Int. J. Hyperthermia, February 2009; 25(1): 3–20

informa
healthcare

HYPERTHERMIA CLASSIC ARTICLE

Arrhenius relationships from the molecule and cell to the clinic*

W. C. DEWEY

Radiation Oncology Research Laboratory, University of California, 1855 Folsom Street, MCB-200, San Francisco, CA 94103, USA

(Received 14 July 1993; Revised 27 October 1993; Accepted 29 November 1993)

Abstract

There are great differences in heat sensitivity between different cell types and tissues. However, for an isoeffect induced in a specific cell type or tissue by heating for different durations at different temperatures varying from 43–44°C up to about 57°C, the duration of heating must be increased by a factor of about 2 (*R* value) when the temperature is decreased by 1°C. This same time–temperature relationship has been observed for heat inactivation of proteins, and changing only one amino acid out of 253 can shift the temperature for a given amount of protein denaturation from 46°C to either 43 or 49°C. For cytotoxic temperatures <43–44°C, *R* for mammalian cells and tissues is about 4–6. Many factors change the absolute heat sensitivity of mammalian cells by about 1°C, but these factors have little effect on *R*s, although the transition in *R* at 43–44°C may be eliminated or shifted by about 1°C. *R* for heat radiosensitization are similar to those above for heat cytotoxicity, but *R*s for heat chemosensitization are much smaller (usually about 1.1–1.2). In practically all of the clinical trials that have been conducted, heat and radiation have been separated by 30–60 min, for which the primary effect should be heat cytotoxicity and not heat radiosensitization. Data are presented showing the clinical application of the thermal isoeffect dose (TID) concept in which different heating protocols for different times at different temperatures are converted into equivalent minutes (equiv) min at 43°C (EM_{43}). For several heat treatments in the clinic, the TIDs for each treatment can be added to give a cumulative equiv min at 43°C, namely, CEM_{43}. This TID concept was applied by Oleson et al. in a retrospective analysis of clinical data, with the intent of using this approach prospectively to guide future clinical studies. Considerations of laboratory data and the large variations in temperature distributions observed in human tumors indicate that thermal tolerance, which has been observed for mammalian cells for both heat killing and heat radiosensitization, probably is not very important in the clinic. However, if thermal tolerance did occur in the clinical trials in which fractionation schemes were varied, it probably would not have been detected because with only the two–three-fold change in treatment time that occurs when comparing one versus two fractions per week, or three versus six total fractions, little difference would be expected in the response of the tumors since both thermal doses were extremely low on the dose-response curve. Data are shown which indicate that in order to test for thermal tolerance in the clinic and to have a successful phase III trial, the thermal dose should be increased about five-fold compared with what has been achieved in previous clinical trials. This increase in thermal dose could be achieved by increasing the temperature about 1.5°C (from 39.5 to 41.0°C in 90% of the tumor) or by increasing the total treatment time about five-fold. The estimate is that 90% of the tumor should receive a cumulative thermal dose (CEM_{43}) of at least 25; this is abbreviated as a CEM_{43} T_{90} of 25. This value of 25 compares with 5 observed by Oleson et al. in their soft tissue sarcoma study. Arguments also are presented that thermal doses much higher than the CEM_{43} T_{90} induce the hyperthermic damage that causes the tumors to respond, and that the minimum CEM_{43} T_{90} of 25 only predicts which tumors that receive a certain minimal thermal dose in <90% of the regions of the tumors will respond. For example, in addition to a minimal CEM_{43} T_{90} of 25 a minimum CEM_{43} T_{50} of about 400 also may be required for a response. Finally, continuous heating for ~2 days at about 41°C during either interstitial low dose-rate irradiation or fractionated high dose-rate irradiation, which we estimate could give a CEM_{43} of 75, should be considered in order to enhance heat radiosensitization of the tumor as well as heat cytotoxicity. In order to exploit the use of hyperthermia in the clinic, we need a better understanding of the biology and physiology of heat effects in tumors and various normal tissues. As an example of an approach for mechanistic studies, one specific study is described which demonstrates that damage to the

W. C. Dewey, 768 Lovell Ave., Mill Valley, CA 94941. E-mail: wcdewey2003@yahoo.com

*This paper is based upon the 1993 Eugene Robinson Memorial Lecture.

ISSN 0265–6736 print/ISSN 1464–5157 online © 2009 Informa Healthcare USA, Inc.
DOI: 10.1080/02656730902747919

centrosome of CHO cells heated during G_1 causes irregular divisions that result in multinucleated cells that do not continue dividing to form colonies. This may or may not be relevant for heat damage in vivo. However, since normal tissues vary in thermal sensitivity by a factor of 10, similar approaches are needed to describe the fundamental lethal events that occur in the cells comprising the different tissues.

Introduction

This article was delivered as a memorial lecture in memory of Dr Eugene Robinson, a pioneer in hyperthermic research for the 20 years preceding his death in 1983. One of his papers [1] which showed that hyperthermia had a selective cytotoxic effect on hypoxic cells at low pH. This paper stimulated many of us to consider the rationale for using hyperthermia in cancer therapy in order to kill the radioresistant hypoxic cells. During the same year that Robinson published this important paper, he organized the first hyperthermia session at the 1974 International Congress of Radiation Research in Seattle, WN, USA. Here plans were laid for the first International Symposium on Hyperthermic Oncology, which Robinson organized in Washington, DC, USA, in 1975. Germane to the topic presented in this memorial lecture is the paper entitled, 'good thermal dosimetry is essential to good hyperthermia research' [2].

This Robinson lecture reviews the relationship between the amount of thermal damage induced by heating and both (1) the magnitude of change in temperature and (2) the duration of the elevated temperature. This relationship led to the development of a thermal isoeffect dose (TID) 'unit', which is the heating duration at a reference temperature, e.g. 43°C, that is calculated to induce the same amount of thermal damage in a specific cell type or tissue as heating for a specified duration at an observed temperature. The applicability of this TID approach for specifying a thermal dose that can be used in describing biological effects versus dose is discussed when heat is delivered by itself or in combination with radiation or chemotherapy. Since the temperature frequently varies during the heating period, the TIDs determined for short periods during heating must be added to obtain the TID for the entire heating period. Factors that modify the equation used to calculate TID, such as a large decrease in the elevated temperature during the heating period, and factors that modify the biological effects induced by heat such as different thermal sensitivities in different cells and tissues, thermal tolerance, and changing intracellular pH are discussed. The retrospective application of the TID concept to a clinical trial is presented to illustrate the validity of TID in interpreting the results of clinical studies completed and in the design of future clinical trials. For example, the TID retrospective analysis led to the conclusion that to demonstrate a beneficial effect of hyperthermia in a phase III clinical study, the thermal doses (TIDs) will have to be about five times higher than those obtained in recent clinical studies. In this context, evidence is presented that the induction of thermal tolerance in the clinical setting is probably not very important and that heating should be pursued vigorosly in several heating sessions, or by heating continuously at relatively low temperatures of 40–41°C over 2–3 days while low dose-rate irradiation is delivered. Finally, biological information on the mechanism of heat cytotoxicity for one particular in vitro, system is presented as an example of what may happen in vitro, but more importantly how mechanistic information might be obtained for other cellular systems, including different tissues in the animal which differ greatly in thermal sensitivity. Hopefully, obtaining such mechanistic information could lead to approaches using thermal sensitizers and protectors, for example, that might improve the therapeutic gain when hyperthermia is delivered in the clinic.

Arrhenius relationships and TID

Background and theory

The simplest physical description of a hyperthermic exposure is temperature as a function of time. If instantaneous temperature transitions occurred between a steady-state normothermic temperature and a specified steady-state elevated temperature, different hyperthermic exposures could be expressed simply as time at the elevated temperature. Also, if time at elevated temperature were fixed, different hyperthermic exposures could be expressed directly as temperature at the elevated state. However, in many cases in which hyperthermia is administered, both time and temperature at the elevated state vary, for which the change (ΔT) in temperature multiplied by the time at the elevated temperature might be considered. For example, raising the temperature from 37 to 42°C (i.e. $\Delta T = 5$°C) and holding at the higher temperature for 10 min, gives 50°-min. However, this will produce much less thermal damage than a transition from 37 to 46°C for 5.55 min [3], which is also 50°-min (Figure 1). Therefore, the simple unit of °-min is not very useful. A consideration of the thermal unit designated in joules, i.e. energy required to raise 1 kg water by 1°C, also does not appear to be biologically meaningful

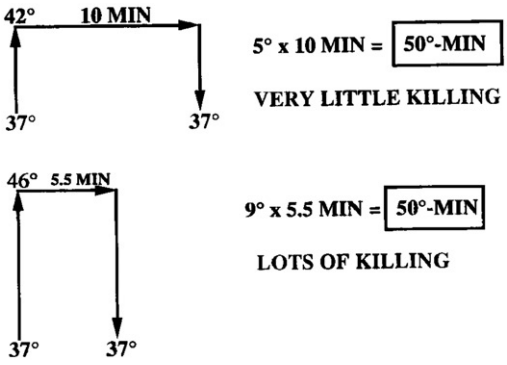

Figure 1. A schematic to illustrate the failure of $\Delta T \times$ duration of heating (expressed in °-min) to predict thermal damage.

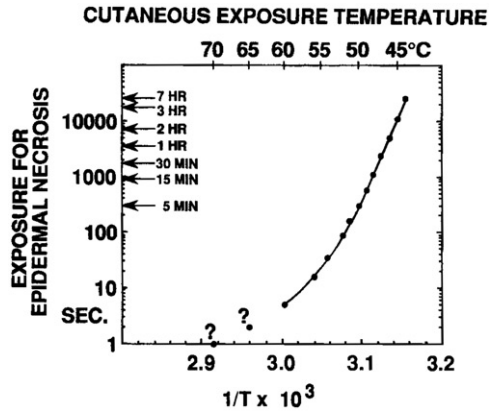

Figure 2. Data were taken from Henriques [12]. Pig skin was exposed to hot water, and the threshold durations of exposure at different temperatures required for complete transepidermal necrosis were determined and tabulated. These values are plotted for the different temperatures, and the activation energy and R were calculated for temperatures between 44 and 52°C. The abscissa at the bottom is the inverse of the absolute temperature in degrees Kelvin. The data for points with question marks were uncertain because of the very short durations of exposure relative to the time required for the temperature of the skin to reach the specified temperature. $R = 2.1$. $\Delta H = 150$ kcal/mol.

since it does not include the duration of time at the elevated temperature, a parameter shown to be very important in the amount of thermal damage induced [3]. Instead, a thermodynamic approach is taken which leads to calculating an equivalent time at some reference temperature, e.g. 43°C, required for an observed biological effect induced by a specified time at a specified temperature [4]. This approach for defining a thermal dose on the basis of a TID was applied to the analysis of a clinical trial involving pet animals [5] and was discussed in a workshop on thermal dose [6]. Examples presented in this article indicate that the generally pessimistic tone that was expressed at this 1987 workshop for using the TID dose in the clinical setting appears to be unwarranted.

