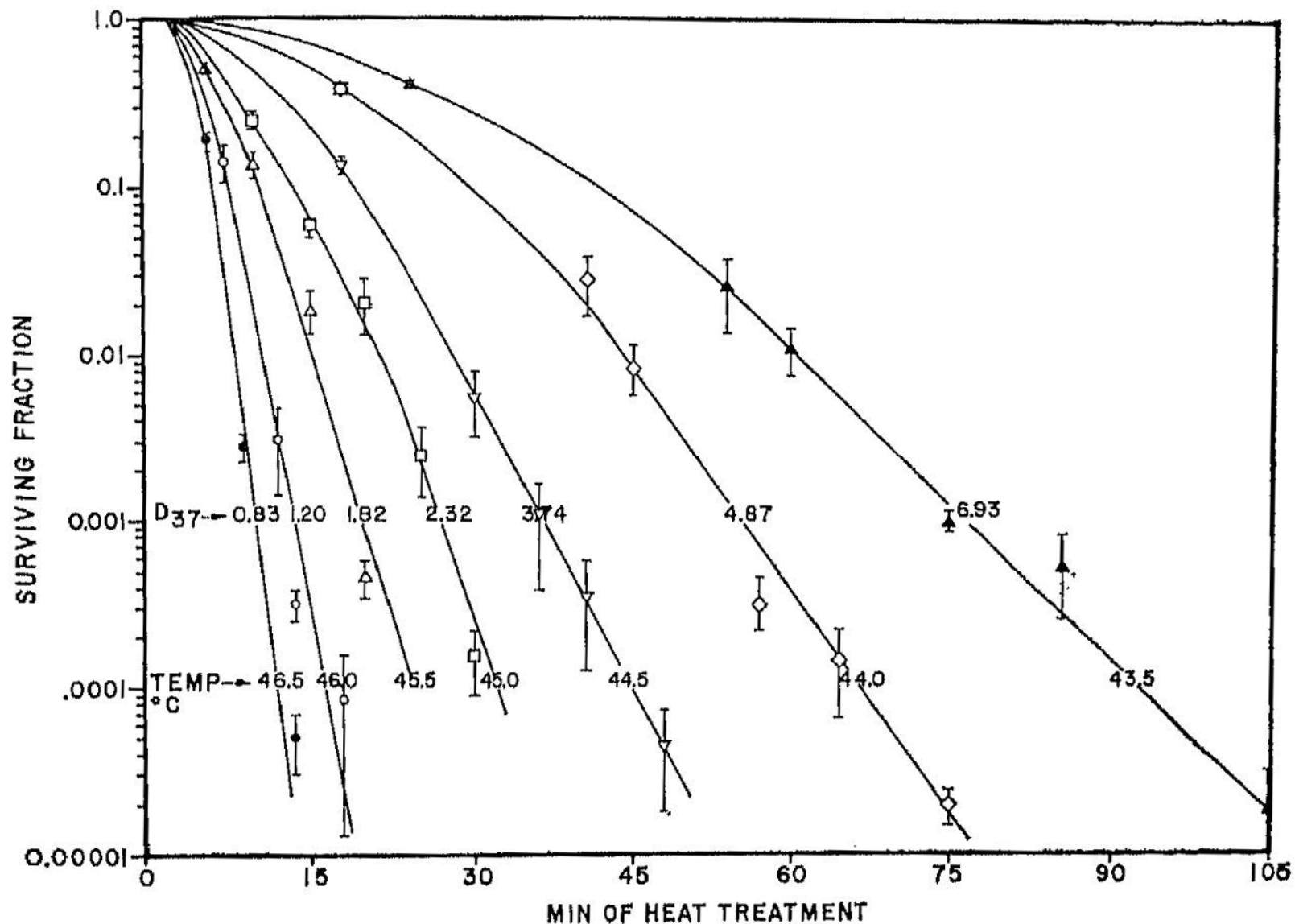


Arrhenius Relationships – from the Molecule & Cell to the Clinic

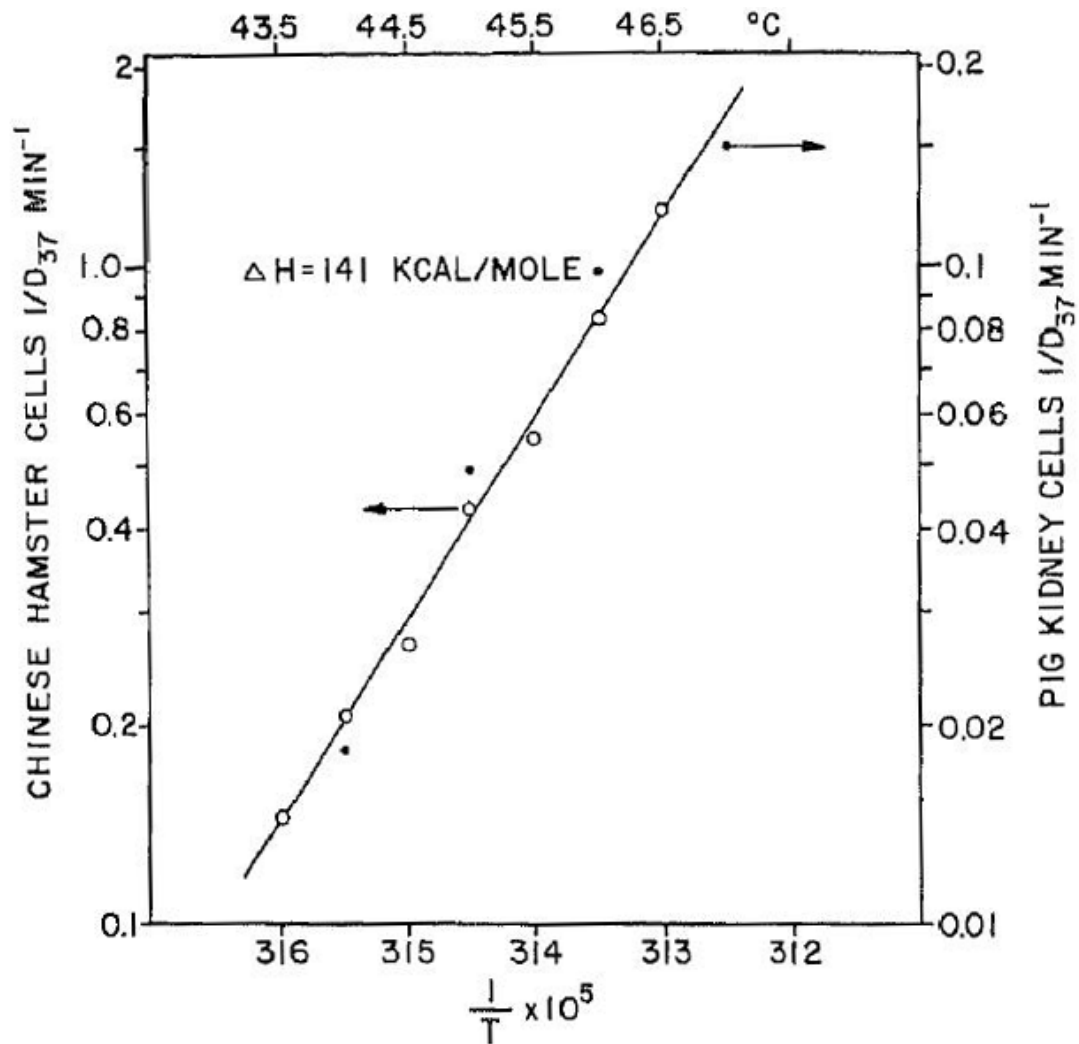
By William Dewey

References

- Westra and Dewey Int. J. Radiat. Biol. Vol 19, pp 467-477, 1971
- Dewey et al Int. J. Radiat. Biol. Vol 20, pp 505-520, 1971
- Dewey et al Radiology Vol 123, pp 463-474, 1977 (citation classic)
- Raaphorst et al Cancer Res. Vol 39, pp 396-401, 1979
- Sapareto and Dewey Int. J. Radiat. Oncology Biol. Phys. Vol 10, pp 787-800, 1984
- Borrelli, Wong, and Dewey J. Cellular Physiol. Vol 126, pp181-190, 1986
