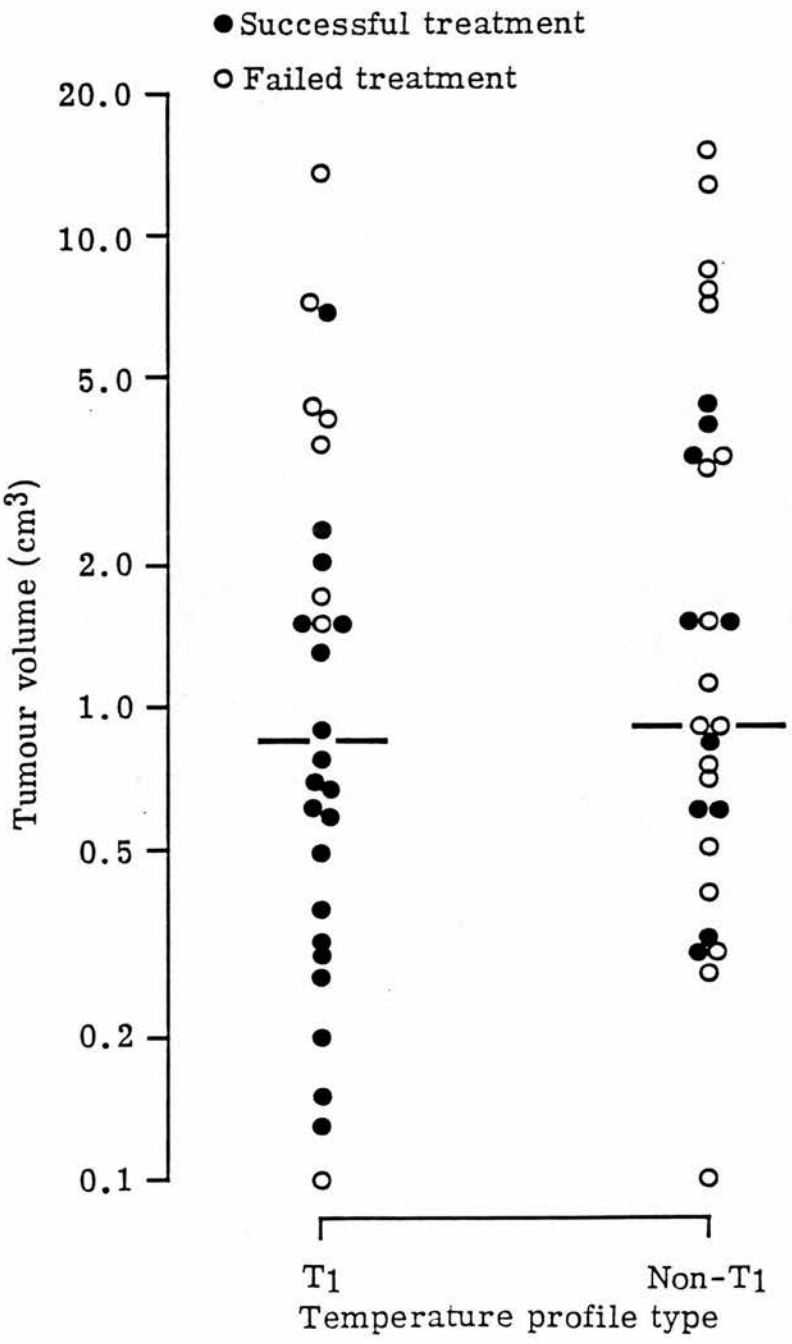
Range of Tumour Volume for Each Temperature Profile Type



Combining all non-$T_1$ temperature profiles for comparison with type $T_1$, the range of values and median volumes are remarkably similar (Fig. 26).

Figure 26

Range of Tumour Volume and Median Values For $T_1$ and Non $T_1$, Temperature Profiles



## 6.4 Influence of Temperature Distribution on Success of Heat Delivery

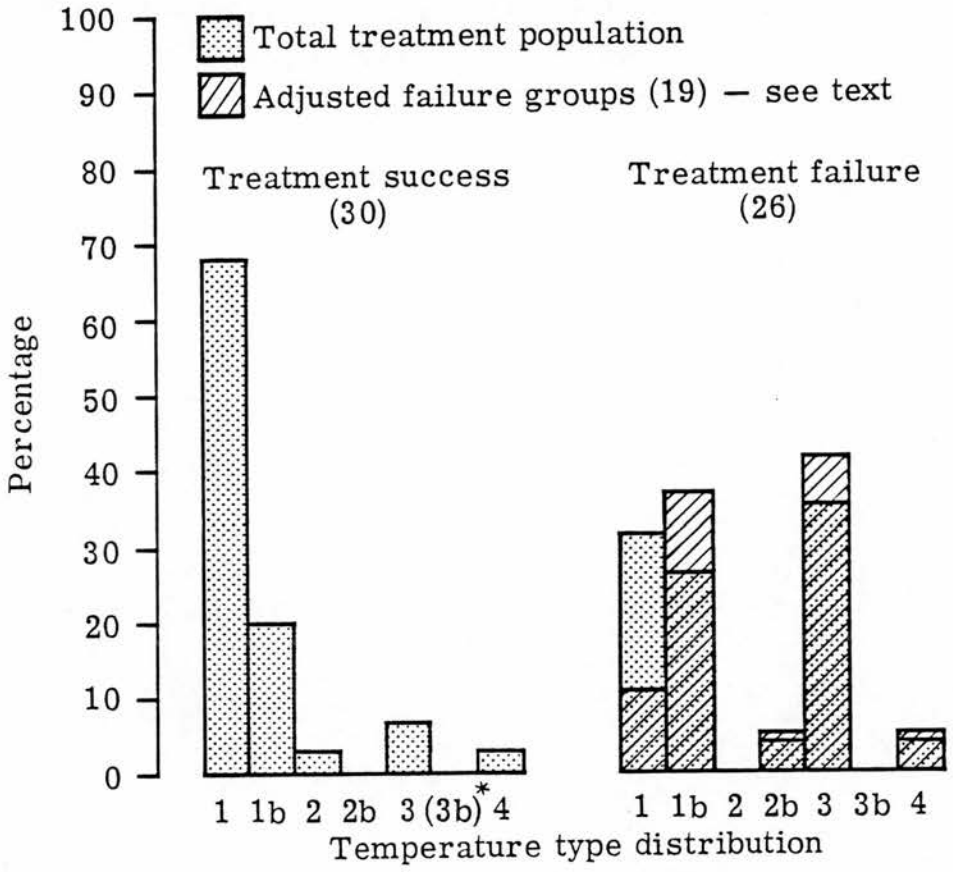
From the definition of success, as described in Table 9, any type of temperature distribution could occur with either a successful or failed heat session, apart from T3b, a distribution pattern which implies failure. Therefore, to examine whether temperature distribution influenced failure, the patterns have been analysed for the two groups (Fig. 27).

A smooth temperature distribution (T1, T1b) occurred in 86% (26/30) of the 30 successful heat deliveries, and of these 77% were type T1. Of the remaining 4 successes, 3 displayed single hot or cold spots in the temperature profiles. There was one completely heterogeneous distribution (T4) which nevertheless fulfilled the criteria for success.

In the 26 failures, smooth temperature profiles were less frequent (58%) and type T3 distribution more prominent (35%), compared with the group of successful heat deliveries (Fig. 27). However, grouping temperature distributions as T1 or non-T1, for the successes and the failures, there was no statistical difference between the two groups ($x^2 = 4.5$, $df = 1$, $p = 0.34$).

Figure 27

Frequency of Temperature Distribution Patterns in Sucessful and Failed Heat Deliveries




*(3b) excluded from success analysis by definition — see Table )

TABLE 10

CHARACTERISTICS OF THE SEVEN EXCLUDED FAILURES

| Reason for discontinuing treatment | Failure type | Temperature distribution pattern |
|---|---|---|
| Non-related symptoms or Inability to maintain position | ( F2 | T1 |
| | ( F2 | T1 |
| | ( F2 | T1 |
| | ( F2 | T3 |
| Excessive sensitivity to low temperature | ( F1 | T1 |
| | ( F1 | T1 |
| | ( F1 | T1 |

Treatment failures were also examined after exclusion of seven patients on the grounds that these failures were not related to temperature distribution. Thus, in 4 patients treatment had to be terminated early because of the onset of non heat-related symptoms and/or the inability to maintain the treatment position after the prescribed minimum temperature had been achieved. By definition these were F2 failures. Table 10 shows that three of the four had smooth distributions (T1) until the power was switched off. In the three other excluded patients, localised discomfort was experienced despite uniform temperature distributions (T1) below the prescribed minimum tumour temperature.

This suggested excessive sensitivity to lower temperatures, as a result of which the treatment aim could not be achieved, making these F1 failures. These seven patients therefore contributed 40% of the T1 distributions in the entire failure group.

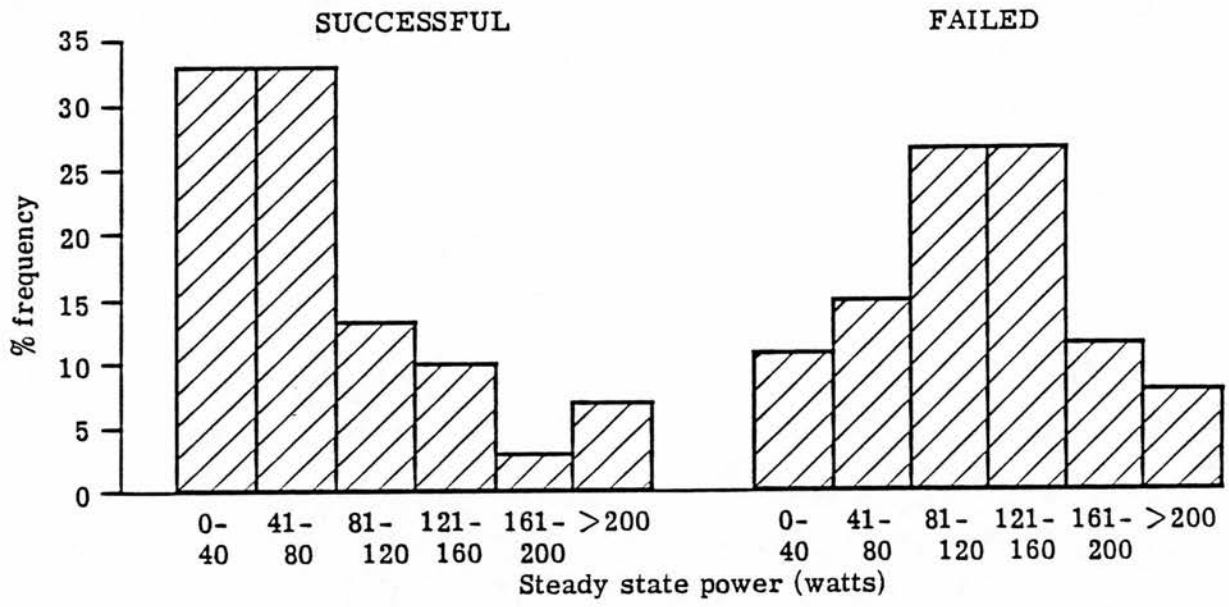
Analysis of temperature distribution in the adjusted failure group reveals only 10% (2/19) T1 distributions, compared with 31% (8/26) in the total failure group. After making the exclusions, T3 distribution is an even more common occurrence (42%). Repeating the analysis by T1 and non-T1 grouping, there was a statistically greater proportion of T1 temperature distributions in the successes compared with the adjusted failure group ($x^2 = 6.84$, df = 1, $p = 0.009$).

## 6.5 Analysis of Heat Delivery by Power Requirements

The steady state power applied during the 56 hyperthermia treatments ranged from 11 to 370 watts. Dividing this range into 40 watt intervals, a comparison has been made between power requirements in the successful and failed heatings (Fig. 28)

Figure 28

Steady State Power Application in Successful and Failed Heat Deliveries




Sixty-six percent of successful heat deliveries achieved the prescribed aim of 43°C (MTT) for 60 minutes with an applied power of 80 watts or less. The majority of the remainder required between 80 and 200 watts, but two successful treatments were achieved after applying 210 and 250 watts respectively.

Anatomical site, adjacent normal tissue structures and histological type have been considered, but in this small group of treatments, no contributory factors have been identified.

Temperature profiles have been examined to see whether a tumour cold spot (Type T3) might have been responsible for the high power application necessary to achieve success. However, only one of the 10 treatments showed a T3 distribution, with T1 and T1b profiles accounting for 8 and 1 treatments respectively.

In 73% (19/26) of failed heat deliveries, power was increased to > 80 watts in an attempt to reach the prescribed temperature. In 6 of the 19, the application of a high power level was in an attempt to elevate a single tumour cold spot (T3) to the prescribed temperature. In five of the failure group treatment temperatures were not achieved despite application of 170-370 watts.

## 6.6 Analysis of Time Taken to Reach Treatment Temperature

Eighty-three percent of successful heat deliveries achieved the prescribed temperature within 10 minutes and the remainder did so in less than 20 minutes. Eighty-four percent (21/25) of rapid temperature achievers ($\leq$ 10 mins.) had smooth temperature distributions ($T_1/T_{1b}$) but one $T_2$, two $T_3$ and one $T_4$ patterns were included in the group.

Only the 10 $F_2$ type failures can be included in this part of the analysis as by definition F1 failures did not reach the treatment temperature. Of these, 50% achieved the prescribed temperature in 10 minutes or less. The remaining 50% reached 43°C between 10 and 20 minutes after turning on the power.

## 6.7 Analysis of Successful Heat Delivery by Tumour Volume

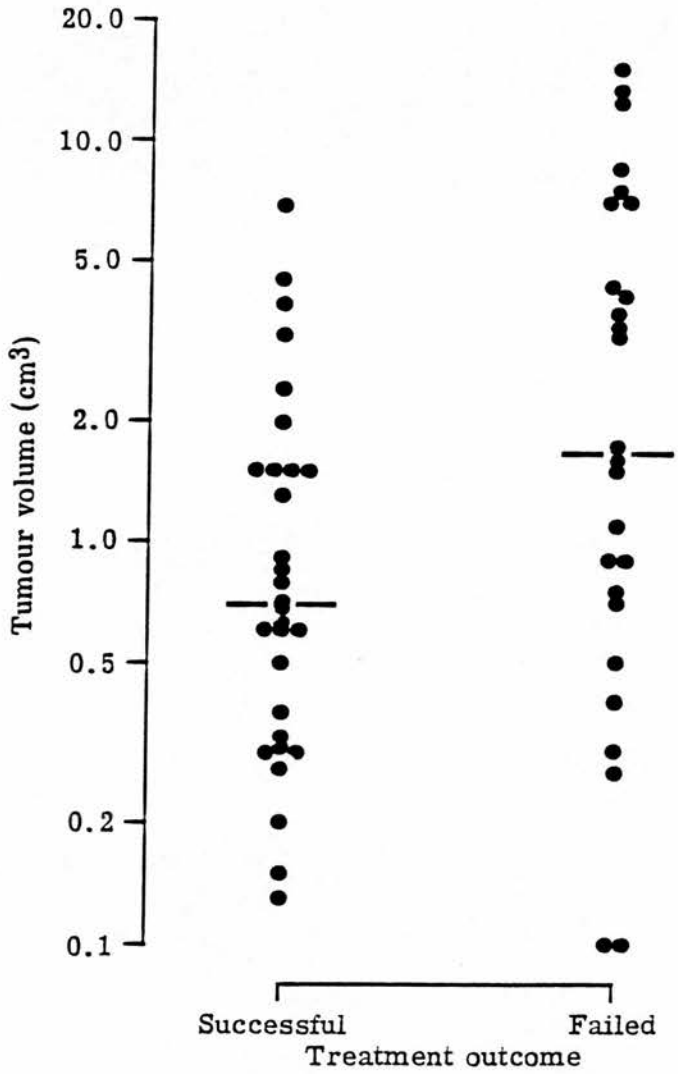
Tumour volumes, calculated from measurements taken on the day of treatment, show a wide overall range from 0.1 $cm^3$ - 15 $cm^3$. The distribution of volumes in the successful and failed heat delivery groups have been analysed and are displayed in Fig. 29.

Comparing the range of values, 0.1 - 6.9 $cm^3$ and 0.1 - 15$cm^3$, with median values of 0.7 $cm^3$ and 1.7 $cm^3$ respectively for successful and failed treatment there was evidence using the Mann Whitney U test ($p = 0.02$) that the tumour volumes were larger in the failure group. However, using the same test to investigate the similarity of the two failure subgroups $F_1$ and $F_2$, there was no difference in the medians of the two groups ($p = 1.00$).

The influence of tumour volume on uniformity of heat delivery and steady state power requirement has been described in Sections 6.3 and 6.5.

Figure 29

Tumour Volume Range and Median Values in Successful and Failed Heat Deliveries




## 6.8 Analysis of Treatment Success by Tumour Site

The majority of the 56 treated lesions were located on the chest wall and there was no other site that predominated in either group.

## 6.9 Analysis of Treatment Success by Tumour Histology

The distribution of histological tumour type was similar between the successful and failed treatments with adenocarcinoma of breast, adenocarcinoma from other primary sites, squamous and small cell carcinoma accounting for 86% and 96% of the two groups. Seventy percent of successful, and 61% of failed, treatments were in metastases from breast cancer.

## 6.10 Patient Variability

Patient variables, such as age, sex, extent of other metastatic disease and general condition did not predict for success of heat delivery. However, patients who were generally anxious, or who were specifically apprehensive at the outset of heating were more likely to be in the failure group.

## 6.11 Discussion

Difficulty in quality assurance in hyperthermia remains one of the major deterrents to evaluation of the modality in Phase III clinical trials (Nussbaum, 1984). It is widely reported that variations in temperature distribution occur not only between tumours, but also between successive treatments in the same tumour, and even during the course of a single treatment session. This inability of currently available heating devices to reproducibly deliver a standard, uniform heat dose is a major complication to the convincing demonstration of a therapeutic gain. However, careful recording and analysis of temperature variation can provide useful physical and biological information which may influence future application. For example, the importance of temperature distribution in predicting response was first recognised in murine systems (Wallen et al, 1981, Gibbs et al, 1981). Subsequently, a correlation between minimum monitored intratumoral temperature and prognosis, for both complete response and long term control, was demonstrated in spontaneous pet animal tumours treated with hyperthermia and radiation (Dewhirst et al, 1984). Human data are also now available which suggest that minimum tumour temperature has a critical influence on biological outcome in clinical hyperthermia (Arcangeli et al, 1985; Oleson et al, 1984).

The rationale for using minimum tumour temperature as a reference temperature in the studies described in this thesis has been presented in Chapter 1. In a further attempt to standardise treatment, the macroscopic junction between

tumour and normal tissue, at the tumour base, was selected as the reference for minimum tumour temperature measurement, and located with the aid of ultrasound imaging.

On the basis of prescribing treatment to a minimum tumour temperature for a specified time, strict criteria have been used to determine success, in terms of heat delivery, and to classify failure. The distinction between $F_1$ and $F_2$ failures is fundamentally important in an analysis of heat delivery. In $F_1$ failures the heating set-up and/or tissue anatomy and physiology prevent adequate tempeature rise at the point of interest. By contrast, in $F_2$ failures the same parameters permit the required minimum tumour temperature to be achieved but, for a variety of reasons, this cannot be sustained for 60 minutes. Thus, analysis of heat delivery by tumour volume showed that failure overall was more likely in larger lesions, an association which could be attributed to the thermometry probe lying at the greater depth. However, this reasoning is inconsistent for $F_2$ failures which achieved the minimum treatment temperature, and raises the possibility that $F_2$ lesions formed a subgroup which were not in fact larger, and the observed difference in volume between successes and failures was due to the impact of large lesions confined to the $F_1$ group. For this reason tumour volume was compared for $F_1$ and $F_2$ heat deliveries, but was found to be similar. This suggested that with this heating system, large lesions are not only less likely to reach the required minimum tumour temperature, but are also less likely to sustain the temperature rise. With only 10 $F_2$ heat

deliveries, it has not been possible to identify why tumour volume might be responsible for the latter, but the larger size nodules may reflect general disease status and thus patient fitness and tolerance of the procedure.

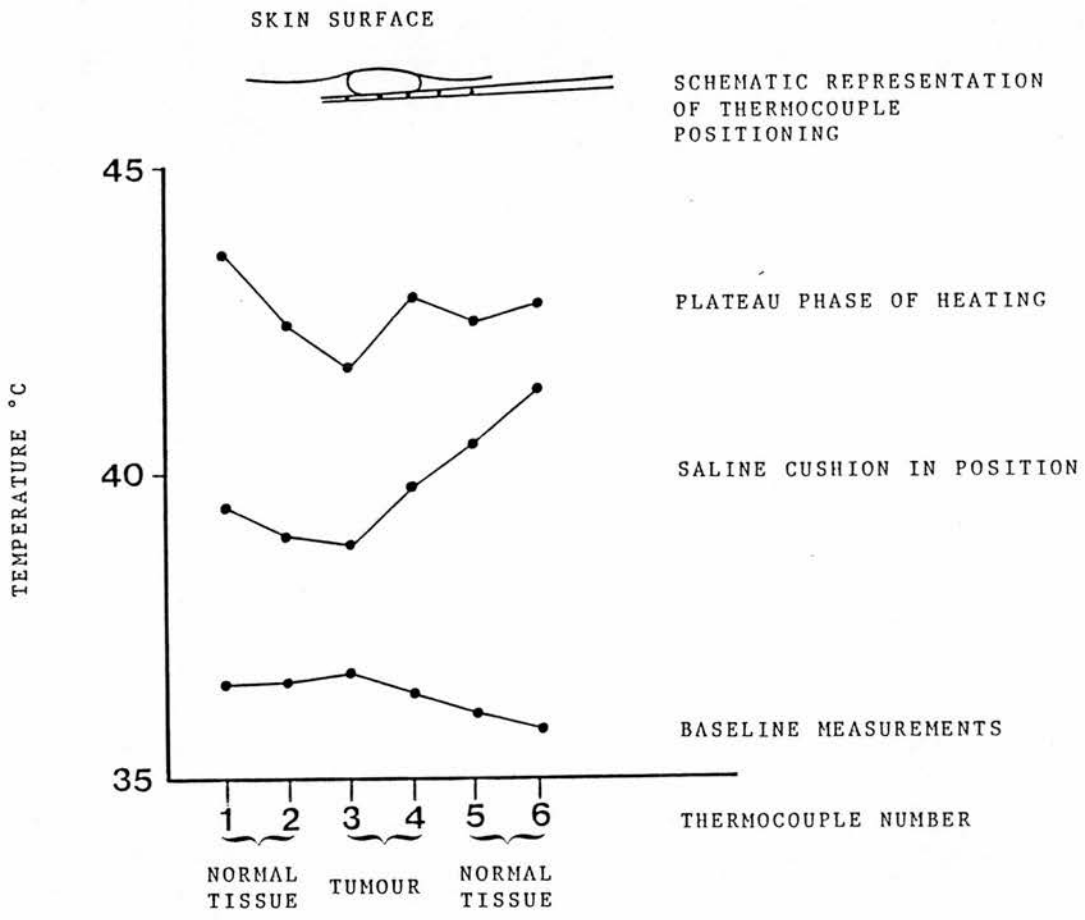
Very little performance data are available in the hyperthermia literature for comparison with the present series in which 54% of small superficial lesions achieved 43°C (MTT) for 60 minutes. In an evaluation of treatment in 116 small superficial tumours, Dunlop et al, (1986) addressed the question of technical performance by calculating minimal thermal dose in terms of minutes equivalent at 43°C (see Chapter 1). Twenty-four percent of treatments reached a level of heating equivalent to 43°C for 60 minutes, of which more than half were not first time treatments, a factor which may bias the outcome towards success. One of the reasons for the difference in success between the two series may be the variety of technical systems employed by Dunlop and his colleagues. The authors considered that the 58% of treatments resulting in the equivalent of 43°C for 20 mins. were "biologically useful". Using the same criteria, 68% of treatments in the present series, all first time treatments, were successful.