The relationship between the inactivation rate in biological systems and temperature is illustrated by an Arrhenius plot in which the logarithm of the inactivation rate is plotted as a function of the inverse of the absolute temperature [7]. A straight line indicates that the same mechanism of inactivation is effective within the temperature range explored. The activation energy, ΔH, is expressed in calories per mole or joules per mole and is determined from the slope of the Arrhenius plot [7, 8]. Lines which are parallel but shifted in temperature indicate that the activation energies are the same, but the entropy values (calorie/degree/mol) for the reactions are different [8]. Thus, the relationship between time and temperature for a given biological effect depends on the activation energy, whereas, the absolute reaction rate depends on both the activation energy and the entropy term. For example, studies of the inactivation of a purified protein, kanamycin nucleotidyl transferase (KNTase) showed that the rate of inactivation of the protein, plotted as a function of 1/absolute temperature, was a straight line with an activation energy of 140 kcal/mol [9]. Furthermore, replacing only one amino acid in the protein by another amino acid could shift the Arrhenius curve by about 3° to a higher or lower temperature, and changing three amino acids could shift the Arrhenius curve by about 9°C to a higher or lower temperature. Thus, changing three amino acids in the 253 amino acid protein to make the protein more thermolabile or thermoresistant caused the temperature for a given amount of inactivation to shift down to 46 or up to 64°C. The inactivation of the protein results from thermal denaturation of the protein, and as expected, this common mechanism for heat inactivation of KNTase produces inactivation curves which are parallel but shifted in temperature as 1–3 amino acids are changed. Inactivation of mammalian cells and heat-induced damage in mammalian tissues also has an activation energy in the range of 140 kcal/mol [8, 10], and a reasonable hypothesis is that the differences in sensitivities between different cell types and between heat resistant and heat sensitive mutants may be related to a change in one or two amino acids in a critical protein(s) that is denatured and aggregated by hyperthermia [11].

One of the first quantitative hyperthermic experiments carried out in a mammalian system [12] determined the duration of thermal exposure required for epidermal necrosis in pig or human skin as a function of temperature (Figure 2). Pig skin was heated with hot water, and for temperatures from 44 up to about 53°C, where the time required for the temperature transition was very short compared with the duration of heating, a straight line Arrhenius plot was obtained with an activation energy of 150 kcal/mol. For temperatures >55°C, the

Arrhenius plot was curved because the time required for the temperature transition became significant relative to the duration of heating. Note that, in this plot 7 h at 45°C caused about the same thermal damage as 5 min at 50°C or about 15 min at 52°C. In other words, for an isoeffect at various temperatures, a decrease of 1°C requires that the hyperthermic exposure be increased by a factor (R) of 2.1. R can be calculated from the activation energy expressed in calories per mole; the natural logarithm of R equals the activation energy divided by two times the absolute temperature squared [8]; i.e. $\ln R = \Delta H/2T^2$. Actually, R is determined experimentally, and ΔH is calculated from the previous equation.

Cell survival studies carried out with mammalian cells in culture in the 1970s and 1980s indicated that a straight line Arrhenius relationship is obtained over the temperature range from 43.5 to 57°C [10, 13], again with an activation energy of about 140 kcal/mol ($R = 2$). This means that 135 min at 43.5°C causes about the same decrease in survival as 2–3 s at 57°C. As was observed for thermal denaturation of molecules of KNTase differing in heat sensitivity [9], the Arrhenius plots for mammalian cells differing in heat sensitivities are parallel but shifted [7]. For example, when a thermal resistant pig kidney cell line is compared with a relatively heat sensitive Chinese hamster line (Figure 3), the inactivation rates differ by a factor of 10, while the activation energies (140 kcal/mol) are the same, thus suggesting that denaturation of a critical protein(s) may be involved and that an alteration in this critical protein(s) may cause the pig kidney cells to become heat resistant. Although the applicability of applying the Arrhenius analysis for establishing mechanisms of heat activation has not been accepted unequivocably [14], there is considerable evidence that heat-induced cell lethality is caused by heat denaturation and aggregation of protein [7, 11, 15].

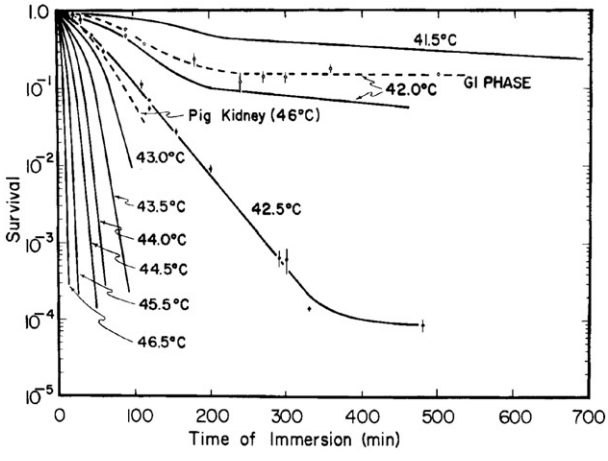

Figure 3. Survival curves for asynchronous Chinese hamster ovary (CHO) cells heated at different temperatures for varying lengths of time. Except for 42.5°C, the individual data points and standard errors of means have been deleted for reasons of clarity. Survival curves for cells heated in the G_1 phase were very similar to those for asynchronous cells, an example of which is indicated for cells heated in the G_1 phase at 42.0°C. To illustrate the wide variation in thermal sensitivity of various cell lines, the dashed line is drawn to show the relative thermal resistance of a pig kidney cell line. The parent line of pig kidney cells was slightly more sensitive, with the 46°C curve similar to that for the 43.5°C curve shown for CHO cells. Data taken from Dewey et al. [8].

TID equations and definition of EM_{43}

When the temperature is reduced below about 43°C, the inactivation rate is reduced considerably with evidence of thermotolerance developing after 1–4 h of heating [3] (Figure 3). Below this critical temperature of about 43°C, R for in vitro and in vivo systems has been observed to vary between 4 and 8 [8, 16]. This variation in $R < 43$°C will depend somewhat on how it is determined, i.e. on the basis of slopes of survival curves or on the basis of isosurvival where R can vary depending on the level of isosurvival that is selected. The Arrhenius plot has a break at about 43°C with the slope between 41.5 and 43°C being steeper than the slope between 43 and 57°C. This biphasic Arrhenius plot leads to the following equation [8];

$$t_2 = t_1 \times R^{(T_1 - T_2)} \quad (1)$$

where t_1, is the time at temperature T_1, t_2 is the time at temperature T_2, and R is about 2 for temperatures >43°C and about 4–6 for temperatures between about 39 and 43°C. With this equation ($R = 4 < 43$°C $< R = 2$), a family of survival curves plotted versus heating time for temperatures between 41.5 and 46.5°C [3] (Figure 3) can be converted into survival as a function of equivalent time at 43°C (t_{43}), where all the points for the different temperatures fall very close to a single curve [4] (Figure 4). Only for heating durations of >3 h and ≤42.5°C where chronic thermotolerance develops, do the points deviate significantly from the single survival curve.

When the temperature varies during a heating period, the time–temperature relationship in Equation 1 can be used to obtain an equivalent time at 43°C for each time interval at a given temperature, and the equivalent times at 43°C for all of the intervals during the heating period can be summed to give the total equivalent time at 43°C for the entire heating period [4]. This TID is given by:

$$\text{equiv min } 43^\circ\text{C} = EM_{43} = \sum_{t=0}^{t=\text{final}} \Delta t \times R^{(T_1 - 43)}, \quad (2)$$

where *R* varies as indicated above for Equation 1 and equiv min means equivalent minutes.

Factors modifying TID and biological effect versus TID

Factors that can influence the Arrhenius plot and thus the TID equation should be discussed. First, as mentioned previously, the break in the Arrhenius curve at about 43°C appears to be caused by the development of chronic thermotolerance. Therefore, preventing the development of chronic thermotolerance should eliminate this break. For example, stepdown heating (SDH) in which a high temperature of about 44–45°C for a few minutes precedes the low temperatures <43°C, sensitizes the cells to the low temperatures and eliminates the break in the Arrhenius curve. This means that during SDH conditions, *R* remains about 2 for hyperthermic temperatures greater and lesser than 43°C [4]. However, SDH probably rarely occurs in the clinic, especially in the cooler regions of the tumour, because of pain tolerance of the patient. However, if anesthesia were used or if heating techniques were improved to provide better uniformity in heating, SDH may play a role clinically. In that event, a correction could be programmed to change *R* from 4 to 2 for those temperature measurements where SDH is observed. Second, the development of thermotolerance in cells and tissues to a second heat dose that is delivered several hours after an initial heat dose has been shown to shift the Arrhenius curve (like that plotted in Figure 2) and the break in the curve by about 1°C towards higher temperatures [17]. In other words, the thermotolerant cells are more heat resistant by about 1°C for a given isoeffect, and the same time–temperature relationship is observed, except that for Equation 1, the temperature at which *R* changes from two to four shifts from 43 to about 44°C. Third, as the heat sensitivity is increased by lowering the pH from 7.4 to about 6.7, the Arrhenius curve shifts toward lower temperatures by about 1°C without much change in the break point [18]. This means that cells heated at low pH are more sensitive by about 1°C, but the shape of the Arrhenius curve is changed very little. However, this shift in Arrhenius curve may not occur for many tumour cells at low pH if they have adapted to low pH over several hours [19]. Such a loss of low pH sensitization to thermal killing has been demonstrated in vitro when rodent cells are maintained at low pH for several hours [20, 21], and occurs because low pH-adapted cells are able to increase their intracellular pH [21]. However, if the intracellular pH is decreased in these low pH-adapted cells by lowering the extracellular pH below the low pH to which the cells have been adapted, the cells are sensitized to heat killing [20, 21]. This can be accomplished in vivo by infusing glucose, for example [19, 22], but this phenomenon of pH adaptation and the ability to override it needs to be demonstrated for human cells, and in particular for cells in human tumors. Fourth, when the heat sensitive S phase is compared with the heat resistant G_1 phase, the Arrhenius plot shifts towards lower temperatures by about 1.5°C [14]; i.e. the absolute sensitivity is about 1.5°C greater for S than G_1, but *R*s used in Equation 1 are not changed appreciably. However, the transition temperature at about 43°C is more apparent for the sensitive S phase than for the resistant G_1, phase because thermal tolerance which did not develop while the cells were heated chronically in S phase did develop as the cells progressed from S into G_2, and the heat resistant G_1 phase after a few hours of chronic heating [23]. Fifth, as mentioned previously, the heat sensitivies of different cell lines can differ by a factor of 10 [24] which shifts the Arrhenius curve by about 3°C, but has little effect on *R*. In other words, the TID equation (1) is not affected, but the biological effect for a given TID differs greatly for the different cell types. This is analogous to different radiation-dose response curves

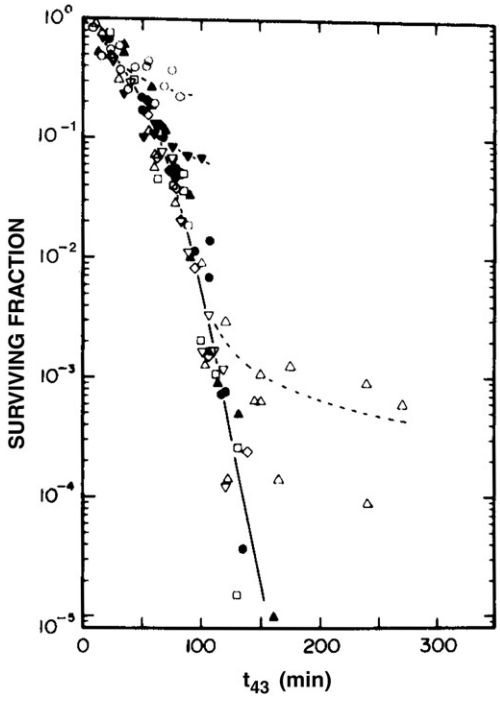

Figure 4. Dose-survival response for asynchronous CHO cells at various temperatures plotted as a function of equiv-min at 43°C. The data at 41.5, 42.0 and 42.5 deviate from a single line. as shown by the dashed lines, due to the development of thermotolerance. Survival curves at the different temperatures are shown in Figure 3. Data are taken from Sapareto et al. [3]. ○, 41.5; ▼, 42.0: △, 42.5; ◆, 43.0; □, 43.5; ▽, 44.0; ◇, 44.5; ●, 45.5; and ▲, 46.5°C.

for radiation sensitive mutants compared with wild-type cells. However, the differences in thermal sensitivities between different cell lines are much greater than the differences in radiation sensitivities [24], and compared with the factors discussed above, probably provide the largest uncertainty in estimating biological damage induced by a TID calculated by Equation (1).