- Dewey Radiat. Res. Vol 120, pp 191-204, 1989
- Dewey Int. J. Hyperthermia Vol 10, pp 457-483, 1994
- Miller et al Int. J. Hyperthermia Vol 18, pp 361-384, 2002
- William C. Dewey, Chris J. Diederich, and Mark. W. Dewhurst. Hyperthermia classic commentary: 'Arrhenius relationships from the molecule and cell to the clinic. Int. J. Hyperthermia, **10**, 457-483, 1994. Publ. Int. J. Hyperthermia, Feb. (2009); 25 (1) 21-24.



Survival curves for asynchronous hamster cells heated at different temperatures for varying lengths of time. Values for the slopes of the exponential portions of the survival curves are indicated as D_{37} in minutes of heat treatment.



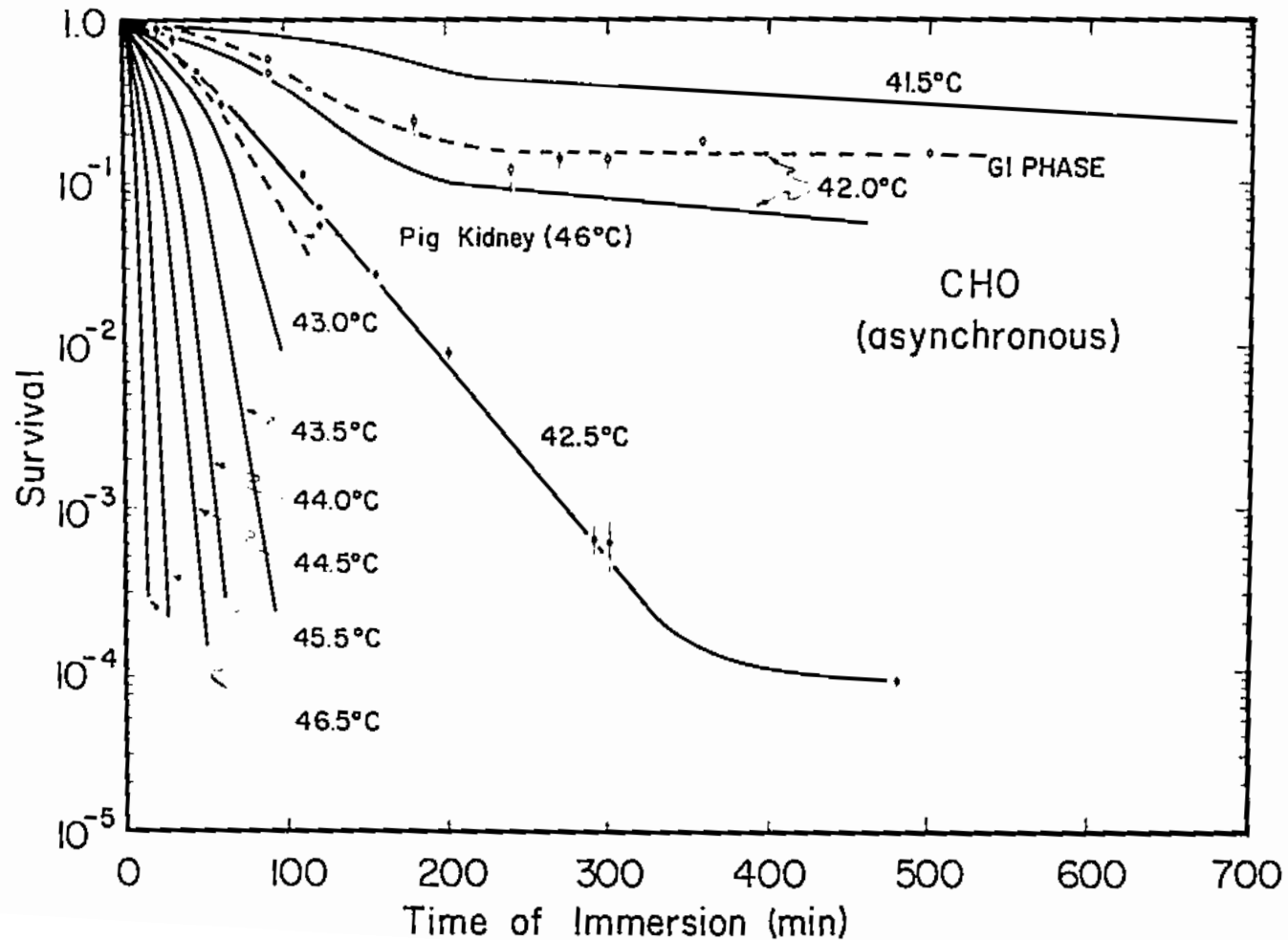
An Arrhenius plot for heat inactivation. On the left ordinate, the reciprocal of the D_{37} values (inactivation rates) obtained from figure 2 are plotted versus the reciprocal of the absolute temperature. On the right ordinate, similar values are plotted from data obtained by Harris (1967) for pig kidney cells in culture. As calculated in the text, the activation energy, μ is 141 kcal/mole for both cell types, but the heat inactivation rates for our hamster cells are a factor of 10 higher than for the pig kidney cells.