The construction of temperature profiles provided an immediate temporal and spatial assessment of each treatment, although the available information was obviously limited by the number of temperature measurement points and their distribution in tissue.

Despite standard positioning of the thermometry probe at the base of the tumour, the selection of an axis of insertion was an arbitrary decision which at certain anatomical sites could have influenced power requirement, subsequent temperature profile, and categorisation as success or failure. Patient comfort and optimisation of equipment positioning were the major considerations taken into account when making this decision. However, visualisation of the thermometry probe, in the treatment position, provided useful information not only indicating the position of each thermocouple with reference to tumour and normal tissue, but also permitting an estimate to be made of the depth of each temperature sensor beneath the skin surface. To minimise temperature gradients, probe alignment would ideally have been parallel to the skin surface. However, as probe insertion was necessarily oblique, there was a gradual increase in depth towards the distal end, the magnitude of which depended on the depth of the lesion. This obliquity was reflected in the range of temperature observed after insertion of the thermocouple wire, before positioning the saline cushion. The extent of the initial temperature rise observed at each thermocouple, as a result of positioning the saline cushion, demonstrated that with a skin surface temperature of 43°C heat transfer, by conduction alone, was very poor and, even over very shallow depths, there was a rapid fall off in temperature. Temperature recordings taken before switching on the power allowed identification of anomalous values which might influence subsequent temperature profiles (Fig 30).

Figure 30

Temperature Profiles Recorded Across Six Thermocouple Junctions Implanted at the Tumour Base and in Adjacent Normal Tissues




Baseline observations indicate lower temperatures at proximal measurement points, in keeping with their superficial situation. Positioning the cushion, perfused with warm saline (43°C), results in a temperature rise which is most marked at the proximal, superficial thermocouples, but at this stage a cold spot is detected at junction 3. The suspicion that vascular influences are responsible for this isolated temperature variation is supported by the persistence of a cold spot, at the same site, throughout the plateau phase of heating.

Temperature distribution was remarkably constant during the plateau phase of the profiles in those tumours successfully heated. As a consequence, the minimum tumour temperature, on which treatment was controlled, remained at the same measurement point throughout treatment in most cases. Inadvertent movement of the applicator was generally responsible for shift in the site of minimum, but the previous distribution could always be restored by simple adjustment of the applicator setup.

A classification of temperature profile was constructed with the aim of examining uniformity of heating and of identifying reasons for treatment failure in terms of temperature distribution. The categories of distribution type were selected to represent the present series, after initial inspection of temperature profiles. For this reason it was generally easy to allocate treatments to distribution type by analysis of initial part of the plateau phase.

Uniformity of heating was remarkably good, with 73% of the 56 treatments showing a smooth temperature profile. Although inhomogeneity of temperature distributions is generally correlated with lesion size, no such tumour volume effect was seen over the range of lesions included in this series. This confirms the adequacy of the heating system in the studies described.

In an attempt to define reasons for treatment failure, in terms of temperature distribution, a number of exclusions

were made from the failure group. This was considered valid as the reasons for failure in the exclusions were well defined in each case, and not related to temperature distribution. Having made this adjustment, non-$T_1$ distributions, in particular $T_3$, were significantly more common in those treatments which failed.

In the present series of small lesions, these isolated cold spots were a significant finding in the heat sessions which failed to deliver the treatment aim. The occurrence of cold spots in larger tumours is presumably just as likely, provided the density of vessels is similar. However, as the density of temperature sampling is unlikely to be as great, a higher proportion of these tumours will be unmonitored, making the detection of cold spots less likely. Because of thermometry limitations, it is not possible to define the exact boundaries of a cold spot, but, from temperature measurement at adjacent points, it seems likely to be only in the order of millimetres. As power deposition problems are unlikely to result in such localised temperature variation, vascular influence remains the most likely explanation.

As yet, the biological significance of cold spots has not been established. Although Dewhirst et al, (1984) clearly demonstrated the impact of minimum tumour temperature on biological outcome, no distinction was made between isolated cold spots and uniformly underheated tumours. As the cold spots described here represented temperature in a small core of tissue, it may be impossible to demonstrate

their influence except at a critical level of cell kill. In addition, when combined with radiation, these underheated areas of tumour should theoretically coincide with the more radiosensitive zones so that in practical terms of clinical application they may be insignificant. However, as the oxygen diffusion distance in tissue is likely to be less in magnitude than the radius of influence of a cold spot the possibility of a biological effect cannot be excluded.

In this study of hyperthermia delivery in 56 small circumscribed superficial tumours, correlation of physical parameters of treatment with biological effect has not been possible because the lesions were pooled from a number of studies designed to examine different effects. The influence of the physical characteristics of heating on biological outcome in terms of growth delay is addressed in Chapter 7.

Analysis of power requirement and time to achieve minimum temperature in successful heat deliveries showed that in 63% of instances < 80 watts was necessary to achieve the presribed tumour temperature of 43°C within 10 minutes. It is therefore possible that a test heating, with this level of power operating for 10 minutes, might discriminate between tumours in which temperatures adequate to produce direct cytotoxicity could be expected, from those tumours in which only lower temperatures, possibly useful for radiosensitisation, will be likely. This information would in turn influence the timing of hyperthermia in relation to irradiation. However, a predictive system employing test

hyperthermia risks the possibility of inducing thermotolerance.

Apart from lesion size, tumour characteristics do not seem to influence treatment success. However, taking the group as a whole, there was little variation in the parameters considered, with most lesions being small, chest wall recurrences from adenocarcinoma of breast. It is therefore unlikely that the present analysis would identify tumour characteristics influencing heat delivery.

Chapter 7

# TUMOUR RESPONSE

## 7.1 Heat Modification of Tumour Response to Single Dose Irradiation

The results have been derived from tumour volume measurements on 96 nodules in 10 evaluable patients. The number of nodules observed in each patient and their characteristics are listed in Table 11.

TABLE 11

NUMBERS AND CHARACTERISTISC OF NODULES STUDIED IN 10 EVALUABLE PATIENTS

| Patient No. | No. of nodules studied | Histology | Lesion type | Median tumour doubling time (days) |
|---|---|---|---|---|
| 1 | 12 | Melanoma | SC | 20 |
| 2 | 12 | Adenocarcinoma (B) | SC | 24 |
| 3 | 7 | Adenocarcinoma (B) | C | 77 |
| 4 | 19 | Adenocarcinoma (B) | C | 28 |
| 5 | 12 | Adenocarcinoma (B) | C | 36 |
| 6 | 9 | Adenocarcinoma (B) | C | 175 |
| 7 | 8 | Adenocarcinoma (B) | C | 33 |
| 8 | 7 | Adenocarcinoma (U) | SC | 64 |
| 9 | 3 | Squamous carcinoma(U) | SC | 40 |
| 10 | 7 | Squamous carcinoma(S) | C | 12 |

(B) = Breast primary
(U) = Unknown primary
(S) = Skin primary

(SC)= Subcutaneous nodule
(C) = Cutaneous nodule

The composition of the nodule group with respect to treatment is shown in Table 12 .

TABLE 12

CLASSIFICATION OF NODULE POPULATION ACCORDING TO TREATMENT

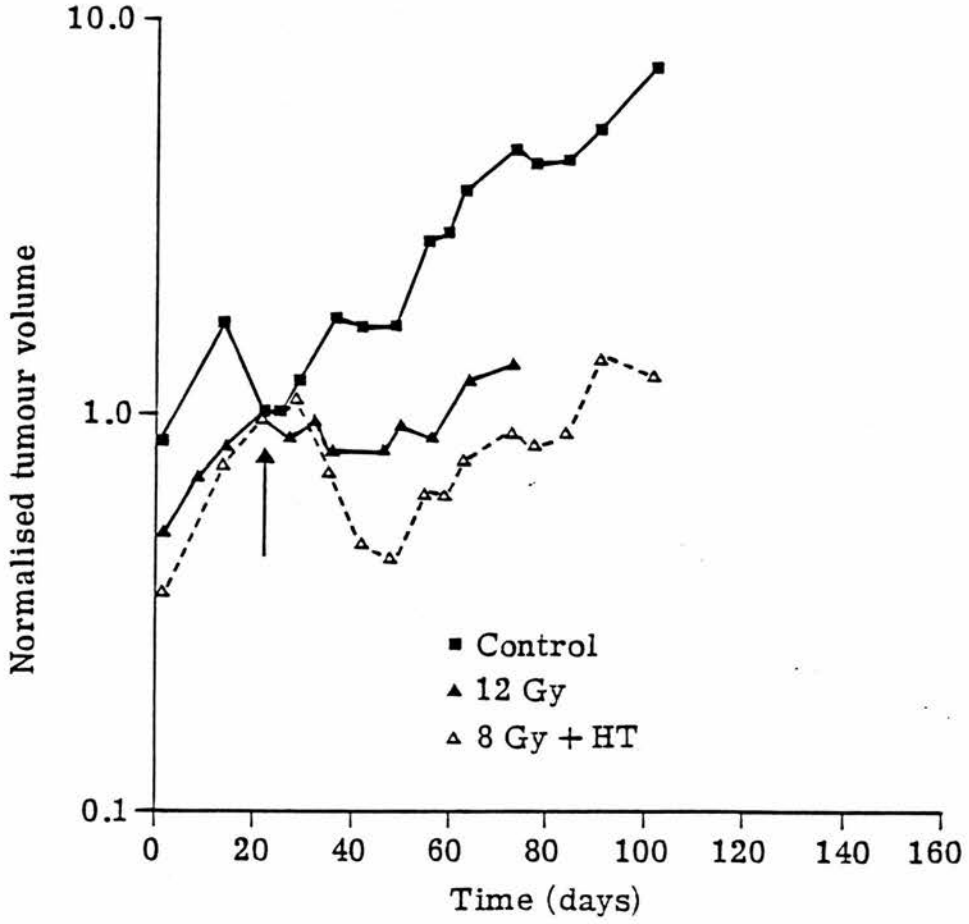
| Treatment | Untreated | Radiation | Heat & radiation | Heat | Total |
|---|---|---|---|---|---|
| No. of nodules | 48 | 31 | 15 | 2 | 96 |

Growth curves were used to quantify the effect of applying heat 3-4 hours after single dose irradiation. Examples of curves constructed from tumour measurements in three patients with melanoma, adenocarcinoma of unknown primary and metastatic breast carcinoma respectively, are shown in Figs. 31, 32, 33. These are patient number 1, 2 and 8 in Table 11.

Figure 31 illustrates 3 of the 12 nodules measured in Patient no. 1. Only two treated lesions and a control are included for clarity but a second nodule treated with 8 Gy plus heat responded very similarly and has a response curve which can almost be superimposed on the heat/radiation nodule shown. A further nodule given 10 Gy alone failed to regress, but growth after treatment slightly deflected from that plotted for control lesions.

Figure 31

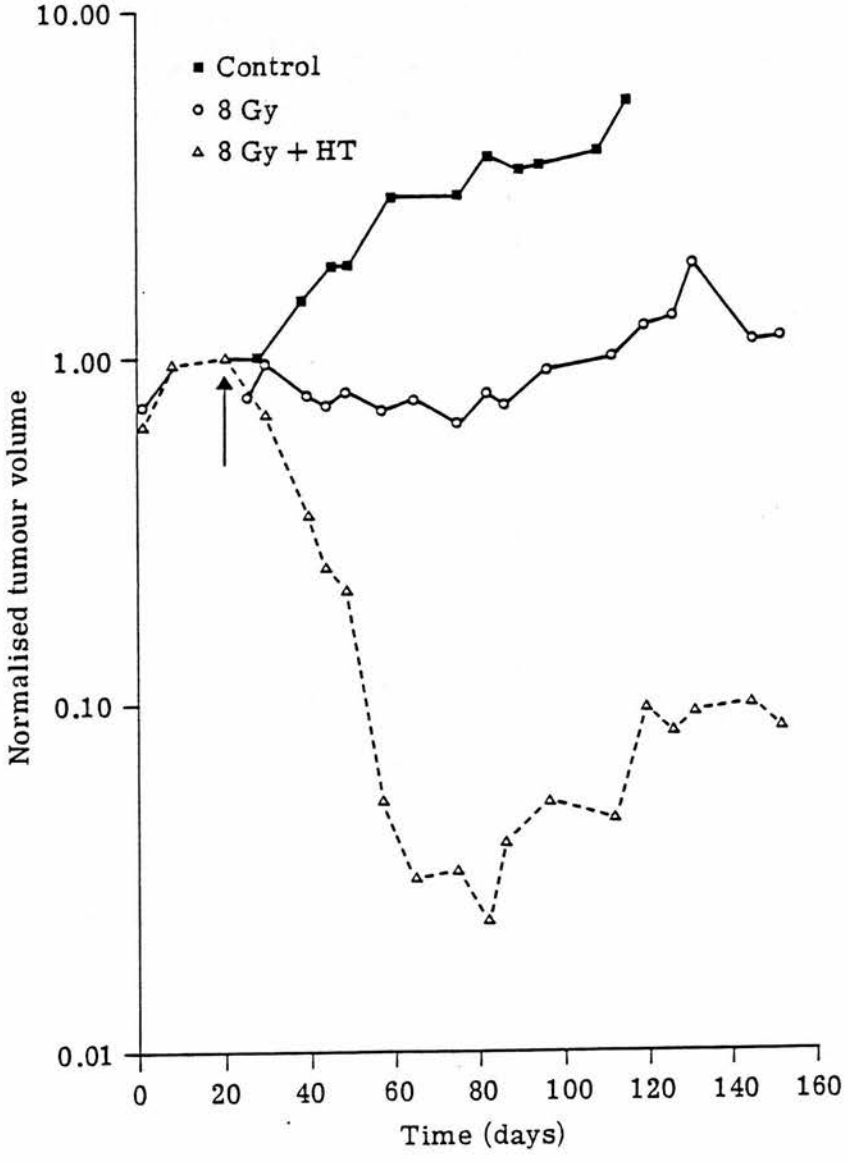
Growth Curves Derived from Tumour Measurements in a Patient with Multiple, Superficial Melanoma Nodules




Arrow indicates date of treatment

Figure 32

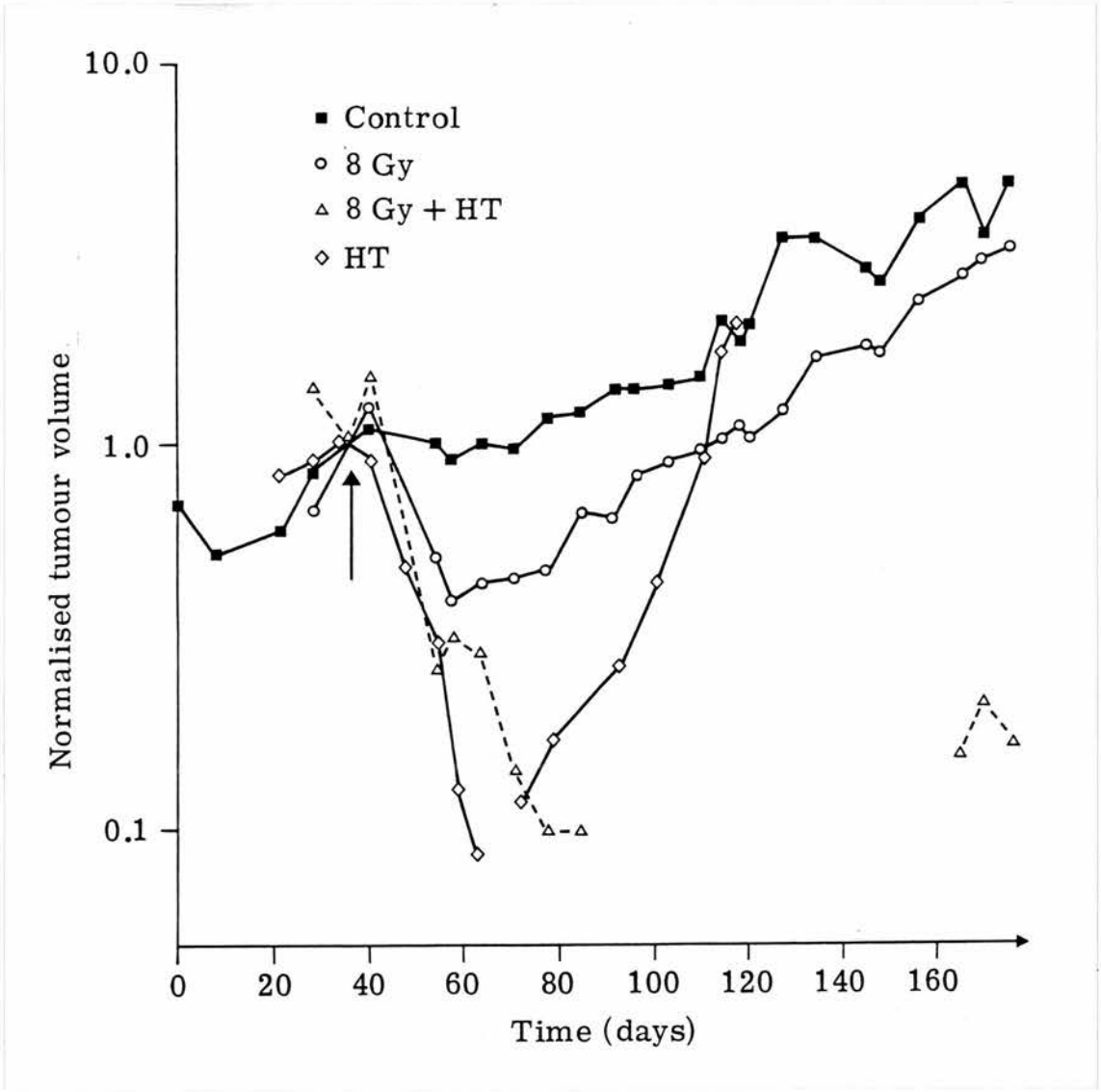
Growth Curves Derived from Tumour Measurements in a Patient with Adenocarcinoma Nodules of Unknown Primary




Arrow indicates date of treatment

Figure 33

Growth Curves Derived from Tumour Measurements in a Patient with Multiple, Superficial Nodules From Breast Adenocarcinoma




Arrow indicates date of treatment

The gross effect on tumour response of adding heat to radiation has been scored as either greater than, equal to, or less than, the effect of radiation alone. In 9 patients the effect was greater with heat, and in the remaining one patient there was no demonstrable effect in terms of tumour volume reduction with either radiation or heat/radiation (Table 13).

TABLE 13

COMPARISON OF THE OVERALL EFFECT OF RADIATION ± HEAT IN MEASUREABLE SUPERFICIAL METASTASES

| Tumour effect | R+H > R | R+H = R | R+H < R |
|---|---|---|---|
| No. of patients | 9 | 1 | 0 |

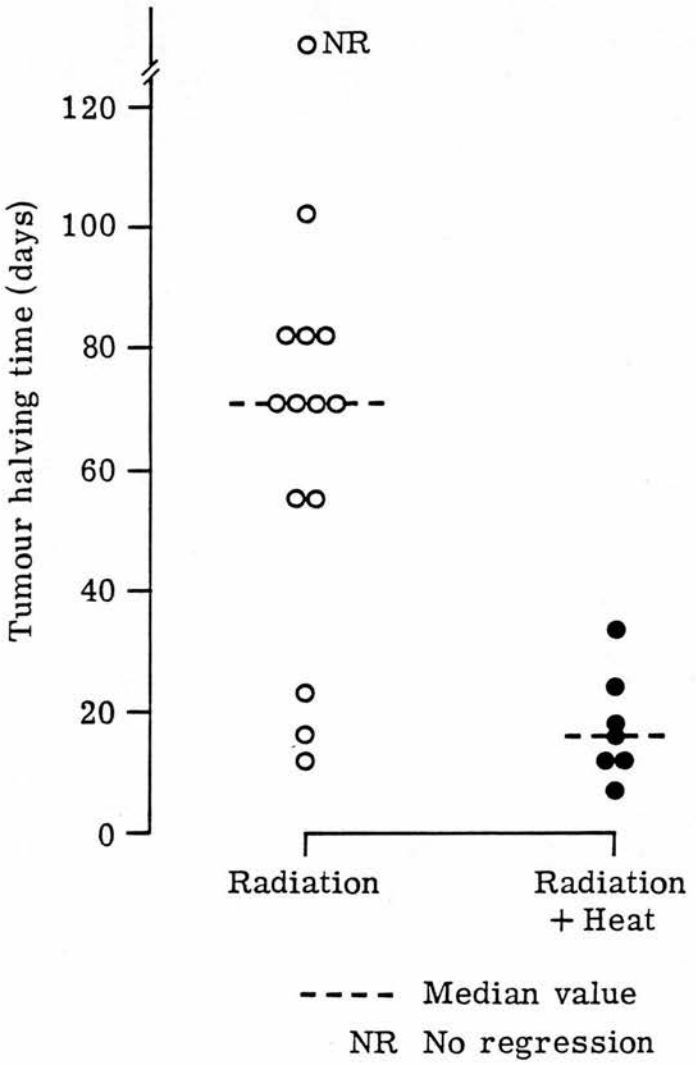
R = Radiation H = Hyperthermia

7.1.i Tumour Volume Halving Time ($t_{\frac{1}{2}}$)

In the seven patients in which tumour volume halving time could be calculated, nodules heated after radiation regressed faster than those treated with radiation alone. For heated nodules, $t_{\frac{1}{2}}$ ranged from 7-34 days with a median value of 15.5 days. Without heat, the range was from 12-102 days with a median of 70 days (Fig. 34). Of the three radiation-only nodules which regressed rapidly, each had a heated counterpart which regressed at an equal or even greater rate. There was no instance where the addition of heat was associated with a slower regression rate.

Figure 34

Tumour Volume Halving Times in Superficial Nodules Treated with the Same Single Dose of Radiation With or Without the Addition of Heat



(ii) Maximum Volume Reduction (MVR)

Six patients could be assessed to compare MVR in nodules irradiated with or without the addition of heat. In all cases a greater maximum volume reduction was recorded in the heated nodules (Table 14).

TABLE 14

MAXIMUM VOLUME REDUCTION OBSERVED IN SUPERFICIAL NODULES TREATED WITH THE SAME DOSE OF RADIATION, WITH OR WITHOUT THE ADDITION OF HEAT 3-4 HOURS LATER

| Patient No. | Ratio of: Pretreatment tumour volume / tumour volume at time of max. response | |
|---|---|---|
| | Radiation | Heat + radiation |
| 1 | 1.25* | 2.8<br>2.4 |
| 2 | 2.5 | 10** |
| 3 | 11 | 14.3 |
| 4 | 1.3<br>1.4<br>1.6<br>1.6 | 2 |
| 8 | 1.6 | 42 |
| 9 | 2.4 | 10 |

* Ratio for 12 Gy - all other data refer to 8 Gy

** Ratio derived from last tumour measurement before nodule became impalpable

(iii) Time to Maximum Volume Reduction (TMR)

Five patients have been analysed to compare TMR in superficial nodules treated with the same single dose of radiation, with or without the addition of heat 3-4 hours later (Table 15). A ratio has been derived from TMR with or without the addition of heat. In each case TMR was slightly longer with the addition of heat resulting in ratios close to one. There was no instance where maximum tumour response was achieved in a shorter time in heated nodules.