When several human cell lines were compared with several rodent cell lines [25], the Arrhenius curves were almost parallel, but the human cells were more resistant, with the Arrhenius curves and their break points shifting by about 0.5°C to higher temperatures for human compared with rodent cell lines. Three human cell lines also were observed to be more heat resistant than one rodent cell line for temperatures of 42–46°C [26]. However, some human cell lines which were more heat resistant than some rodent cell lines >43°C were more heat sensitive at temperatures <43°C than the rodent cell lines [27]; this should result in less of a break in the Arrhenius curve for these human cell lines compared with the rodent cell lines (discussed later). More work is needed for different cell lines, and in particular, for different tissues in animals and humans in order to obtain more reliable *R*s at different temperatures > and <43°C. Although the various factors discussed above can modify the TID equation and the biological effect versus TID, the evidence indicates that the most important factor in determining biological effect versus calculated TID is the intrinsic thermal sensitivity. Therefore, as a first approximation, the TID calculated by Equations (1) and (2) should apply for estimating thermal effects as a function of TID for a specific tissue, tumour, or cell line.

TID for heat and chemotherapy

I shall not discuss in any detail the activation energies for hyperthermia combined with chemotherapy. However, several studies have indicated that the activation energy for heat chemosensitization is much smaller than that for heat killing or heat radiosensitization [28, 29]. In general, these values for ΔH range from about 20–50 kcal per mol, giving *R*s of 1.11–1.22. These activation energies depend on the effects of hyperthermia on reaction rates and on the formation of reactive intermediates [28]. These points have been reviewed [29], and the reported activation energies were found to be (kcal/mol): 51 for BCNU, 44 for *cis*platin, 35 for bleomycin, and 22 for 5-fluorouracil.

TID for heat und radiation

A question to be considered is whether *R*, when heat is combined with radiation to produce heat radiosensitization, is the same as *R* for heat killing. For heat radiosensitization, heat and radiation need to be given simultaneously or with heat being given within 5–10 min of the radiation dose [30–32]. In practically all clinical studies reported, the separation between heat and radiation has been ≥30–60 min so that primarily only heat cytotoxicity would have been obtained with no or very little heat radiosensitization. For example, the therapeutic gain observed in both mouse tumors and human melanoma when hyperthermia was administered 4 h after irradiation was attributed to heat cytotoxicity and not heat radiosensitization in tumour [32, 33]. However, heat radiosensitization studies that have been carried out in mammalian cell culture systems and in animals [30–32, 34] indicate that for temperatures >43°C, *R*s for heat radiosensitization are similar to those observed for heat cytotoxicity, i.e. about 2, whereas, *R*s <43°C range from 4 to 8 for heat cytotoxicity and only 2–3 for heat radiosensitization.

More work is needed in evaluating *R* for heat radiosensitization compared with heat cytotoxicity when heat is given immediately before or after irradiation, but especially when radiation is delivered during the heating interval at temperatures <43°C or for very short intervals >43°C where there may be considerable heat radiosensitization without much heat cytotoxicity [31]. Under these conditions, heat may not be inhibiting repair processes, for example, but may be accelerating biochemical reactions involved in converting potentially lethal lesions into lethal lesions [8].

Thermal tolerance for heat radiosensitization

As we have discussed, thermotolerance can shift the Arrhenius curve for heat cytotoxicity. What effect can thermotolerance have on heat radiosensitization? Although thermotolerance has not always been observed for heat radiosensitization [35–39] it has been observed in several in vitro and in vivo systems [33, 37, 40–50]. An example of thermotolerance for heat radiosensitization is shown in Figures 5 and 6 in which CHO cells in the G_1 phase were heated at 45.5°C and then either immediately irradiated or were allowed to develop thermotolerance during an 18-h incubation period before they were heated a second time and immediately irradiated. In these experiments, the heat-induced delay in progression from G_1 into S caused the cells to remain in G_1, during the 18-h incubation period; therefore, there was no complication related to cell cycle progression. The data clearly indicate that thermotolerance was observed for heat radiosensitization; i.e. the thermal enhancement ratio (TER) increased to 8–9 as the non-tolerant cells were heated up to 21 min at 45.5°C before they were irradiated, but *TER* reached a maximum of only 3 when the preheated

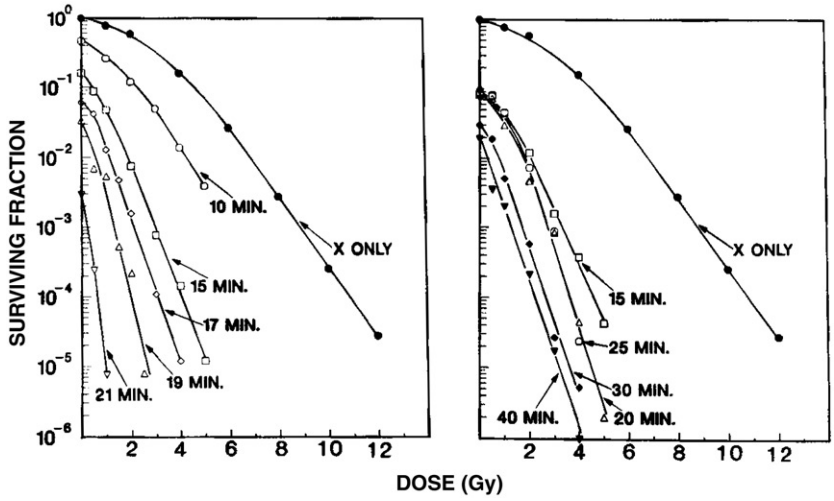

Figure 5. Survival versus radiation dose for non-tolerant (left) and thermal tolerant (right) CHO cells. For the non-tolerant cells, synchronous cells in G_1 were either irradiated at 37°C or were heated (△) for 10–21 min at 45.5°C and then incubated for 10 min at 37°C before they were irradiated at 37°C. For the thermal tolerant cells, synchronous cells in G_1 were heated at 45.5°C for 15 min and then incubated at 37°C for 18 h before they were either irradiated at 37°C or were heated (△) for 15–40 min at 45.4°C and then incubated for 10 min at 37°C before they were irradiated at 37°C. During the 18-h period, heat-induced delay in G_1 prevented the cells from progressing into S phase. Left, ΔX; right 45.5°C-15 min – (18 h) ΔX.

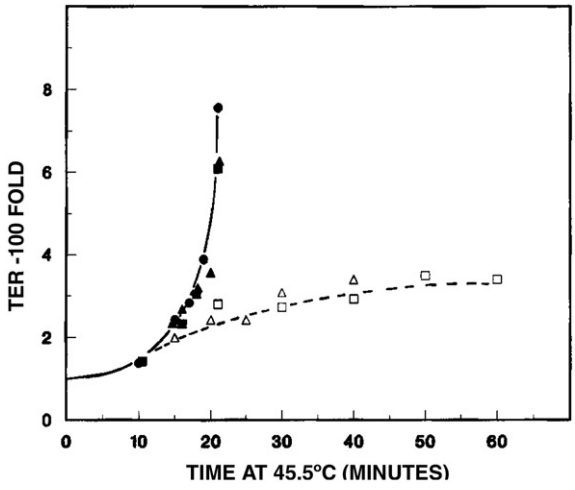

Figure 6. TER for heat radiosensitization was determined from data in Figure 5 plus additional data (not shown) when the second heat treatment was delivered 18 h after an initial treatment of 15 min at 45.5°C. To obtain the TER, radiation survival curves were first normalized to 1.0 to eliminate the effect of heat killing. Then, when radiation reduced survival 100-fold, TER was calculated as the dose for radiation treatment alone divided by the radiation dose required for a 100-fold reduction in survival when it was delivered 10 min after heating. $X/\Delta X$ (closed symbols) is the TER for non-tolerant cells and $X/\Delta - \Delta X$ (open symbols) is the TER for thermal tolerant cells. The time on the abscissa is the duration of the second heat dose in the case of the thermal tolerant cells and the duration of the single heat dose for the non-tolerant cells.

thermotolerant cells were heated up to 60 min at 45.5°C before they were finally irradiated. However, in the clinical use of hyperthermia, thermotolerance for heat radiosensitization is probably not important because a considerable amount of heat is required for the thermotolerance effect to become appreciable. Note in Figure 6 that heat doses of >15 min of heating at 45.5°C would be required for any appreciable difference in the TER for non-tolerant and thermal tolerant cells. In the clinic, thermal doses during a single treatment that are equivalent to 15 min at 45.5°C (85 EM_{43} which is equivalent to 85 min at 43°C) are rarely, if ever achieved in a significant fraction of the tumour volume.

Applications of TID to clinical studies

Definition of T_{90}, T_{50}, and T_{10}, applied to EM_{43}

Attempts have been made to apply TID, expressed in terms of equiv min at 43°C, to the clinical applications of hyperthermia combined with radiation. Since the temperatures obtained at various points in the tumour differ by as much as 4–5°C [51] and sometimes by as much as 8–10°C (Stauffer, personal communication) during a heat treatment of 60 min, there is a need to establish a means of integrating each of these temperatures obtained at different points in the tumour over the time of treatment by using the TID formula. For example, during a 1-min interval in time, the distribution of the temperatures at several points in the tumour (possibly as many as 30) are obtained in terms of the percentage of points that are greater than a certain temperature. The criteria [51] are frequently expressed as T_{90}, the temperature which 90% of the measured points exceed; T_{50}, the temperature which 50% of the measured points exceed; and T_{50},

the temperature which only 10% of the measured points exceed. Then, for each 1-min interval, the T_{90}, T_{50}, and T_{10}, are converted into equiv min at 43°C by Equation (1). The equiv min at 43°C calculated for each 1-min interval are then summed over the duration of heating (Equation 2) in order to obtain a total equiv min 43°C (EM_{43} for T_{90}, T_{50}, and T_{10} for the entire heat treatment.

