

Thermal Biophysics Lectures

Rajiv Chopra

chopra@sri.utoronto.ca

Rm C713, Imaging Research

Sunnybrook Health Sciences Centre

2075 Bayview Avenue, Toronto, Ontario, M4N3M5

Big Picture

- **Lecture 1: Biology/Rationale/Nomenclature**
- Lecture 2: Blood Flow/Modelling
- Lecture 3: Energy Delivery
- Lecture 4: Thermometry/Treatment monitoring

Thermal Therapy

- Therapeutic use of heat
 - Primary: Cryotherapy, hyperthermia, thermal coagulation
 - Combination: Heat + Radiation/Drugs
- Treatment of localized disease
- Many methods/technologies for heating tissue
- Site-specific

Important physical concepts

- Energy absorption and temperature rise
- Redistribution of heat through conduction/heat loss
- Physical Constants: thermal conductivity, specific heat capacity, blood flow, perfusion
- Many tissue properties are temperature dependent, and spatially varying

Important biological concepts

- Cellular response to heat is time and temperature dependent
- Tissue type is an important consideration with respect to thermal sensitivity & absorption properties
- Vascular response to heat is dynamic
- Local/regional/systemic responses to heat injury

Tools of the trade...

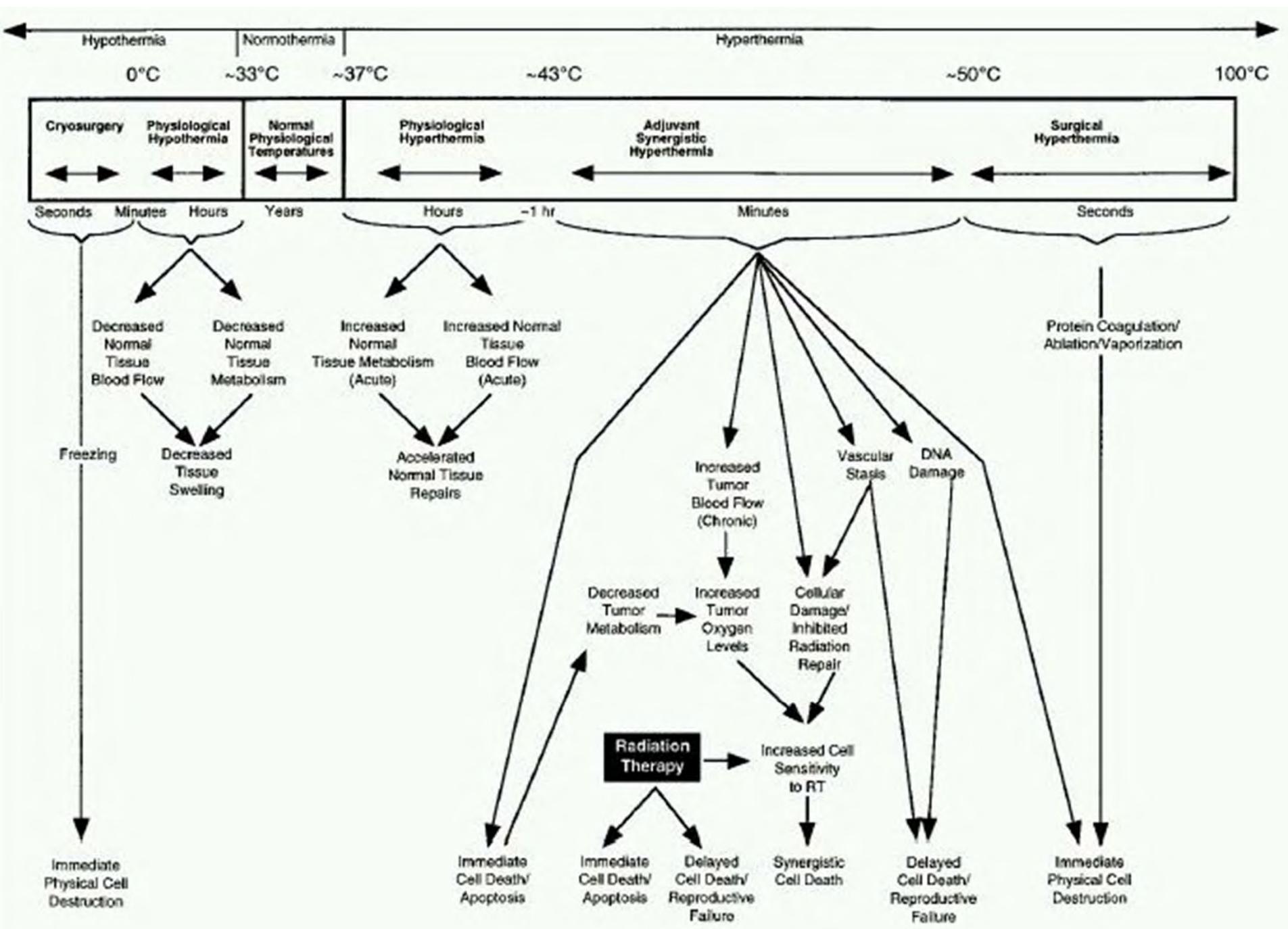
- Energy sources:
 - Optical, Microwave, RF, ultrasonic
- Temperature measurement
 - Invasive, non-invasive, image-based
- Computer modelling
 - Bio-Heat transfer equation

Unique advantages to heat...

- Normal tissue damage is not cumulative
 - Enables repeat treatments of tissue
- Non-linear response between temperature and cell death
- Heat triggers a number of host responses that are thought to be beneficial in treating a tumour
- Possibility of producing arbitrarily shaped treatment volumes with sharp margins (2-3mm)
- Non-invasive thermometry provides quantitative dosimetric information in the target volume
- Biological effects are evident on imaging immediately after treatment

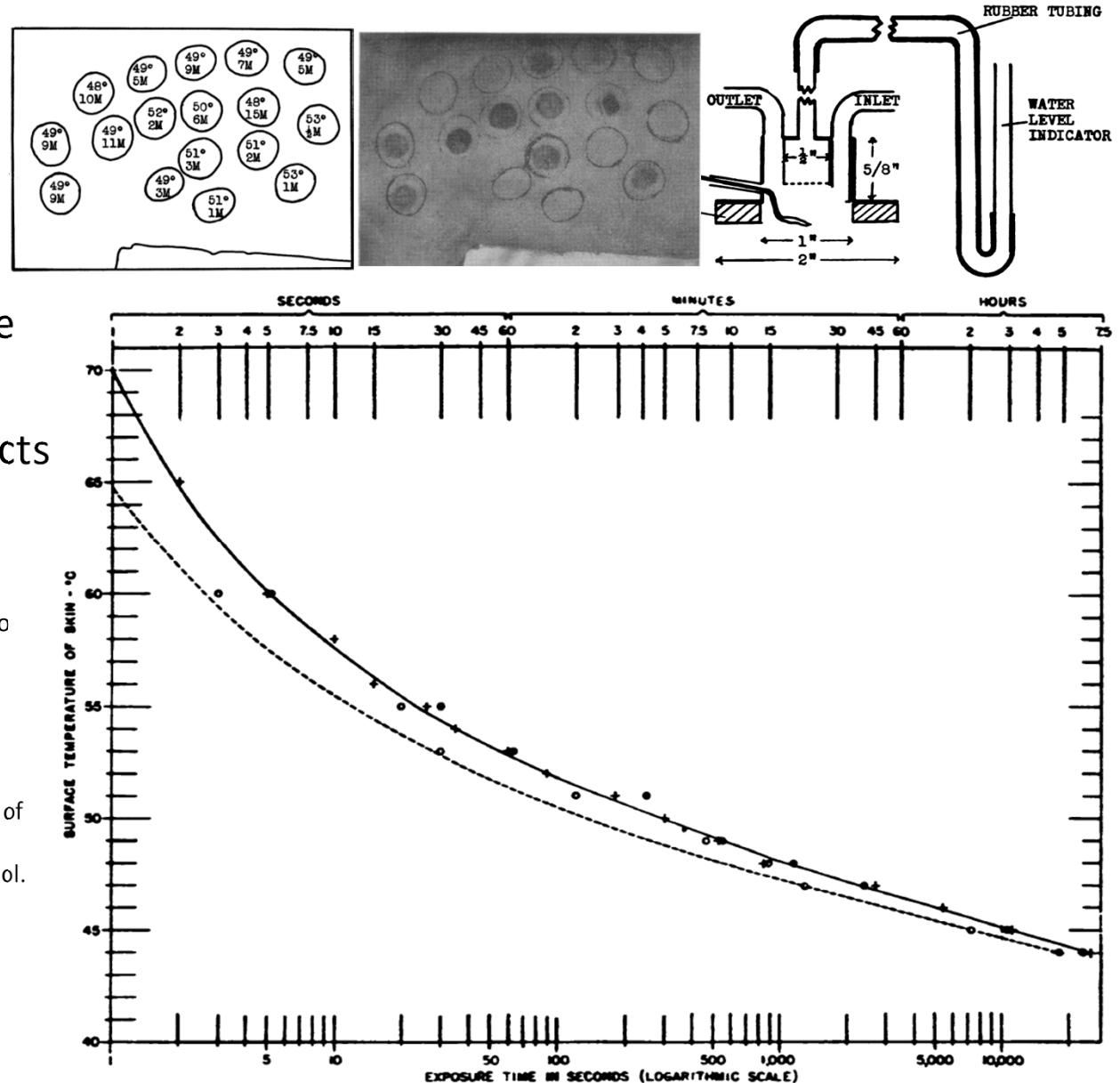
Unique challenges to heat...

- Tissue changes (blood flow, absorption properties) are dynamic during therapy
 - Challenging for uniform delivery of energy
- Tissue thermal and absorption properties have wide range in the literature, and difficult to measure



Biological effects of heat: Early Studies

- Tissue coagulation
- Edema
- Hemostasis, Hemorrhage
- Temperature and exposure duration dependent
- Immediate & delayed effects



Studies of Thermal Injury: I. The Conduction of Heat to and through Skin and the Temperatures Attained Therein. A Theoretical and an Experimental Investigation. Henriques FC, Moritz AR. Am J Pathol. 1947 Jul;23(4):530-49.

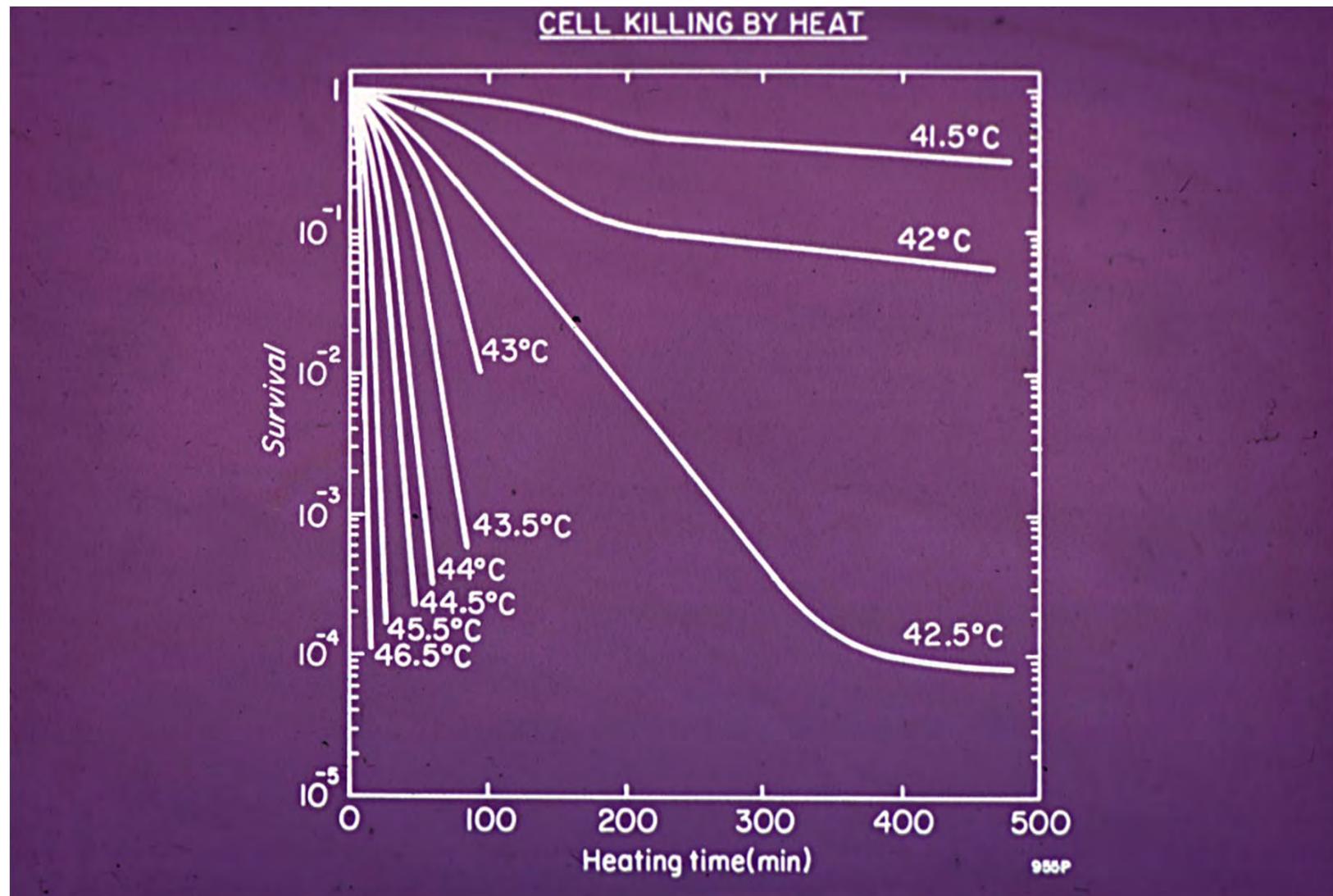
Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns. Moritz AR, Henriques FC. Am J Pathol. 1947 Sep;23(5):695-720.