TABLE 15

TIME IN DAYS FROM TREATMENT TO MAXIMUM VOLUME REDUCTION IN SUPERFICIAL NODULES TREATED WITH THE SANE SINGLE DOSE OF RADIATION, WITH OR WITHOUT THE ADDITION OF HEAT 3-4 HOURS LATER

| Patient No. | Radiation | Heat & radiation | Ratio: $\frac{\text{time H \& R}}{\text{time R}}$ |
|---|---|---|---|
| 1 | 19 | 24<br>25 | 1.2 |
| 2 | 29<br>29 | 56 | 1.9 |
| 3 | 34 | 45 | 1.4 |
| 4 | 35<br>35<br>35<br>28 | 42 | 1.2 |
| 8 | 54 | 61 | 1.1 |

(In Patient 1 the radiation only dose (12Gy) was not the same as for the heated nodules (8Gy), in all other cases the same doses were employed)

(iv) Growth Delay

Twenty-one nodules in 6 patients provided information on growth delay either by direct measurement or by extrapolation from the regrowth phase of the growth curves. Table 16 outlines the values obtained for nodules treated with single doses of radiation (6-12 Gy) with or without the addition of heat.

TABLE 16

GROWTH DELAY IN SUPERFICAL NODULES TREATED WITH SINGLE DOSES OF RADIATION WITH OR WITHOUT THE ADDITION OF HEAT 3-4 HOURS LATER

| | Growth delay (days) | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Radiation | | | | Heat & radiation | |
| Radiat' dose Gy | 6 | 8 | 10 | 12 | 6 | 8 |
| Patient 1 | - | N.R.* | N.R. | 36 | - | 58<br>60 |
| Patient 2 | - | 65 | 65 | - | - | 224** |
| Patient 4 | - | 42 | - | - | - | >105° |
| Patient 5 | - | 93<br>170<br>215<br>250 | - | - | - | 185 |
| Patient 8 | - | 64 | - | - | - | 270** |
| Patient 9 | 44 | - | - | - | 84 | - |

* NR = no regression
** Estimated by extrapolation from regrowth phase to treatment volume
° Nodule remained impalpable to last observation date (day 105)

In all instances growth delay was greater with heat/radiation than with radiation alone. Where more than one nodule received the same treatment in any individual patient, there was good internal consistency of response. The exception to this was Patient 5 where 8 Gy alone produced growth delays ranging from 93-210 days.

(v) Thermal Enhancement Ratio (TER)

A thermal enhancement ratio for tumour response has been calculated in 6 patients (Table 17). A TER of > 1.6 was assumed in patient 1 as the only nodule treated with radiation alone which regressed received a higher dose (12 Gy) compared with the two nodules heated after irradiation (8 Gy). Discounting Patient 5, in which there was marked heterogeneity of response to radiation alone making interpretation of TER difficult, the range of values was from > 1.6 to 4.2. In Patients 2 and 8 extrapolation of the regrowth phase in heated nodules was necessary to derive a growth delay. In these patients, with inadequate follow-up, TER has also been calculated from the date of the last observation on heated nodules not yet regrown to pretreatment volume. These values, included in parentheses show that a considerable TER is still obtained.

TABLE 17

THERMAL ENHANCEMENT RATIOS DERIVED FROM GROWTH DELAY IN TUMOURS IRRADIATED WITH OR WITHOUT THE ADDITION OF HEAT

| Patient No. | TER |
|---|---|
| 1 | >1.6 |
| 2 | 3.4* (>2.1)° |
| 4 | 2.5<br>2.5* (>2.0)° |
| 5 | 1.3 (>1.0)°<br>1.1 |
| 8 | 4.2* (>2.0)° |
| 9 | 1.9 |

* Estimation of growth delay in heated nodules by extrapolation from regrowth curve

° TER estimated from latest observation in heated nodules not yet regrown to treatment volume

## 7.2 Correlation of Heat Modification of Radiation Response with Physical Parameters of Heat Delivery

Clinical outcome in the 50 nodules heated after irradiation was examined for correlation with physical parameters of heat delivery (Table 18). According to the criteria for successful and failed heat delivery (see Chapter 6), 10 were classified as successful, two as $F_1$ failures and two as $F_2$ failures. One further nodule was unclassifiable because of inadequate thermometry, and assessment in this lesion was complicated by a wide range of growth delay in the four other nodules in the same patient, given the same dose of radiation, without the addition of heat.

As the measured biological effect of hyperthermia in assessable patients varied, with TER values ranging from 1.6 - > 4.2, it was difficult to make comparisons between treatments in different patients in terms of heat delivery and clinical outcome. However, four patients each had two heat-radiation nodules which allowed a more direct evaluation. One patient (Patient 7) showed no response to 8-12 Gy irradiation alone, and the addition of neither an inadequate, nor a successful heat delivery altered this. There was discordance between physical parameters and biological effect in one patient (Patient 2) who had two successful heat deliveries of which only one produced a definite clinical effect. In the remaining two patients where was good correlation between heat "dose" and tumour response. Lack of influence of an isolated cold spot on outcome is demonstrated in one patient (Patient 2) who had a $T_3$ temperature profile.

TABLE 18

CORRELATION OF HEAT MODIFICATION OF RADIATION RESPONSE WITH PHYSICAL PARAMETERS OF HEAT DELIVERY

| Patient No. | Nodule | Heat delivery classification | Temp Profile | Power application (watts) | Successful clinical effect |
|---|---|---|---|---|---|
| 1 | J | S | $T_1$ | 110 | Yes |
| | H | S | $T_1$ | 45 | Yes |
| 2 | K | S | $T_3$ | 40 | Yes |
| | P | S | $T_1$ | 170 | No |
| 4 | A | S | $T_1$ | 20 | Yes |
| | O | $F_1$ | $T_1$ | 20 | Marginal |
| 7 | A | S | $T_{1b}$ | 70 | No |
| | G | $F_1$ | $T_{2b}$ | 140 | No |
| 5 | C | * | * | ) 80 | N.A. |
| | D | $F_2$ | $T_4$ | ) | Yes |
| 3 | H | S | $T_1$ | 20 | Yes |
| 6 | A | S | $T_4$ | 40 | Yes |
| 8 | A | S | $T_1$ | 250 | Yes |
| 9 | 1 | S | $T_1$ | 130 | Yes |
| 10 | C | $F_2$ | $T_{1b}$ | 25 | Yes |

S = Success

N.A. = Not assessable

$F_1$) = Failure
$F_2$)

* = Limited thermometry

Despite failing to fulfill the prescribed time-temperature requirements, the two $F_2$ heat deliveries produced a greater clinical effect compared with the same dose of radiation alone. In the two $F_1$ failures, one produced a response which was marginally superior compared with radiation alone, and the other occurred in a patient in which there was no response to any of the treatments given.

## 7.3 Tumour Response to 8Gy Irradiation

The homogeneity of response to 8 Gy single irradiation was explored in a group of superficial nodules of one tumour type, breast cancer. Ten of the 18 breast cancer patients who provided doubling time data in the tumour measurement study (see Chapters 4 and 9) collectively contributed 58 superficial tumours in which growth rate could be correlated with radiation response.

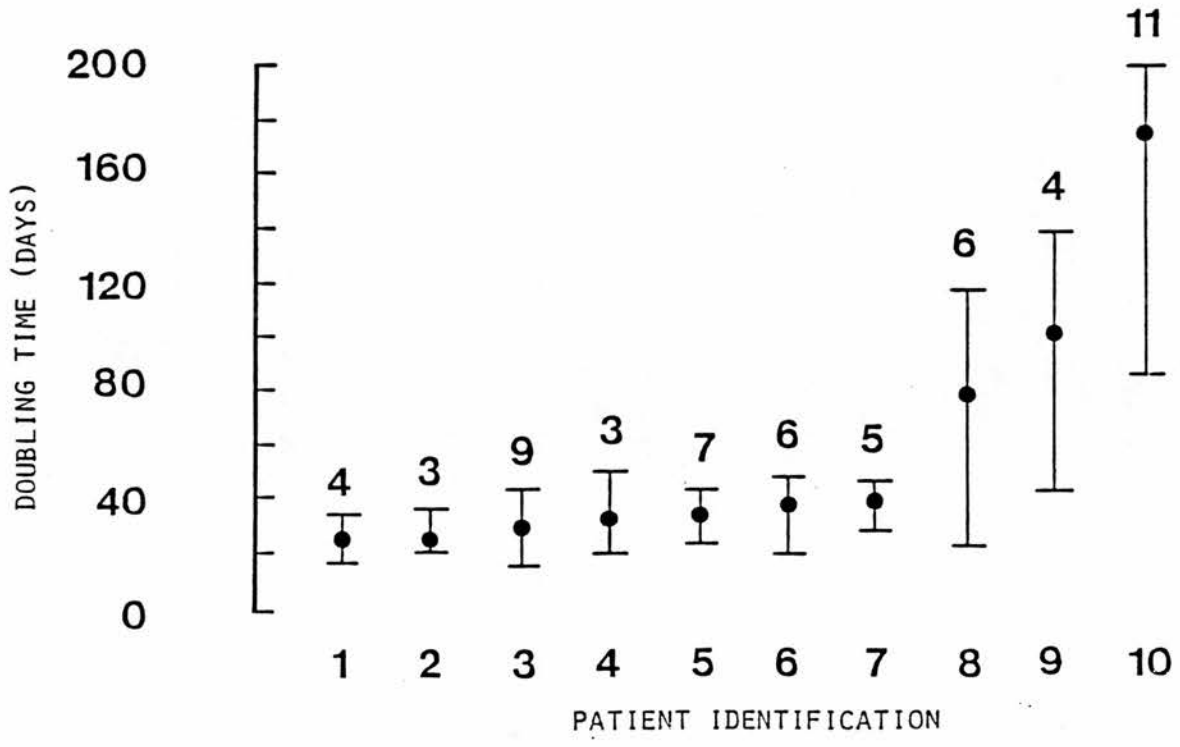
### 7.3.i Tumour Doubling Time and Clinical Features in Breast Cancer Nodules

The range, and median value of tumour volume doubling time for each of the ten patients is shown in Fig. 35. Although the overall range varied between 16 and 206 days, seven of the patients had remarkably similar median doubling time values with little intra-patient variation. Seven of the 10 patients (patients 1-6 and 8) had clinically comparable lesions in terms of size, cutaneous involvement and anatomical location on the breast or chest wall. Of the

remaining three patients, one had subcutaneous lesions, one had both cutaneous and subcutaneous lesions of a wider size range and the third had cutaneous lesions situated on the back.

Figure 35

Range and Median Tumour Volume Doubling Time in 10 patients with Superficial Metastases from Breast Cancer

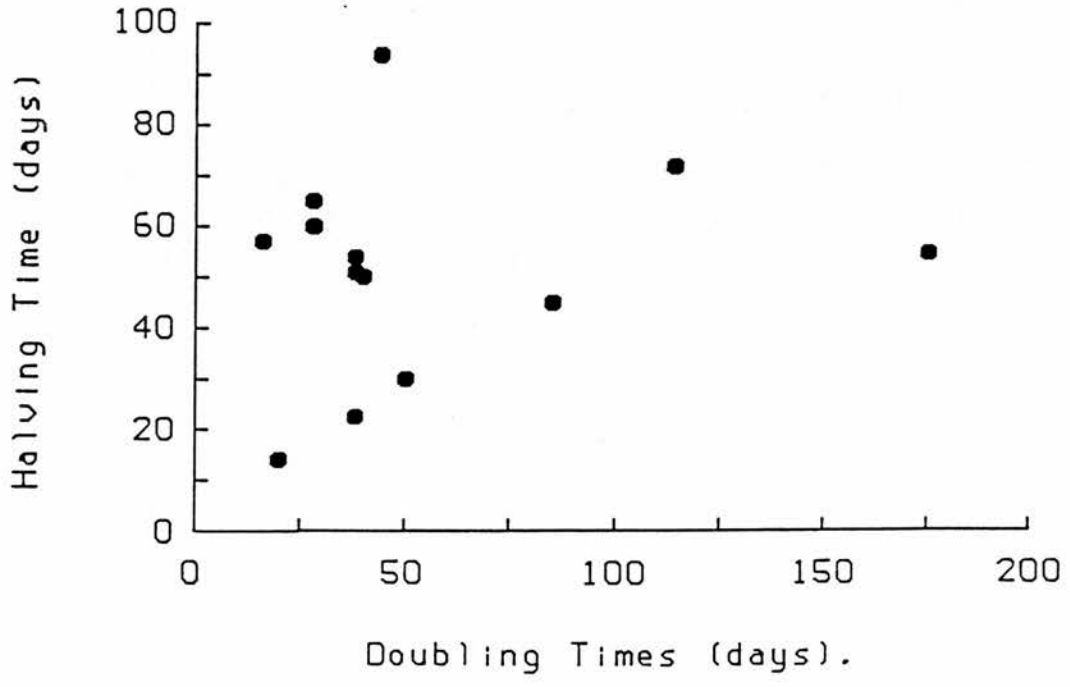


### 7.3.ii Correlation of Tumour Doubling Time with Parameters of Radiation Response

Tumour response was measured by rate of regression, expressed as tumour volume halving time, and growth delay. There was no correlation between pretreatment doubling time and regression rate following 8 Gy single dose irradiation. As shown in Fig. 36, the more rapidly growing nodules, with doubling times of 50 days or less, had half time values ranging from 14-94 days with a median of 51 days.

Figure 36

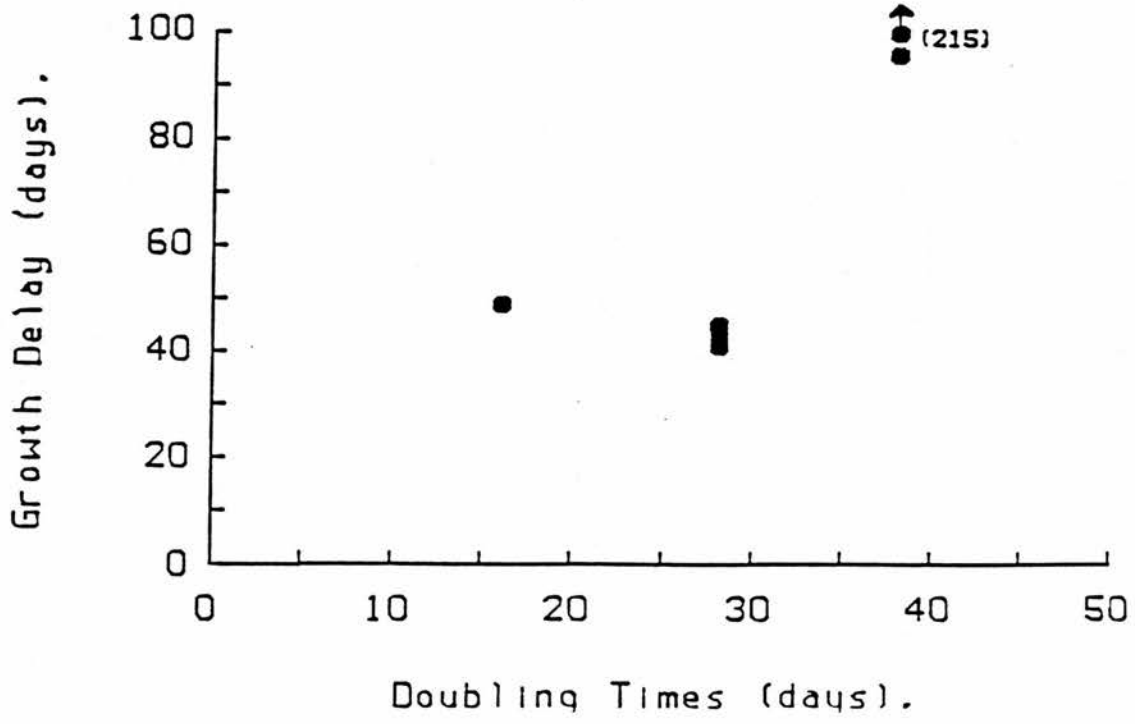
Correlation of Tumour Volume Halving Time Following 8 Gy Irradiation with Pretreatment Volume Doubling Time



Growth delay was calculated for the six nodules which regrew to pretreatment volume after irradiation. Correlating these values with pretreatment doubling time there appears to be a trend towards longer growth delay with slower growing lesions but the numbers are small (Fig. 37).

Figure 37

Correlation of Growth Delay Following 8 Gy Irradiation with Pretreatment Volume Doubling Time



### 7.3.iii The Contribution of the Components of Response to Growth Delay

The components of growth delay, tumour regression and regrowth were examined independently to assess the influence of each on the calculated value for growth delay. In this nodule population, there was no correlation with halving time (Fig. 38) but growth delay did correlate with tumour regrowth doubling time (correlation coefficient 0.81) (Fig. 39).

Figure 38

Correlation of Growth Delay with Tumour Volume Halving Time after 8 Gy Irradiation

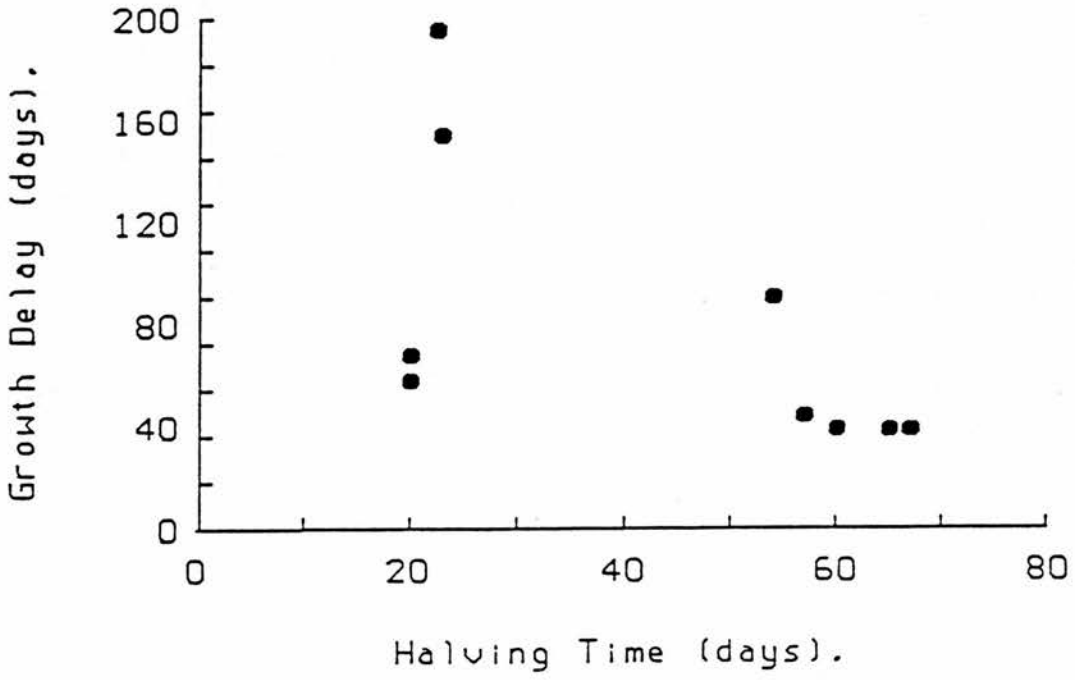
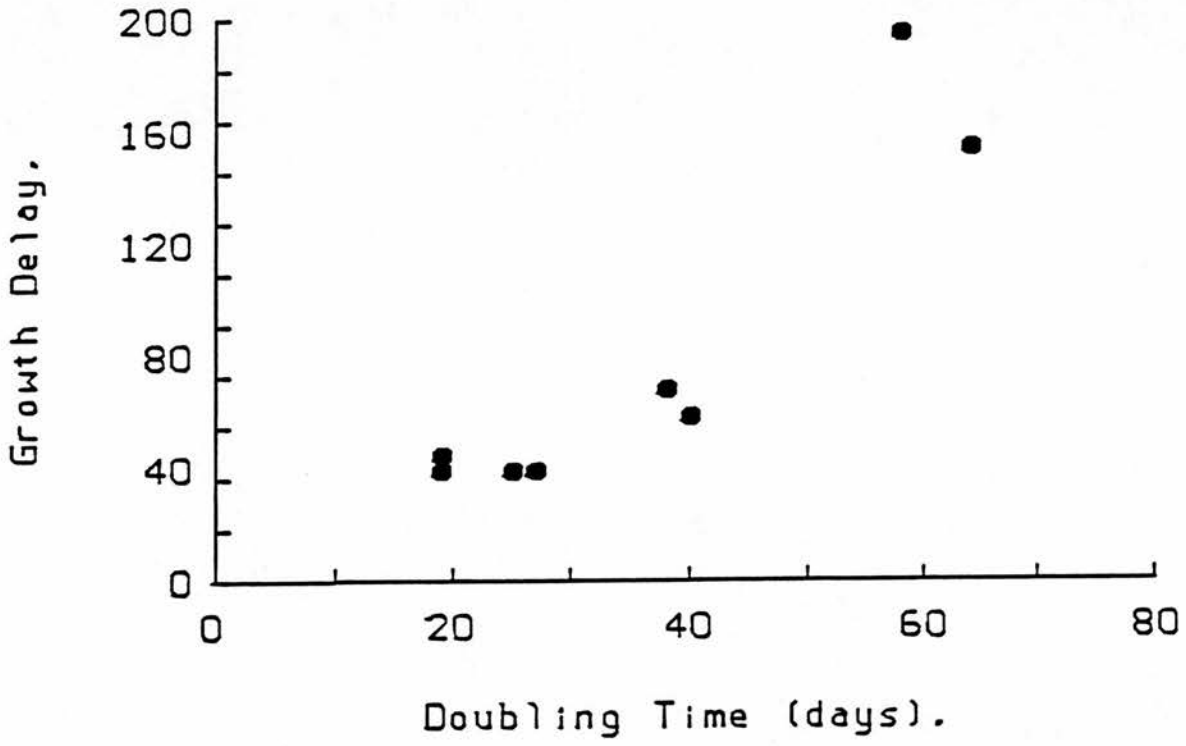


Figure 39

Correlation of Growth Delay with Tumour Regrowth Doubling Time after 8 Gy Irradiation



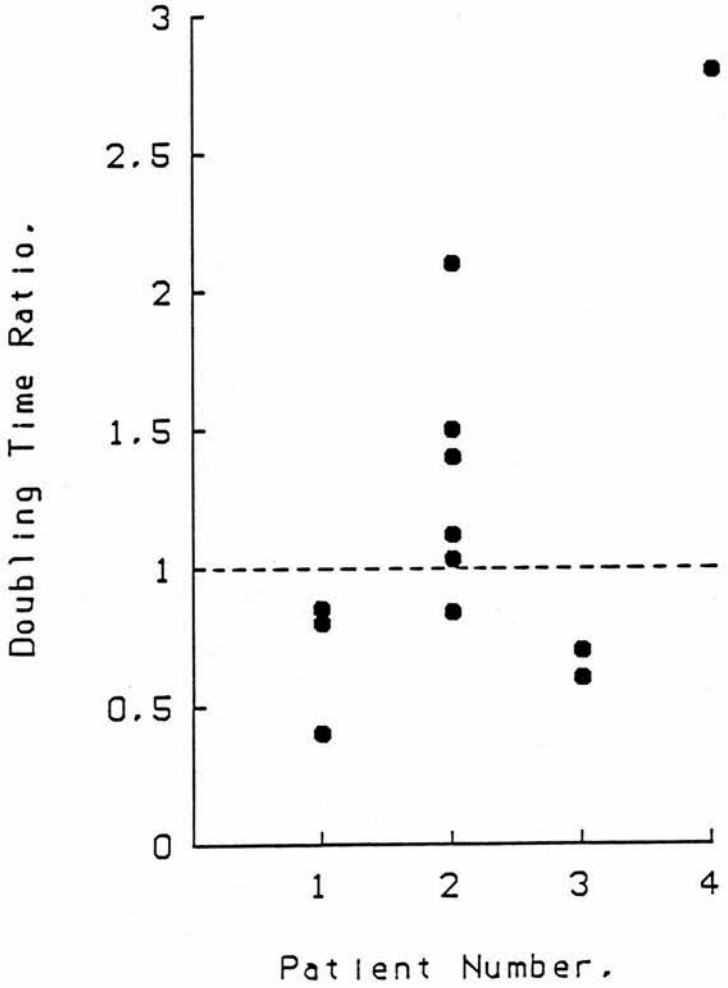

7.3.iv Investigation of a Tumour Bed Effect Following 8 Gy Irradiation

Regrowth doubling time data was available for 15 nodules from five patients and ranged from 19-65 days. In 12 of these nodules serial measurements prior to irradiation also provided a measurement of pretreatment doubling time which varied between 16 and 98 days. In these 12 nodules, from four patients, the ratio of pre to post-treatment doubling time was calculated and is shown in Fig. 40. Within the errors of clinical measurement and growth curves analysis

there was no consistant change in growth rate. Further, nine of the 12 nodules regrew at approximately the same rate pre and post-irradiation.