A clinical study evaluating response versus CEM_{43} T_{90} and CEM_{43} T_{50}

Oleson et al. [52] have used the TID approach to evaluate the effect of hyperthermia when combined with radiation (radiation delivered 5 days per week with a 1-h treatment of hyperthermia delivered once or twice per week within 30–60 min after 5 or 10 of the radiation treatments). As mentioned previously, the only heat damage to be expected when heat and radiation are separated by 30–60 min is heat cytotoxicity without any significant heat radiosensitization. In their study of superficial adenocarcinomas and non-adeno- carcinomas combined (mean vol of 100 cm), the median T_{90} was 39.4°C. The observed T_{90} and T_{50} during each 1-min interval were converted (Equation 1 with $R=2$ for temperatures >43°C and $R=4$ for temperatures between 37 and 43°C) into equiv min 43°C for T_{90}, and equiv min 43°C for T_{50}, and these values were added (Equation 2 to give EM_{43}) for the entire 60-min treatment. Then, EM_{43} for T_{90} (EM_{43} T_{90}) was added for each treatment to obtain for each patient a summation for all 5–10 1-h treatments. For brevity and simplification in presentation, this summation for all treatments is abbreviated as follows:

$$\text{Cumulative equiv min } 43^\circ\text{C for } T_{90} = CEM_{43}\ T_{90} \tag{3}$$

$$\text{Cumulative equiv min } 43^\circ\text{C for } T_{50} = CEM_{43}\ T_{50} \tag{4}$$

In the study, no difference in clinical response was observed for one or two heat fractions per week; therefore, the data were combined. For 105 patients, the median CEM_{43} T_{90} was 2.6 and the median CEM_{43} T_{50} was 43.1. Dose responses were obtained for two groups-non-adenocarconomas (57 patients) (Figure 7) and both adenocarcinomas and non-adenocarcinomas combined [52]. The probabilities of complete response were plotted as a function of CEM_{43} T_{90}. The median CEM_{43} T_{90}, for the non-adenocarcinomas was only 2.1 (median T_{90} of 38.8°C), which increased the probability of response from about 15% for radiation alone (assumed to be the probability for the CEM_{43} T_{90} of 0.1) to about 30% for hyperthermia combined with radiation

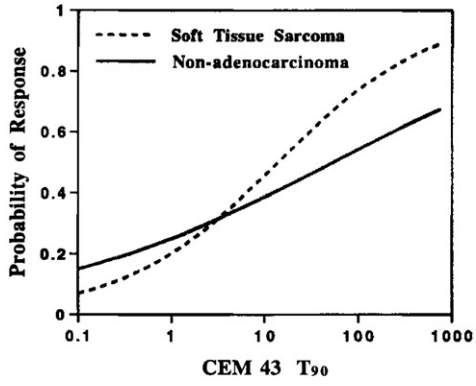

Figure 7. Data from Oleson *et al.* [52] and M. Dewhirst, (personal communication) for probability of complete response of 57 superficial non-adenocarcinomas and probability of necrosis (≥80% necrotic) of 44 soft tissue sarcomas as a function of cumulative equiv min at 43°C for the T_{90} of 5–10 1-h hyperthermic treatments delivered once or twice per week 30–60 min after the radiation doses were delivered 5 days per week for a total dose of 50 Gy. For 5–10 hyperthermia treatments (Mean of 7), the median cumulative equiv in 43°C T_{90} (CEM_{43} T_{90}) was 5.4 for soft tissue sarcomas and 2.1 min for superficial tumours. The curves were derived from empirical equations obtained from the clinical data base.

(Figure 7). The median T_{50} was only 40.8°C, and the median CEM_{43} T_{50} was 42.8. Indeed, the thermal dose was quite low in most of the tumors as indicated by a median T_{10} of only 42.7°C.

A similar analysis was performed (Figure 7) for necrosis in soft tissue sarcomas (≥80% necrosis) as evaluated in 44 patients 4–6 weeks after the end of treatment, consisting again of 5 or 10 1-h hyperthermic treatments delivered once or twice per week 30–60 min after a radiation treatment. In this study, the median T_{90} was 39.5°C, the median T_{50} was 41.6°C, and the median T_{10} was 43.0°C. The median CEM_{43} 43 T_{90} for this sarcoma study was 5.4 and the median CEM_{43} 43 T_{50} was 80, with a median CEM_{43} 43 T_{50} of 237 observed in those patients manifesting ≥80% necrosis. The plot of the probability of ≥80% necrosis as a function of CEM_{43} T_{90} is shown in Figure 7 for one heat fraction per week for the soft tissue sarcomas (STS) and indicates that the curve for STS (500–800 cm^3 in vol.) is steeper than the curve for complete response for superficial non-adenocarcinomas (100 cm^3 in vol.), possibly because the thermal sensitivity of the group of tumors is more homogeneous for the STSs than for the miscellaneous non-adenocarcinomas (M. Dewhirst, Personal communication).

CEM_{43} T_{90} and CEM_{43} T_{50} required for clinical efficacy and hyperthermia clinical trials

An important conclusion that Oleson et al. have drawn from their studies is that in order to conduct

Table 1. Time–temperature thresholds for chronic damage in various tissues[a]

Tissue	Species	Temperature damage threshold	Lesion
Muscle and fat	Pig	(240 min) 46.0°C, 30 min	Necrosis, fibrosis
Skin	Man, rat, mouse	(210 min) 46.5°C, 45 min	Epidermal necrosis
Skin	Man, rat, mouse	(120 min) 44°C, 60 min	Epilation
Oesophagus	Pig	(120 min) 45°C, 30 min	Fibrosis
Cartilage	Rat, mouse	(120 min) 44°C, 60 min	Necrosis
Bladder	Dog, rabbit	(80 min) 46.5°C, 8 min	Mucosal necrosis
Small intestine	Rat, mouse	43°C, 40 min	Crypt loss
Colon	Pig, rabbit	43°C, 30 min	Fibrosis
Liver	Dog	43°C, 30 min	Hepatocyte loss, fibrosis
Brain	Cat, dog	(25 min) 42.5°C, 50 min	Neuronal pyknosis
Kidney	Mouse	43°C, 20 min	Necrosis of glomeruli and tubules

Notes: [a]Temperatures and times that were observed as the thresholds required for the induction of the thermal damage were compiled by Dr Luis Fajardo (personal communication, 1989) from data that he published (see text). When the temperatures were not at 43°C, they were converted to an equivalent time at 43°C by using the TID equation (1) in the text; these values for equiv min 43 (EM_{43}) are in parenthesis.

a phase III trial to show with statistical significance that hyperthermia combined with radiation is more effective than radiation alone, the CEM_{43} T_{90} would have to be increased by a factor of about 5, i.e. from about 5 to about 25 for soft tissue sarcomas. This corresponds to an increase of about 1.5°C (from 39.5 to 41 0°C) for the median T_{90} during each of 5–10 hyperthermia treatments, and from the plot in Figure 7 might be expected to increase the probability of complete response for soft tissue sarcomas from about 35% for a median T_{90} of 39.5°C to about 60% for a median T_{90} of 41.0°C. Another important conclusion reached from these thermal dose relationships is that a schedule of one versus two hyperthermia treatments per week or a total of two versus six hyperthermia treatments, i.e. a two or three-fold increase in cumulative thermal dose, would result in differences in complete response of only 5–8%, respectively [52], since both cumulative doses are low on the dose–response curve (Figure 7). Therefore, a phase III trial to detect this very small difference in response between one and two treatments per week or between two and six total treatments would require about 1000 patients per arm for a sensitivity of 0.05 [52]. In other words, the trials that have been conducted of this type have had an insufficient number of patients to determine any difference between one and two treatments per week.

Oleson et al. believe they have been successful in showing that the approach to thermal dosimetry using descriptors of the frequency distribution of temperatures in a TID formula is useful in a retrospective analysis, and that such a thermal dosimetry model should be used prospectively to set thermal goals of treatment. Furthermore, they concluded that temperatures presently achieved correspond to extremely low doses on the thermal dose–response curves. In fact, Oleson et al. suggested, as is inferred from the plots in Figure 7, that a more stringent requirement for at least a 95% confidence of success in a phase III trial to obtain high freedom from relapse rates, might have a treatment goal of a median T_{50} of 42.5°C and a median T_{90} of 44.6°C. For a mean number of seven treatments of 60 min each, this would give a CEM_{43} T_{90} of about 220 and a CEM_{43} T_{50} of about 1300. From the plots in Figure 7, this amount of heat might be expected to increase the probabilities of complete responses from 10 to 15% for radiation alone to about 60 and 80% for non-adenocarcinomas and STSs, respectively. In fact, this might be translated into an optimal criterion of achieving a CEM_{43} T_{90} of about 100 when hyperthermia is used in the clinic. This criterion would correspond in Figure 7 to a probability of complete response of 50–70%.

I suspect that the CEM_{43} T_{50} parameter, which is expressing the minimum thermal dose in the 50% of the tumour that is the hottest, is contributing more towards enhancing the complete response than the lower CEM_{43} T_{90}. This hypothesis is based on the relatively large amount of heat required both to kill cells in vitro and to damage normal tissues and tumors in animal model systems [10] and in humans [54]. Data compiled and tabulated in Table 1 [53] indicate that 20–240 equiv min 43°C (20–240 EM_{43}) are required as thresholds to induce damage in different normal tissues in various species, including man. Furthermore, data [54] from heating human breast carcinomas and surrounding normal tissue indicate that a thermal dose (averaged from 18–28 points in the tissue) of 100–200 equiv min 43°C (100–200 EM_{43} in the hottest of 5–10 heat fractions is required to induce vascular damage and necrosis in tumour and normal tissue. For a total of five fractions this should be a threshold cumulative thermal dose of 500–1000 equiv min 43°C for the mean dose in the tissue, which should be comparable with a CEM_{43} T_{50} of 500–1000. Also, data for human tumors [55] suggest that a minimum CEM_{43} of 40–80 which is probably comparable with a CEM_{43} T_{50} of 40–80 is required to obtain a high probability for a complete response when

hyperthermia is combined with radiation. Finally, the plots of probability of response versus thermal dose expressed as CEM_{43} T_{50} [51,52] suggest that relatively large thermal doses are needed for tumour response.

CEM_{43} T_{90} as a predictor of response and CEM_{43} T_{50} as a cause of response

If the hypothesis is true that tumour response is caused by the high temperatures in the tumour, one would expect the CEM_{43} T_{50} to correlate with the response rate better than, or at least as well as, the CEM_{43} T_{90}. This may be true for necrosis of STSs [52], but, in several studies, the response rate correlates best with the CEM_{43} T_{90} [52] or with the lower temperatures measured in the tumour [5]. Having the CEM_{43} T_{50} or median T_{50} for the hyperthermia treatments to a tumour exceed a certain threshold in thermal dose may be a necessary but not a sufficient condition for thermal response, with the additional requirement for response being that the CEM_{43} T_{90} or median T_{90} also reach a certain threshold in thermal dose (Oleson, personal communication). In other words, in tumors that manifest a thermal response, both CEM_{43} T_{50} and CEM_{43} T_{90} would have reached the required thresholds, whereas, in some tumors that do not manifest a thermal response, the CEM_{43} T_{50} but not the CEM_{43} T_{90} would have reached the required thresholds. A possibility is that a CEM_{43} T_{90} of a certain threshold value serves as an index of how uniformly or effectively the viable cells in the tumour have been heated, which, e.g. could depend on the blood flow related to the amount and type of microvasculature and amount of necrosis in the tumour. These properties of the tumour could cause changes in the metabolic parameters in the tumour, such as pH, energy charge, and nutrient levels, which in turn could effect the thermal sensitivity of the viable tumour cells. But even more importantly, tumors with a lot of necrosis might be expected to have the high temperatures contributing to the T_{50}, in necrotic regions which should be the hottest because of low blood flow; then, many viable tumour cells in vascularized regions might be expected to be at temperatures $<T_{50}$. Thus, for a group of tumors that have heterogeneity in their physiologic and metabolic parameters and amount of necrosis, a certain minimum threshold for CEM_{43} T_{90} reached during heating might serve as an index that the regions of the tumour reaching a required threshold value for CEM_{43} T_{50} contain viable tumour cells and not mostly non-clonogenic cells in necrotic regions.