Studies of Thermal Injury: III. The Pathology and Pathogenesis of Cutaneous Burns. An Experimental Study. Moritz AR. Am J Pathol. 1947 Nov;23(6):915-41.

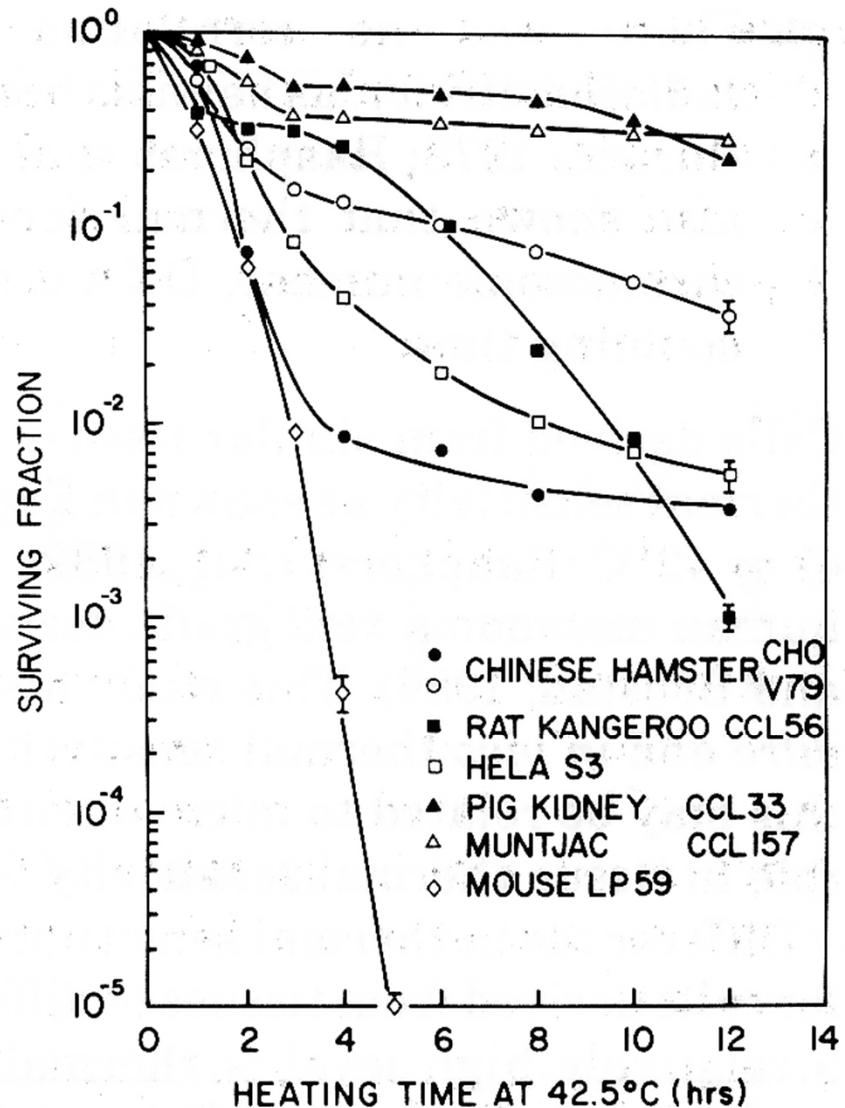
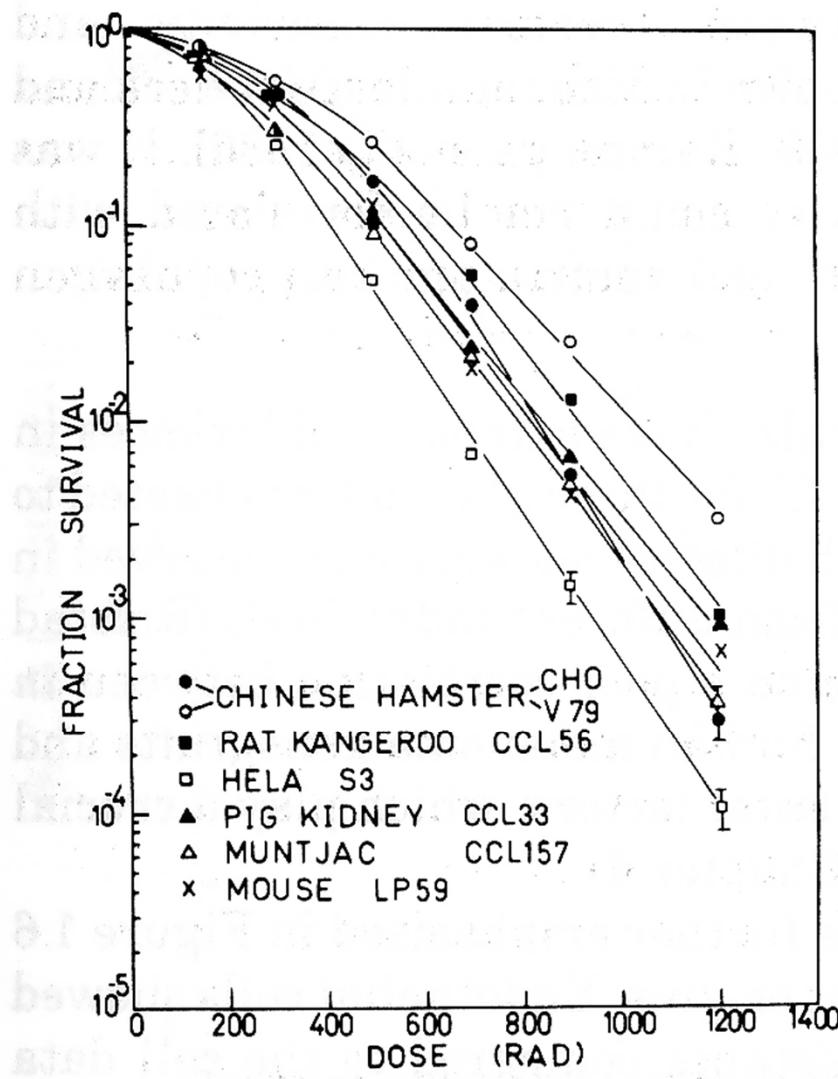
Hyperthermia

- Application of heat between 42-46°C for durations of minutes to hours
- Cellular responses to heat studied extensively in late 70's
- Cell death is stochastic, with higher probability of dying with increasing temperature and time of exposure

Cell survival curves



Variability in cell kill / cell line



Interaction between heat and radiation

- Complimentary damage
 - Hyperthermic damage in addition to radiation-induced damage
- Synergy
 - Inhibition of cellular repair to radiation damage
 - Improved tumour oxygenation due to increased blood flow
 - Cell cycle sensitivity differs

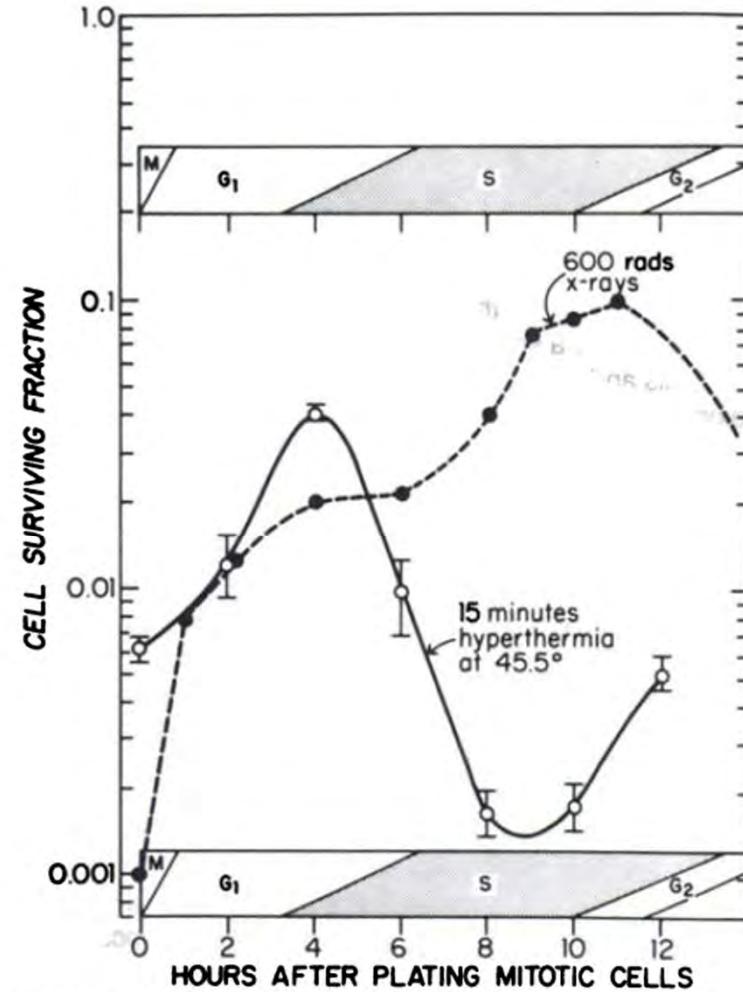


Chart 2. Comparison of the age response function, i.e., the pattern of sensitivity through the cell cycle, for cells exposed to heat or X-rays. Redrawn from Westra and Dewey (32).

Dewey et al, Radiology 1977

Hypoxia and thermal sensitivity

- Hypoxic cells tend to be more radioresistant
- In contrast hypoxic cells are slightly more heat sensitive
- Could be related to pH

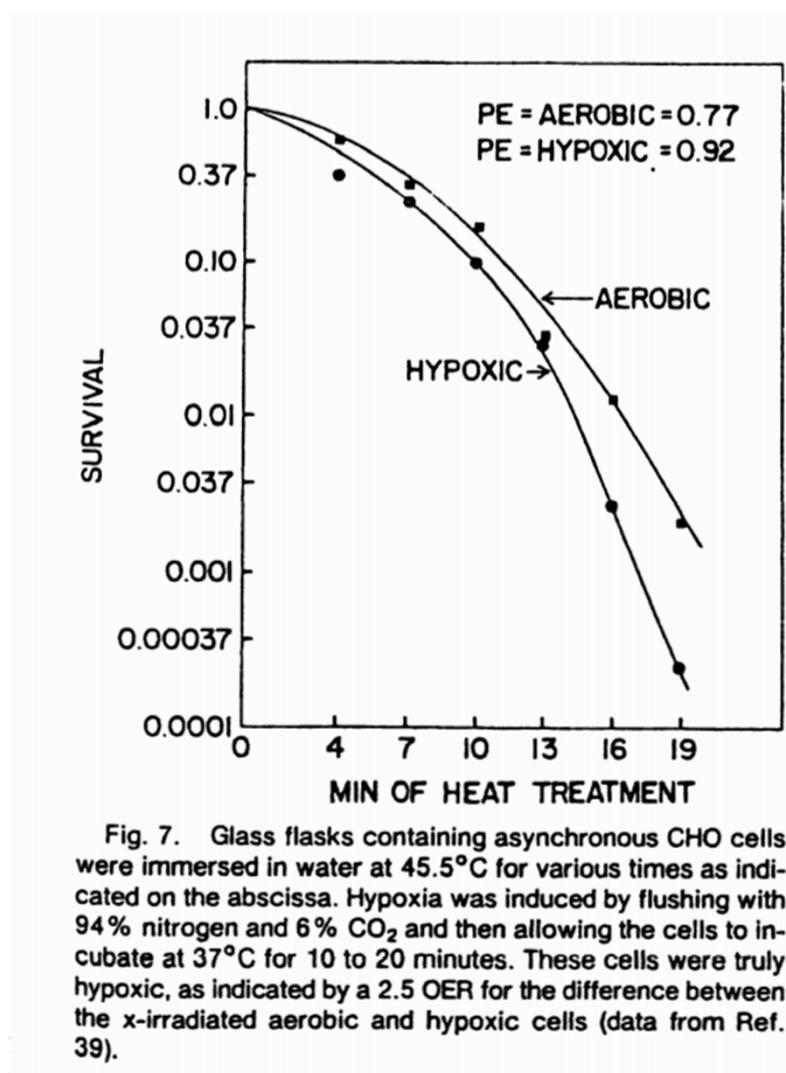


Fig. 7. Glass flasks containing asynchronous CHO cells were immersed in water at 45.5°C for various times as indicated on the abscissa. Hypoxia was induced by flushing with 94% nitrogen and 6% CO₂ and then allowing the cells to incubate at 37°C for 10 to 20 minutes. These cells were truly hypoxic, as indicated by a 2.5 OER for the difference between the x-irradiated aerobic and hypoxic cells (data from Ref. 39).

Effect of pH

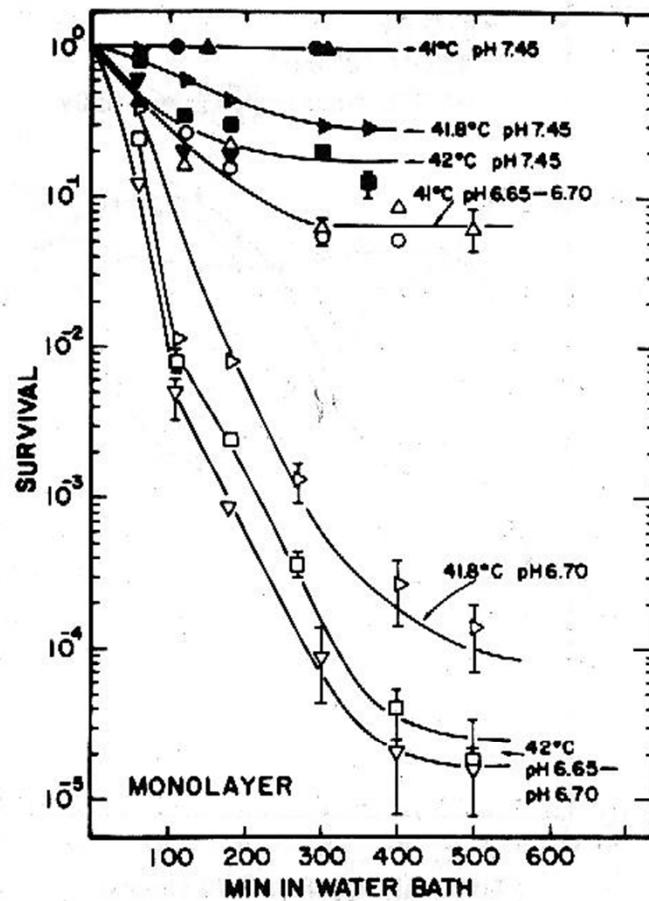


Figure 1.8 Survival of Chinese hamster ovary (CHO) cells heated in alkaline and acidic media. The pH was changed 2h before heating. (Data from Freeman et al. (1981) with permission.)