Figure 40

Ratio of Pre to Post Treatment Doubling Time in 12 Nodules from 4 Patients



### 7.3.v Investigation of the Influence of Tumour Volume on Response to 8 Gy Irradiation

In individual patients, the range of tumour volume for irradiated nodules was generally not sufficiently great to permit investigation of a volume effect.

## 7.4 Discussion

This study aimed to demonstrate a quantitative and qualitative difference in tumour response, as documented by tumour growth curves, when irradiated nodules were heated after an interval of 3-4 hours. Despite the difficulties involved in clinical heat delivery, and the problems encountered with tumour measurements and their subsequent utilisation to derive clinical endpoints, an overall greater effect was observed for heated nodules in 9 out of the 10 patients studied. This initial encouraging impression was consolidated by the more specific demonstration of therapeutic enhancement ratios ranging from > 1.6 - 4.2. However, the term TER is used with caution on two accounts. Firstly, because radiation and heat were applied sequentially, with a 3-4 hour interval between them, it is likely that the two modalities were non-interactive and that there was no true modification of radiation response by, for example, a heat-mediated inhibition of repair. Secondly, in experimental animal tumours, values for TER are derived from ratios of radiation doses giving the same biological effect in the absence, or presence, of heat (Robinson, 1974). However, construction of dose-response curves from clinical data is difficult, and TER values for human tumours have often been derived by comparison of tumour responses to the same dose of radiation, with or without the addition of heat (Arcangeli et al, 1983, Kim et al, 1982, Scott et al, 1982). This derivation of TER has been employed in the present study, but a TER determined in this way may be greater than

that based on a uniform biological response (Gillette, 1982, 1984).

In the present internally controlled study, TER values were calculated in the six patients for whom growth delay could be measured, directly or by extrapolation. However, in one of these patients the heterogeneous response observed in four nodules treated with 8 Gy alone made the derivation of TER from the single heated nodule meaningless. In the remaining five patients, values for TER ranged from > 1.6 - 4.2 suggesting that the advantages of combining heat with radiation may not be the same for all tumours. However, there is some evidence to suggest that a dose response exists for hyperthermia alone (Maher & Carnochan, 1986) and it seems likely therefore that, in part, this variation can be attributed to the problem of administering a reproducible heat treatment. The influence of the physical parameters of heat delivery on biological outcome was examined in the present series. The possibility of variation in biological effect between different tumour types makes analysis of this data difficult. However, examination of those patients with more than one heat-radiation nodule provides a better basis for comparison. Unfortunately, there were only five such patients and in one of these inadequate thermometry prevented definite allocation by physical parameters. From this small number it was not possible to determine a direct correlation, but individual patients provided useful information. There is scope for expansion of this area of investigation with the

aim of establishing the importance of achieving uniform heating in clinical practice.

Despite the variables encountered in this study, evidence of thermal enhancement was obtained in breast cancer, melanoma, squamous carcinoma and in an adenocarcinoma of unknown primary. This suggests that hyperthermia may improve the radiotherapeutic management of a wide range of tumours, but more extensive information is required to define the optimum utilisation of the two modalities in specific cancers.

Table 19 summarises available data on the TER values for human tumours derived from both radiation dose ratios and tumour response ratios. As predicted by Gillette (1982, 1984), TERs derived from iso-effective radiation doses (Overgaard & Overgaard, 1984) tend to be low compared with values derived from response ratios. The treatment and endpoint variables encompassed in the studies summarised probably account for the range of TERs obtained. Archangeli (1983) has demonstrated the variation in thermal enhancement obtained by modifying the therapeutic protocol in a study which compared four different schedules of combined radiotherapy and hyperthermia. The influence of the tumour endpoint selected has been emphasised by Scott (1982), who demonstrated a decrease in TER from 2 to 1.3 when initial complete response rate and that after 6 months were used as endpoints for calculation of TER in the same group of tumours. Radiation dose also influences the degree of

TABLE 19

THERMAL ENHANCEMENT RATIOS (TER) IN HUMAN TUMOURS

| Ref | Tumours | Heat treatment Temp°C/time in mins | No. of heat treatments | Sequencing of H* + R** | Size R Fraction (Gy) | Total R Dose Gy | Ratios used to derive TER | TER |
|---|---|---|---|---|---|---|---|---|
| Scott (1982) | Superficial epithelial, melanomas | 42.5 - 43.5°/45 | 12 | H 30 mins. post R 2H/week | 1.8 - 2 | 50 - 60 | Initial CRR+ | 2.2 |
| | | | | | | | CRR at 6/12 | 1.3 |
| Kim (1982) | Superficial and lymph node metastases from melanoma | 42 - 43.5°/30 | 13 | H immediately 2H/week | 3.3 | 42.9 | CRR | 2.3 |
| | | | 10 | | 4 | 40 | | 1.4 |
| | | | 7 | H immediately pre R 1H/week | 5.5 | 38.5 | | 1.5 |
| | | | 6 | | 6.6 | 39.6 | | 1.4 |
| Arcangeli (1983) | Carcinomatous neck node metastases | 42.5°/45 | 7 | H immediately post R 3H/week | 1.5 - 2 | 60 | | 1.74 |
| | Superficial recurrent tumours | | 8 | H immediately post R 2H/week | 5 | 40 | Initial CRR | 2.05 |
| | Superficial recurrent tumours | | 8 | H 4 hrs. post R 2H/week | 5 | 40 | | 1.79 |
| | Superficial recurrnt tumours | 45°/30 | 6 | H immediately post R 2H/week | 6 | 30 | | 2.63 |
| Overgaard & Overgaard (1984) | Melanoma - recurrent primary - superficial & lymph node metastases | 43°/30 | 3 | H immediately post R 3H in 8 days | 5 - 10 | 15 - 30 | 11 doses required for persistent CRR | 1.5 |
| | | | 3 | H 3-4 hrs. post R 3H in 8 days | 5 - 10 | 15 - 30 | | 1.3 |

H* - Hyperthermia

R** - Radiation

+ CRR - Complete response rate

thermal enhancement, with suboptimal radiation likely to produce a higher TER value (Scott et al, 1983). The effect of radiation fraction size is probably dependent on tumour type. For example, in Arcangeli's study the greatest effect was seen with conventional radiation fraction size whereas, in a series of melanomas large fractions induced the greatest enhancement (Kim et al, 1982).

Only 18 of the 56 patients on whom tumour measurements were initiated were considered suitable for entry into the hyperthermia protocol, and of these only six contributed growth delay information. Patient deterioration or introduction of systemic treatment were the main reasons for exclusion. In those proceeding to the treatment protocol, acquisition of measurement data appropriate for estimating growth delay was difficult. For example, the radiation doses selected were those judged necessary to induce tumour regression while permitting subsequent regrowth within the estimated available follow-up period. However, as individual tumour radiosensitivity to single dose irradiation and patient prognosis could only be guessed at, there was a high probability that the available information would be inadequate. Further, as discussed in Chapter 9, tumour measurement pretreatment provided doubling time information, valuable to the design and assessment of treatment in individual patients. But time devoted to this was always at the expense of post-treatment observation. However, the value of estimating tumour growth rate pretreatment is indicated by the range of doubling times obtained from

multiple metastases in individual patients (Chapter 9). This information allowed selection of comparable nodules for entry into the treatment protocol and to act as untreated controls. All but two of the 10 evaluable patients had more than five nodules from which to make this selection, and most had considerably more. Of the two patients with only three nodules, one developed further lesions after treatment which provided confirmation of the uniformity of nodule growth rate in this instance.

Allocation of nodules to treatment type or control group was non-random and selection was based on increasing the likelihood of achieving successful heat delivery. As the entire heating procedure, from insertion of thermometry probe at the start to collection of cooling data on completion of heat delivery could take up to two hours it was essential that patients were able to lie comfortably. As this was generally best achieved in a supine position, anteriorly situated lesions were usually selected for heating. A further consideration was the provision of good contact between skin and saline cushion, which implied avoiding lesions in body folds.

Substantial thermal enhancement of the radiation response provided further obstruction to the successful application of growth delay as an endpoint. In the present study, of the six patients with measurable growth delay, all nodules receiving only radiation regrew to their pretreatment volumes within the observation period. Amongst the

heat/radiation nodules in the same patients, the magnitude of the effect was such that one nodule remained permanently inpalpable and three others, though regrowing, did not reach the pretreatment volume within the follow-up period. For these regrowing nodules, growth delay was calculated by extrapolation from the observed regrowth phase, with the assumption that regrowth would continue at the same rate. This was considered valid as, in the group as a whole, treated nodules regrew at a constant rate equivalent to their pretreatment doubling time. The only exception to this was one of the heat only nodules in which very rapid regression was followed by a regrowth rate greater than that observed pretreatment (Fig 33). From the available period of follow-up it is not possible to determine whether this increased rate was sustained or whether the pretreatment rate was re-established after a "catch-up" phase.

There was no convincing evidence of a tumour bed effect in nodules irradiated with single doses of 6-12 Gy without the addition of heat. This finding was supported by observation in the breast cancer subgroup, in nodules irradiated with 8 Gy. This may not be surprising as the effect is dose dependent and animal studies have similarly failed to demonstrate growth retardation after small dose irradiation with 8 Gy (Yerushalmi & Weinstein, 1979). In addition, other clinical growth delay studies have not shown a tumour bed effect after single dose irradiation with 4-11.2 Gy (Thomlinson et al, 1976, Ash et al, 1979, Yarnold et al, 1986). However, as a result of an enhanced radiation

response, Yerushalmi & Weinstein (1979) observed an appreciable TBE when 8 Gy radiation was combined with heat. The fact that this was not the case in the present study supports the supposition that a 3-4 hour interval between irradiation and heat application provides selective enhancement of tumour effect, and thus allows a therapeutic gain. This scheduling of the two modalities mainly relies on the additive effect of their direct cytotoxicities and probably prevents any true radiosensitisation. As thermal cytotoxicity is enhanced by the microenvironmental conditions commonly found in tumour, a selective tumour effect can be expected.

Conversely, acceleration of tumour growth rate following irradiation has been observed in animal tumours after both single dose and fractionated radiation (Barendson & Broerse, 1969, 1970). This has also been documented in human lung metastases three to six days after irradiation, with gradual return to pretreatment doubling time (Van Peperzeel, 1972). In the present study there was no evidence of a similar period of accelerated growth rate in the irradiated nodules, with or without the addition of heat. In Van Peperzeel's study histological tumour type and lung site probably accounted for the extremely rapid reduction in volume, which was maximal at 3-6 days after irradiation. By contrast, the maximum response in soft tissue nodules in the present study was usually not seen until at least 20 days after treatment, and was often considerably later. Therefore, any initial acceleration of repopulation was

likely to be masked by the slower resolution of these nodules. However, measurement of labelling indices in human tumours biopsied after irradiation has also failed to demonstrate accelerated repopulation (Courdi et al, 1980).

Despite the problems inherent in deriving and interpreting growth delay as a clinical endpoint, as yet there is no better alternative for quantifying the effect of treatment in terms of clonogenic potential. Local regrowth of tumours from surviving clonogenic cells is the only available means of attempting to detect differences in the numbers of survivors, and although a greater growth delay does not necessarily indicate greater cell kill it is a valid endpoint in its own right by virtue of the conferred clinical benefit.

Tumour shrinkage was documented by calculating the extent (maximum volume reduction - MVR) and rate ($t_{\frac{1}{2}}$) of regression. The clinical impression that heated tumours regress faster than their irradiated counterparts has previously been anecdotal (Scott, 1984). However, the $t_{\frac{1}{2}}$ values calculated in the present study demonstrated more rapid tumour shrinkage with the addition of heat. Although some irradiated nodules regressed rapidly, they were always equalled or exceeded in their regression rate by their heated counterpart.

The rapid shrinkage observed in these nodules is most likely to represent the enhancement of the process of

resorption. Although the extent of cell kill does not seem to influence the rate of tumour shrinkage (Thomlinson & Craddock, 1967), the timing of expression of cell damage may be important since resorption is presumably initiated by the presence of dying cells. Following irradiation, there is evidence that cells do not die immediatly but often undergo one or more divisions before dying in mitosis (Trott, 1972). By contrast, cell death as a result of hyperthermic cytotoxicity is much more abrupt, with cells in $G_1$ dying before entering mitosis (Coss & Dewey, 1983). Although cells heated in S phase lyse following mitosis, damage is nevertheless usually expressed after a period of hours (Leeper, 1984). Cell resorption may therefore be initiated early, leading to more efficient tumour clearance. Alternatively, or additionally, the effect of heat on the tumour microcirculation may contribute to rapid tumour shrinkage. In animal tumours, depending on the time and temperature of heating, a fall in tumour blood flow has been observed with a concurrent reduction in the vascular compartment (Eddy, 1980, Emami et al, 1980, Song, 1980a). However, it is unlikely that this mechanism was important in the present study, as it was not possible to demonstrate a decrease in tumour blood flow, using the thermal clearance technique, following single hyperthermia treatment at 43°C for 60 minutes in similar, small, superficial metastases (Chapter 8).

In breast cancer nodules given 8 Gy radiation alone, tumour regrowth seemed to be a more important determinant of

growth delay than rate of regression. On the other hand, in the hyperthermia study, despite equivalent regrowth rates, heat-radiation nodules had greater growth delay compared with their radiation only counterparts. However, in general, heated nodules not only regressed faster but also achieved a greater volume reduction, with the maximum response occurring at approximately the same, or slightly later, time after treatment compared with unheated nodules. A greater degree of cell kill in the heated nodules is implied and is in agreement with the demonstration of prolonged growth delay.

For all nodules measured in the present studies, calculation of tumour doubling time and regression rate relied on lines drawn by eye through data points, as there was no available measure of the variation which might be expected at individual points. As data collection in this study could not be standardised, the application of complex statistical techniques were considered irrelevant. Thus, the number of nodules studied in each patient, their allocation to treatment type and dose, and the frequency of observation were all individualised according to disease status and patient fitness. However, the chance of observing a greater effect in the heated nodules in 9 out of 10 patients would be very remote if there were in fact no true difference between the two treatment alternative.

Chapter 8

# BLOOD FLOW MEASUREMENT

## 8.1 Reproducibility of Xenon Clearance as a Technique for Measuring Blood Flow in Small Superficial Human Tumours

Two sequential xenon clearance measurements were performed in 31 tumours; 18 in cutaneous or subcutaneous malignant deposits, and 13 in lymph node metastases. Paired blood flow values for the two groups, expressed in ml/min/100g, are shown in Tables 20 and 21 respectively.

Initial blood flow measurements varied widely with values ranging from 0.34 - 31.04 ml/min/100g for superficial metastases and 0.63 - 62.27 ml/min/100g for lymph node metastases, the median values being 6.3 and 20.1 ml/100gm/min respectively. Applying a Mann Whitney U test and confidence interval to the median values of the two groups, there is a marginally significant difference in flow between the two types of lesion at $p = 0.0475$. Tumour volume and histological type did not influence the measured value of blood flow.

TABLE 20

XENON$^{133}$ CLEARANCE MEASUREMENT OF BLOOD FLOW IN SUPERFICIAL, CUTANEOUS/SUBCUTANEOUS, METASTATIC HUMAN TUMOURS

| Tumour histology | Normal tissue site | Tumour volume (mls) | $Flow_1$ mls/min/100g | $Flow_2$ mls/min/100g | Flow change as % of mean |
|---|---|---|---|---|---|
| Chordoma | S/C | 18.2 | 0.34 | 1.54 | + 128 |
| Adenocarcinoma (B) | S/C | 18.4 | 0.56 | 0.97 | + 71 |
| Chordoma | S/C | 10.0 | 0.60 | 0.30 | - 150 |
| Melanoma | S/C | 59.7 | 0.82 | 1.42 | + 54 |
| Adenocarcinoma (U) | S/C | 17.97 | 3.32 | 4.26 | + 25 |
| Adenocarcinoma (B) | C | 0.65 | 4.96 | 9.58 | + 63.5 |
| Adenocarcinoma (B) | S/C | 6.5 | 5.10 | 6.80 | + 29 |
| Adenocarcinoma (B) | S/C | 56.12 | 6.07 | 17.35 | + 96 |
| Myeloma | S/C | 8.18 | 6.21 | 7.42 | + 18 |
| Adenocarcinoma (U) | S/C | 5.58 | 6.36 | 2.64 | - 83 |
| Carcinoid | S/C | 13.44 | 7.07 | 2.77 | - 87 |
| Melanoma | S/C | 1.44 | 9.40 | 21.80 | + 79.5 |
| Adenocarcinoma (B) | C | 0.75 | 16.62 | 6.9 | - 83 |
| Adenocarcinoma (O) | S/C | 1.77 | 18.98 | 15.17 | + 22 |
| Adenocarcinoma (B) | C | 1.29 | 19.45 | 10.5 | - 60 |
| Clear cell carcinoma | S/C | 4.29 | 19.98 | 10.81 | + 60 |
| Adenocarcinoma (B) | C | 17.97 | 29.58 | 31.53 | + 6 |
| Adenocarcinoma (B) | S/C | 28.73 | 31.04 | 12.01 | - 88 |

(B) = Breast S/C = Subcutaneous + = $Flow_2 > Flow_1$

(U) = Unknown primary C = Cutaneous - = $Flow_2 < Flow_1$

(O) = Ovary

TABLE 21

XENON $^{133}$ CLEARANCE MEASUREMENT OF BLOOD FLOW IN SUPERFICIAL METASTATIC LYMPH NODES

| Tumour histology | lymph node site | $Flow_1$ mls/min/100g | $Flow_2$ mls/min/100g | Flow change as % of mean |
|---|---|---|---|---|
| Lymphoma | Groin | 0.63 | 2.34 | + 115 |
| Adenocarcinoma (B) | Axilla | 2.06 | 0.32 | - 145 |
| Adenocarcinoma (B) | Neck | 4.63 | 15.5 | + 108 |
| Adenocarcinoma (B) | Neck | 9.17 | 19.58 | + 72.4 |
| Adenocarcinoma (B) | Groin | 12.82 | 14.89 | + 14.9 |
| Adenocarcinoma (B) | Axilla | 16.52 | 14.24 | - 14.8 |
| Adenocarcinoma (B) | Neck | 20.1 | 18.96 | - 5.8 |
| Lymphoma | Neck | 20.12 | 22.33 | + 10 |
| Squamous carcinoma (H & N) | Neck | 28.21 | 38.85 | + 32 |
| Adenocarcinoma | Groin | 42.98 | 55.24 | + 25 |
| Squamous carcinoma (H & N) | Neck | 43.13 | 8.64 | - 133 |
| Squamous carcinoma (H & N) | Neck | 58.83 | 55.92 | - 5 |
| Lymphoma | Axilla | 62.27 | 48.19 | - 25 |

(B) = Breast

(H & N) = Head and neck

+ = $Flow_2 > Flow_1$

- = $Flow_2 < Flow_1$

Of the total 62 Xenon clearance washout curves carried out in 31 tumours, 37 showed mono-exponential and 25 bi-exponential decay. Only one tumour showed a difference in pattern between the two sequential measurements (Table 22).

TABLE 22

PATTERNS OF EXPONENTIAL FIT IN 62 XENON WASHOUT CURVES FROM 31 TUMOURS

| Exponential fit in 2 sequential clearance measurements | B.B. | M.M. | M.B. |
|---|---|---|---|
| No. of tumours | 12 | 18 | 1 |

B. = bi-exponential
M. = mono-exponential

Significantly lower flow values were obtained from mono-exponential, compared with bi-exponential curves ($p = < 0.005$), and the range of values for each is shown in Tables 23 and 24 respectively. Other tumour characteristics (histology, volume and site) included in these tables demonstrate a preponderance of lymph node metastases (76%) in the bi-exponential group compared with the mono-exponential group (24%).