MRS parameters related to T_{90}, CEM_{43} T_{90} and tumour response

MRI/MRS studies underway in the Oleson and Dewhirst groups with human and canine STSs [56,57] are attempting to determine if metabolic parameters can predict both the ability to heat tumors and their response to hyperthermia and radiation combined. Briefly, the results indicate that tumors that are alkaline and energetic (high ATP/Pi) respond better to heat and radiation than those that are acidic and less energetic. Also, tumors that are energetic heat better, as measured by T_{90}, than those that are less energetic. These relationships appear to be consistent with the hypothesis that less necrotic tumors heat more uniformly and have a higher T_{90} when a threshold T_{50} is reached than the tumors that are more necrotic. The relationship between pH and ability to heat, as measured by T_{90}, was not reported in their studies. An unlikely possibility is that these metabolic parameters as well as ability to reach a threshold T_{90} simply indicate or predict which tumors would respond best to radiation alone. In other words, a possibility that needs to be excluded in future studies is that the dose-response relationship shown in Figure 7 is not caused by an increase in thermal damage, but instead is due to an increase in CEM_{43} T_{90} correlating with some physiological parameter such as a reduction in the amount of tumour hypoxia that increases the ability of heterogenous tumors to respond to radiation alone. Results arguing against this possibility are evident in the randomized study of pet animals [5] in which those animals having tumors that received a certain minimum thermal dose (probably comparable with a CEM_{43} T_{90}) survived significantly longer than those animals with tumors receiving radiation alone as well as those receiving a lower thermal dose. Ideally, physiological parameter(s) that would predict the ability to heat tumors to a higher threshold T_{90} (e.g. a CEM_{43} $T_{90} > 25$) could be used to select patients who would be randomized to radiation alone or radiation plus hyperthermia. A reasonable criterion for such a study is that the median T_{90} and CEM_{43} T_{50} should be increased from the values reported by Oleson et al. (39.5°C and 5, respectively) to 41.0°C and 25, respectively, while noting that the median T_{50} and median CEM_{43} T_{50} should also be increased from about 41.6°C and 80 to about 43.0°C and 400, respectively. According to our hypothesis, the CEM_{43} T_{50} must be much higher than the CEM_{43} T_{90} in order to have sufficient cytotoxicity in the hotter regions of the tumour containing viable tumour cells. However, as heating techniques are improved to provide more uniform tumour heating, I predict that the threshold CEM_{43} T_{90} will have to be raised above what we have been proposing in order to

have a sufficiently high CEM_{43} T_{50} to kill the viable tumour cells throughout the tumour.

Additional data that TID needs to be increased in clinical hyperthermia

The conclusion that thermal doses larger than those obtained in recent clinical trials are needed is supported by other clinical data [55] indicating that a minimum TID dose of 20 equiv min 43°C (probably similar to 20 EM_{43} T_{90}) in small 10 cm^3 superficial tumors was needed for each of 2–4 hyperthermic treatments in order to obtain a complete response. These data suggest that for a high probability of complete response, a CEM_{43} T_{90} of 40–80 would be required. Therefore, the recent RTOG phase III interstitial trial (8419) with only one 1-h of heating before and after interstitial radiation probably had thermal doses which were too low to show a significant beneficial effect of hyperthermia. For example, in one series of six patients undergoing 10 treatments at UCSF with interstitial implants in gynecological tumors heated with a ferromagnetic seed heating system, the tumour temperature distributions as sampled in tissue midway between implants showed a median T_{90} of only 40.0 ± 0.5°C (Stauffer, personal communication). Thermal dosimetry of heat treatments with other interstitial techniques has shown similar results. For a similar group of 10 patients that received 15 1-h heat treatments with RF needle implants in gynecological tumors, the median T_{90} was similarly 40.2 ± 0.7°C when temperatures were measured predominantly inside the heated needles. The median T_{50} was only 41.9 ± 0.6°C. Clearly, for these large GYN tumors, there is always a significant volume of tissue at the periphery that can not be heated much higher than 40°C without inducing pain. Converting these values into equiv min at 43°C gives an estimated CEM_{43} T_{90} of only 2 and a CEM_{43} T_{50} of only 20, and when most of the radiation dose is given several hours before or after heating, the rapid decay in the interaction between heat and radiation discussed previously, strongly suggests that heat cytotoxicity with very little if any heat radiosensitization would be the primary effect to be expected from the hyperthermic treatments. Thus, the RTOG randomized phase III interstitial study will probably have a median thermal dose much lower than a CEM_{43} T_{90} of 25, which we estimate would be the minimum required to detect a significant difference between radiation and radiation combined with hyperthermia.

In a phase I/II study at UCSF [58], hyperthermia was combined with interstitial radiation to treat brain tumors (recurrent anaplastic astrocytoma (AA) and recurrent glioblastoma multiforme (GM)); 1 h of heating occurred at the beginning and end of treatment, but primarily because of lack of pain, the median T_{90} of 41.2°C was 1.0–1.2°C higher than for the GYN cases mentioned above. The median T_{90} of 41.2°C, which equates to an estimated CEM_{43} T_{90} of ~10, is close to the minimum CEM_{43} T_{90} of 25 suggested from Oleson's studies for demonstrating a benefit of hyperthermia combined with radiation. Indeed, the GM patients who had a $T_{90} \geq 41.2$°C showed a significant ($p = 0.008$) increase in survival of 64 weeks compared with 37 weeks for those who had a $T_{90} < 41.2$°C (Figure 8). Also, the AA patients who had a $T_{90} \geq 41.2$°C showed a non-significant ($p = 0.36$) beneficial effect of hyperthermia compared with those who had a $T_{90} < 41.2$°C. Of the AA patients who had a $T_{90} \geq 41.2$°C, 60% survived for >l00 weeks, compared with 60 weeks for patients who had a $T_{90} < 41.2$°C. The thermal dose-response relationships in Figure 7, however, suggest that the thermal dose of ~10 for the CEM_{43} T_{90} achieved in this brain tumour study is only minimal, and while indicative of improved response, should be increased 2–5 times to greatly improve the effect of hyperthermia on tumour, hopefully without the induction of significant deleterious effects on normal brain. In future studies, CEM_{43} T_{50} and CEM_{43} T_{10} also should be evaluated.

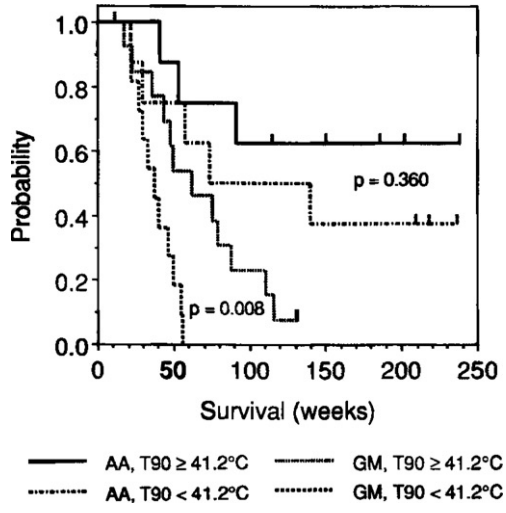

Figure 8. Actuarial survival following brain implant with interstitial hyperthermia (calculated by the product limit method of Kaplan and Meier) for patients with recurrent anaplastic astrocytoma (AA) and recurrent glioblastoma multiforme (GM). Separate curves are shown for eight patients with AA and $T_{90} \geq 41.2$°C, eight patients with AA and $T_{90} < 41.2$°C, 14 patients with GM and $T_{90} \geq 41.2$°C, and 11 patients with GM and $T_{90} < 41.2$°C. Note the significantlyimproved survival ($p = 0.008$) for patients with GM who achieved a T_{90} of at least 41.2°C. This study was carried out as described by Penny Sneed, Phil Gutin and Paul Stauffer at UCSF [58].

An important point to be made is that the CEM_{43} analysis should be made for different tumour types and different normal tissues because response versus CEM_{43} from Oleson's study may be quite different for different tumour types and different heating-radiation protocols. Furthermore, the technique for sampling temperature distributions should be [58] specified and standardized [59] because with heterogeneities in temperature distributions in tumours, the measured distributions, e.g. T_{90} and T_{50}, can be different for: (a) sampling uniformly along one or more catheters through the centre of the tumour, (b) sampling at random throughout the tumour; and (c) sampling near the periphery where there is more of the tumour volume compared with the centre [59] and where there may be an increase in highly perfused tumourhormal tissue at lower temperatures.

Role of thermal tolerance in clinical hyperthermia

Because of the potential of thermal tolerance greatly reducing the effect of the thermal response, the possibility of thermotolerance when hyperthermia is delivered in the clinic should be considered. When various fractionation schemes are reviewed [52], there is no evidence of thermotolerance, although as discussed above, thermotolerance probably could not have been detected if it did occur. In fact, in Oleson's study of large STSs, a greater effect of hyperthermia was observed for the same CEM_{43} T_{50} when two treatments per week were used instead of one. The investigators (M. Dewhirst, personal communication) thought this occurred because of tumour reoxygenation or perhaps an increased probability of heating more tumour cells when the number of heat treatments was increased.