Effect of heat on cells (hyperthermia)

- Primary target is proteins, however chromosomes, cytoskeleton, membrane receptors also affected
- Onset of denaturation is 41-42°C in mammalian cells
 - Denaturation of ~5% of total cell protein required for onset of cell death
 - 95% of cells killed at 10% denaturation
- Activation energy for proteins is approximately 120-150 kcal/mol

INT. J. HYPERTERMIA
VOL. 19, NO. 3 (MAY-JUNE 2003), pp. 252-266



Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage

JAMES R. LEPOCK*

Department of Medical Biophysics, University of Toronto, and Ontario Cancer Institute, Princess Margaret Hospital, 610 University Avenue, Toronto, Ontario M5G 2M9 Canada

Thermotolerance

- Decrease in rate of cell killing as a function of time
- Observed primarily at temperatures below 43°C
- Can be induced at higher temperatures if cells previously exposed to temperatures for short duration
- Caused by induction of heat shock proteins
- Long lasting

Thermotolerance

[CANCER RESEARCH 38, 1843-1851, July 1978]
0008-5472/78/0038-0000\$02.00

Heat Fractionation and Thermotolerance: A Review¹

Kurt J. Henle and Lyle A. Dethlefsen²

Department of Radiology, University of Utah Medical Center, Salt Lake City, Utah 84132

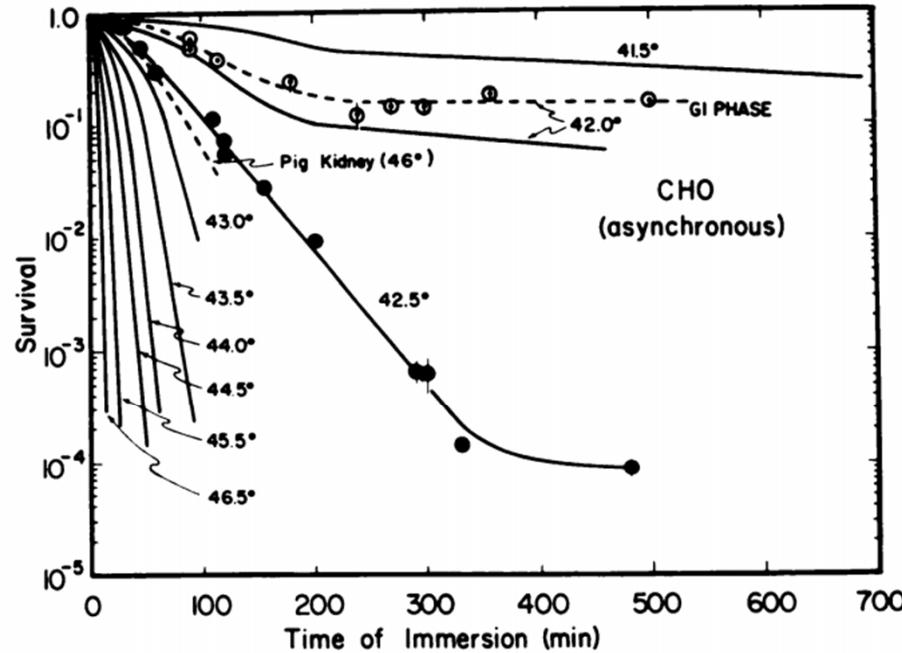


Chart 2. Development of thermotolerance in asynchronous CHO cells at 41.5–42.5° is illustrated by the appearance of biphasic survival curves during continuous hyperthermia. The survival curve for synchronized G₁ cells at 42° is similar to that for asynchronous cells [data with permission of the authors (12)].

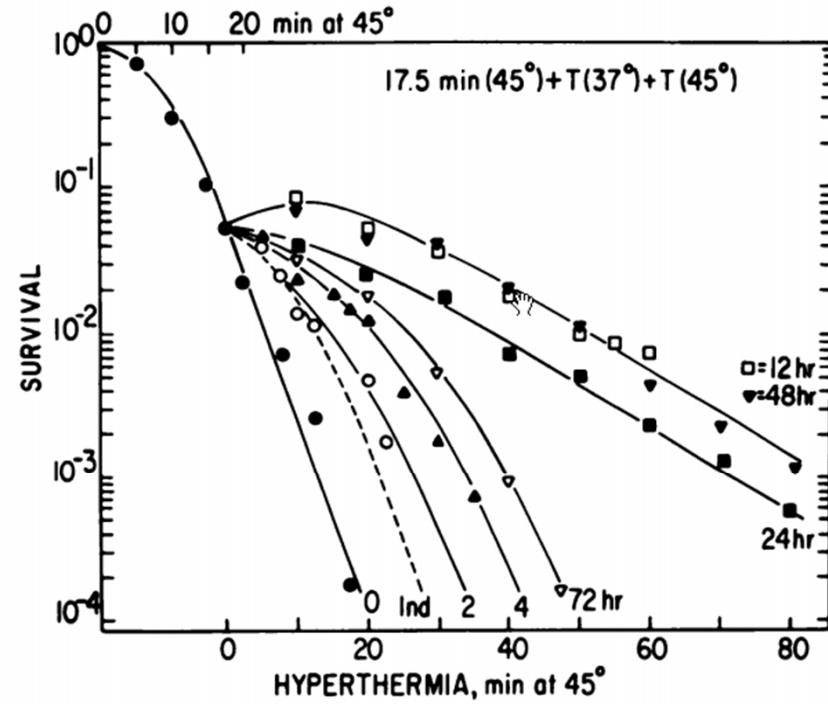


Chart 1. Development of thermotolerance in asynchronous CHO cells is illustrated by a series of survival curves obtained at various times after heat conditioning at 45° for 17.5 min. Top abscissa, duration of hyperthermia for the single treatment control curve; bottom abscissa, that for second treatment survival curves. The fractionation intervals are indicated in hr. The independent (Ind) curve represents the unconditioned control curve displaced downward for comparison (data from Ref. 33).

Thermotolerance

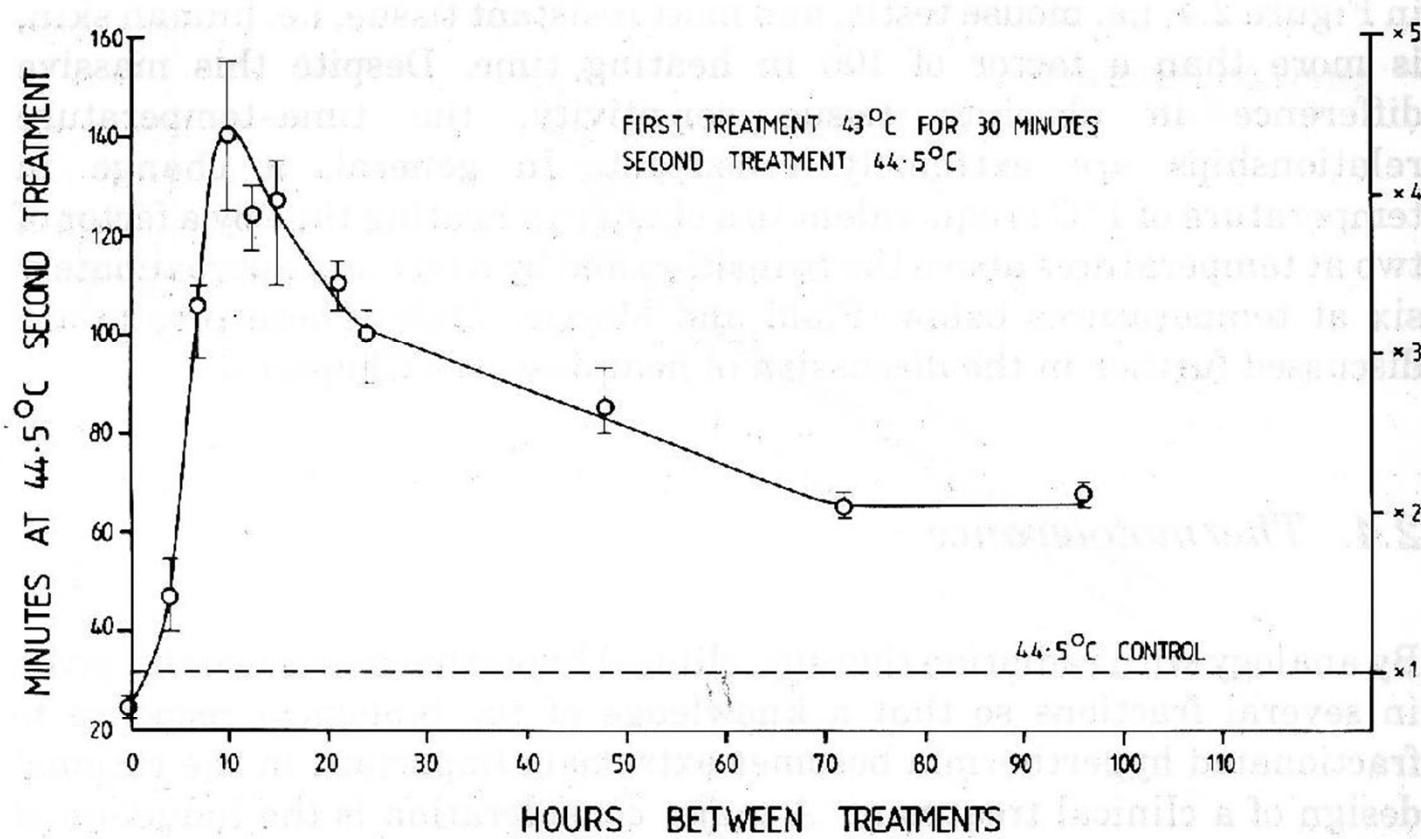


Figure 2.5 Thermotolerance in the rat tail following 43°C for 30 min, tested by measuring the time required to cause a given level of necrosis at 44.5°C at various intervals after the initial treatment. The fine line shows the effect in the absence of prior treatment. (From Field and Morris (1985).)

High Temperature Thermal Therapy

- Elevate tissue temperatures to levels sufficient to cause rapid irreversible damage
- Generally less sensitive to effects of blood flow
- Primary mechanism is thermal coagulation (55°C – 90 °C)
- Required heating times < 1 minute
- Tissue vaporization, boiling, charring occurs at higher temperatures
 - Usually results in undesirable changes in tissue properties

Thermal Coagulation: Macroscopically

Microscopic

- Denaturation and aggregation of cellular components

Physiological:

- fibrosis – formation of extra fibrous tissue in an organ as a mechanism of repair
- collapse of vessels -> no more blood flow to tissue
- ultimate result = cell and tissue necrosis

Physical:

- changes in optical properties: scattering, absorption
- changes in density and elasticity



Biological effects of heat

High temperature thermal therapy:

- **Acute Response:** Tissue inflammation leading to edema & swelling
- **Chronic response:** Removal of necrosed tissue by body; scar formation; tissue shrinkage

Example: Prostate Tissue

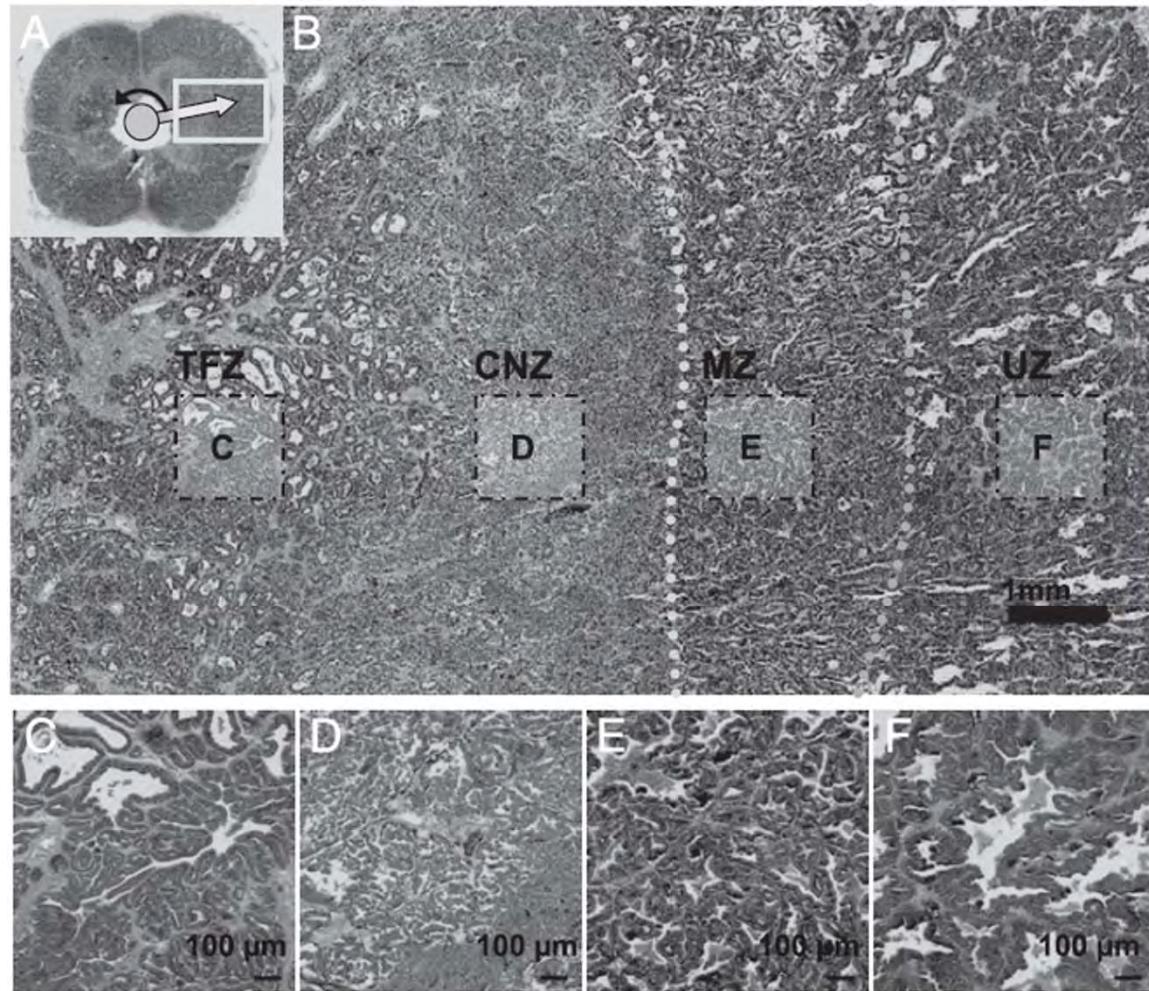


FIG. 5. A, whole mount 5 μm section of canine prostate stained with H & E. Graphic indicates device position in urethra and rotation direction. Rectangle indicates area of B. B, thermal damage boundaries. C to F, details of areas in B. C, TFZ. D, CNZ. E, MZ. F, UZ.