TABLE 23

BLOOD FLOW MEASUREMENTS DERIVED FROM SINGLE EXPONENTIAL XENON$^{133}$ WASHOUT CURVES

| $Flow_1$ mls/min/100g | $Flow_2$ mls/min/100g | Histology | Volume (mls) | Tumour site |
|---|---|---|---|---|
| 0.34 | 1.54 | Chordoma | 18.2 | S/C |
| 0.56 | 0.97 | Adenocarcinoma (B) | 18.90 | S/C |
| 0.6 | 0.3 | Chordoma | 10.0 | S/C |
| 0.63 | 2.34 | Lymphoma | -* | L.N. |
| 0.82 | 1.42 | Melanoma | 59.7 | S/C |
| 2.06 | 0.32 | Adenocarcinoma (B) | - | L.N. |
| 3.32 | 4.26 | Adenocarcinoma (U) | 17.97 | S/C |
| 4.63 | 15.50 | Adenocarcinoma (B) | - | L.N. |
| 4.96 | 9.58 | Adenocarcinoma (B) | 0.65 | C |
| 5.10 | 6.80 | Adenocarcinoma (B) | 6.50 | S/C |
| 6.07 | 17.35 | Adenocarcinoma (B) | 56.12 | S/C |
| 6.21 | 7.42 | Myeloma | 8.18 | S/C |
| 6.36 | 2.64 | Adenocarcinoma (U) | 5.58 | S/C |
| 7.07 | 2.77 | Carcinoid | 13.44 | S/C |
| 9.4 | 21.80 | Melanoma | 1.44 | S/C |
| 16.62 | 6.90 | Adenocarcinoma (B) | 0.75 | C |
| 19.45 | 10.50 | Adenocarcinoma (B) | 1.29 | C |
| 20.10 | 18.95 | Adenocarcinoma (B) | - | L.N. |
| 4.63 | Bi-exponential | Adenocarcinoma (B) | - | L.N. |

B = Breast
U = Unknown

L.N. = Lymph node
S/C = Subcutaneous
C = Cutaneous

* = volume not assessable

TABLE 24

BLOOD FLOW MEASUREMENTS DERIVED FROM BI-EXPONENTIAL

$XENON^{133}$ WASHOUT CURVES

| $Flow_1$ mls/min/100g | $Flow_2$ mls/min/100g | Histology | Volume (mls) | Tumour site |
|---|---|---|---|---|
| 9.17 | 19.88 | Adenocarcinoma (B) | - | L.N. |
| 12.82 | 14.89 | Adenocarcinoma (B) | - | L.N. |
| 16.52 | 14.24 | Adenocarcinoma (B) | - | L.N. |
| 18.98 | 15.17 | Adenocarcinoma (O) | 1.77 | S/C |
| 19.98 | 10.81 | Clear cell | 4.19 | S/C |
| 20.12 | 22.33 | Lymphoma | - | L.N. |
| 28.21 | 38.95 | Squamous carcinoma | - | L.N. |
| 31.04 | 12.01 | Adenocarcinoma (B) | 28.73 | S/C |
| 42.98 | 55.24 | Adenocarcinoma (?) | - | L.N. |
| 43.13 | 8.64 | Squamous carcinoma | - | L.N. |
| 58.83 | 55.92 | Squamous carcinoma | - | L.N. |
| 62.27 | 48.19 | Lymphoma | - | L.N. |
| Mono-exponential | 15.5 | Adenocarcinoma (B) | - | L.N. |

B = Breast
O = Ovary

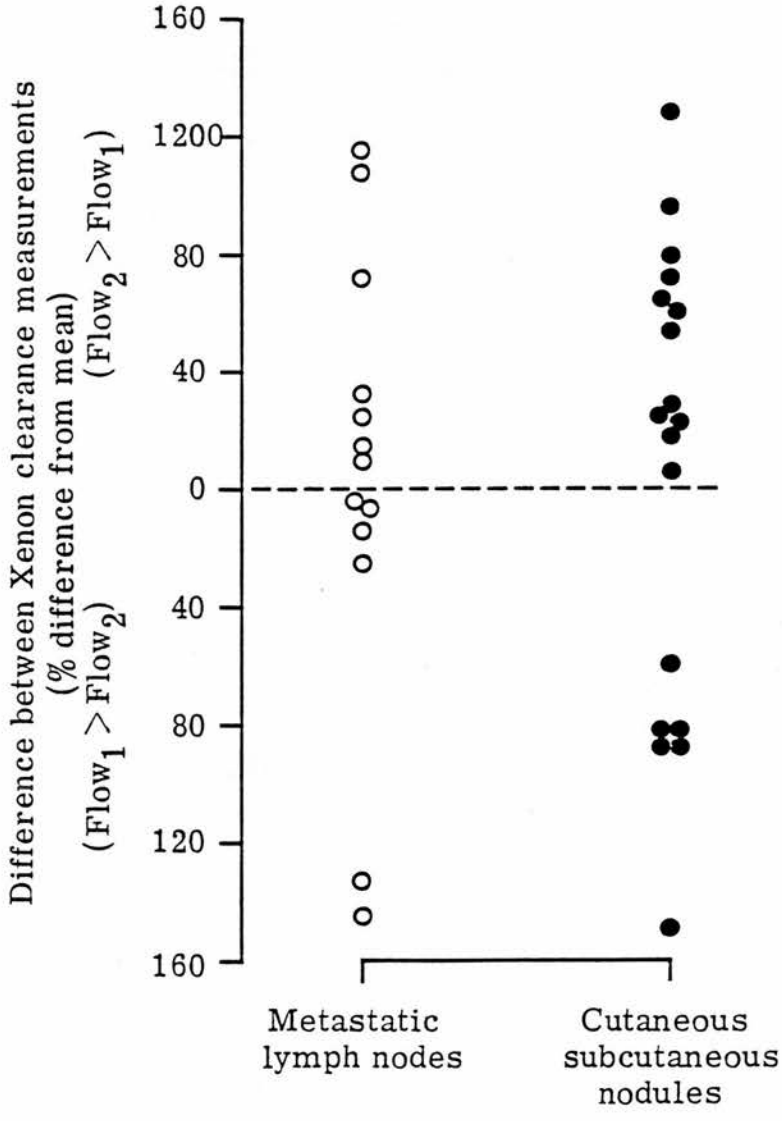
L.N. = lymph node
S/C = Subcutaneous
C = Cutaneous

The difference between the two flow measurements in the reproducibility study was expressed as a percentage of the mean of the two readings (Fig. 41) and calculated as follows:-

$$\frac{2\ (Flow_2 - Flow_1)}{Flow_2 + Flow_1} \times 100$$

Figure 41

Comparison of Two Sequential Xenon Clearance Measurement in Superficial Human Tumours Expressed as Percentage Difference From the Mean




Wide disparity between the two blood flow measurements was observed regardless of tumour histology, site or volume.

## 8.2 Reproducibility of Thermal Clearance as a Technique for Measuring Blood Flow in Small Superficial Tumours

The procedure for measuring thermal clearance was repeated up to 5 times in 10 patients, providing 74 observations in a total of 6 tumour and 13 normal tissue sites (Table 25 ). A wide distribution of values for the decay coefficient beta (B) was obtained, with tumour and normal tissue observations ranging from $7.14 - 23.2 \times 10^{-3}$/sec and $6.99 - 19.6 \times 10^{-3}$/sec respectively (Table 24). The median beta values for the two groups were $14.1 \times 10^{-3}$/sec for tumour and $11.6 \times 10^{-3}$/sec for normal tissue. Having allowed for variation between patients and using analysis of variance techniques, beta values were significantly greater ($p < 0.001$) in tumour compared with normal tissue.

The variation in beta values obtained at each measurement site has been expressed as a percentage of the mean of the first and last measurement (Fig. 42), and calculated from:-

$$\frac{2\ (B_1 - Bx)}{B_1 + Bx} \times 100$$

where $B_1$ is the decay coefficient for the first thermal washout, and Bx the decay coefficient for the last thermal washout.

TABLE 25

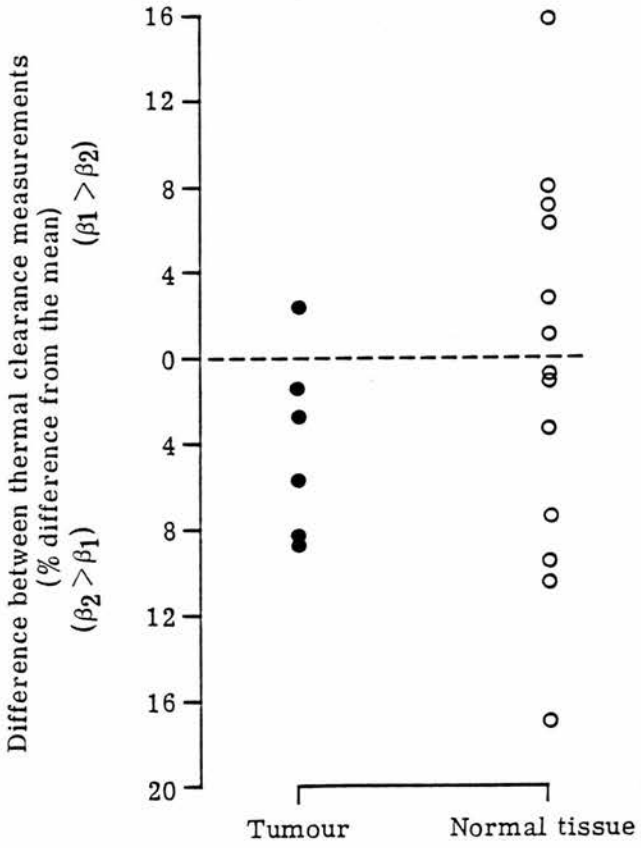
BETA VALUES DERIVED FROM THERMAL WASHOUT CURVES OBTAINED IN NINETEEN, TUMOURS AND NORMAL TISSUE, MEASUREMENT POINTS IN TEN PATIENTS

| | POINT$_1$ (T/NT)* | | | | | | | POINT$_2$ (NT) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient No. | T/NT | $B_1$ | $B_2$ | $B_3$ | $B_4$ | $B_5$ | $\bar{B}$ | $B_1$ | $B_2$ | $B_3$ | $B_4$ | $B_5$ | $\bar{B}$ |
| 1 | T | 8.13 | 7.14 | 8.33 | - | - | 7.87 | 6.99 | 7.14 | 7.19 | - | - | 7.11 |
| 2 | T | 14.1 | 12.7 | 13.0 | 13.3 | - | 13.3 | 10.2 | 9.26 | 10.3 | 9.17 | - | 9.74 |
| 3 | - | - | - | - | - | - | - | 12.0 | 12.5 | 13.2 | - | - | 12.6 |
| 4 | T | 8.13 | 7.87 | 8.00 | 8.06 | - | 8.02 | 8.13 | 8.33 | 7.87 | 8.26 | - | 8.15 |
| 5 | T | 14.1 | 14.1 | 13.9 | - | - | 14.0 | 11.6 | 11.6 | 12.3 | 11.5 | - | 11.8 |
| 6 | NT | 19.6 | 18.5 | 17.9 | 18.2 | - | 18.5 | 18.5 | 18.2 | 18.5 | 18.9 | - | 18.5 |
| 7 | NT | 10.2 | 10.6 | 10.1 | - | - | 10.3 | 12.3 | 11.6 | 11.9 | - | - | 11.9 |
| 8 | NT | 7.81 | 7.69 | 8.4 | 7.81 | 7.87 | 7.92 | 8.85 | 8.33 | 7.19 | 8.77 | 7.46 | 8.12 |
| 9 | T | 14.7 | 15.9 | 16.1 | 14.3 | - | 15.2 | 13.5 | 15.1 | 14.9 | 14.5 | - | 14.5 |
| 10 | T | 22.2 | 20.0 | 23.2 | 21.7 | 20.4 | 21.5 | 16.4 | 15.4 | 15.6 | 15.1 | 15.4 | 15.6 |

$\bar{B}$ = mean beta value  *T = Tumour  NT = Normal tissue

Figure 42

Variation in Thermal Clearance Measurement in Superficial Human Tumours Expressed as Percentage Difference from the Mean




The variation in beta was greater in normal tissue than in tumour, although the number of tumour observations was small. Overall, the percentage differences were considerably less than with Xenon clearance. There was no consistent pattern of change from the first to the last thermal clearance measurement.

Additionally, an overall estimate of reproducibility was derived from the pooled mean beta value for each patient point and their deviations, giving $0.53 \times 10^{-3}$ $sec^{-1}$ for use as a guide to the significance of any changes in beta observed in prospective studies of heat treated tissue.

## 8.3 The Effect of Hyperthermia on Thermal Clearance in Tumour and Normal Tissue

Patterns of thermal clearance, at specified intervals following a prescribed dose of heat, were documented and compared with pre-treatment thermal washout in 10 additional patient volunteers. In seven of these patients, the protocol could be followed exactly so that four thermal clearance measurements were obtained for each patient (i.e. immediately before hyperthermia and at 20 minutes, 4 hours and 24 hours after hyperthermia). Of the remaining three patients, one volunteered for three measurements only, and in the other two the probe was accidentally damaged after only two measurements. Lesions ranged in ellipsoidal area (estimated from caliper measurements) from 0.6 - 8.2 $cm^2$ (median 3 $cm^2$), permitting 2-4 intratumoural thermal clearance measurements to be made per patient (probe: Design 2). Tumour characteristics for these ten patients, together with details of temperatures achieved during a prescribed heat treatment, are given in Table 26. Five of the treatments fulfilled the time/temperature aim of a minimum tumour temperature of 43°C for 60 minutes. In the remainder, patient discomfort limited temperature elevation in three treatments.

TABLE 26

CLINICAL AND HYPERTHERMIA TREATMENT DETAILS

| Tumour site | Primary | measured temperature range (0°C) (6 points in total) | | Duration at specified temperature (min) |
|---|---|---|---|---|
| | | tumour base | surrounding tissue | |
| Chest | ca. breast | 43.0-43.5 | 43.5-44.0 | 60 |
| Chest | ca. breast | 43.0-45.0 | - | 60 |
| Chest | ca. breast | 41.5-42.0* | 40.0 | 60 |
| Chest | ca. breast | 43.0-44.0 | 44.0 | 60 |
| Chest | ca. breast | 43.0 | 42.0-45.0 | 60 |
| Neck | ca. H & N | 41.0-43.0 | - | 30* |
| Chest | ca. breast | 42.0* | 40.0-41.0 | 60 |
| Scapula | ca. breast | 41.0-43.0 | - | 60 |
| Chest | ca. breast | 43.0-43.5 | 40.0-43.5 | 60 |
| Chest | ca. breast | 40.5-42.5* | 42.0-43.0 | 30* |

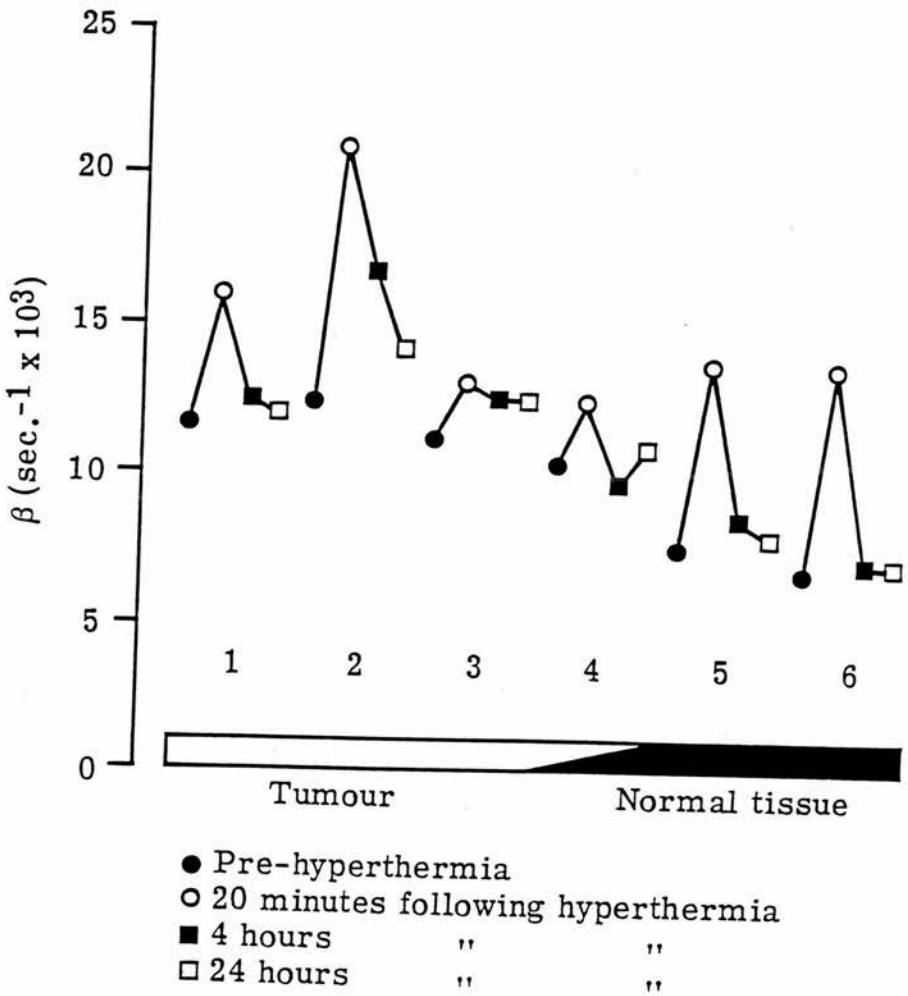
H & N = Head and neck

* = limited by patient discomfort

Figure 43 illustrates the effect of hyperthermia on thermal clearance in a single patient experiment.

Figure 43

Beta Values (B) at Six Measurement Points in Tumour and Adjacent Normal Tissue; Pre-hyperthermia and at Intervals of 20 Minutes, 4 Hours, and 24 Hours Following Hyperthermia

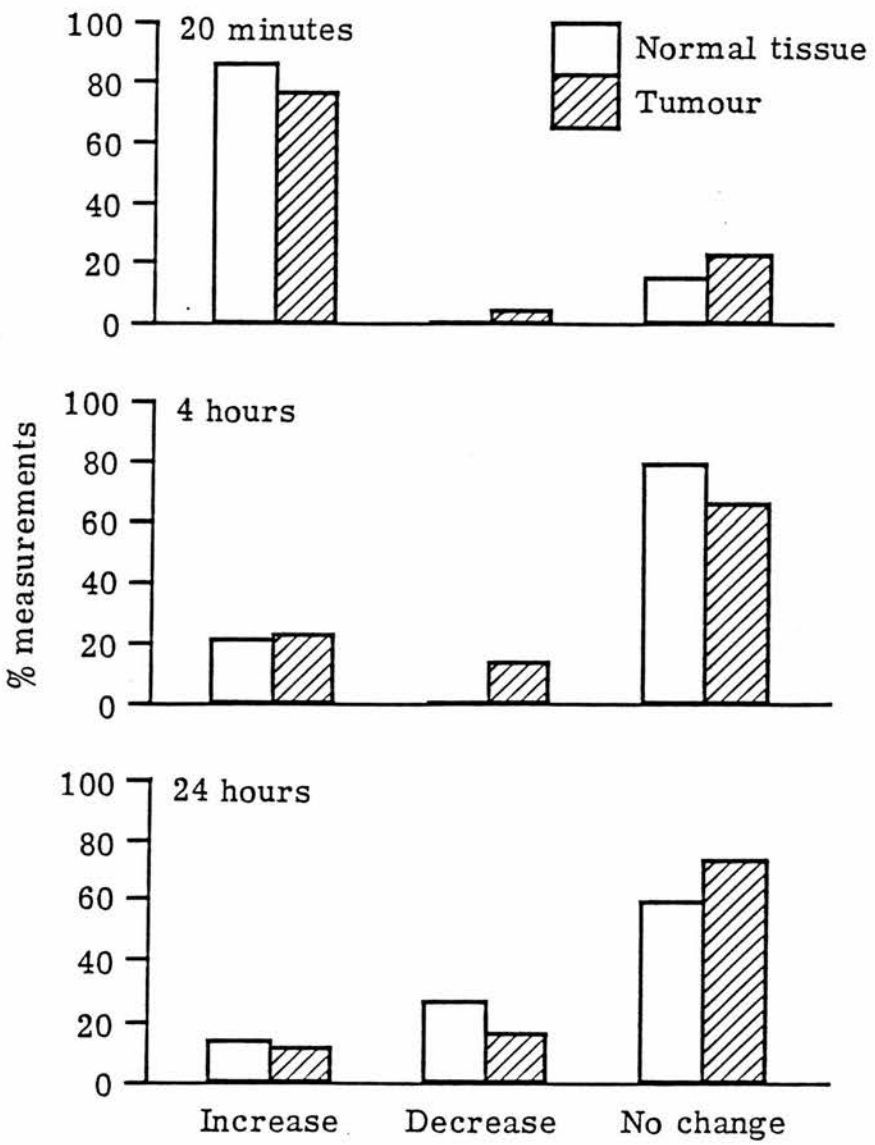


As indicated, the first three observation points (1-3) on the thermal clearance probe were positioned within tumour, the last two (5 & 6) within normal tissue, and the fourth point was estimated to be at the boundary between tumour and normal tissue. For each observation point, the sequence of beta values derived from thermal clearance measurement before, immediately following, and at 4 and 24 hours after hyperthermia are displayed. Each of the 24 data points plotted represents the arithmetic mean of the beta values derived from two clearance measurements (± 1 S.D.). In the example shown in Fig 43, an increase in beta is seen at all six measurement points immediately following hyperthermia.

The significance limit for immediate change (20 minutes post hyperthermia) was taken to be the separation of deviations associated with successive pairs of observations, or a change in beta of $> 10^{-3}$ $sec^{-1}$. The initial increase in beta was deemed to be sustained if the magnitude of change was reduced by < 50% at the four hour post-hyperthermia measurement. However, where there was a substantial return towards the pretreatment value (> 50% reduction), the initial increase was considered transient. At 24 hours the change was classified as either an overall increase in beta, a decrease, or a return to pretreatment values. Observations obtained from the 10 patients in the hyperthermia treatment study have been summarised according to these criteria and are illustrated in Fig. 44.

Figure 44

Percentage Beta Measurements Showing an Increase, Decrease or No Change in Thermal Clearance, with Reference to Pretreatment Values, at 20 minutes, 4 Hours and 24 Hours After Hyperthermia



Immediately following hyperthermia, there was an increase in beta in the majority of normal tissue (86%) and tumour (76%) points. A decrease in the magnitude of beta occurred at only one tumour point, and the remainder of observations, 14% and 21% for normal tissue and tumour points, showed no significant change from pretreatment values.

The initial increase in beta was generally not sustained, with 79% of normal tissue and 65% of tumour points returning to pretreatment values by 4 hours after hyperthermia. After an interval of 24 hours even fewer observation points, 13% of normal tissue and 11.5% tumour points, demonstrated an increase in beta compared with pretreatment values. Overall, there was no difference in the pattern of change of beta in tumour and normal tissue.

## 8.4 Discussion

Ideally, the Xenon clearance reproducibility study would have been best carried out by performing a series of measurements on the same tumour so that an average and standard deviation could be established. However, ensuring reproducibility of patient positioning, external conditions, injection technique and radioactivity monitoring involves the patient in a time-consuming technique which is difficult to justify performing repeatedly on an experimental basis. Additionally, the time scale required for a greater number of observations would make it more difficult to control other parameters which might affect blood flow, such as patient medication.

The flow values derived in the present study were in the same general range as previously reported clinical observations using $Xe^{133}$ clearance (Table 27 ).

It has not been possible to stratify the results of the present study according to tumour histology, as the majority of lesions (17/31) were adenocarcinoma, the remainder being distributed among seven different histological groups. However, Mantyla demonstrated significantly higher flow values in lymphomas compared with superficial metastatic differentiated or anaplastic carcinomas (Mantyla, 1979a, 1982). This finding may be related more to local differences between lymph nodes and other sites rather than histological type. The results of the present series would support this

view, with the lymph node metastases showing a marginally significant greater flow than the cutaneous/subcutaneous deposits.