$$1/\tilde{D}_0 = K' \text{ sec}^{-1} = 2.05 (10)^{10} T e^{\Delta S/2} e^{-\Delta H/2 T}$$

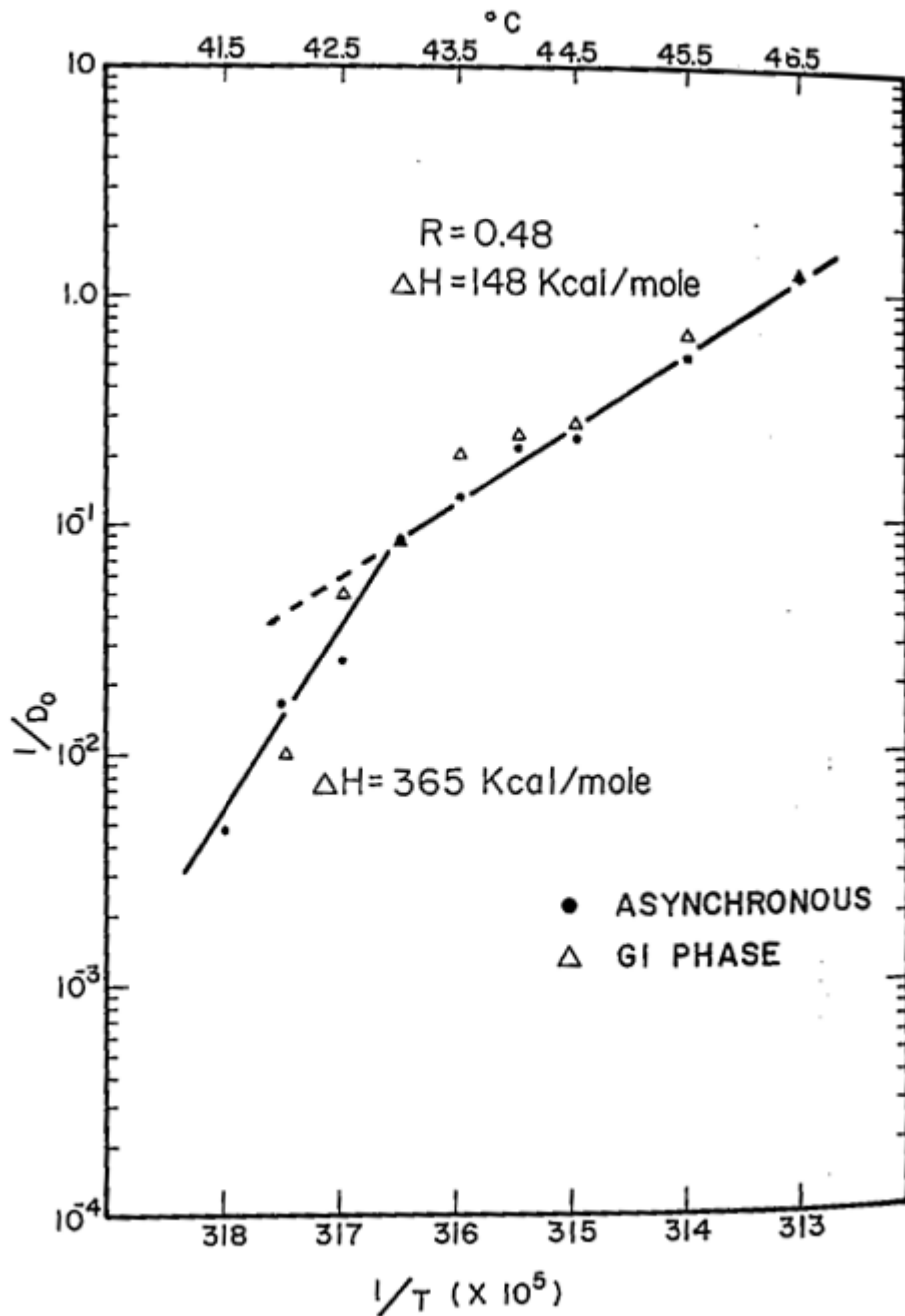
$$A = 2.05(10)^{10} T e^{\Delta S/2}$$

$$1/\tilde{D}_0 = A e^{-\Delta H/2 T}$$

$$\ln 1/\tilde{D}_0 = \ln A - (\Delta H/2) (1/T)$$



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 the means have been deleted for reasons of clarity. The survival curves for cells heated in the G₁ phase were very similar to those for the asynchronous cells, an example of which is indicated for cells heated in the G₁ phase at 42.0°C. To illustrate the wide variation in thermal sensitivity of various cell lines, the dashed line is drawn to illustrate the relative thermal resistance of a pig kidney cell line (3). The parent line of pig kidney cells (2) was slightly more sensitive with the 46°C curve, similar to that for the 43.5°C curve shown for CHO cells.

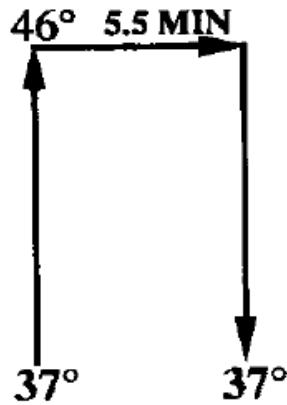


An Arrhenius plot for heat inactivation of CHO cells. On the ordinate, the reciprocal of the D_0 values (inactivation times) obtained from Figure 1 are plotted versus the reciprocal of the absolute temperatures. Separate data points are shown for asynchronous cells and for cells heated in the G_1 phase. See the text for definitions of D_0 and R . For different mammalian cellular systems with different inactivation rates, the line in the Arrhenius plot would have about the same slope, but would be shifted up or down. There would be a downward shift by a factor of 10, for example, when the inactivation rate is 10 times slower [see (Ref. 13) for pig kidney cells].



$$5^{\circ} \times 10 \text{ MIN} = \boxed{50^{\circ}\text{-MIN}}$$

VERY LITTLE KILLING



$$9^{\circ} \times 5.5 \text{ MIN} = \boxed{50^{\circ}\text{-MIN}}$$

LOTS OF KILLING

A schematic to illustrate the failure of $\Delta T \times$ duration of heating (expressed in $^{\circ}\text{-min}$) to predict thermal damage.