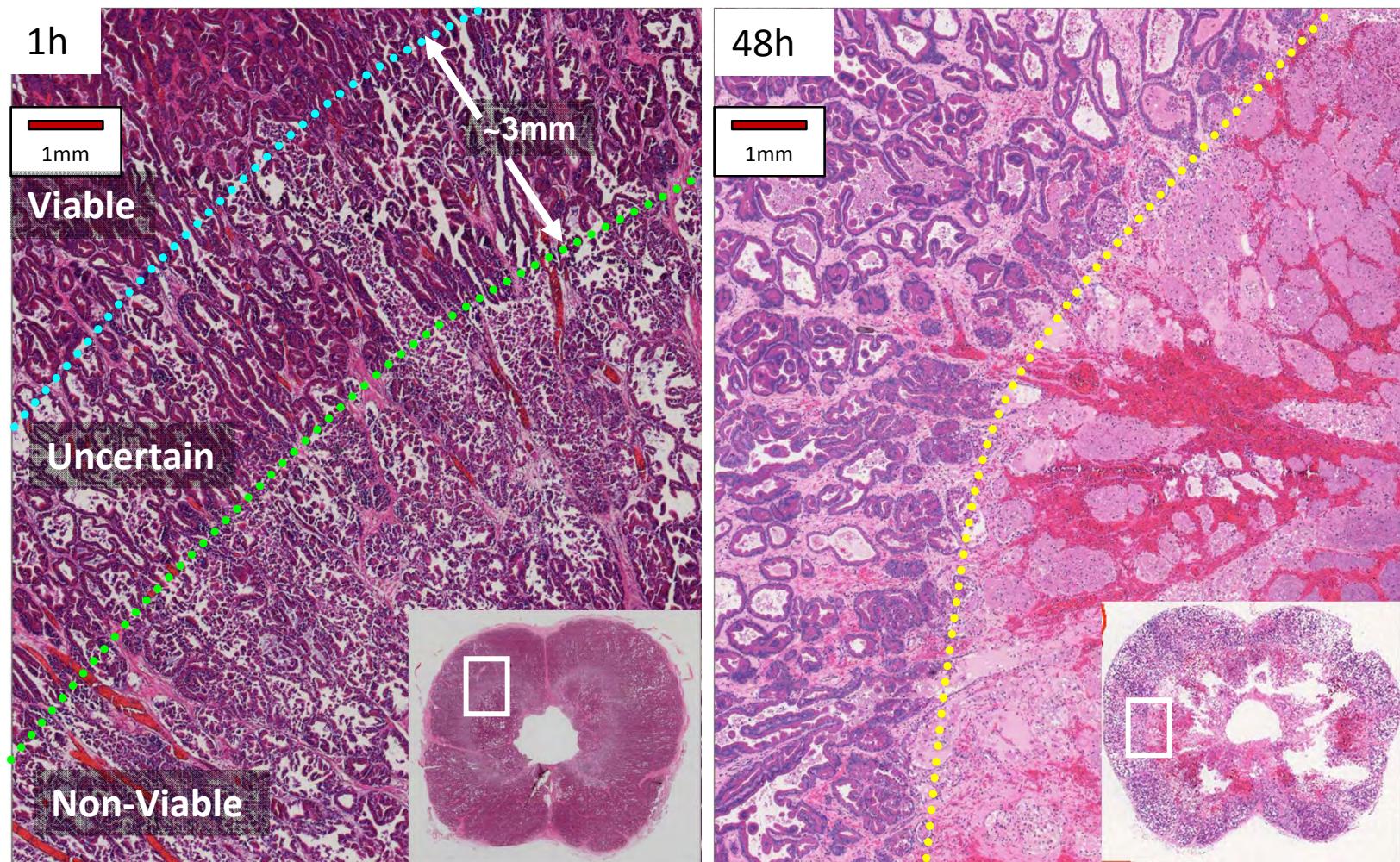
Boyes et al, J Urol, 2007



Thermal Biology

Sharp transition from thermal coagulation to normal tissue

- Margin of 2-3 mm subsides 48 hours after treatment



Chronic Effects

ENHANCED IRREGULARITY OF LESION

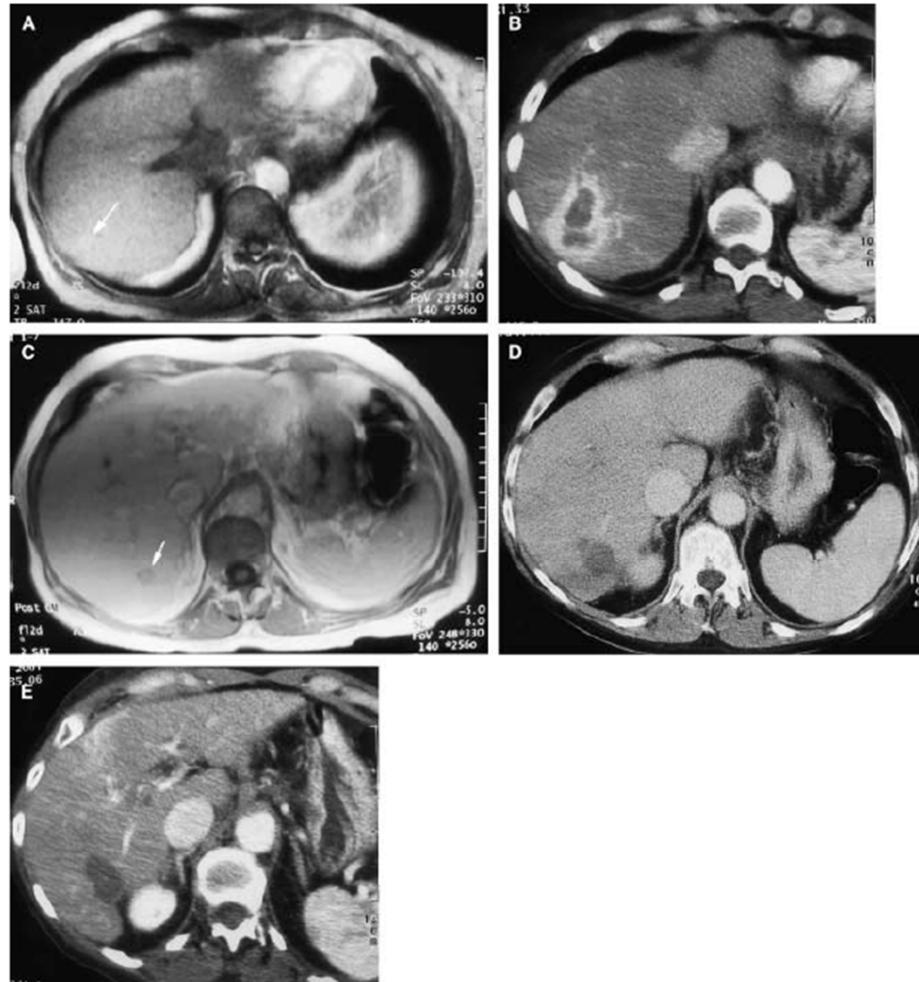


Fig. 1. Imaging studies of patient 1. (A) Magnetic resonance image, showing an enhancing 0.9-cm lesion in segment 7 (arrow – lesion 2). (B) Computer tomography scan, showing lesion in Fig. 1A immediately after ablation. Note the larger size of the lesion, as compared with the initial observed lesion (Fig. 1A). (C) Magnetic resonance image of the first lesion before ablation (arrow). (D) Computer tomography scan of the first treated lesion, taken 4 months after ablation of the first lesion. (E) Computer tomography scan of the first treated lesion, taken 8 months after ablation of the first lesion. This first lesion remained stable in size and does not demonstrate areas of enhancement.

Coad et al, Clinical Transplantation, 2003

Thermal Fixation

- Preservation of cellular architecture resembling normal tissue, but not viable
- Can be mistaken for untreated tissue
- Tends to happen at the highest temperatures
- Appears to result from denaturation of structural and enzyme protein constituents, resulting in resistance to breakdown by body's repair pathways
- Often results in preserved scar tissue within the treated region

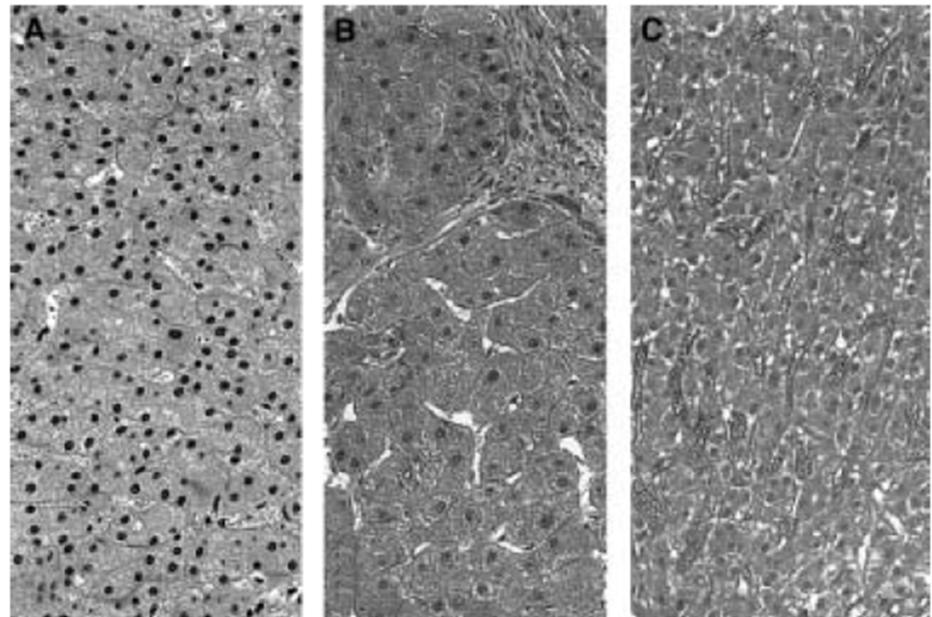
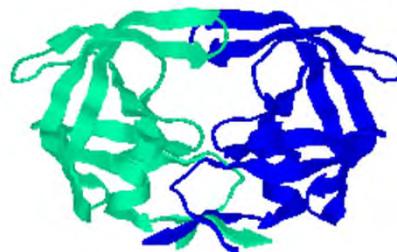


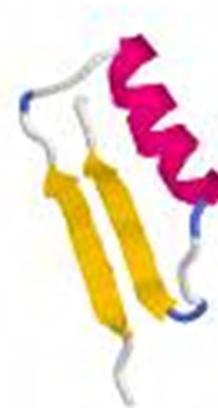
Fig. 3. Photomicrographs demonstrating 'thermal fixation' with preservation of both architectural and cytologic detail of the central tumor at: (A) 4 d after ablation (patient 1), (B) 9 months after ablation (patient 1) and (C) 14 months after ablation (patient 4). The cellular staining characteristics slowly faded post-ablation, but the structural features of the tissue components were preserved without signs of breakdown.