TABLE 27

HUMAN TUMOUR BLOOD FLOW VALUES DERIVED FROM XENON$^{133}$ CLEARANCE

| Author | $Xe^{133}$ application | Tumour type | Tumour volume (ml) | Mean blood flow (ml/min/100g) |
|---|---|---|---|---|
| Mantyla 1979a | Intratumoral injection | Lymphoma | 0.3 - 440 | 34.6 ± 21 (SD) |
| | | Differentiated cancers | | 22.8 ± 14.9 (SD) |
| | | Anaplastic carcinoma | | 15.4 ± 11.4 (SD) |
| Mantyla 1982 | Intratumoural injection | Lymphoma | 0.3 - 400 | 33.0 ± 24.4 |
| | | Differentiated cancers | | 20.0 ± 14.7 |
| | | Anaplastic carcinomas | | 10.2 ± 8.2 |
| Olch 1983a | Intratumoural injection | Sarcoma, Melanoma, Carcinoma | 65 | 1.6 - 6.6 |
| Wheeler, 1986 | Intratumoural injection | Squamous carcinoma - head and neck | Not given | 13.6 ± 6.7 |

Both single and bi-exponential equations have been used to fit Xenon clearance data in previous studies (Gelin, 1968, Daly, 1980, Mantyla, 1979a, Handel, 1976). Where the curve is biphasic, blood flow can be calculated from the initial phase (Daly & Henry, 1980, Handel et al, 1976, Lassen, 1967), from the later steady state component (Sekins et al, 1980) or from a combination of the two (Mantyla, 1979a). It can be argued that the initial portion of the curve may be the appropriate determinant of blood flow where the injected tissue has a significant fat content, and consequently a high

Xenon affinity. Alternatively, avoidance of this early phase of the curve may be advisable if it represents a transient needle-induced hyperemia rather than steady state blood flow.

Analysis of the present data showed a preponderance of mono-exponential fits (60%), and the consistency within individual tumours (98%) suggests stability of the clearance mechanism. The finding of significantly lower blood flow values from mono-exponential, compared with bi-exponential, curves parallels Mantyla's observations (Mantyla, 1982). This is probably not surprising as a bi-exponential curve implies incorporation of an initial rapid phase into the blood flow calculation. However, it is interesting that there was a preponderance of lymph node metastases in the bi-exponential group, and it seems plausible to suggest that the vascular architecture of lymph nodes provides a different mechanism of clearance. The initial rapid portion of a curve may be a reflection of preservation of normal vasculature and its functional capacity to vasodilate in response to needle injection.

The technique, as described here, was not sufficiently reproducible for use as a measure of change of flow following hyperthermia, and possible contributing factors have been considered. Firstly, the volume of fluid injected (0.1ml) represents a considerable proportion of the volume of small lesions (< 3ml) and might be expected to disturb the micro-environment with disruption of the capillary system.

This would be particularly likely where considerable pressure was necessary to introduce Xenon into the tumour substance.

Furthermore, preservation of normal vessels capable of vasodilation would permit a needle-induced hyperemic reaction in small lesions whereas the structural and functional changes that occur in incorporated vessels with tumour expansion would prevent reactivity in larger lesions. As previous use of the technique has generally involved lesions of volume $\geq$ 10ml, the present series was adapted to include larger cutaneous/subcutaneous lesions and lymph node metastases. However, the present results indicate that tumour volume had no influence on the reproducibility of the technique. Conversely, decreasing the injected volume to 0.01 ml. might reduce interstitial injury, but varying the volume between 0.1 and 0.2 ml. in larger lesions did not affect the outcome in Mantyla's series.

Secondly, interpretation of the washout curve is difficult and therefore susceptible to error. A variable initial accumulation of activity reflects the non-uniform rate of interstitial injection of $Xe^{133}$ and is followed by a decay curve which can be either mono or bi-exponential. A biphasic clearance curve is interpreted as indicating the presence of two mechanisms of Xenon clearance. The initial rapid phase may reflect the influence of a transient hyperemic response at the site of injection, whereas Xenon diffusing beyond this region may be cleared by steady state blood flow. In previous studies, both portions of the decay

curve have been examined to determine blood flow. A criticism of the method is that the point beyond which decay follows an exponential curve is selected arbitrarily. In the present study, great care was taken to reproduce patient position, external conditions, the site of injection, and the means of detecting radioactivity. As a result, the technique was time consuming but could have been incorporated into a hyperthermia programme had this seemed appropriate.

There are no detailed reproducibility studies on the application of this technique in human tumours in the literature. It is important to be aware of the problems of the technique, and to emphasise the extent of variation that can occur, before incorporating Xenon clearance into protocols designed to investigate blood flow change as a result of therapeutic intervention (Olch, 1983a). Mantyla refers to the reproducibility of the method as checked by repeated measurements in eight patients, but no further details are given. The present results do not concur with the finding of a deviation from the mean of $< \pm 10\%$.

In the present study, a thermal method for the qualitative description of heat induced blood flow change has been evaluated as to its suitability for clinical application in small volume tumours and surrounding normal tissue. The aim was to employ the technique to investigate changes in blood flow after a moderate hyperthermia treatment (43°C for 60 minutes) such as is commonly attempted in the clinic.

Comparison of thermal clearance in tissue with that in non-perfused phantom models supports the suggestion that both conductive and convective mechanisms of heat transfer contribute to clearance measurements in vivo. However, no attempt has been made to quantify the rate of influence of these components in the present comparative, qualitative study, which aimed to demonstrate whether flow changes do occur and, if so, to assess roughly their magnitude and duration. Much of the controversy associated with the use of thermal clearance stems from interpretive difficulties inherent in applying simple mathematical models, particularly the "bio-heat" equation (Eberhart et al, 1980), to estimate absolute blood flow (Sandhu, 1986), and is therefore not relevant to the present application of the technique.

In using thermal clearance as a qualitative index of heat-induced blood flow change, two important assumptions have been made. Firstly, as the heat-induced vascular damage reported in animal studies is predominantly a capillary phenomenon, any meaningful index of blood flow must be capable of reflecting changes at this level of vessel calibre. The assumption is generally made that convective heat transfer within tissues takes place at the capillary bed, a situation analogous with the exchange of other metabolites such as oxygen. However, recent theoretical modelling and experimental studies (Chen & Holmes, 1980, Lagendijk, 1985, Weinbaum & Ji Ji, 1985) fail to support this view, and suggest that patent vessels having diameters in the range 40-300μm are of much greater significance. These

studies have been exclusively concerned with normal tissues, and the findings may not be relevant in tumours, where the vasculature differs both morphologically and rheologically (Warren, 1979). Within the context of the present study, therefore, it is reasonable to suggest that the biological significance of thermal clearance measurement rests upon the assumption that flow at the level of terminal arterial branches down to arterioles is affected by heat, either directly or influenced by flow changes at the capillary level (i.e. imperfect arteriovenous shunting). This is supported in a recent report illustrating the relationship between tissue temperatures and mean volume blood flow assessed by a microsphere method, during water bath immersion heating of rat normal tissues (Lokshina et al, 1985). Secondly, parameters influencing thermal conduction, such as tissue water content, are assumed to be constant throughout the series of measurements in each patient. This assumption is probably valid as considerable changes in tissue water content would be necessary to alter thermal conduction and have not been reported to occur in response to hyperthermia within the 24 hour time scale considered here.

The practicality of introducing the thermal clearance probe and securely maintaining the desired position was established before proceeding to the reproducibility study. In this study, manipulation of the probe within normal tissues was always achieved painlessly, but penetration into the tumour substance was prevented by discomfort in four of the ten patients. In three of these, both measurement points

were positioned in adjacent normal tissue, but in the fourth only one of the points could be accommodated. Aside from insertion of the probes, all volunteers considered the procedure to be painless and consequently patient positioning was easily maintained. As the sites studied were superficial, room temperature was controlled and heat transfer from skin surface minimised by a covering of insulating material.

Considering both tumour and normal tissues, beta values overall ranged from 6.99 to 23.2 x $10^{-3}$ $sec^{-1}$. There are no directly analogous derivations of beta values from thermal clearance in human tissues with which to compare the present series. However, the small multi-junction heating probe used here provided thermal washout curves not dissimilar to those derived from cooling data in our series of 56 heat treatments in superficial tumours. In addition, beta values obtained from animal data are in general agreement with the result of this study (Cater et al, 1965).

Although the clinical data in the literature are not adequate to establish that tumour blood flow is generally lower than normal tissue blood flow, the aberrant morphological features of tumour vasculature and the phenomenon of selective tumour heating suggest that this is the case. In the present study however, beta values obtained in tumour were significantly greater than in adjacent normal tissue The small size of the lesions studied may account for this finding as, at least in experimental tumours, blood flow

decreases with increasing tumour size (Vaupel, 1977). A lack of differential blood flow between small lesions and surrounding normal tissue has been implied by Kim et al (1982), who were unable to demonstrate selective tumour heating in metastatic melanoma lesions of < 10 mls.

There were too few tumour observations to make any comment on the effect of tumour size, site or histology on the beta values derived from thermal washout curves.

The reproducibility of the technique has been assessed by calculating the percentage difference from the mean of two observations, in the same way that the Xenon clearance technique was evaluated. As 3 - 5 measurements of beta were obtained at each observation point, the first and last measurements were used to derive this expression, being those considered most likely to reflect any inconsistency. The percentage difference between beta values was much less than that obtained with two sequential Xenon clearance measurements, although the direction of change was equally unpredictable. For thermal clearance, the percentage differences ranged from + 16 to - 17, whereas the corresponding values for Xenon clearance were +130 and -150. On the basis of this, thermal clearance was considered sufficiently reproducible for investigation of changes in blood flow associated with hyperthermia. However, assessment of reproducibility also provides an index of sensitivity of a technique as some fluctuation in the parameter under investigation is inevitable. The lack of consistent

variation between sequential Xenon clearance measurements, despite careful control of conditions, makes it unlikely that this is a useful reflection of sensitivity.

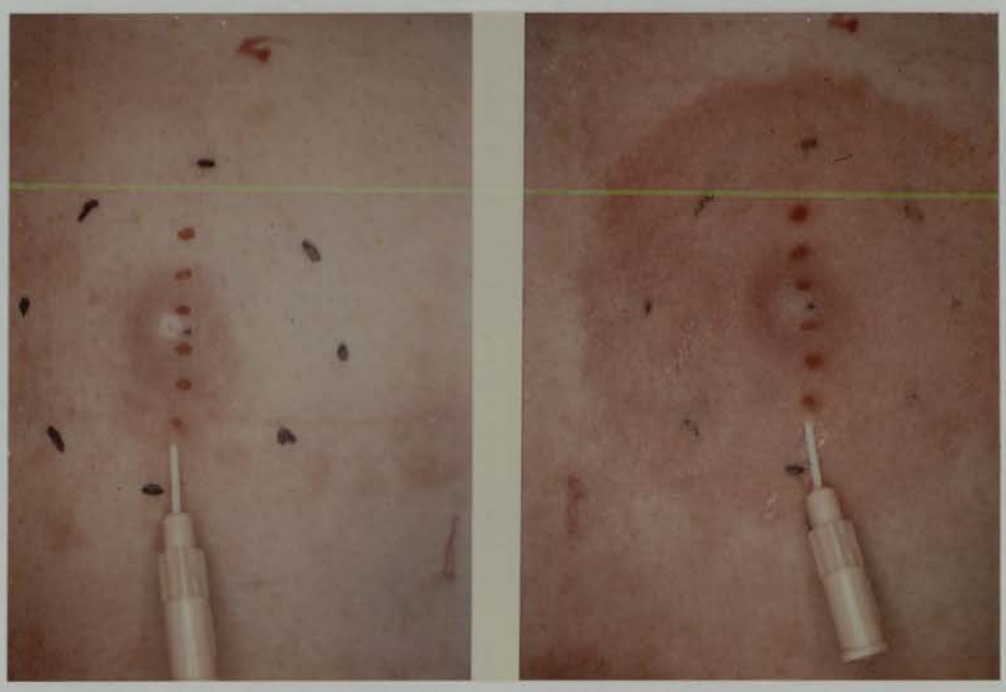
Using the technique, a consistent pattern of heat-induced blood flow change has been observed. After moderate heating, a clear majority of thermal clearance measurements imply an increase in flow through tumours (T) and their surrounding tissues (NT) (T:76% and NT:86% of points sampled.) A substantial reduction in this change was often seen after an interval of four hours (T:65%, NT:79%). This was in agreement with the subjective attenuation of hyperemic skin reactions observed within the hyperthermia treatment field (Fig. 45). A small number of measurements showed no immediate flow change (11/48), and of those approximately one half (6/11) showed no later evidence of a delayed change. A return to pretreatment flow values within 24 hours was observed in 73% and 60% of tumour and normal tissue measurements respectively. The small group of observations showing an overall flow reduction (T:4/26, N:4/15) was influenced considerably by one case (T:3, N:2) following the lowest HT dose achieved in the series (T:40.5-42.5°C, N:42-43°C x 30 minutes). In this patient, the consistent reduction in flow at 24 hours (5/6 points) was striking, and the preceding pattern of flow change was qualitatively similar to other cases. The significance of this anomalous pattern of flow change remains in doubt in the absence of control data from unheated tissues in the same patient. A comparison between the tumour and normal tissue

responses summarised in Fig. 44 showed no significant difference in each group (single tailed $x^2$ test, $p = 0.05$ level). This result was also supported by a careful inspection of temperature data recorded during hyperthermia, which did not reveal any significant relative changes between tumour and normal tissue temperatures throughout the heating period.

Figure 45

Transient Erythema Following Heating of the Skin to 43°C for 60 Minutes

Prehyperthermia Posthyperthermia



Although the present data are consistent with experimental studies in normal tissues (e.g. Song 1984), there is an apparent contradiction between the observed human tumour response and the general conclusions drawn from transplantable animal tumour studies. Of the 18 reports reviewed by Song (1984), 15 suggested that doses less than 43°C for 60 minutes were sufficient to achieve vascular stasis within tumours during the period of heating. It is possible that a differential vascular response may occur in human tumours taken to a higher heat dose. However, from clinical experience, hyperthermia treatment of 43°C for 60 minutes is close to the upper practical limits of tolerance for a minimum tumour dose delivered in a single treatment. Increasing thermal dose beyond this level raises the probability of normal tissue toxicity, as demonstrated by the substantial damage sustained in rat skin following 45°C for 60 minutes (Okumura & Reinhold, 1978). Increased heat dosage is therefore likely to require a fractionated regime which introduces the possibility of significant thermotolerance effects (Overgaard and Nielsen, 1983). Therefore, the present data suggest that enhanced tumour cell killing, mediated by microvascular damage, is unlikely to contribute to the hyperthermic effect, at least in its current clinical application.

The present series of observations on carefully selected small superficial tumours does not permit general statements concerning vascular response of human tumours, and the need for further investigation with regard to the effect of tumour

size, site and histological type is emphasised. However, some of the commonly reported features of clinical hyperthermia, such as lack of selective tumour heating in small lesions (Kim et al, 1982), are consistent with the present findings.

Chapter 9

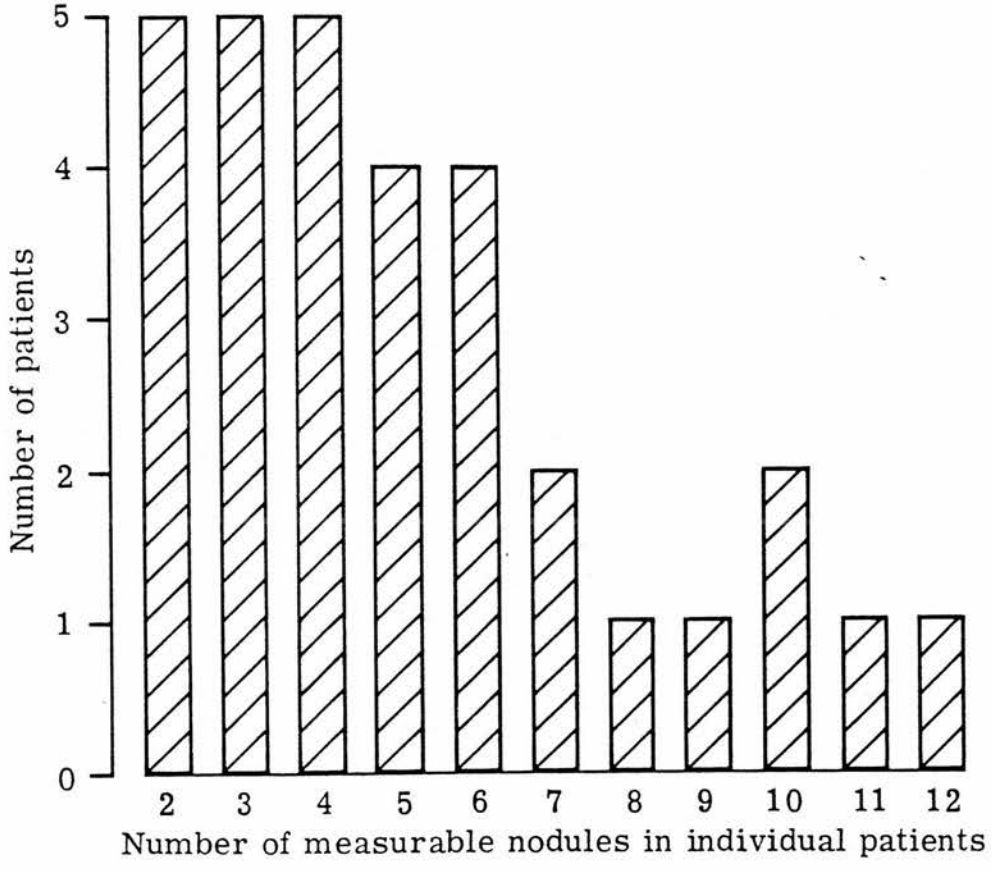
# TUMOUR GROWTH

9.1 Tumour Doubling Time in Human Metastases.

Tumour doubling times have been calculated for 163 metastatic nodules in 31 patients contributing between 2 and 12 nodules each (Fig. 46). The number of measurable nodules available per patient has important implications for study design in terms of inclusion of control lesions and the range of dose levels investigated.

Figure 46

Frequency Distribution for Number of Measureable Nodules per Patient




Classification of nodules by histology showed a preponderance of adenocarcinoma of primary breast origin (101 nodules) with the remainder distributed as shown in Table 28.

TABLE 28

CLASSIFICATION OF NODULE POPULATION BY HISTOLOGICAL TYPE

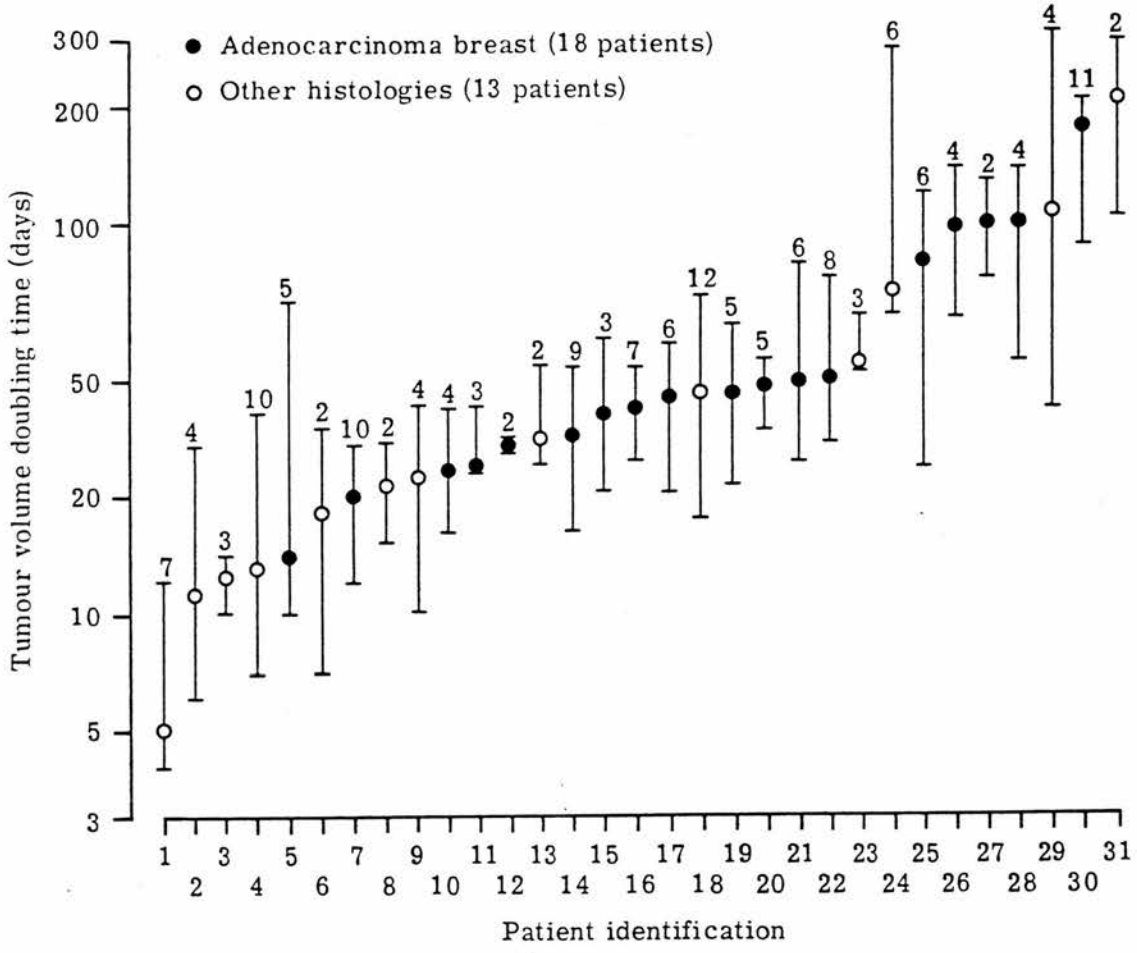
| Histological type | | No. nodes | No. of patients |
|---|---|---|---|
| ADENOCARCINOMA | - breast | 101 | 18 |
| | - bronchus | 11 | 2 |
| | - unknown origin | 3 | 1 |
| | - corpus uteri | 4 | 1 |
| SQUAMOUS CARCINOMA | - bronchus | 5 | 2 |
| | - skin | 3 | 1 |
| ANAPLASTIC CARCINOMA | | 10 | 1 |
| MELANOMA | | 22 | 4 |
| CARCINOID | | 4 | 1 |
| | | 163 | 31 |

Data analysis has been carried out on the entire population (163 nodules) and, separately on the breast carcinoma lesions (101 nodules) to investigate growth characteristics in this specific tumour type.

Overall, nodule doubling time varied between 4 and 310 days and the range of values and median for each patient are displayed, ranked in order of increasing median doubling time, in Fig. 47.