In addressing the question of thermotolerance in the clinical setting, we should consider the magnitude and duration of thermotolerance as a function of the amount of heat delivered during the initial heating. Data for mouse mammary carcinoma turnours in vivo [32] and data for cells in culture [60] indicate that the magnitude and duration of thermotolerance are reduced greatly as the amount of heat delivered during the initial heating is reduced. For example, in Overgaard's in vivo studies, in which small mouse tumors were heated quite uniformly and then studied for growth delay induced by heat alone, the thermotolerance ratio increased to about 3 and then decayed by about 24 h when the initial heating was only 30 min at 42.5°C. However, when the initial heating was 44.5°C for 30 min, the thermotolerance ratio increased to about 10 and did not decay until about 6 days. Therefore, as shown in Figure 9, which models a simple case for thermotolerance, one would not expect much thermotolerance in the cooler regions of the tumour that received a low TID

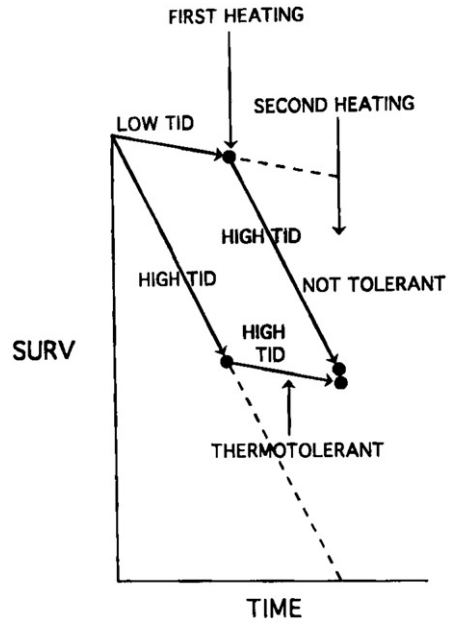

Figure 9. A schematic to illustrate that a region of a tumour that received a low thermal dose (low TID) should contain many viable non-thermotolerant cells after the first heat dose and that these viable cells should be inactivated if they could be heated with a second high heat dose (high TID). In contrast, a few viable thermotolerant cells might remain in the region of the tuniour that received a first high thermal dose, but the thermal resistance of these few cells to a second heat treatment should be of little consequence to the overall survival due to the relatively high thermal sensitivity of the many viable non-thermotolerant cells remaining in the region that received a low thermal dose during the first treatment.

close to a T_{90} of only 2–5 EM_{43} for one dose, and if thermotolerance were induced, it should decay very quickly. However, in the hotter regions of the tumors that received a TID >60 EM_{43}, thermotolerance might be expected if the second heat dose were given within 1–2 days after the first heat dose. But, when complete response is being evaluated in clinical trials, thermotolerance in regions that are effectively heated to temperatures >44°C, for example, would be expected to have very little significance compared with a lack of thermotolerance in the cooler regions where the tumour cells would be expected to survive the initial treatment. Then, if viable non-thermotolerant cells remaining in the cooler regions after the first heat treatment could be heated better with subsequent treatments, due to differences in the power deposition patterns or changes in blood flow, the non-thermotolerant cells might be killed and not influenced by thermotolerance that was induced during previous treatment(s) only in the hottest parts of the tumour. In fact, thermal tolerance may serve as a biological dose modifier [32] which helps (as suggested by P. Stauffer) to homogenize the

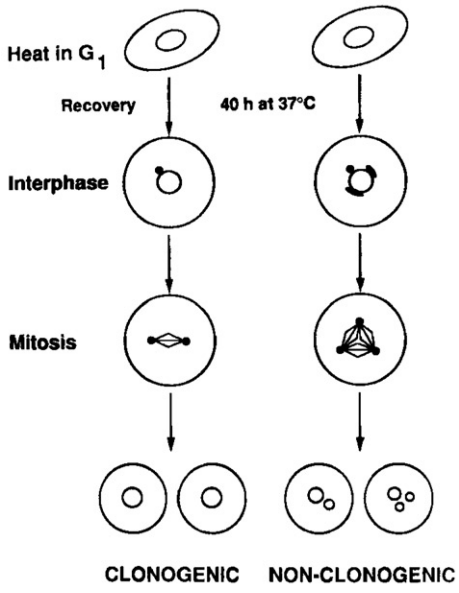

Figure 10. A schematic of results [73] illustrating that the centrosome is a critical target for the killing of CHO cells heated (EM_{43} of 52) in G_1 to result in survival levels >10%. The lack of staining of centrosomes with antisera is observed in all G_1 cells immediately after heating, but recovery occurs in some of the cells which then undergo a normal bipolar division and form macroscopic colonies. The cells which do not recover a normal centrosome, but instead have a fragmented centrosome, undergo an aberrant multipolar division that results in non-clonogenic multinucleated cells.

effective thermal dose accumulated over a number of fractionated treatments.

Thus, as tumour response versus thermal dose is evaluated, we conclude that as many good heat treatments as possible should be delivered in order to achieve a minimum CEM_{43} T_{90} of about 25. Furthermore, development of thermotolerance between treatment fractions is unlikely to present a problem clinically because of the low thermal doses achieved in regions of the tumors that would be expected to contain the viable cells after a hyperthermic treatment.

Heating at 40–41°C during irradiation: clinical possibilities suggested from in vitro studies

Another approach that is being considered is to heat continuously at 41°C simultaneously with interstitial low dose-rate irradiation or fractionated irradiation [61, 62]. Twenty years ago, Ben-Hur et al. [63] showed that heating cells at 41°C during low dose-rate irradiation eliminated recovery from sublethal radiation damage and thus for a given total radiation dose caused the survival from low dose-rate irradiation to decrease to about the same level as that for an acute dose of radiation at 37°C. Although thermotolerance has been observed during the first few hours of heating at 41–42°C, thermotolerance was lost after heating for about 18–20 h as the cells surviving in G_1 moved into the heat-sensitive S phase [64, 65]. Also, CHO cells heated at 42°C in S phase did not develop thermotolerance while they remained in S phase [23]. Furthermore, HeLa cells which were not blocked in G_1 did not manifest any significant amount of thermotolerance during the first few hours of heating at 41.5°C [66]. There are also data indicating that for some human cell lines, heat cytotoxicity at 41°C is higher for these human cell lines than for rodent cell lines, although at 43°C, the rodent cell lines are more heat sensitive than the human cell lines [27]. For their human cell lines, R between 43 and 45°C was 2.2–2.6 with a mean of 2.4, and for heating for 48–72 h at 41–43°C, R was 3.7–8.5 with a mean of 6.2. The comparable data for rat 9L and CHO cells gave Rs for 41–43°C of 9.5 and 12.6, respectively. As discussed previously, Rs are expected to increase <43°C because of the development of chronic thermotolerance during heating; therefore, the lower $R < 43$°C for human compared with rodent cells reflects less chronic thermal tolerance for human compared with rodent cells. However, even if some chronic thermotolerance does develop during prolonged heating at 41°C, heating for as long as 48 h at a temperature of 41°C should give a significant amount of killing without any appreciable sparing effect from thermotolerance; e.g. for an R of 6.3, as observed for the human cells mentioned above, heating for 48 h at 41°C would give a CEM_{43} of 75. But, most importantly, maintenance of 41°C for about 34 h while low dose-rate irradiation was delivered simultaneously with heat [67] resulted in survival values for low dose-rate irradiation that were much lower than without heat and almost identical to those for an acute single dose of irradiation at 37°C. This thermal radiosensitization occurred primarily because recovery of sublethal damage was eliminated by maintaining the cells at 41°C during low dose-rate irradiation. Also, heating at 41°C for about 4 h before and after high dose-rate irradiation was delivered every day resulted in a survival value that was much lower than without heat and was almost identical to survival from a single dose of high dose-rate irradiation at 37°C [62]. In other words, prolonged heating at 41°C effectively eliminates the sparing effect or reduction in radiation damage observed with both low dose-rate irradiation and fractionated irradiation compared with a single high dose of irradiation; at a given survival level, the fractional reduction in total dose caused by heating is expressed as a TER. Therefore, there may be a good rationale for combining continuous heating at about 41°C with low dose-rate irradiation or fractionated

irradiation in order to give a *TER* of ~2 [61] and thus enhance the radiation damage to the heated tumour. But, a caveat is that radiation damage to heated normal tissue should also be increased and thus could eliminate or reduce the desired sparing of radiation damage to normal tissue associated with low dose-rate irradiation and fractionated irradiation. Therefore, for a preferential hyperthermic effect on tumour versus normal tissue, this approach will probably require selective heating of the tumour, unless biological and physiological factors will lead to a greater enhancement of thermal damage in the tumour than in the infiltrating and surrounding normal tissue. Such modifying biological factors have been studied for hyperthermia combined with high dose-rate irradiation, but little information is available for hyperthermia combined with low dose-rate irradiation. As a practical approach, I recommend selective heating of the tumour while attempting to heat the regions of the tumour that have the lowest radiation doses in order to have the highest TERs in the tumour regions that receive the lowest radiation doses.

Since it is easier to selectively irradiate most of the tumour relative to surrounding normal tissue than to selectively heat most of the tumour relative to surrounding normal tissue, I recommend animal and human trials in which the entire region or body is heated at 40–41°C (104–105.8°F) for several hours before, during, and after daily 2-Gy doses to see if a therapeutic gain can be obtained when both the normal tissue and tumour are at the same temperature. In this case, we would be determining if there are biological aspects of hyperthermic effects that might allow for preferential killing or destruction of tumour cells relative to cells in normal tissue. These biological aspects [10] include: selective heat sensitization [7, 68–70] and heat radiosensitization [71] of radioresistant S phase cells, increased heat sensitivity and heat radiosensitization at low pH [18, 19, 72], and possibly hyperthermia-induced reoxygenation in the tumour (M. Dewhirst, personal communication), Possibly, whole-body heating techniques could be used, but another approach that might be considered is the use of pyrogens, if they could be developed, to safely reset the body thermostat every day for a few hours before, during, and after the dose of radiation is delivered.

Mechanisms of heat cytotoxicity

Killing of CHO cells heated in G_1

Consideration of the approach involving biological and physiological differences in the tumour relative to normal tissue leads us to the importance of obtaining a better understanding of the mechanisms of how heat kills mammalian cells. As an example of how mechanistic information can be obtained, I would like to describe in some detail a mechanistic study that identified a fundamental mechanism for killing CHO cells heated in G_1 with a clinically relevant heat dose, i.e. a thermal dose that killed 50–90% of the cells. In this study [73], time-lapse video microscopy was used to study the progression of heated cells through their first division followed by either cell death or the formation of macroscopic colonies. Vidair et al. observed that the heated cells consisted of both regular and irregular dividers; irregular dividers are defined as those with at least one daughter cell having a multinucleated morphology, and regular dividers are defined as those with two daughter cells, each of which has only one nucleus. In a high percentage of cases, the regular dividers formed colonies, whereas, the irregular dividers with a multinucleated morphology did not.

To investigate the mechanism behind the formation of non-clonogenic multinucleated cells, Vidair et al. studied the effects of heat on the centrosome, which has been shown previously to be altered by relatively low heat doses [74]. The centrosome, which consists of a centriole pair with its associated pericentriolar material (PCM), exists as a single organelle in G_1. Following replication during interphase and separation in late G_2, the two centrosomes function to organize the bipolar mitotic spindle. The PCM nucleates the spindle microtubules that ultimately contact and pull apart the sister chromatids, resulting in the segregation of one set of chromatids to each daughter cell. Vidair et al. observed immediately after the cells were heated in G_1 for about 13 min at 45°C (52 EM_{43} calculated by Equation 1), that centrosomes no longer stained with antisera directed against PCM proteins. As the cells were incubated at 37°C, they recovered from their cell cycle delay and progressed through the cycle into G_2, with some of the cells having normal-looking centrosomes and others acquiring fragmented centrosomes. The cells which entered mitosis with a normal bipolar spindle (organized by two normal centrosomes) underwent a normal division followed by formation of a macroscopic colony. However, the cells that entered mitosis with fragmented centrosomes had multipolar spindles that led to the formation of multinucleated cells that were nonclonogenic.