Thermal Coagulation: Microscopically

- denaturation of the intracellular and extracellular proteins
- disruption of cells' activity
- aggregates formed of both native and denatured proteins
- dissolution and melting of cells' membranes



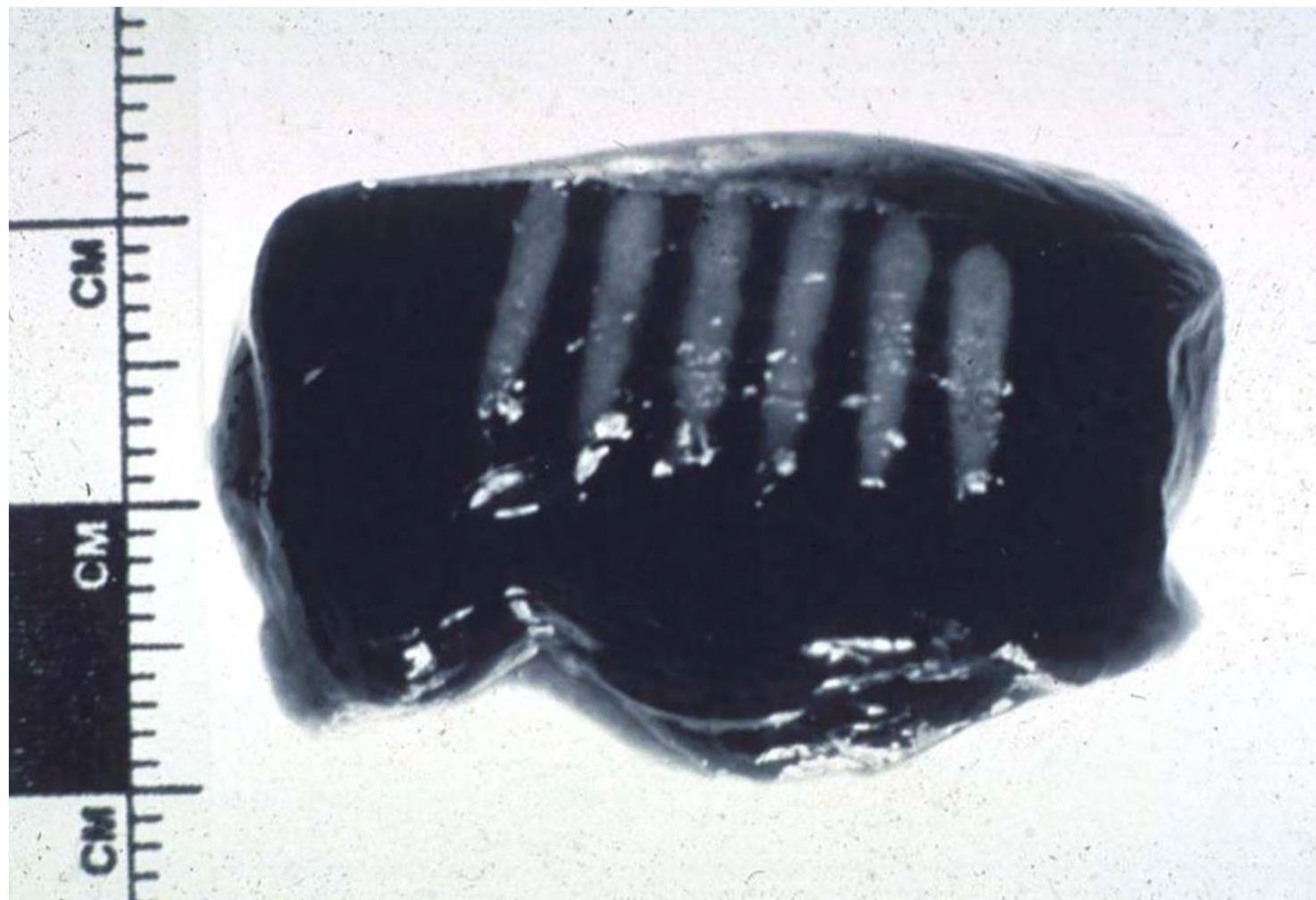
Quaternary structure:
-subunits dissociated
-spatial arrangement of subunits disrupted



Tertiary structure:
-breaking of covalent interactions between aa sidechains
(ie. disulfide bonds)
-dipole-dipole interactions
-van der-Waals interactions

Secondary structure:
-alpha helices and beta pleated sheets dissociated

Coagulation by HIFU



Quantifying cell killing with heat

- Eyring (Arrhenius) equation – relates reaction rate to temperature

$$k = \frac{k_b T}{h} e^{-G/RT} = A e^{-E_a/RT}$$

$$\ln(k) = \ln(A) - \left(\frac{E_a}{R} \right) \frac{1}{T}$$

- k (reaction rate) = units sec⁻¹
- R (universal gas constant) = 1.98 cal/K/mol
- Ea (inactivation energy) = units of kcal/mol
- T (temperature) = units of K
- A (constant) = assumed constant over temperature range of study;

Cell Survival vs. Heating

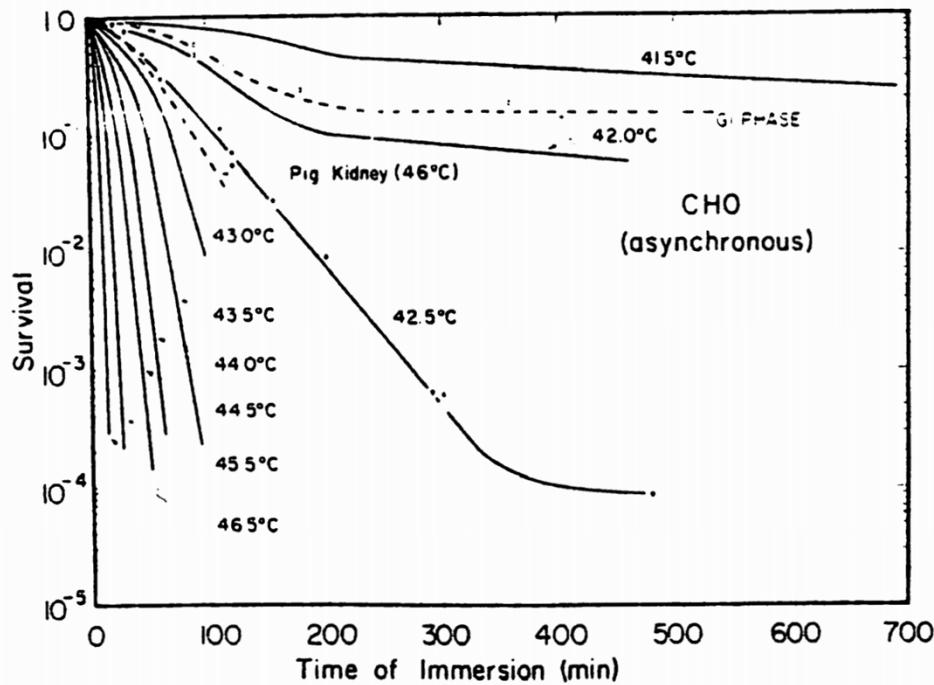


Fig. 1. Survival curves for asynchronous Chinese hamster ovary (CHO) cells heated at different temperatures for varying lengths of time.

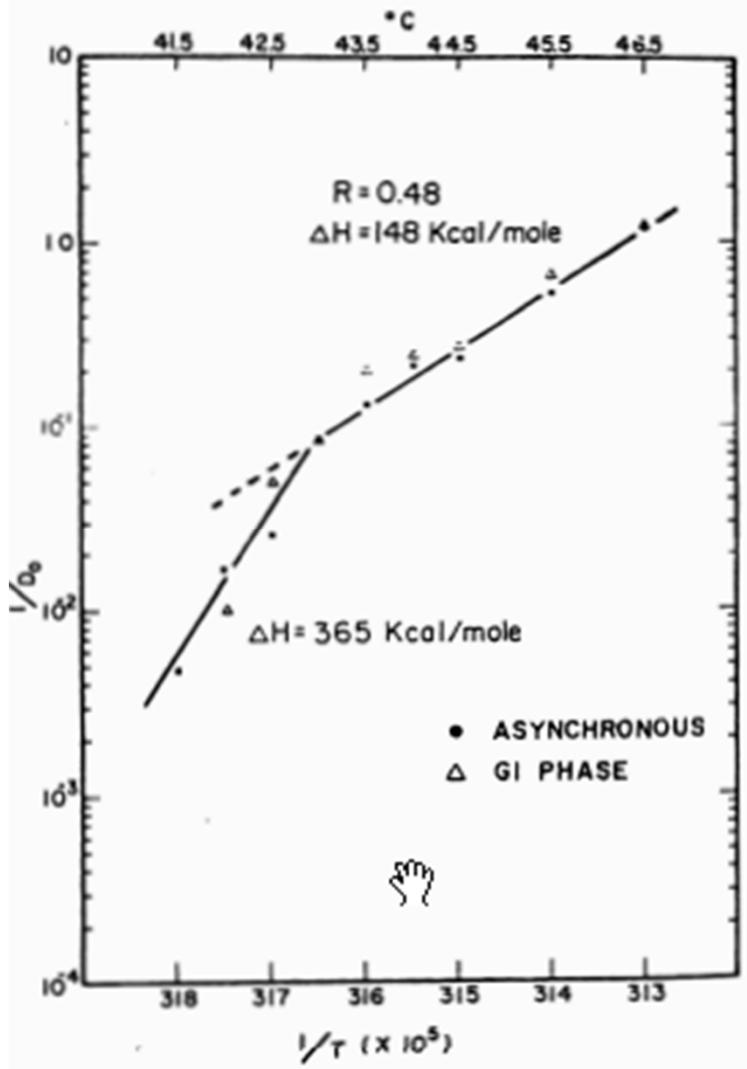
$$k = A e^{-E_a/RT}$$

- Define D_o = time required to reduce survival by $1/e$; $k = 1/D_o$

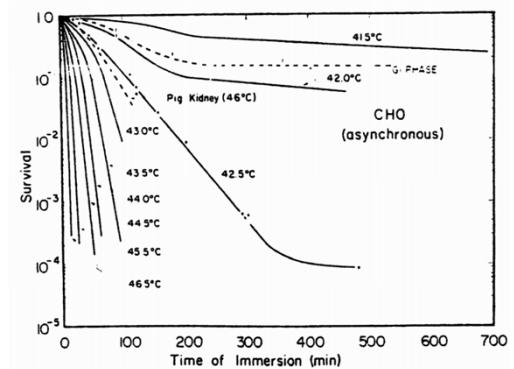
$$\ln(k) = \ln(A) - \left(\frac{E_a}{R} \right) \frac{1}{T}$$

$$\ln\left(\frac{1}{D_o}\right) = \ln(A) - \left(\frac{E_a}{R} \right) \frac{1}{T}$$

Arrhenius Plots

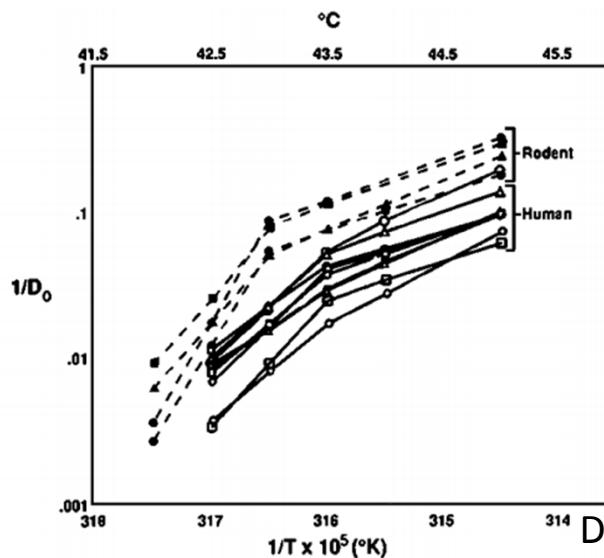


$$\ln\left(\frac{1}{D_o}\right) = \ln(A) - \left(\frac{E_a}{2}\right)\frac{1}{T}$$



$$R = \frac{D_o^{T+1}}{D_o^T} = \frac{e^{E_a/2(T+1)}}{e^{E_a/2(T)}} = e^{-E_a/(2T(T+1))}$$