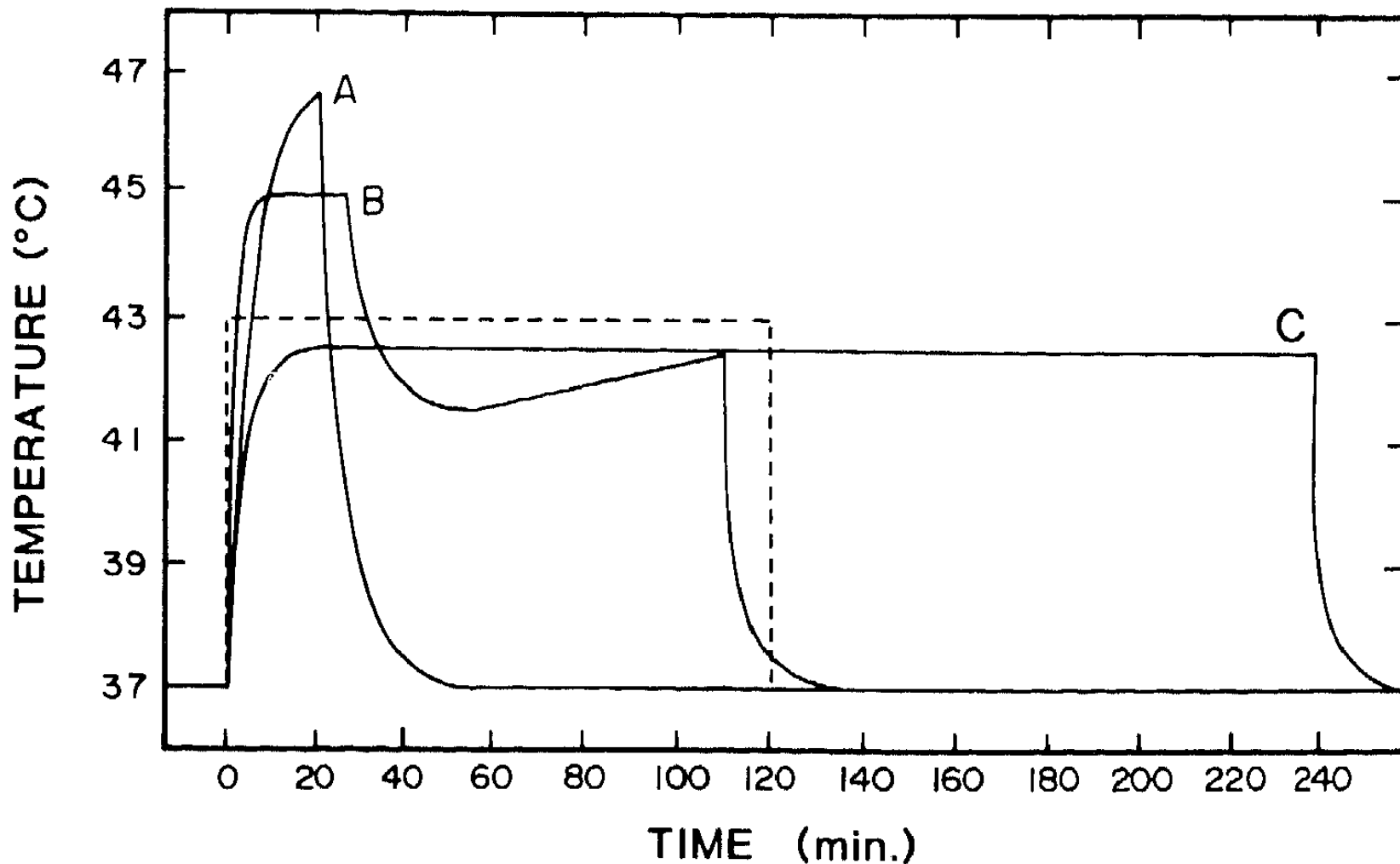
$$t_1 = t_2 R^{(T_1 - T_2)}$$

$$R = e^{-\Delta H / (2T(T+1))}$$