Figure 47

Median and Range of Tumour Volume Doubling Times in Multiple Metastases from 31 Patients



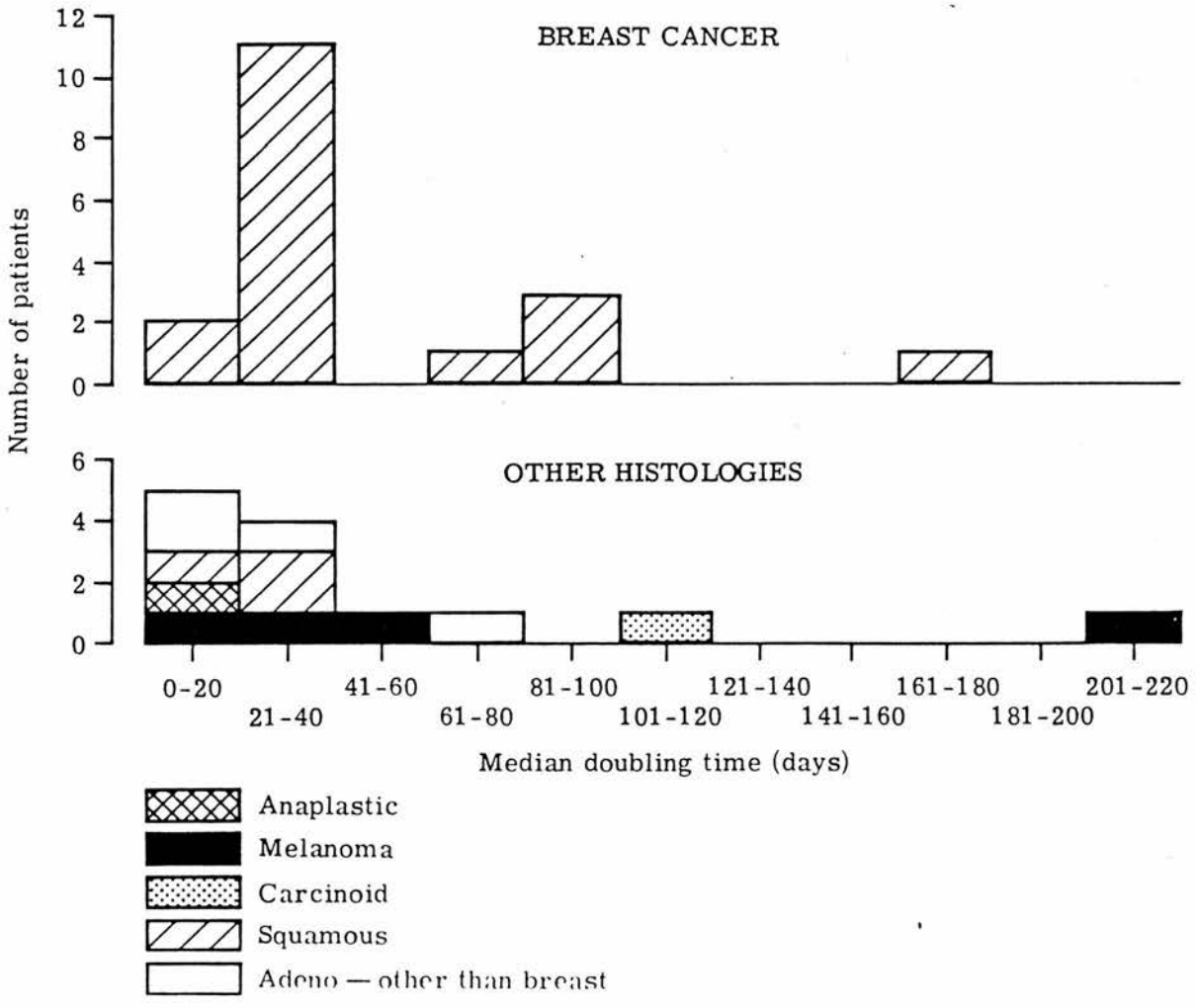

The number of nodules measured in each patient is indicated above the corresponding range of doubling time values.

In 71% (22/31) of patients the median doubling time was less than 40 days.

In breast cancer patients median tumour doubling time ranged from 14 to 175 days and was less than 40 days in 13/18 (72%) patients (Fig. 48). In the remainder, median values ranged from 5 to 206 days with a similar proportion, 9/13 (69%), being less than 40 days (Fig. 48). There was a suggestion that the growth rate observed in breast cancer patients was relatively homogeneous since 11/18 (61%) of the median values fell within the range of 21-40 days.

Figure 48

Frequency Distribution of Median Tumour Volume Doubling Time in Relation to Histology



## 9.2 Consistency of Tumour Growth Rate in Multiple Metastases

Measurement of more than one tumour nodule in each patient allowed uniformity of growth rate to be assessed. As some patients had only two or three nodules, the application of rigorous statistical tests was inappropriate in evaluating uniformity. For this reason an index of uniformity was derived from the ratio of maximum to minimum doubling times in individual patients. A ratio of one indicated uniform growth rate in different nodules in the same patient, whereas values > 1 suggested heterogeneity of doubling time. The uniformity ratio, and the median doubling time, for each patient is shown in Fig. 49. There was a suggestion of greater intra-patient uniformity in breast tumours, where 13/18 (72%) patients had ratios of less than 3.0, compared with 4/13 (31%) in other tumour types. There did not appear to be any correlation between the uniformity of tumour doubling time for each population of nodules and the median doubling time for that population.

An example of uniform growth rate in 10 nodules from a patient with breast cancer is shown in Fig. 50.

Figure 49

Uniformity of Tumour Volume Doubling Time in Multiple Metastases in Relation to Median Doubling Time for Individual Patients

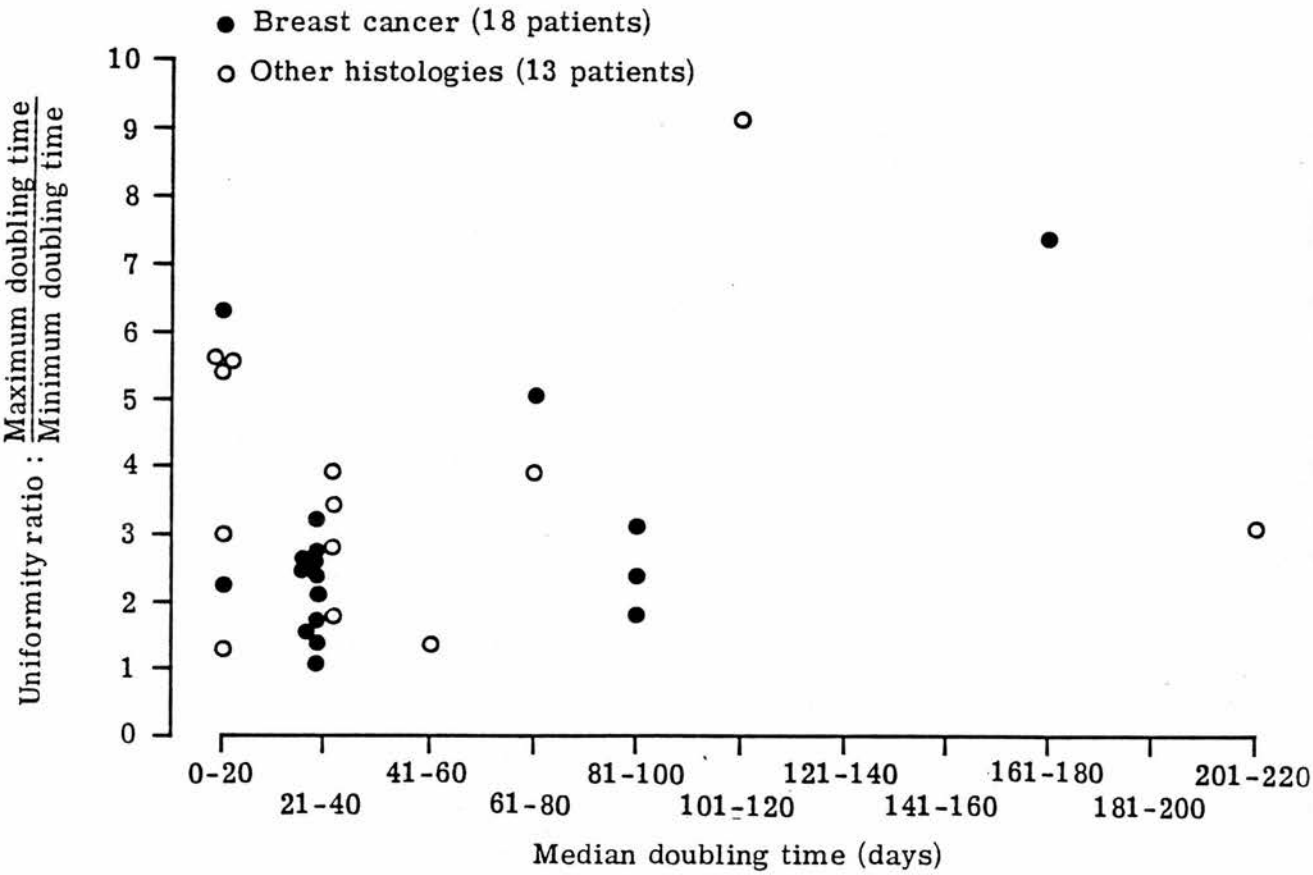
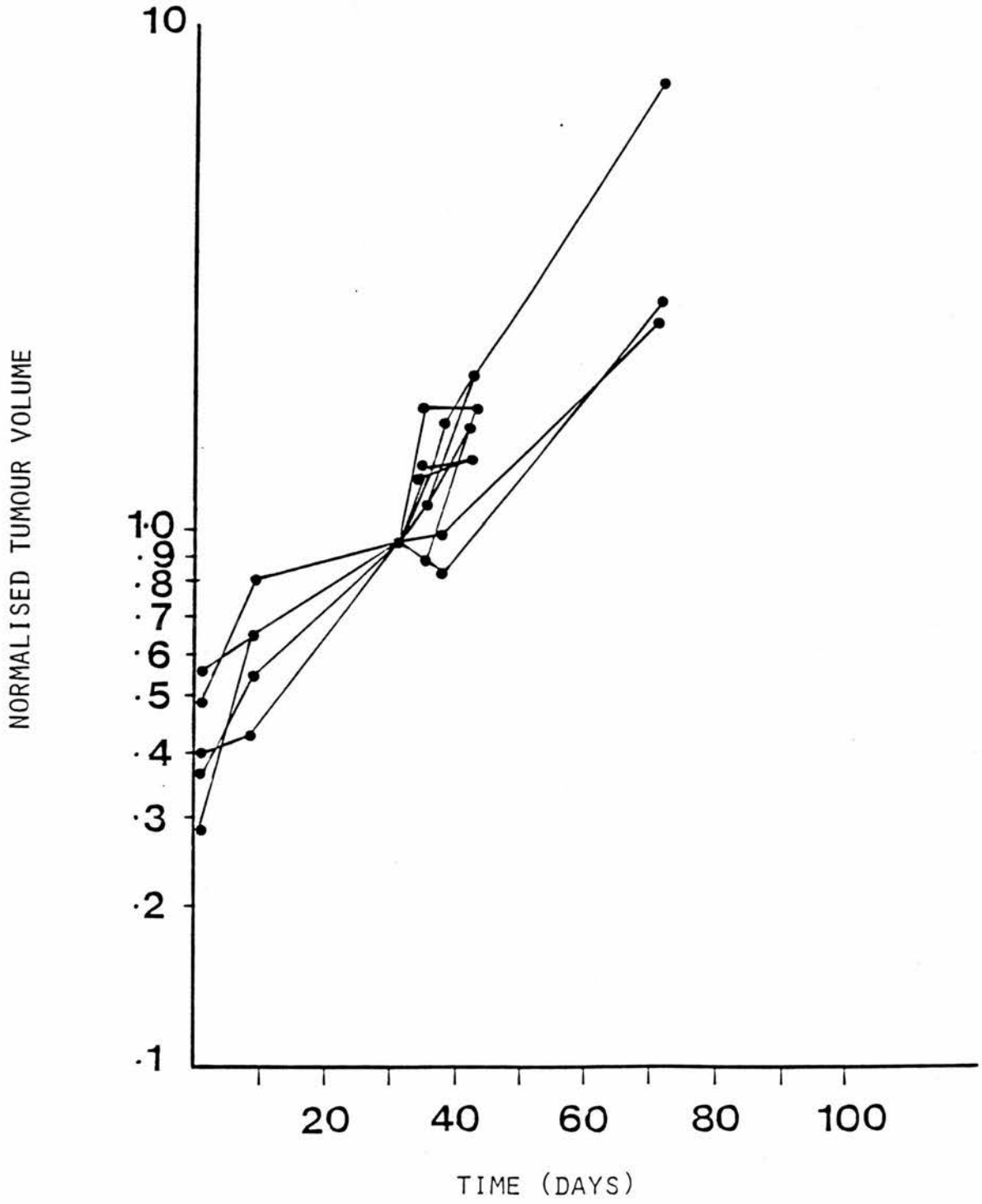


Figure 50

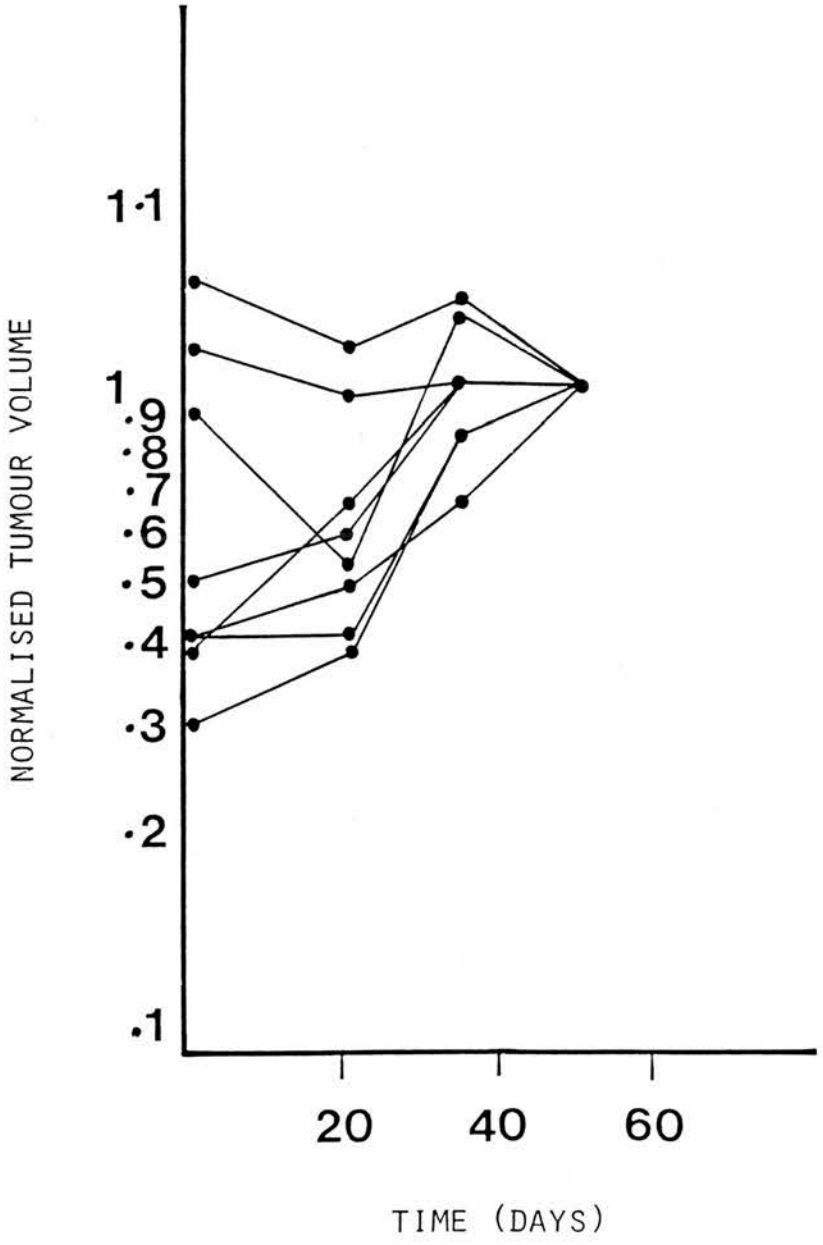
Growth Curves from 10 Nodules in a Patient with Breast Cancer



In patients with nodules growing at different rates, such as the example shown in Fig. 51, tumour volume and site were considered as possible factors contributing to non-uniformity of growth rate:-

Figure 51

Growth Curves From 8 Nodules in a Patient With Breast Cancer



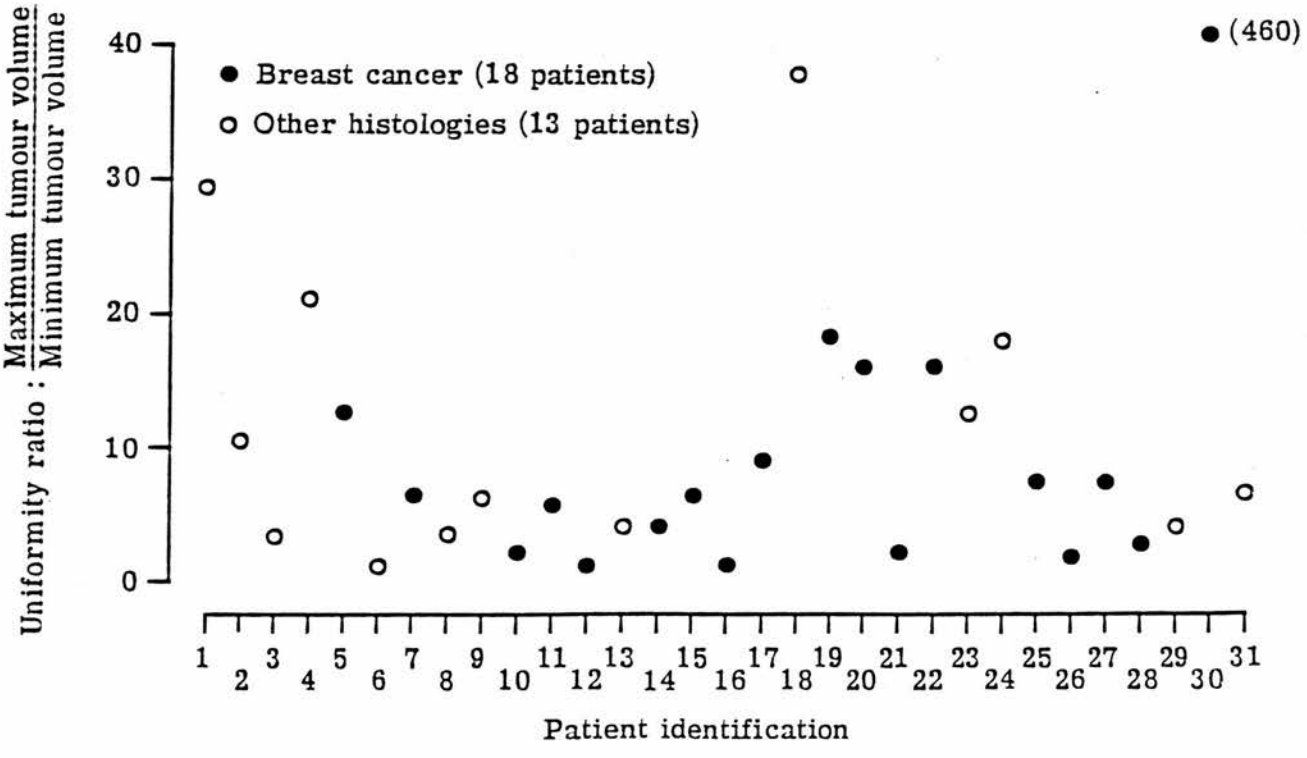
## 9.3 Factors Influencing Tumour Doubling Time in Multiple Metastases in Individual Patients

### 9.3.1 Tumour Volume

The ratio of initial tumour volume, in the largest and smallest nodule, has been used as an indicator of uniformity of tumour size within individual patients (Fig. 52).

Figure 52

Uniformity of Nodule Size Within Individual Patients Expressed as the Ratio of Maximum to Minimum Tumour volume

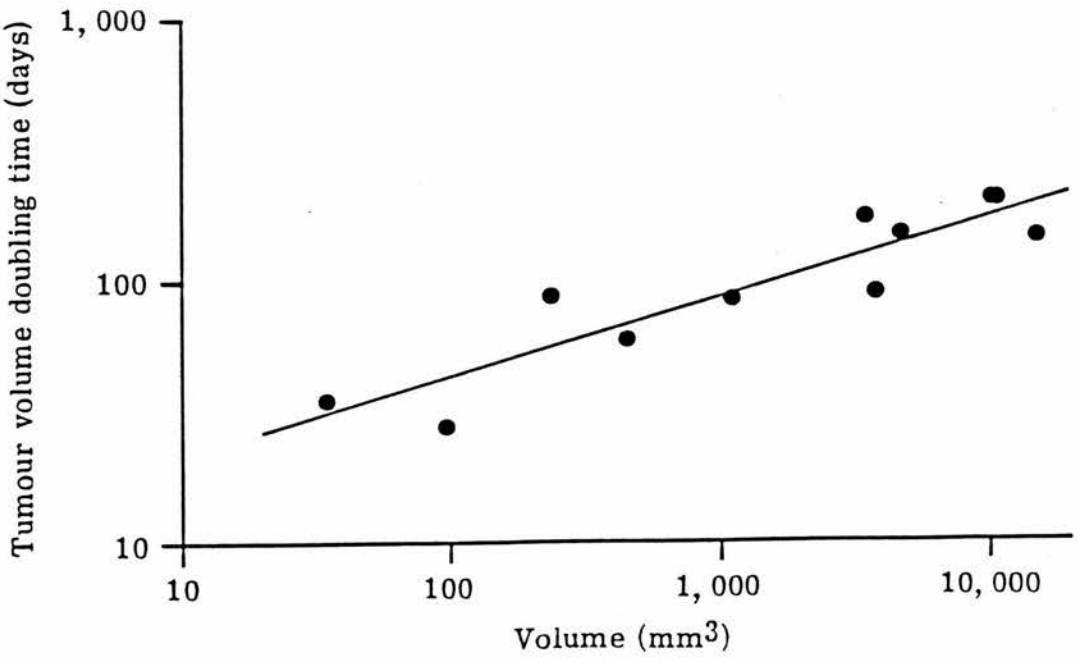



The 11 patients who showed wide variation of nodule size as demonstrated by a ratio of greater than 10, were analysed to investigate the effect of volume on doubling time in the same individual. In three of these 10 patients, heterogeneity of doubling time was demonstrated, with a

uniformity index of greater than four. However, only one of the three (patient 30) showed a significant correlation between volume and growth rate (Fig. 53). In this patient, 11 nodules with initial volumes ranging from 34 $mm^3$ - 15,492 $mm^3$ provided doubling time data. In the other two patients (patient 4 and 24), with 11 and 6 nodules respectively, despite a wide range of volumes and doubling times, correlation coefficients were not significant (Table 29 and Fig. 54).