= 0.5 for $T > 43$, 0.25 for $T < 43$



Dewey et al, Radiology, 1977

Biological Variability

- Similar trends seen in multiple tissue types

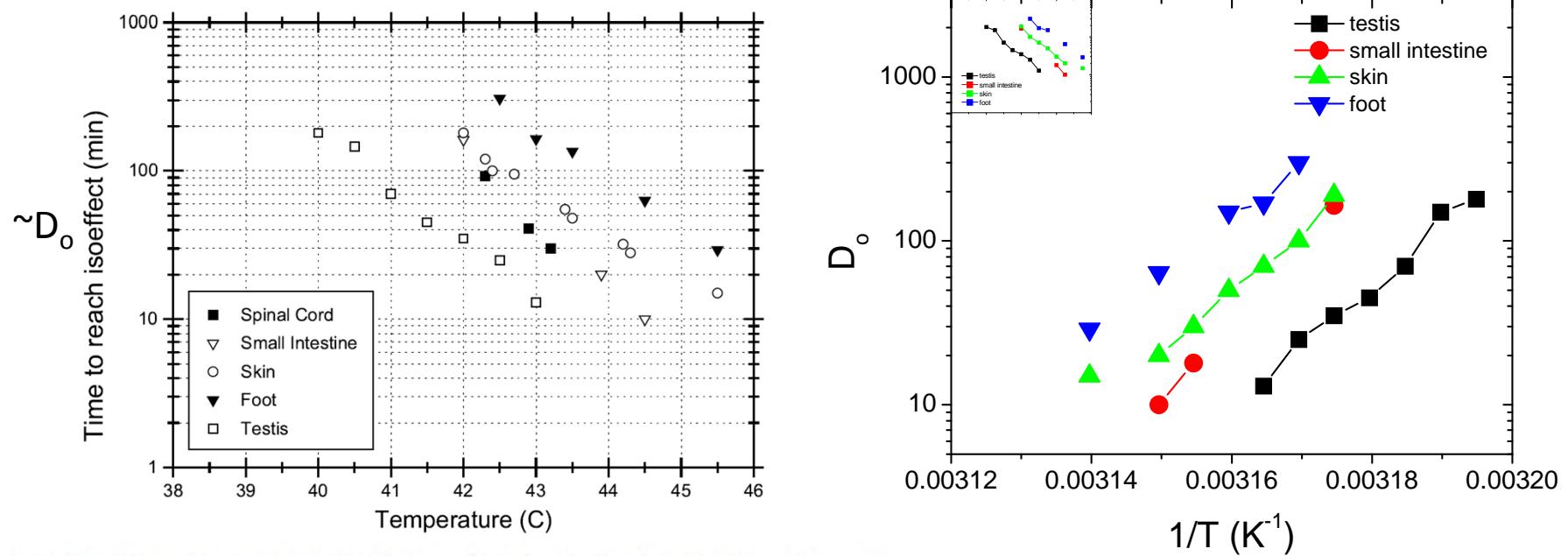


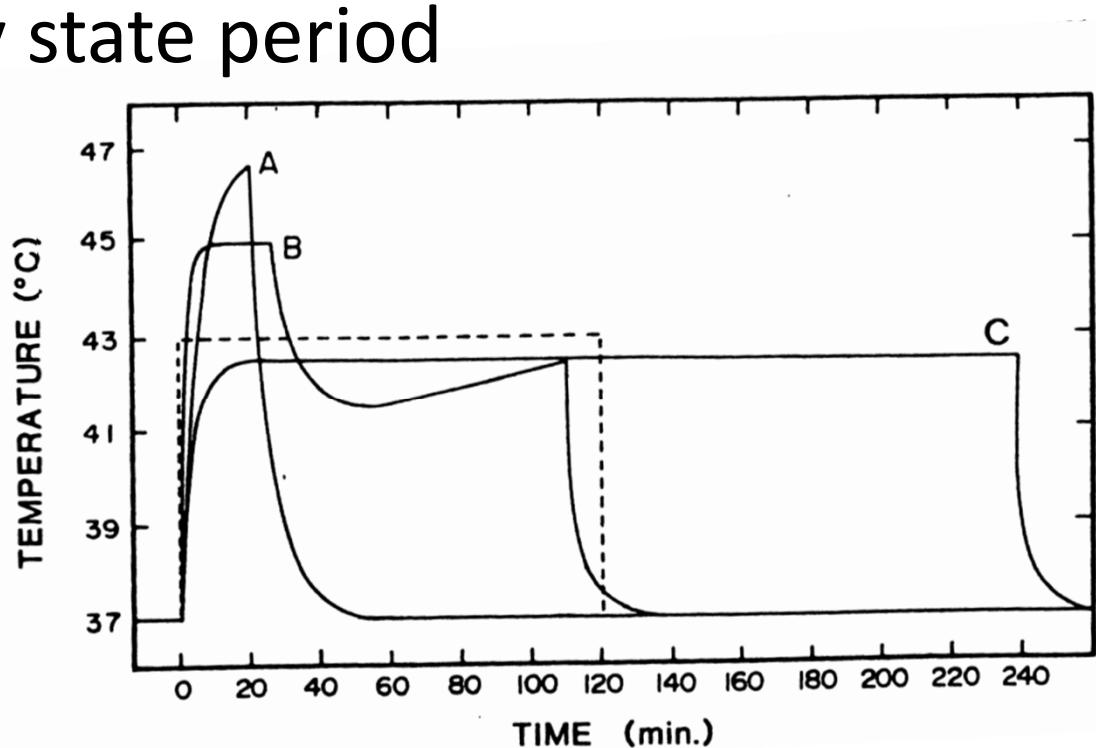
Figure 5. Time-temperature relationships to achieve isoeffective thermal damage in several mouse tissues. Note that the slopes for these isoeffects are parallel, but some tissues appear more sensitive than others (i.e. the thresholds for thermal damage vary from one tissue to the next). There are multiple reasons for this, some of which may not relate to actual differences in tissue sensitivity (see text for details).

Effect of heat on cells

- Exponential relationship between time & temperature both *in vitro* and *in vivo*
- The ***increase*** in the rate of cell killing with temperature is relatively constant (for $T>43$, $T<43$)
- These properties seem to be conserved across multiple cell types, even though sensitivity to heat will differ

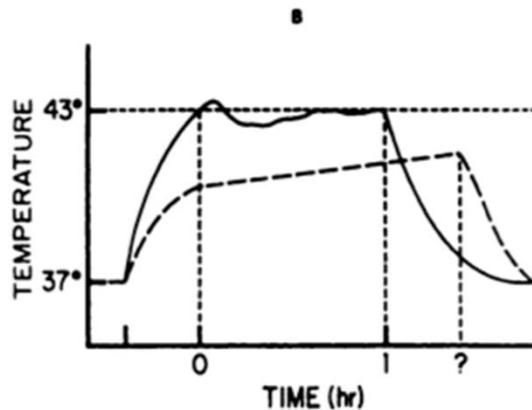
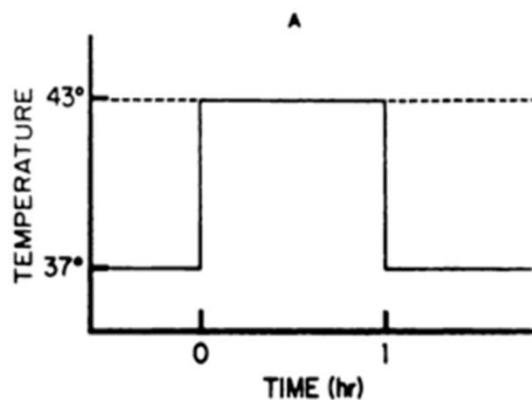
Thermal Dose

- Sapareto & Dewey, IJROBP 1984
- Clinical situations involve ramp-up of temperatures, cool down, fluctuations during steady state period



Thermal Dose

- Goal of this formulation is to relate all time-temperature curves back to a single temperature, chosen arbitrarily as 43°C
- Not the only way to perform thermal dosimetry, but has become widely adopted and the standard in the field



$$\left(\frac{1}{D_o} \right) = A \exp\left(\frac{E_a}{2T} \right)$$

Assume two treatments, first at T1, resulting in isosurvival time D1, and another at T2 with D2

$$\frac{D_1}{D_2} = \exp\left(\left[\frac{E_a}{2} \right] \left[\frac{1}{T_2} - \frac{1}{T_1} \right] \right)$$

$$D_1 = D_2 \exp\left(\frac{E_a}{2} \left[\frac{T_1 - T_2}{T_1 T_2} \right] \right)$$

$$t_1 = t_2 R^{(T_1 - T_2)}$$

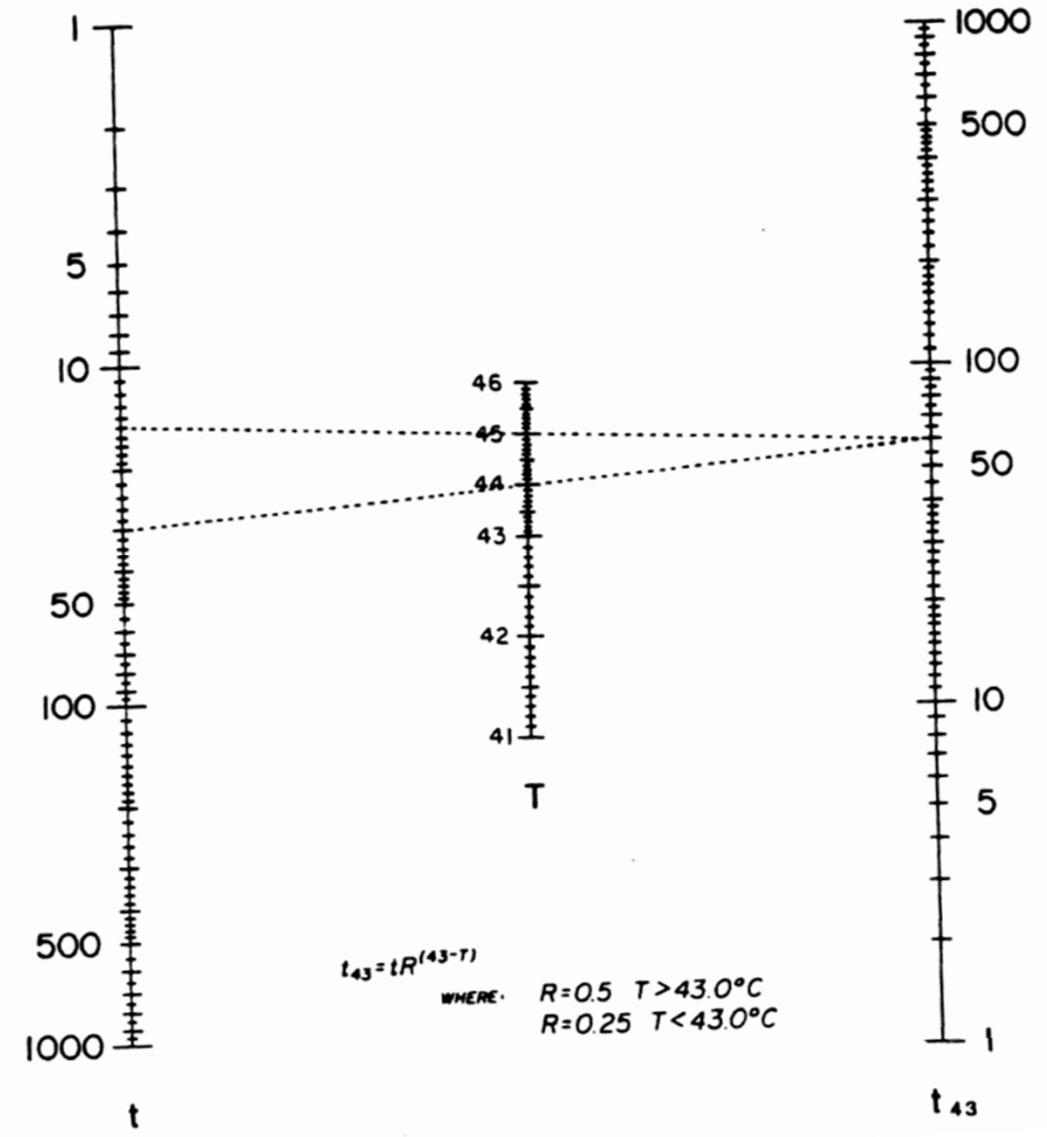
$$R = e^{-E_a/(2T_1 T_2)} \approx e^{-E_a/(2T(T+1))}$$

$$R = \begin{cases} 0.5, & T \geq 43^\circ C \\ 0.25, & T < 43^\circ C \end{cases}$$

Thermal Dose

$$t_1 = t_2 R^{(T_1 - T_2)}$$

- Ex 1: $T_2 = 44^\circ\text{C}$, $t_2 = 30 \text{ min}$; how long is required at $T_1 = 45^\circ\text{C}$?
 - $t_1 = 30 * (0.5)^{(45-44)} = 15 \text{ minutes}$.
- Ex 2: $T_2=44^\circ\text{C}$, $t_2=30 \text{ min}$; What is the time required at 43°C ?
 - $30 = t_2 * (0.5)^{(44-43)} = 60 \text{ minutes}$
- Nomogram developed for straightforward calculation of iso-effective dose



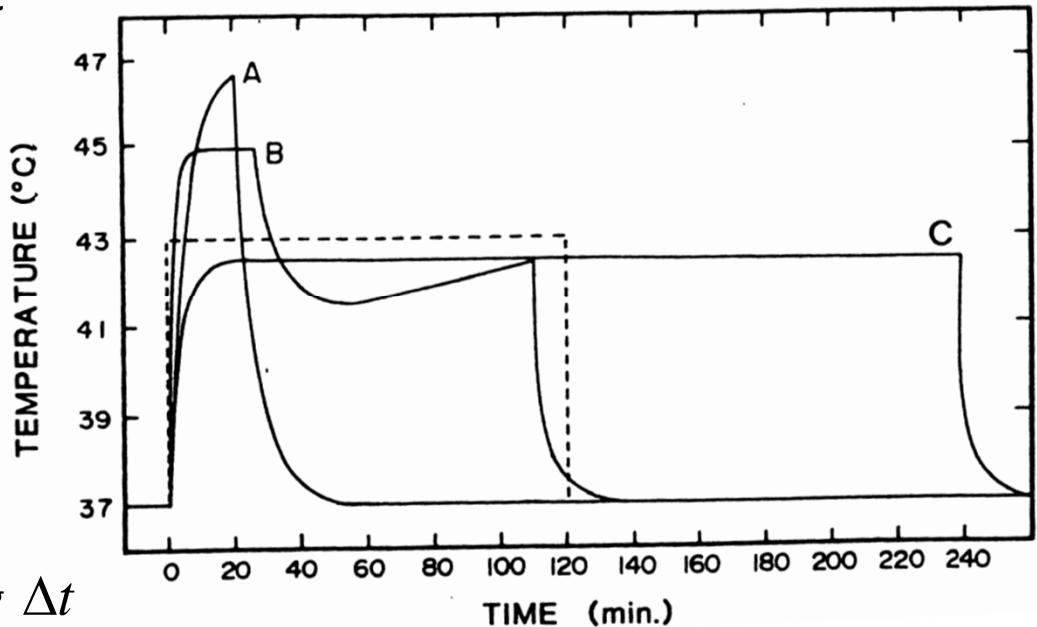
Thermal Dose

- Important extension is to time-varying heating profiles
- Discretize temperature profile into small time steps (Δt)