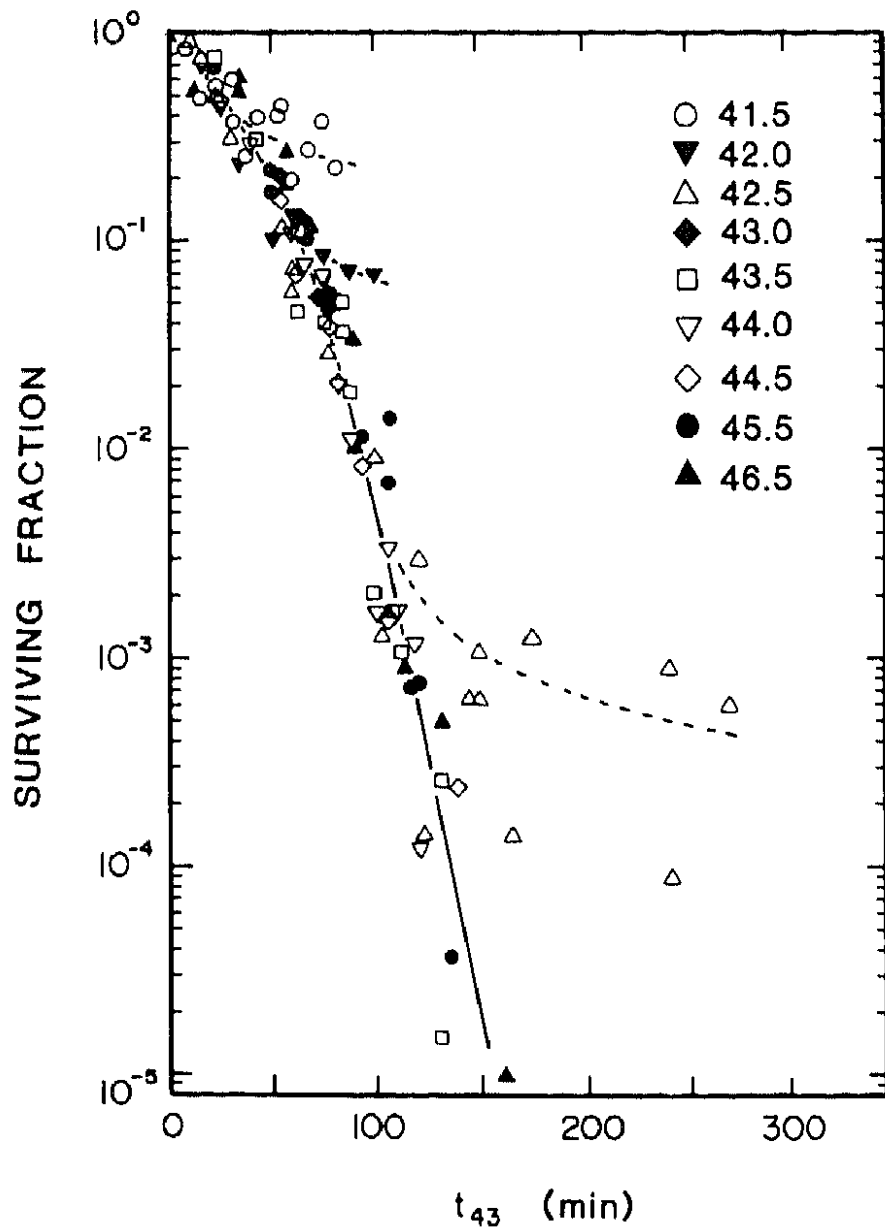
$$t_{43} = \sum_{t=0}^{t=\text{final}} R^{(43 - \hat{T})} \Delta t$$