Serial sectioning indicated that the heat-induced fragmentation of centrosomes was not due to breakage of centrioles; rather, disorganization of the PCM was the cause. The questions that need to be addressed are: (I) What are the molecular interactions altered by heat which cause disorganization of PCM proteins?; (2) Can these heat-sensitive alterations to the PCM be used as models for developing

better thermal sensitizers and protectors?; and (3) Why are thermotolerant cells better able to reorganize the proteins of the PCM during the post-heating period?

Different heat sensitivities and mechanisms of heat killing for different cells and tissues

I wish to emphasize that the one example I have presented is just one of the many possible mechanisms of heat killing that need to be investigated for many different cell types and tissues. For example, when CHO cells are heated in the heat sensitive S phase with thermal doses that reduce their survival to values between 10 and 100%, cell lethality correlates very well with the induction of chromatid aberrations [70, 75]. Furthermore, for certain cell types, apoptosis occurs [76, 77] probably involving the activation of the apoptotic gene at relatively low hyperthermic temperatures. Also, as shown in Table 1, the heat sensitivity for obtaining a threshold of thermal damage in various tissues [53, 78–80] varies greatly from values as low as 20–30 equiv min at 43°C (20–30 EM_{43}) for kidney, brain, liver and colon, to values as high as 240 EM_{43}, for muscle and fat. We need to determine which cells are involved in these different tissues and how they die. Will there be any common underlying principles such as observed for radiation [81], in which the tissues with rapidly dividing cells manifest more radiation damage because rapidly dividing cells die primarily from a mitotic-linked death associated with chromosomal aberrations? Are there any underlying rules of this type for heat cytotoxicity? As for radiation, the importance of vascular damage relative to parenchymal damage needs to be considered, including heat effects on tumour angiogenesis [82]. Data in which vascular damage and necrosis are plotted as a function of EM_{43}, suggest that both vascular damage leading to secondary necrosis and necrosis resulting from primary heat damage in parenchymal cells appear to be occurring [54]. Both need to be considered and carefully evaluated as endpoints of damage are evaluated as a function of thermal dose.

Considerations and challenges for the future of clinical hyperthermia

Selected sentences are quoted from an article [83] published in the Proceedings of the 9th International Congress of Radiation Research.

'*Difficulty in randomized trials in radiation oncology*: Technical advances in radiation oncology, such as supervoltage irradiation or treatment-planning simulators, were thought, intuitively, to be so superior to prior practice that randomized trials appeared unnecessary. Other modalities in the realm of radiation oncology are in increasing use without the benefit of a randomized trial, including intraoperative RT, high dose rate remote afterloading brachytherapy, particle radiation, stereotactic radiosurgery, photodynamic therapy, and three-dimensional RT treatment planning. Phase III trials that compared outcome with and without each of these modalities have, to date, been less successfully executed than those for hyperthermia.

Competing clinical trials: The priority assigned to trials with competing eligibility is usually based on political rather than scientific grounds.

Interpretation of phase III trials. Because of cost, patient discomfort, and risk of complications, should hyperthermia trials be designed only if they have the potential to improve survival and not merely response rates?

It is likely that interest in clinical hyperthermia will wane during the early 1990s. This will occur not because a lack of efficacy was demonstrated but because proof of efficacy was not pursued with sufficient vigour. The burden of proof may now fall upon a smaller band of investigators. We will eagerly await results of their work.'

The challenge is for us to deliver a sufficient thermal dose to the tumour when hyperthermia is combined with radiation and then to rigorously quantify the effects in tumour and normal tissue as a function of thermal dose. This approach hopefully will lead to a well designed phase III study that will test the clinical efficacy of hyperthermia combined with radiation when the thermal dose administered has been shown in a phase II study to give a high probability of success in a phase III study. The need for establishing an 'optimal' therapy based on a quantifiable treatment that is reproducibly related to treatment outcome and tolerated by surrounding normal tissues is paramount [84]. In my opinion, with the development of improved equipment for delivering hyperthermia in the clinic and/or with hyperthermia treatments delivered either every day or continuously, a CEM_{43} T_{90} of ~25 and a CEM_{43} T_{50} of ~400 as minimal criteria can be achieved in selected tumour sites. In fact, continuous heating with a T_{90} at 41°C for 48 h might be expected to give a CEM_{43} T_{90} of ~75 (see §4). Once data are available in a phase II trial that show acceptable TID criteria can be met without unacceptable patient discomfort and/or normal tissue toxicity, a phase III study should be possible in which cure rate or disease free survival is the end point.

Acknowledgements

The research that was conducted in my laboratory was supported in part by NCI Grants CA 08618, 31813 and 09215. Appreciation is expressed to Louise Thompson for technical assistance and or making several of the figures, and to Lisa Raskulinec for typing the manuscript. For assistance in conducting these studies and developing concepts of thermal dose, I would like to acknowledge many students and colleagues at Colorado State University and the Radiation Oncology Research Laboratory, University of California, San Francisco. In particular, I would like to thank Steve Sapareto for his creative work and ideas regarding thermal dose concepts, Mark Dewhirst for many discussions concerning thermal dose in the clinical setting, and Mark Dewhirst, Jim Oleson, Peter Corry, Woody Armour, Luis Fajardo, Paul Stauffer and Penny Sneed for sharing their unpublished results with me. In addition, the many suggestions that were given by Paul Stauffer from his critical and thorough reading of the manuscript are greatly appreciated; they resulted in many changes that hopefully clarified many statements and conclusions made in the text. Furthermore, he suggested the acronym, $CEM_{43}\ T_{90}$, that is introduced here.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Robinson JE, Wizenbekg MJ, Mcready WA. Combined hyperthermia and radiation, an alternative to heavy particle therapy for reduced oxygen enhancement ratios. Nature 1974;251:521–522.
2. Robinson JE, Harrison GH, Mccready WA, Samaras GM. Good thermal diosimetry is essential to good hyperthermia research. Brit J Rad 1978;51:532–534.
3. Sapareto SA, Hopwood LE, Dewey WC, Raju MR, Gray JW. Hyperthermic effects on survival and progression of CHO cells. Cancer Res 1978b;38:397–400.
4. Sapareto SA, Dewey WC. Thermal dose determination in cancer therapy. Int J Rad Oncol, Biol Phys 1984;10:787–800.
5. Dewhirst MW, Sim DA, Sapareto S, Connor WG. Importance of minimum tumor temperature in determining early and long-term responses of spontaneous canine and feline tumors to heat and radiation. Cancer Res 1984;44:43–50.
6. Sapareto SA. A workshop on thermal dose in cancer therapy: introduction. Int J Hyperthermia 1987;3:289–290.
7. Westra A, Dewey WC. Variation in sensitivity to heat shockduring the cell cycle of Chinese hamster cell *in vitro*. Int J Rad Biol 1971;19:467–477.
8. Dewey WC, Hopwood LE, Sapareto SA, Gerweck LE. Cellular responses to combinations of hyperthermia and radiation. Radiology 1977;123:463–474.
9. Matsumura M, Yasumura S, Shuichi A. Cumulative effect of intragenic aminoacid replacements on the thermostability of a protein. Nature 1986;323:356–358.
10. Dewey WC, Freeman ML, Raaphorst GP, Clark EP, Wong RSL, Highfield DP, Spiro IJ, Tomasovic CP, Denman DL, Coss RA. Cell biology of hyperthermia and radiation. In: Meyn RE and Withers HR, editors. Radiation biology in cancer research. New York: Raven Press; 1980. pp 589–621.
11. Dewey WC. Failla memorial lecture: the search for critical cellular targets damaged by heat. Rad Res 1989;120:191–204.
12. Henriques FC. Studies of thermal injury V. The predictability and the significance of thermally induced rate processes leading to irreversible epidermal injury. Arch Pathol 1947;43:489–502.
13. Borrelli MJ, Thompson LL, Cain CA, Dewey WC. Time-temperature analysis of cell killing of BHK cells heated at temperatures in the range of 43.5°C to 57.0°C. Int J Rad Oncol Biol Phys 1990;19:389–399.
14. Mackey MA, Dewey WC. Time temperature analyses of cell killing of synchronous G_1 and S phase Chinese hamster cells *in vitro*. Rad Res 1988;113:318–333.
15. Rosenberg B, Kemeny G, Switzer RC, Hamilton TC. Quantitative evidence for protein denaturation as the cause of thermal death. Nature 1971;232:471–473.
16. Field SB, Morris CC. The relationship between heating time and temperature: Its relevance to clinical hyperthermia. Radiother Oncol 1983;1:179–186.
17. Bauer KD, Henle KJ. Arrhenius analysis of heat survival curves from normal and thermotolerant CHO cells. Rad Res 1979;78:251–262.
18. Freeman ML, Raaphorst GP, Hopwood LE, Dewey WC. The effect of pH on cell lethality induced by hyperthermic treatment. Cancer 1980;45:2291–2300.
19. Hiraoka M, Hahn GM. Comparison between tumor pH and cell sensitivity to heat in RIF-1 tumors. Cancer Res 1989;49:3734–3736.
20. Hahn GM, Shiu EC. Adaptation to low pH modifies thermal and thermo-chemical responses of mammalian cells. Int J Hyperthermia 1986;2:379–387.
21. Chu GL, Dewey WC. The role of low intracellular or extracellular pH in sensitization to hyperthermia. Rad Res 1988;114:154–167.
22. Thistlethwaite AJ, Alexander GA, Moylan DJ, Leeper DB. Modification of human tumor pH by elevation of blood glucose. Int J Rad Oncol Biol Phys 1987;13:603–610.
23. Mackey MA, Dewey WC. Cell cycle progression during chronic hyperthermia in S phase CHO cells. Int J Hyperthermia 1989;5:405–415.
24. Raaphorst GP, Romano SL, Mitchell JB, Bedford JS, Dewey WC. Intrinsic differences in heat and/or X–ray sensitivity of seven mammalian cell lines cultured and treated under identical conditions. Cancer Res 1979;39:396–401.
25. Roizin-Towle L, Pirro JP. The response of human and rodent cells to hyperthermia. Int J Rad Oncol, Biol Phys 1991;20:751–756.
26. Hahn GM, Ning SC, Elizaga M, Kapp DS, Anderson RL. A comparison of thermal responses of human and rodent cells. Int J Rad Biol 1989;56:817–825.
27. Armour EP, McEachern D, Wang Z, Corry PM, Martinez A. Sensitivity of human cells to mild hyperthermia. Cancer Res 1993a;53:2740–2744.
28. Dewey WC. Interaction of heat with radiation and chemotherapy. Cancer Res 1984;44(Suppl):4714s–4720s.
29. Urano M, Majima H, Miller R, Kahn J. Cytotoxic effect of 2,3 bis (2-chloroethyl)-N-nitrosourea at elevated temperatures: arrhenius plot analysis and tumour response. Int J Hyperthermia 1991;7:499–510.
30. Sapareto SA, Hopwood LE, Dewey WC. Combined effects of x-irradiation and hyperthermia on CHO cells for various temperatures and orders of application. Rad Res 1978a;73:221–233.