$$t_{43} = \sum_{t=0}^{t=final} R^{(43-\bar{T})} \Delta t$$

t_{43} : Equivalent time at $43^\circ C$

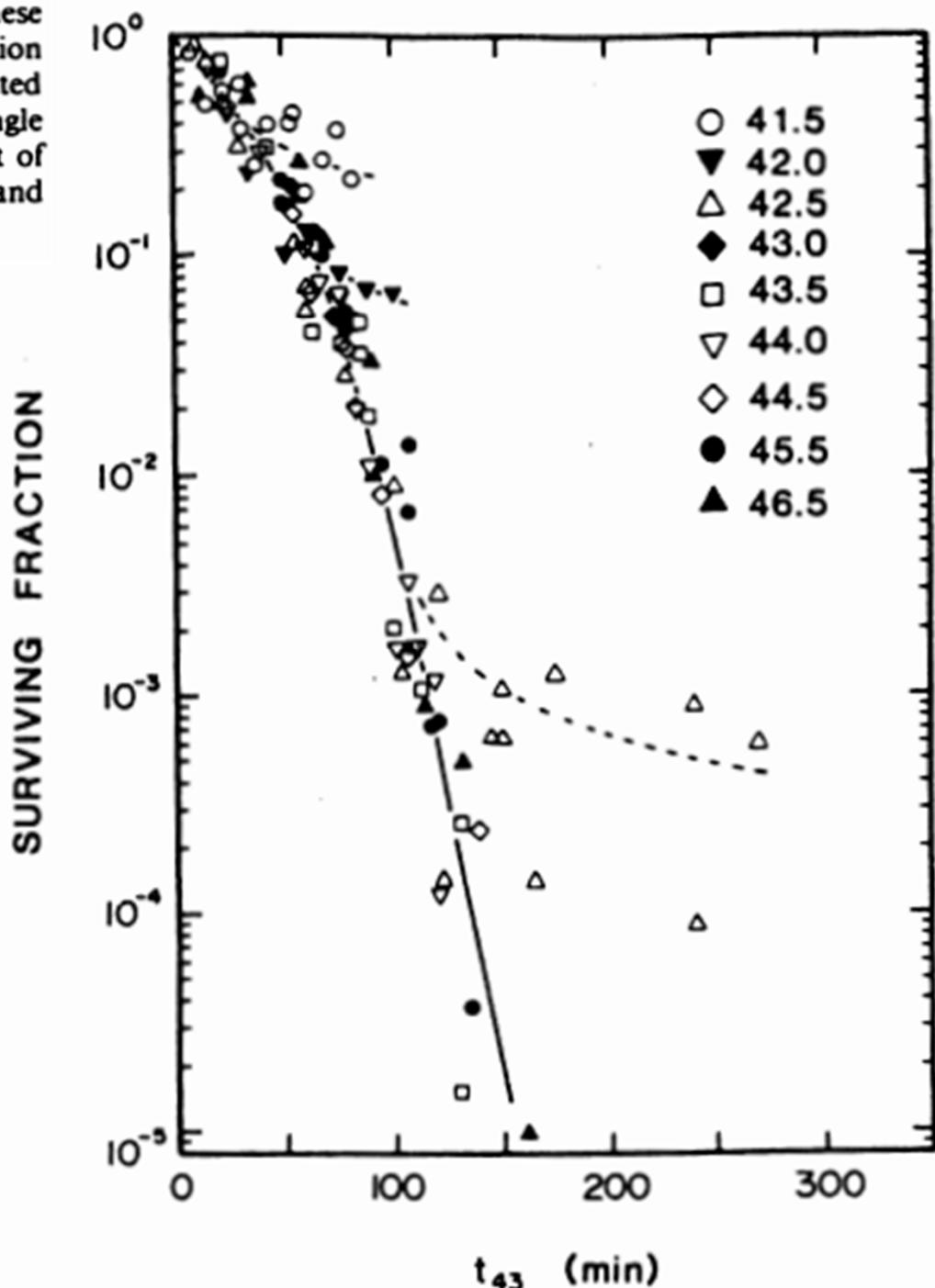
\bar{T} : Average temperature during Δt



- All of the above temperature profiles have a t_{43} of 120 minutes.

Fig. 5. The dose survival response for asynchronous Chinese hamster ovary cells at various temperatures plotted as a function of equivalent-minutes at 43°C. Error bars have been omitted for clarity. 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. Actual data are taken from reference 29 and replotted.

- Valid for tissues with different thermal sensitivity
- Only the threshold thermal dose required for cell death changes



Higher temperatures?

- Heated baby hamster kidney cells for different durations at temperatures between 43.5 and 57°C
- Measured colony formation after heating to evaluate cell survival
- Logarithmic time-temperature relationship observed at all temperatures
 - Surviving fraction of 0.001 achieved in 3-4 seconds at 55°C
- Used to support validity of thermal dose model at elevated temperatures

Borrelli et al, Time-temperature analysis of cell killing of BHK cells heated at temperatures in the range of 43.5 degrees C to 57.0 degrees C, IJROBP, 1990

TIME-TEMPERATURE ANALYSIS OF CELL KILLING OF BHK CELLS HEATED AT TEMPERATURES IN THE RANGE OF 43.5°C TO 57.0°C

M. J. BORRELLI, PH.D.,^{1,3} L. L. THOMPSON, M.S.,¹
C. A. CAIN, PH.D.² AND W. C. DEWEY, PH.D.¹

I. J. Radiation Oncology • Biology • Physics August 1990, Volume 19, Number 2

- Heated BHK cells for different durations at temperatures between 43.5 and 57°C
- Logarithmic time-temperature relationship observed at all temperatures
 - Surviving fraction of 0.001 achieved in 3-4 seconds at 55°C
 - E_a found to be 120-126 kcal/mol
- Used to support validity of thermal dose model at elevated temperatures

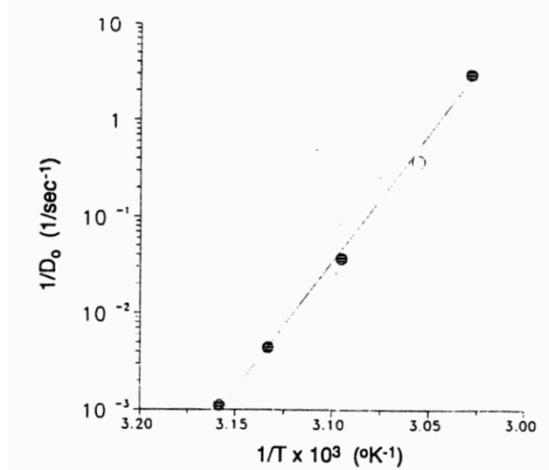
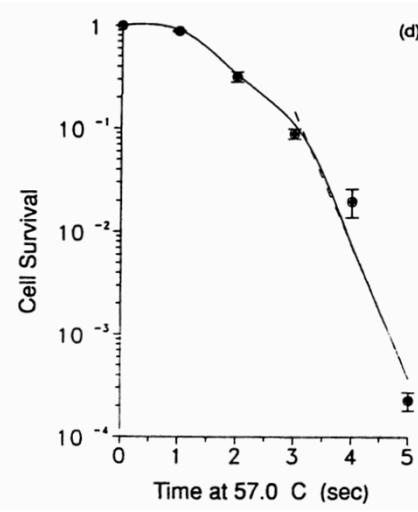
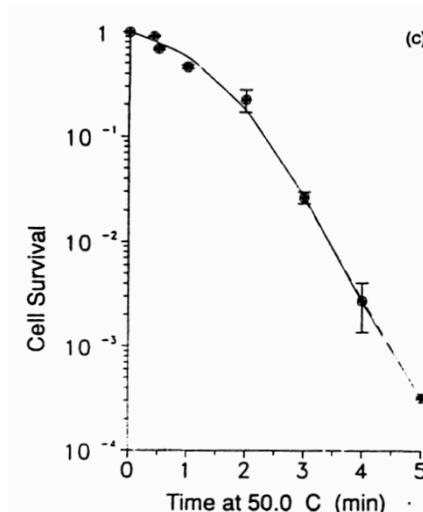
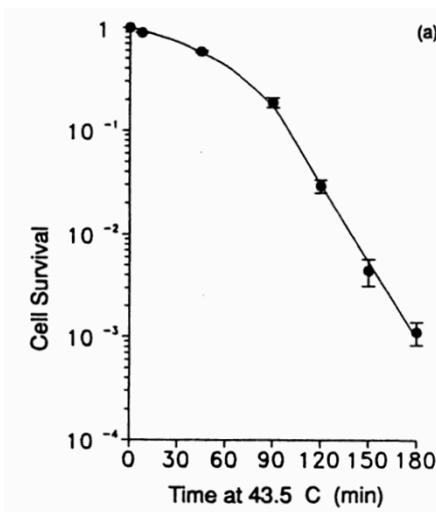


Fig. 5. Time-temperature relationship plot of the logarithm of $1/D_0$ versus $1/T$. The data points are larger than the standard error of the mean. The open circle represents datum from one experiment at 54.0°C.

TIME-TEMPERATURE ANALYSIS OF CELL KILLING OF BHK CELLS HEATED AT TEMPERATURES IN THE RANGE OF 43.5°C TO 57.0°C

M. J. BORRELLI, PH.D.,^{1,3} L. L. THOMPSON, M.S.,¹
C. A. CAIN, PH.D.² AND W. C. DEWEY, PH.D.¹

I. J. Radiation Oncology • Biology • Physics

August 1990, Volume 19, Number 2

- Heated BHK cells for different durations at temperatures between 43.5 and 57°C
- Logarithmic time-temperature relationship observed at all temperatures
 - Surviving fraction of 0.001 achieved in 3-4 seconds at 55°C
 - E_a found to be 120-126 kcal/mol
- Used to support validity of thermal dose model at elevated temperatures