Figure 53

Correlation of Volume Doubling Time with Nodule Size in a Patient with Breast Cancer (Patient 30)



Correlation coefficients for tumour volume against doubling time were calculated for the 21 patients who had four or more nodules. These are shown in Table 29, together with the number of nodules per patient on which the analysis was based and the volume ratio for each individual. In the group with volume ratios of less than 10, two patients (Patient 9 and 28) showed a significant correlation ($p < 0.005$). In those patients with more variation in tumour size, volume ratios greater than 10, a further two individuals (patients 1 and 30) demonstrated a significant correlation between tumour volume and doubling time ($p < 0.001$)

TABLE 29

CORRELATION COEFFICIENTS FOR TUMOUR VOLUME AGAINST DOUBLING TIME IN PATIENTS WITH FOUR OR MORE NODULES

| Volume ratio < 10 | | | | Volume ratio > 10 | | | |
|---|---|---|---|---|---|---|---|
| Patient No. | No of Nodules | Volume ratio | Correlation Significance | Patient No. | No. of nodules | Volume ratio | Correlation coefficient |
| 7 | 10 | 6.5 | 0.486 | 1 | 7 | 29.5 | 0.979*** |
| 9 | 4 | 6.1 | 0.988* | 2 | 4 | 10.5 | 0.863 |
| 10 | 5 | 2.2 | 0.365 | 4 | 10 | 22.1 | 0.234 |
| 14 | 9 | 4.1 | 0.191 | 5 | 5 | 12.6 | 0.553 |
| 16 | 7 | 1.4 | -0.308 | 18 | 12 | 37.7 | 0.263 |
| 17 | 6 | 9.2 | 0.704 | 19 | 5 | 18.2 | 0.574 |
| 21 | 6 | 2.1 | 0.501 | 20 | 5 | 16.3 | 0.213 |
| 25 | 6 | 7.4 | 0.116 | 22 | 8 | 16.2 | 0.605 |
| 26 | 4 | 1.8 | 0.194 | 24 | 6 | 18.2 | -0.053 |
| 28 | 4 | 2.9 | 0.965* | 30 | 11 | 460 | 0.913*** |
| 29 | 4 | 4.1 | 0.267 | | | | |

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Figure 54

Lack of Correlation of Tumour Doubling Time with Nodule Size in 2 patients Showing a Wide Range of Both Parameters (Patient 4 and 24)

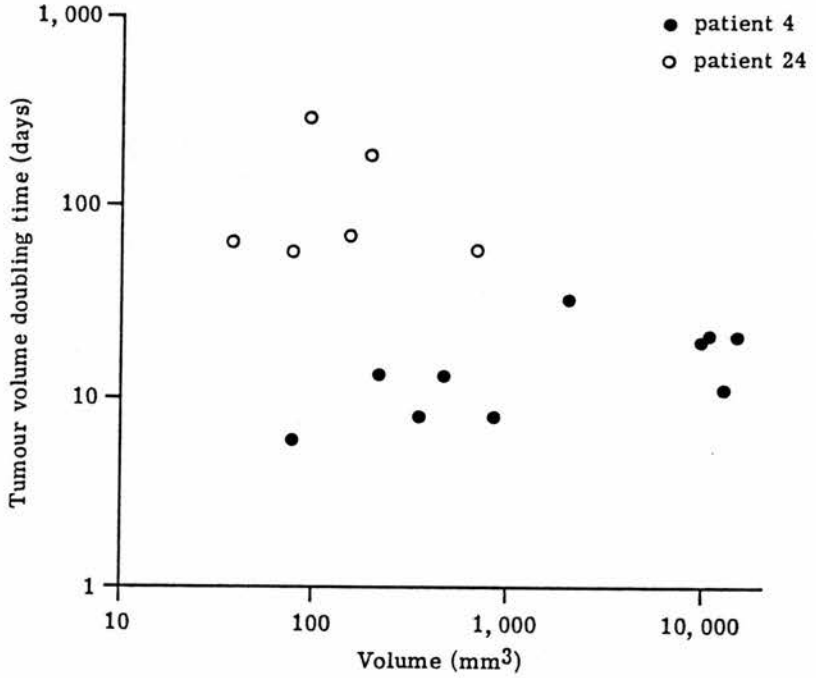
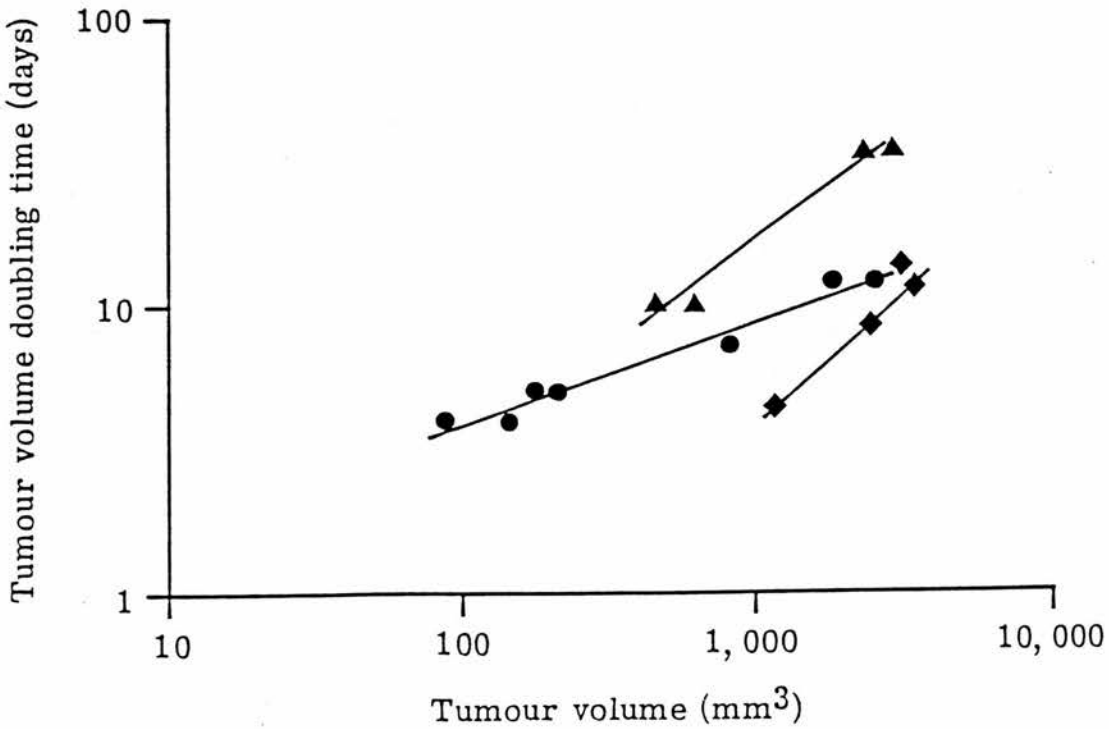



Figure 55

Correlation of Volume Doubling Time with Nodule Size in 3 Patients (Patient 1, 9 and 28



### 9.3.ii The Effect of Tumour Site on Doubling Time

Breast cancer nodules usually occurred in a localised cluster on the breast or chest wall (15/18 patients) and were therefore unsuitable for analysis by site except in three patients. In a further six patients with other histologies, the distribution was adequate for investigation of a site effect. Of these nine patients, only one (patient 9) showed any variation of growth rate by site with two subcutaneous neck nodules doubling in 34 days compared with a 10 day doubling time in two subcutaneous abdominal wall lesions. Two patients (patient 1 and 4) had lesions widely scattered over the torso without any detectable effect on growth, and the same was the case in a third patient (patient 18) who also had limb lesions.

## 9.4 DISCUSSION

The reason for investigating tumour growth in the present study was to assess variation in growth rate between individual metastases in the same patient and to see whether nodule size or geographical site influenced volume doubling time. Ideally, standard deviation from the mean would have best reflected variation in doubling time in each nodule sample, but the small number of observations (2 and 3) in 10 of the patients precluded this approach. For this reason the ratio of maximum to minimum doubling time, within individual patients, was used as an index of growth rate uniformity. Applying this index of uniformity, variation in nodule doubling time was three-fold, or less, in 19/31 (61%) patients. A greater than three-fold variation was due to a single nodule in only three patients, and in the remainder the values were spread fairly evenly within the individual's range. Consistency of growth rate was not related to the number of nodules studied nor to the median doubling time of the sample.

A similar expression was used for evaluation of uniformity of nodule size within individual patients, i.e. the ratio of maximum to minimum tumour volume. Again, the small number of observations prohibited the use of more stringent statistical methods. The most significant correlation ($p < 0.001$) between tumour volume and doubling time was detected in two patients with volume uniformity ratios of $> 10$, i.e. 460 and 29.5, but there was no

significant correlation in the other 8 assessable patients in this group. Only two patients in the group with less volume variation demonstrated a correlation and this was at a lower level of significance. In one of these, patient 28, the correlation may have been misleading as ultrasound was not available for depth measurement in plaque-like lesions making it likely that the volume range obtained from two-dimensional measurements was exaggerated. The results suggest that in patients with lesions suitable for entry into the measurement study, the variation in volume is unlikely to affect the calculated doubling times unless the size range is extreme. Likewise, nodule site did not appear to have any influence on growth rate within individual patients.

The breast cancer tumour volume doubling time data derived in the present study demonstrates wide biological heterogeneity, with an overall doubling time range of 10 - 206 days and median values varying between 14 and 175 days. However, the finding that 72% of patients had median doubling time values within the range 14 - 39 days and that 62% of all breast nodule doubling times were between 14 and 40 days indicated a more uniform subset of tumours. In addition, considering the crudeness of the technique for establishing doubling time, consistency within individual patients was reasonable with a less than three-fold variation in doubling time range in 72% of the patients.

Consistent with previous reports on breast cancer metastases, growth was considered rapid, with 78% of nodules

doubling in volume in 60 days or less (Spratt & Spratt, 1964, Lee & Spratt, 1972). The slower growth rate reported in primary breast cancers (Ingleby & Moore, 1956, Ingleby et al, 1958) seems likely to reflect selected biological behaviour in that data is only available for indolent primary tumours not requiring immediate therapeutic intervention, and there are no reports of serial measurement of both primary tumour and metastases in the same patient. The tumours studied in the present series were also highly selected in that they were recurrent or metastasising, resistant to conventional therapy and therefore biologically aggressive. Shackney (1978) has used the discrepancy between growth rate of primary breast cancer (advanced disease) and that of mastectomy scar recurrence (early disease) as evidence of growth retardation with advancing tumour age. Although this is an attractive approach, the two tumour groups cannot be considered biologically comparable in terms of growth potential. In the present study, measurement of variably sized metastases within individual patients has provided an opportunity to assess doubling time at different stages of tumour growth. Only two of the 18 breast cancer patients displayed a significant correlation between doubling time and tumour size and it seems likely that over the range of tumour volumes encountered in suitable patients with superficial multiple metastatic nodules, variation in doubling time is not sufficiently great to influence results.

Other series also report a wide range of doubling time values both within the same tumour type and between

histological types. Batterman (1981) reported on a series of pulmonary metastases from different primary tumours and was unable to show any correlation with tumour type. There was however, a significant relationship between histological grade and volume doubling time.

In this series, histological type did not appear to influence volume doubling time. For example, the four patients with malignant melanoma had median doubling times of 18, 46, 56 and 206 days respectively. The general range of values was consistent with those obtained in other series (Batterman et al, 1981; Steel, 1977), despite the fact that most reported observations have been made on pulmonary metastases.

Multiple measurable metastases provide a potentially useful model for obtaining quantitative information on the modification of tumour response to radiation and have been employed to investigate neutrons and radiosensitizers (Battermann et al, 1981; Thomlinson et al, 1976; Dawes et al, 1978; Ash et al, 1979; Urtasun et al, 1980). The system permits direct comparison of different treatments on like tumours and allows observation of untreated control lesions for reference. However, success of the technique relies on the assumption that multiple metastases grow at the same rate and behave similarly. Careful selection of patients with similarly sized nodules, growing in the same tissue, at the same site increases the likelihood of biological uniformity,

but in practice such patients are rare and less ideal candidates must be considered.

Pre-treatment serial tumour measurements allow calculation of doubling time in individual nodules and thus provide a basis for selecting comparable tumours in which to evaluate the effect of treatment. In addition, doubling time information allows calculation of specific growth delay. In the treatment protocol presented in this thesis, where possible serial tumour measurements were obtained 3-4 weeks prior to treatment (see Chapter 4). This permitted selection of nodules growing at a similar rate in which to compare the effect of adding heat to radiation (see Chapter 7).

Accuracy of Tumour Measurement

Provision of a "measurement clinic" allowed optimum conditions, in terms of time and space, for obtaining accurate, reproducible, quantitative data. Individual patient tumour measurements took up to 1½ hours depending on the number of nodules, ease of visualisation on ultrasound, patient mobility etc. Incorporation of studies of this sort into routine clinical practice is therefore not recommended. The frequency of attendance at the clinic was determined by patient factors such as time required off work, distance between hospital and home, general patient condition etc., but where possible weekly observations were made to increase the reliability of measurement.

The accuracy of caliper and ultrasound measurement have previously been evaluated in superficial lesions similar in type to those included in the present study (Yarnold et al, 1986). The data showed that ultrasound provided an accurate measure of tumour depth, but tended to underestimate the lateral margins. Conversely, caliper measurement tend to over-estimate tumour dimensions partly because of the unavoidable inclusion of two layers of skin and subcutaneous tissue. The importance of incorporating tumour depth into serial volume calculations is emphasised by ellipsoid lesions which demonstrate that tumour growth is not necessarily equal in all dimensions. Likewise, regression in superficial nodules after treatment is often more pronounced in the depth than in the transverse dimension so that tumour shrinkage may be underestimated by caliper measurement alone. In the present study calipers were therefore used to obtain longitudinal and transverse lateral dimensions and, where, possible, ultrasound provided a measure of tumour depth. In general, subcutaneous lesions were easier to visualise on ultrasound than cutaneous nodules but the depth of the latter could always be precisely measured above a depth of 2 mm.

The tendency for cutaneous nodules to occur in clusters and to become confluent with progressive growth necessitated careful identification of study lesions. An example of extensive breast cancer metastases becoming confluent over the chest wall is shown in Fig. 56. The development of new lesions within the area of interest provided further difficulty in this respect. Ulceration of cutaenous lesions,

with subsequent central necrosis or crust formation further hampered tumour measurement. The patient in Fig. 57 had a fore-quarter amputation for extensive axillary nodal disease secondary to a squamous carcinoma on the dorsum of the hand. Discrete superficial nodules, covered by intact skin, appeared adjacent to the scar and each evolved in a similar way with central necrosis occurring at tumour dimensions greater than 4mm. Lesions continued to enlarge but volumes calculated from linear measurements are difficult to interpret. In addition, ultrasound images were not able to determine the depth of the lesion because of the shadow cast by the ulcer crater.

Figure 56

Cutaneous Metastases Becoming Confluent Over the Chest Wall

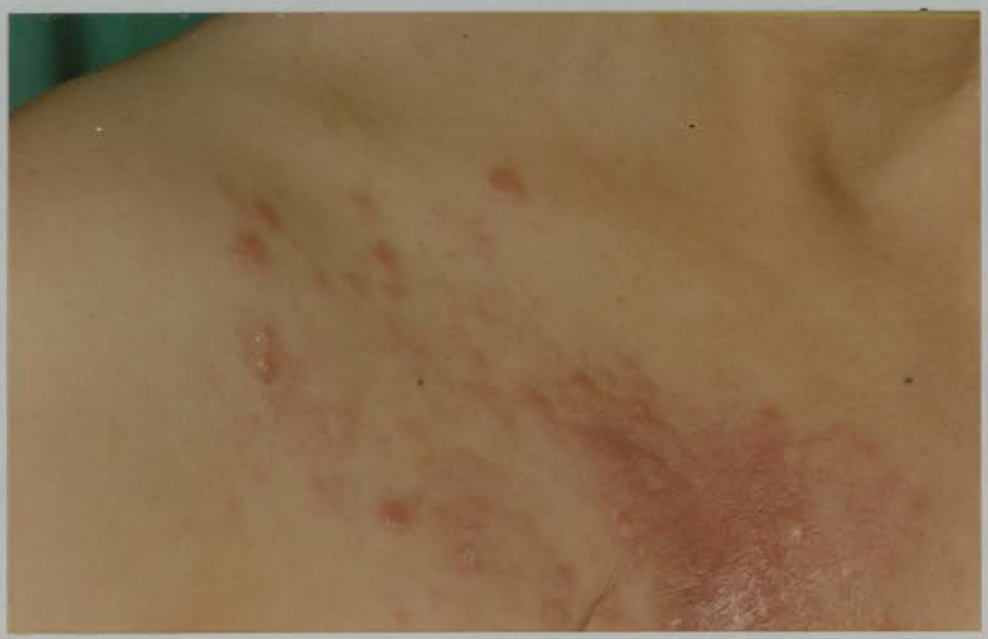
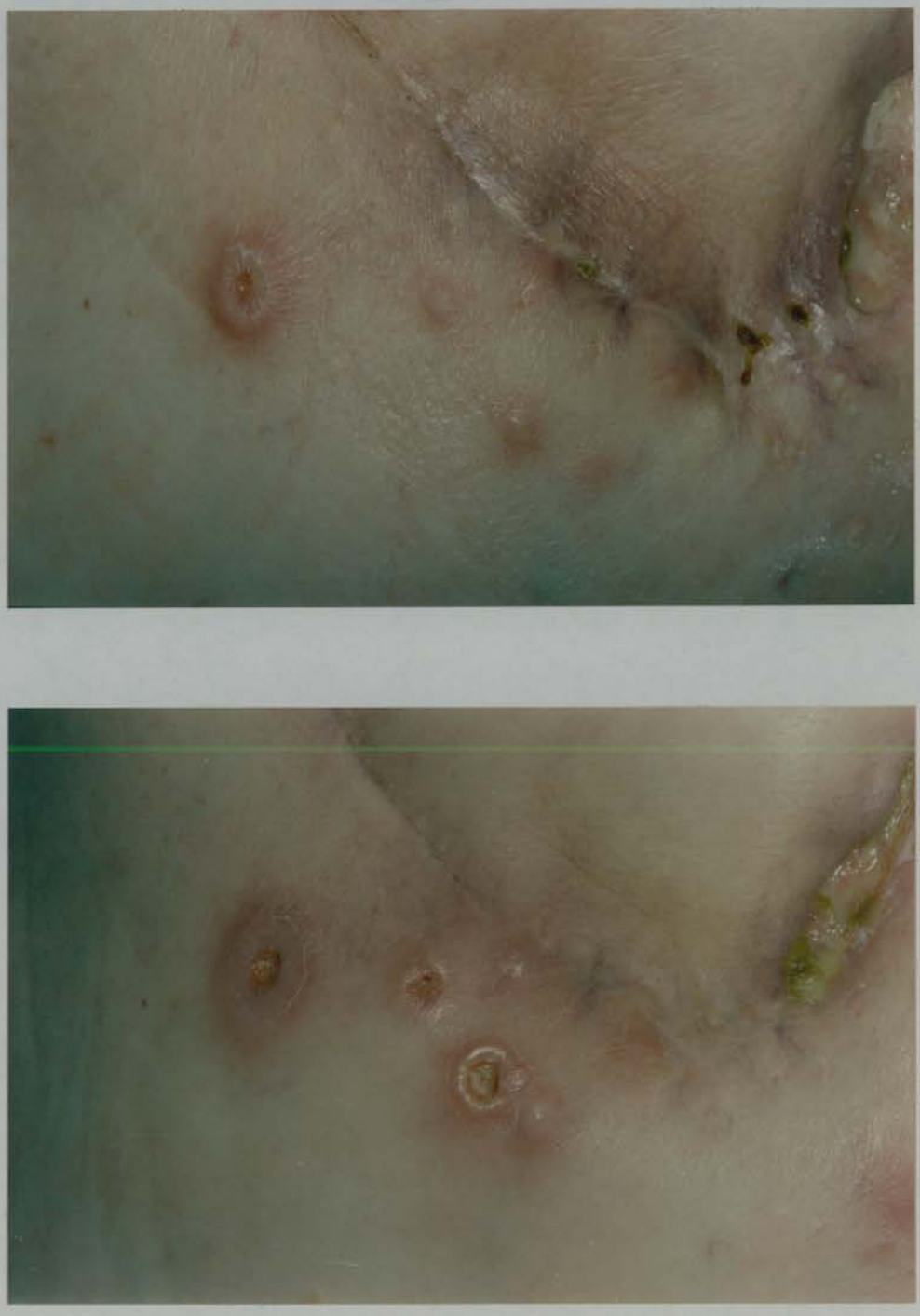


Figure 57

Evolution of Ulceration in Nodules of Squamous Carcinoma Around an Amputation Scar



Chapter 10

# SUMMARY AND CONCLUSIONS

Technical inadequacies in heat delivery and thermometry remain major problems in the clinical evaluation of hyperthermia, but are perhaps overstated. Non-uniformity of dose distribution for example, is not unique to heat delivery but is a significant consideration in other non-surgical approaches to cancer treatment. A close parallel can be made with chemotherapy where drug delivery, like heat, is probably very dependent on tumour physiology, in particular blood flow, and where the variation in dose throughout a tumour is likely to be large. The comparison can be further extended to the inability to measure drug levels throughout the tumour volume in individual patients. Thus, power application for a hyperthermia treatment and drug dose administered in a chemotherapy regime may be considered similarly remote from tumour cell "dosage". By contrast, the dose variation within a radiotherapy treatment volume is dependent on anatomical considerations rather than physiological parameters and these are generally static apart from exceptions such as bowel mobility and lung expansion. This allows the absorbed radiation dose at any particular point to be predicted and the setup adjusted to optimise uniformity. However, although dose can be expressed in physical terms, microenvironmental variations, such as hypoxia, may introduce incalculable variation in effect.

The shortcomings in chemotherapeutic dosimetry have not prevented attempts of the evaluation of this modality in clinical trials. The ensuing experience with chemotherapy can either be taken as an inducement or a deterrent to

accepting shortcomings of hyperthermia, and embarking on a clinical trial using the best technical facilities available.

There is a further biological rationale for accepting temperature inhomogeneity within a treatment volume, at least when heat is used in combination with radiation. Thus, temperature cold spots within tumour are likely to correspond to well-oxygenated radiosensitive areas, and greater temperatures are likely to be achieved in poorly perfused regions which may harbour radioresistant cells.

A major concern in applying hyperthermia with only limited thermometry is the occurence of undetected normal tissue hot spots and risk of consequent damage. Total exclusion of normal tissues from the treatment volume is rarely possible even with interstitial or implantation techniques. However, when used in combination with radiation the scheduling of the two modalities may help to reduce this hazard. Thus, delaying hyperthermia by more than 3-4 hours after irradiation probably minimises radiosensitisation, and direct heat cytotoxicity is likely to be the dominant mechanism. As intratumoural microenvironmental conditions favour heat toxicity, this allows the possibility of a therapeutic gain. Although simultaneous application of heat and radiation produce a greater tumour effect by implicating both radiosensitisation and direct toxicity, the magnitude of normal tissue effect detracts from an overall therapeutic gain. The sequential scheduling employed in the present study provided considerable enhancement of the tumour

response to radiation and justifies optimism regarding the clinical role for heat in improving local tumour control by radiation.

Spacial limitations in thermometry necessitate that the available temperature measurement points be used to best advantage. This requires selection of biologically relevant reference points on which to control treatment temperature, and accurate location of the thermometry equipment at the specified site. So far, collection of clinical thermometry data has tended not to be systematic but this approach is essential in attempting to deliver standardised heat treatments in a clinical trial.

Performance analysis for heat delivery is another area which requires careful scrutiny. Essentially, failure of heat delivery implies either inadequate temperature rise or an inability to maintain treatment temperature for the prescribed time, but the biological implications of the two are likely to be very different. Analysis of temperature profiles, in successful and failed heat delivery, was attempted in the present study and indicated that an isolated cold spot in tumour was a significant cause of failure to deliver the prescribed heat treatment. The contribution of this phenomenon to biological outcome is an important question in terms of defining what can be considered a clinically useful heat treatment. Unfortunately, in the present work there were inadequate numbers of heated nodules with a standardised biological outcome in which to make this

correlation. However, this would seem to be an important area of study which has not yet been addressed in human heating.

Hyperthermia and blood flow are inextricably linked. Current interest in tumour blood flow will, one hopes, be of benefit to hyperthermia and may supply mechanisms for controlling this physiological variable which has such an influence on heat delivery. The other side of the coin, heat-induced vascular change may critically determine the role of hyperthermia in clinical oncology, if the observations on animal tumours reflect events in clinical cancers. In the present study, tolerable heat treatments did not induce the vascular status reported in transplantable animal tumour studies. This is encouraging, as it reduces some of the constraints for scheduling hyperthermia when used in combination with radiation or drugs. However, further investigation is necessary to establish whether tumour type influences vascular susceptibility to heat, and whether higher temperatures, such as may be possible with implantation techniques, can promote predictable blood flow changes.

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