31. Sapareto SA, Raaphorst GP, Dewey WC. Cell killing and the sequencing of hyperthermia and radiation: Brief Communication. Int J Rad Oncol Biol Phys 1979;5:343–347.
32. Overgaard J. Some problems related to the clinical use of thermal isoeffect doses. Int J Hyperthermia 1987;3:329–336.
33. Overgaard J. Fractionated radiation and hyperthermia: experimental and clinical studies. Cancer 1981;48:1116–1123.
34. Dikomey E, Jung H. Thermal radiosensitization in CHO cells by prior heating at 41–46°C. Int J Rad Biol 1991;59:815–825.
35. Nielsen OS, Overgaard J. Hyperthermic radiosensitization of thermotolerant tumour cells *in vitro*. Int J Rad Biol 1979;35:171–176.
36. Nielsen OS. Influence of thermotolerance on the interaction between hyperthermia and radiation in L1A2 cells *in vitro*. Int J Rad Biol 1983;43:665–673.
37. Raaphorst GP, Azzam EI. Thermal radiosensitization in Chinese hamster (V79) and mouse C3H IOT 1/2 cells. The thermotolerance effect. Brit J Cancer 1983;48:45–54.
38. Jorritsma JBM, Burgman P, Kampinga HH, Konings AWT. DNA polymerase activity in heat killing and hyperthermic radiosensitization of mammalian cells as observed after fractionated heat treatments. Rad Res 1986;105:307–319.
39. Jorritsma JBM, Kamp!inga HH, Scaf AHJ, Konings AWT. Strand break repair, DNA polymerase activity and heat radiosensitization in thermotolerant cells. Int J Hyperthermia 1985;1:131–145.
40. Henle KJ, Tomasovic SP, Dethlefsen LA. Fractionation of combined heat and radiation in asynchronous CHO cells. I. Effects on radiation sensitivity. Rad Res 1979;80:369–377.
41. Law MP. Some effects of fractionation on the response of the mouse ear to combined heat and X-rays. Rad Res 1979;80:360–368.
42. Law MP, Ahier RG, Field SB. The effect of prior heat treatment on the thermal enhancement of radiation damage in the mouse ear. Br J Radiol 1979;52:315–321.
43. Haveman J. Influence of pH and thermotolerance on the enhancement of X-ray induced inactivation of cultured mammalian cells by hyperthermia. Int J Rad Biol 1983; 43:281–289.
44. Nielsen OS, Overgaard J, Kamura T. Influence of thermotolerance on the interaction between hyperthermia and radiation in a solid tumour *in vivo*. Bri J Radiol 1983; 56:267–273.
45. Overgaard J, Nielsen OS. The importance of thermotolerance for the clinical treatment with hyperthermia. Radiother Oncol 1983;1:167–178.
46. Overgaard J, Nielsen OS. Influence of thermotolerance on the effect of combined hyperthermia and radiation in a C3H mammary carcinoma *in vivo*. In: Overgaard J, editor. Proceedings of the IV International Symposium on Hyperthermic Oncology. London: Taylor & Francis; 1984. pp 227–230.
47. Hartson-Eaton M, Malcolm AW, Hahn GM. Radiosensitivity and thermosensitization of thermotolerant Chinese hamster cells and RIF-1 tumors. Rad Res 1984;99: 175–184.
48. Van Rijn J, Van Den Berg J, Schamhart DHJ, Van Wijk R. Effect of thermotolerance on thermal radiosensitization in hepatoma cells. Rad Res 1984;97:318–328.
49. Holahan PK, Wong RSL, Thompson LL, Dewey WC. Hyperthermic radiosensitzation of thermotolerant Chinese hamster ovary cells. Rad Res 1986;107:332–343.
50. Dikomey E, Jung H. Effect of thermotolerance and step-down heating on thermal radiosensitization in CHO cells. Int J Rad Biol 1992;61:235–242.
51. Leopold KA, Dewhirst M, Samulski T, Harrelson J, Tucker JA, George SL, Dodge RK, Grant W, Clegg S, Prosnitz LR, Oleson JR. Relationships among tumor temperature, treatment time, and histopathological outcome using preoperative hyperthermia with radiation in soft tissue sarcomas. Int J Rad Oncol Biol Phys 1992;22:989–998.
52. Oleson JR, Samulski TV, Leopold KA, Scott TC, Dewhirst MW, Dodge RK, George SL. Sensitivity of hyperthermia trial outcomes to temperature and time: implications for thermal goals of treatment. Int J of Rad Oncol, Biol Phys 1993; 25:289–297.
53. Fajardo LF. Pathological effects of hyperthermia in normal tissues. Cancer Res 1984;44(Suppl.):4826s–1835s.
54. Lyng H, Monges OR, Bohler PJ, Rofstad EK. Relationships between thermal dose and heat-induced tissue and vascular damage after thermoradiotherapy of locally advanced breast carcinoma. Int J Hyperthermia 1991;7:403–415.
55. Dunlop PRC, Hand JW, Dickinson RJ, Field SB. An assessment of local hyperthermia in clinical practice. Int J Hyperthermia 1986;2:39–50.
56. Sostman HD, Prescott DM, Dewhirst MW, Dodge RK, Thrall DE, Page RL, Tucker JA, Harrelson J, Reece G, Leopold KA, et al. MR Imaging and spectroscopy for prognostic evaluation in soft tissue sarcomas. Radiol 1994; 190:269–275.
57. Prescott DM, Charles HC, Sostman HD, Dodge RK, Thrall DE, Page RL, Tucker A, Harrelson JM, Leopold KA, Oleson JR, et al. Therapy monitoring in human and canine soft tissue sarcomas using magnetic resonance imaging and spectroscopy. Int J Rad Oncol, Biol Phys 1994;28:415–423.
58. Sneed PK, Gutin PH, Stauffer PR, Phillips TL, Prados MD, Weaver KA, Suen S, Lamb SA, Ham B, Ahn D, et al. Thermoradiotherapy of recurrent malignant brain tumors. Int J Rad Oncol Biol Phys 1992;23:853–861.
59. Dewhirst MW, Phillips TL, Samulski TV, Stauffer P, Shrivastava P, Paliwal B, Pajak T, Gillim M, Sapozink M, Myerson R, et al. RTOG quality assurance guidelines for clinical trials using hyperthermia. Int J Rad Oncol Biol Phys 1990;18:1249–1259.
60. Li GC, Hahn GM. A proposed operational model of thermotolerance based on effects of nutrients and the initial treatment temperature. Cancer Res 1980;40:4501–4508.
61. Armour EP, Wang Z, Corry P, Martinez A. Equivalence of continuous and pulse simulated low dose rate irradiation in 9L gliosarcoma cells at 37° and 41°C. Int J Rad Oncol Biol Phys 1992;22:109–114.
62. Armour EP, Wang Z, Corry PM, Chen PY, Martinez A. Hyperthermic enhancement of high dose-rate irradiation in 9L gliosarcoma cells. Int J Rad Oncol Biol Phys 1993b;28: 171–177.
63. Ben-Hur E, Elkind MM, Bronk BV. Thermally enhanced radioresponse of cultured Chinese hamster cells: inhibition of repair of sublethal damage and enhancement of lethal damage. Rad Res 1974;58:38–61.
64. Read RA, Bedford JS. Thermal tolerance. Brit J Radiol 1980;53:920–921.
65. Dewey WC, Esch JL. Transient thermal tolerance: cell killing and polymerase activities. Rad Res 1982;92:611–614.
66. Mackey MA, Anolik SL, Roti JLR. Cellular mechanisms associated with the lack of chronic thermotolerance expression in HeLa S3 cells. Cancer Res 1992;52:1101–1106.
67. Wang Z, Armour EP, Corry PM, Martinez A. Elimination of dose-rate effects by mild hyperthermia. Int J Rad Oncol Biol and Phys 1992;24:965–973.
68. Palzer RJ, Heidelberger C. Studies on the quantitative biology of hyperthermic killing of HeLa cells. Cancer Res 1973;33:415–421.
69. Bhuyan BK, Day KJ, Edgerton CE, Olufunso O. Sensitivity of different cell lines and different phases in the cell cycle to hyperthermia. Cancer Res 1977;37:3780–3784.

70. Wong RSL, Kapp LN, Krishnaswamy G, Dewey WC. Critical steps for induction of chromosomal aberrations in CHO cells heated in S Phase. Rad Res 1993;133:52–59.
71. Dewey WC, Sapareto SA, Betten DA. Hyperthermic radiosensitization of synchronous Chinese hamster cells: Relationship between lethality and chromosomal aberrations. Rad Res 1978;76:48–59.
72. Holahan EV, Highfield DP, Holahan PK, Dewey WC. Hyperthermic killing and hyperthermic radiosensitization in Chinese hamster ovary cells: Effects of pH and thermal tolerance. Rad Res 1984;97:108–131.
73. Vidair CA, Doxsey SJ, Dewey WC. Heat shock alters centrosome organization leading to mitotic dysfunction and cell death. J Cell Physiol 1993;154:443–455.
74. Barrau MD, Blackburn GR, Dewey WC. The effects of heat on the centrosomes of CHO cells. Cancer Res 1978;38:2290–2294.
75. Dewey WC, Westra A, Miller HH, Nagasawa H. Heat-induced lethality and chromosomal damage in synchronized Chinese hamster cells treated with 5-bromodeoxyuridine. Int J Rad Biol 1971;20:505–520.
76. Harmon BV, Corder AM, Collins RJ, Gobe GC, Allen J, Allan DJ, Kerr JFR. Cell death induced in a murine mastocytoma by 42–47°C heating *in vitro*: Evidence that the form of death changes from apoptosis to necrosis above a critical heat load. Int J Rad Biol 1990;58: 845–858.
77. Harmon BV, Takano YS, Winterford CM, Gobe GC. The role of apoptosis in the response of cells and tumors to mild hyperthermia. Int J Rad Biol 1991;1991:489–501.
78. Meshorer A, Prionas SD, Fajardo LF, Meyer JL, Hahn GM, Martinez AA. The effects of hyperthermia on normal mesenchymal tissues. Arch Pathol Lab Med 1983; 107:328–334.
79. Fajardo LF, Meyer JL, Meshorer A, Prionas S, Martinez AA, Hahn GM. Thermal injury and thermotolerance in mesenchymal tissues. In: Vaeth JM, editor. Frontiers of radiation therapy and oncology. Basel: Karger; 1984. pp 144–152.
80. Prionas SD, Taylor MA, Fajardo LF, Kelly NI, Nelson TS, Hahn GM. Thermal sensitivity to single and double heat treatments in normal canine liver. Cancer Res 1985;45: 4791–4797.
81. Bergonie J, Tribondeau L. Interpretation de quelques resultats de la radiotherapie et essai de fixation d'une technique rationnelle. Compt Rend Acad Sci 1906;143:983–985 (Translation published in Radiation Research, 11, 32, 1960.).
82. Fajardo LP, Prionas SD, Kowalski J, Kwan HH. Hyperthermia inhibits angiogenesis. Rad Res 1988;114: 297–30612.
83. Curran WJ, Goodman RL. Hyperthermia 1991: A critical review. In: Dewey WC, Edington M, Fry RJ, Hall EJ, and Whitmore GF, editors. Radiation research: a twentieth-century perspective. San Diego: Academic; 1992. pp 883–888.
84. Dewhirst MW, Griffin TW, Smith AR, Parker RG, Hanks GE, Brady LW. Commentary: Intersociety council on radiation oncology essay on the introduction of new medical treatments into practice. J Nat Cancer Instit 1993; 85:951–957.