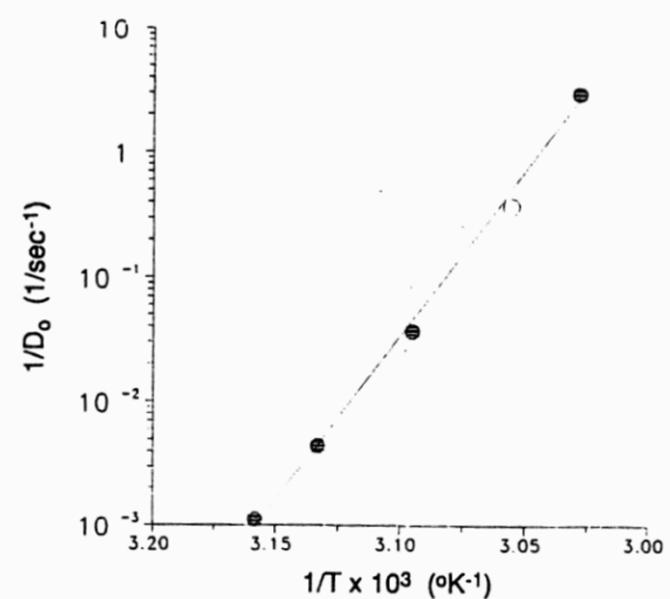
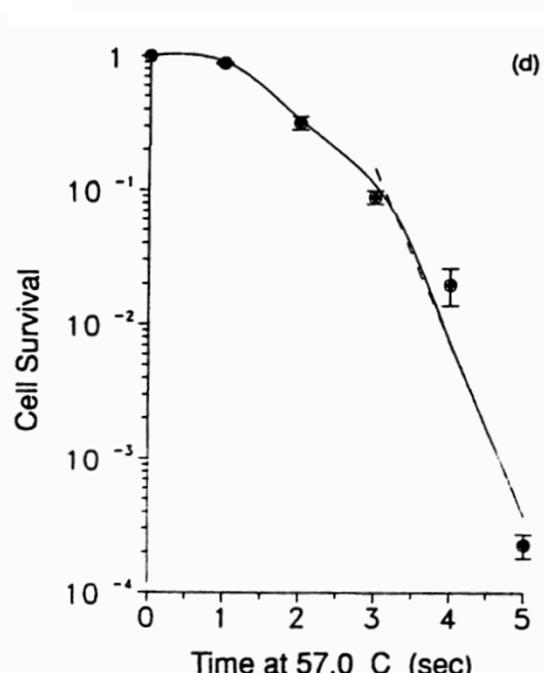
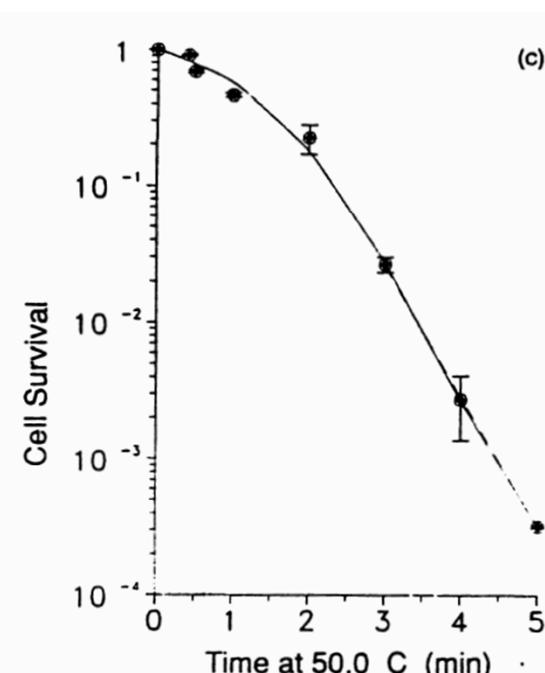
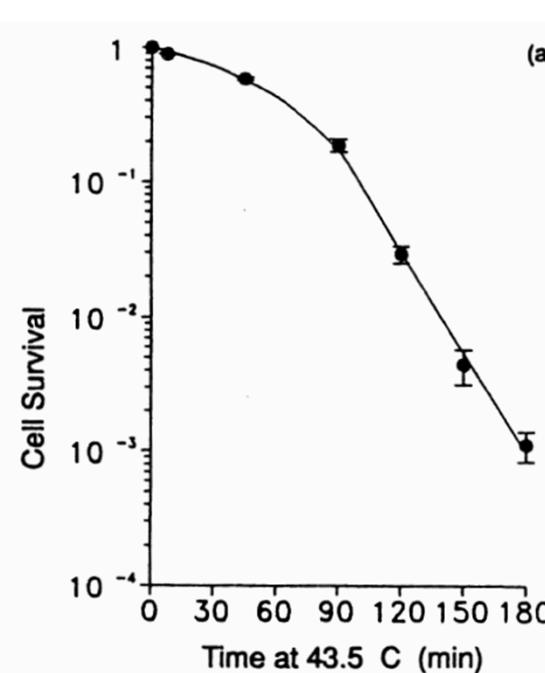
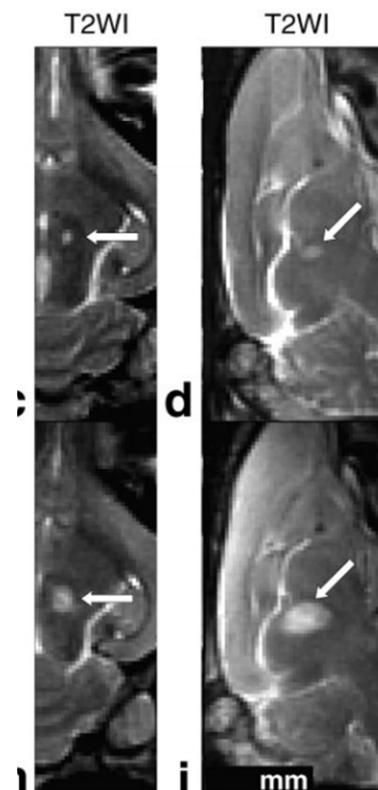
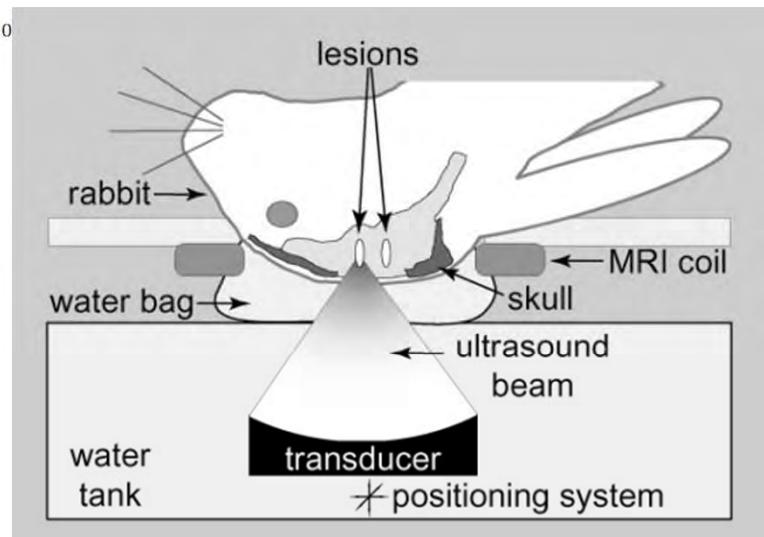
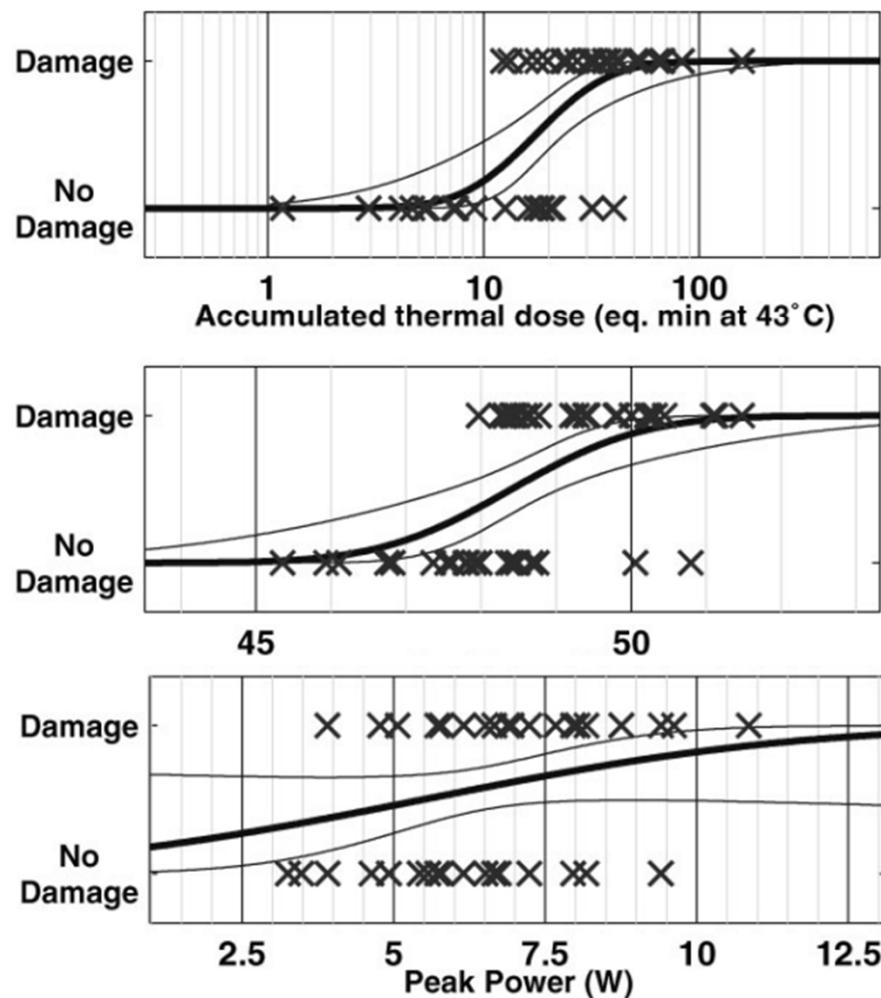
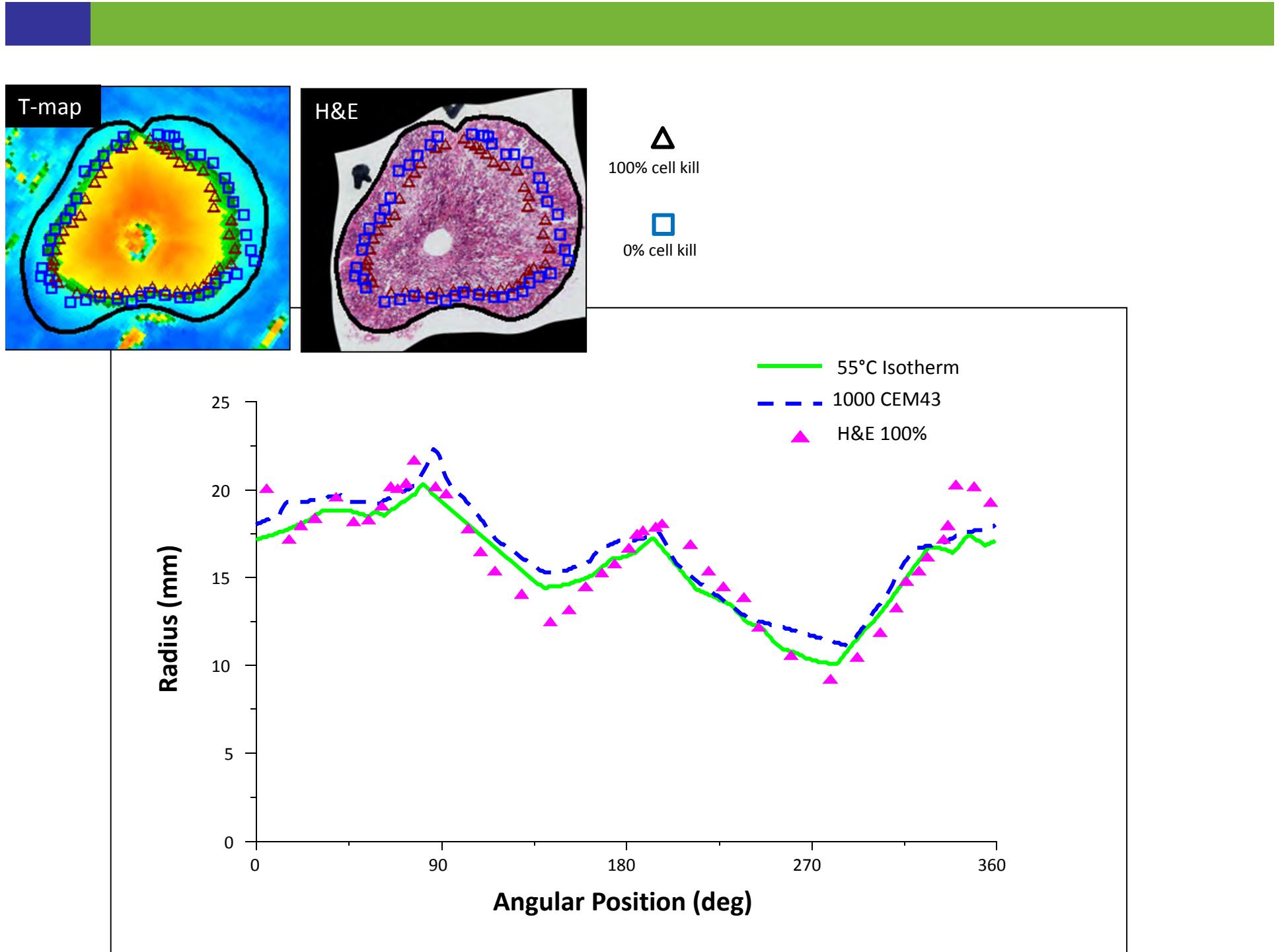


Fig. 5. Time-temperature relationship plot of the logarithm of $1/D_0$ versus $1/T$. The data points are larger than the standard error of the mean. The open circle represents datum from one experiment at 54.0°C.

MRI Investigation of the Threshold for Thermally Induced Blood–Brain Barrier Disruption and Brain Tissue Damage in the Rabbit Brain

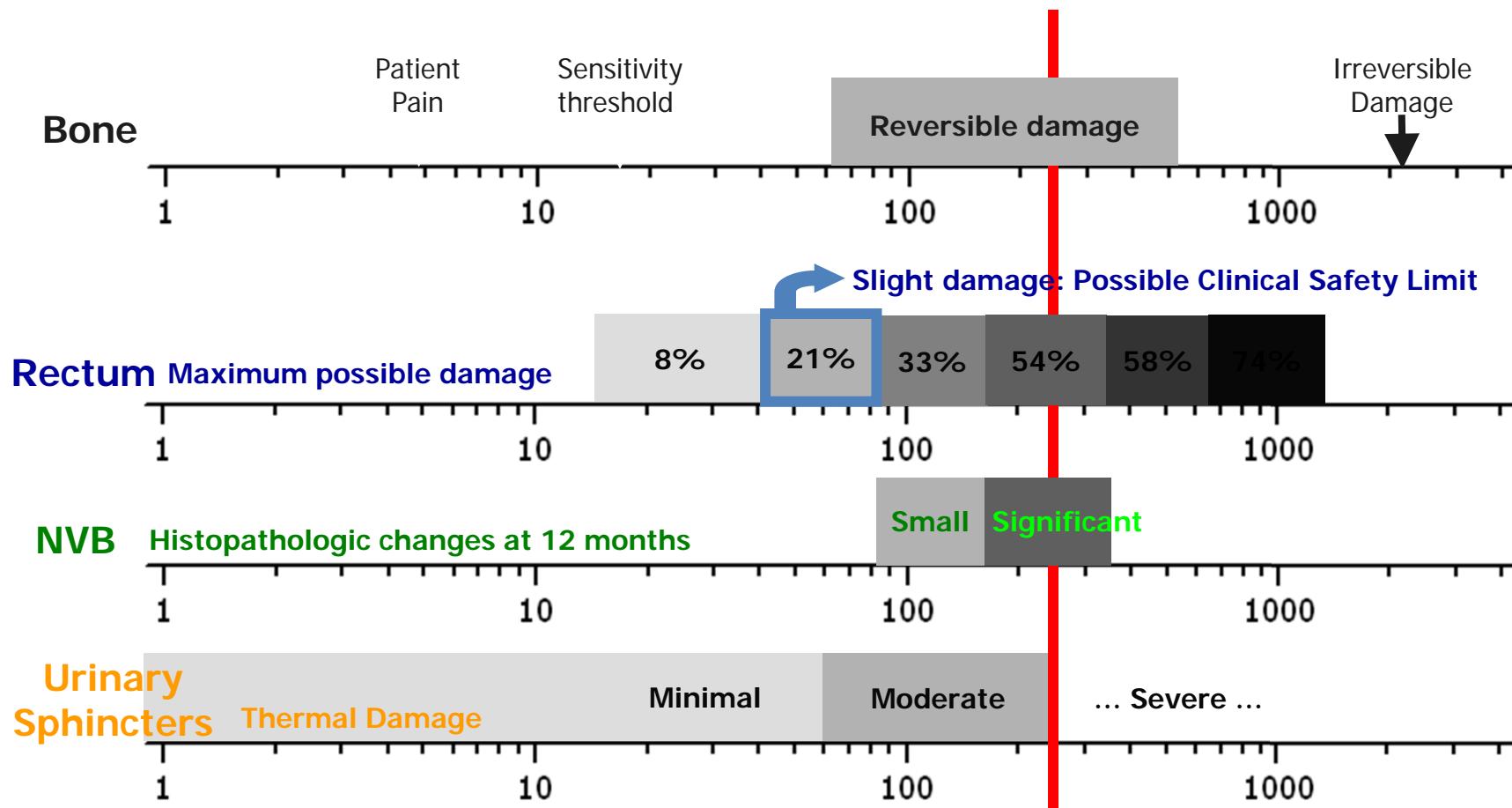
Nathan McDannold,* Natalia Vykhotseva, Ferenc A. Jolesz, and Kullervo Hynynen





Thermal Dose Thresholds in Prostatic and surrounding tissues

- Units of CEM43



“The biology is with us” *

- Well-defined relationships between heating and cell death
- Complementary cell sensitivity between radiation and heating
- Cells at low pH, hypoxic, nutrient deficient more sensitive to heat
- Vascular response in tumours deficient as compared with normal tissues

“The physics is against us” *

- Energy absorption highly non-uniform from initial devices (microwave)
- Temperature monitoring initially done with implanted temperature sensors which provided insufficient spatial information
- Dynamic changes in blood flow create challenging control problem
- Requirement to elevate and maintain tissue temperature within 1°C across an entire tumour volume for minutes to hours is extremely difficult