For $T > 43$ C,
 $\Delta H = 141$ kcal/mole
 $R = 0.5$

For $T < 43$ C,
 $\Delta H = 365$ kcal/mole
 $R = 0.25$

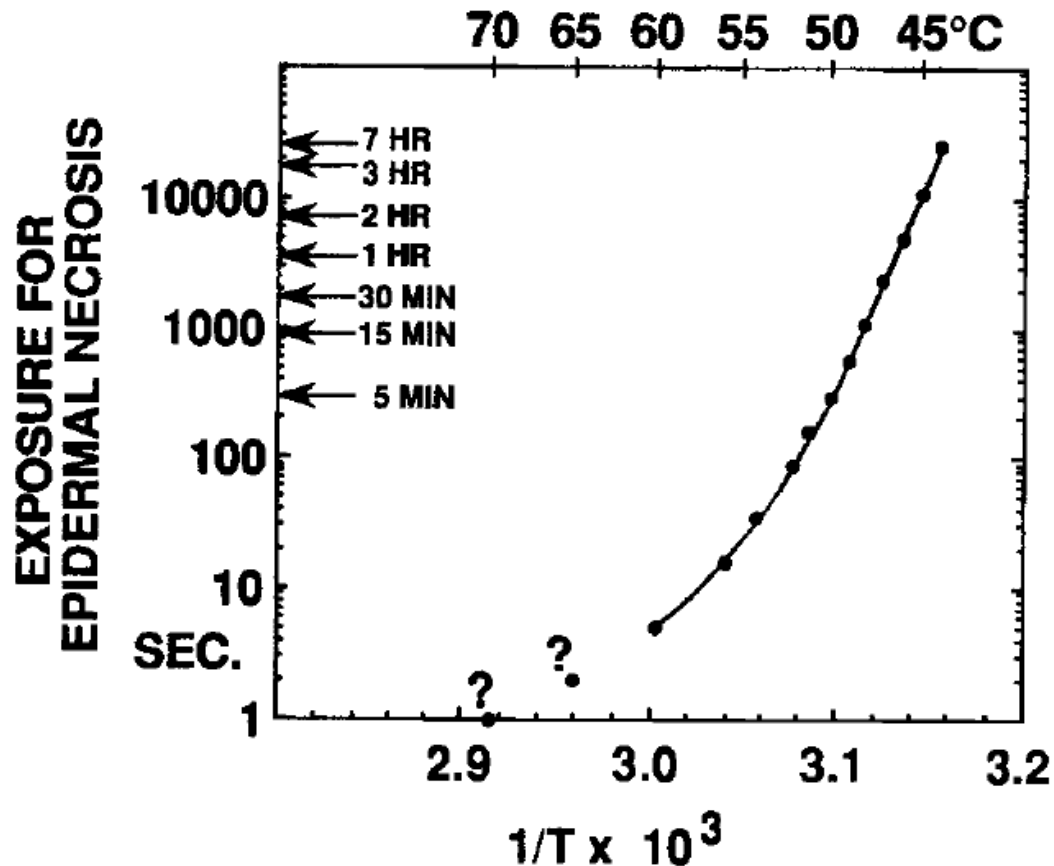


Theoretically generated temperature profiles as a function of time. The ideal temperature profile is shown by the dashed line and represents 120 minutes at 43°C by equivalent-minute calculation. As can be seen, the areas under each curve are *not* equal.

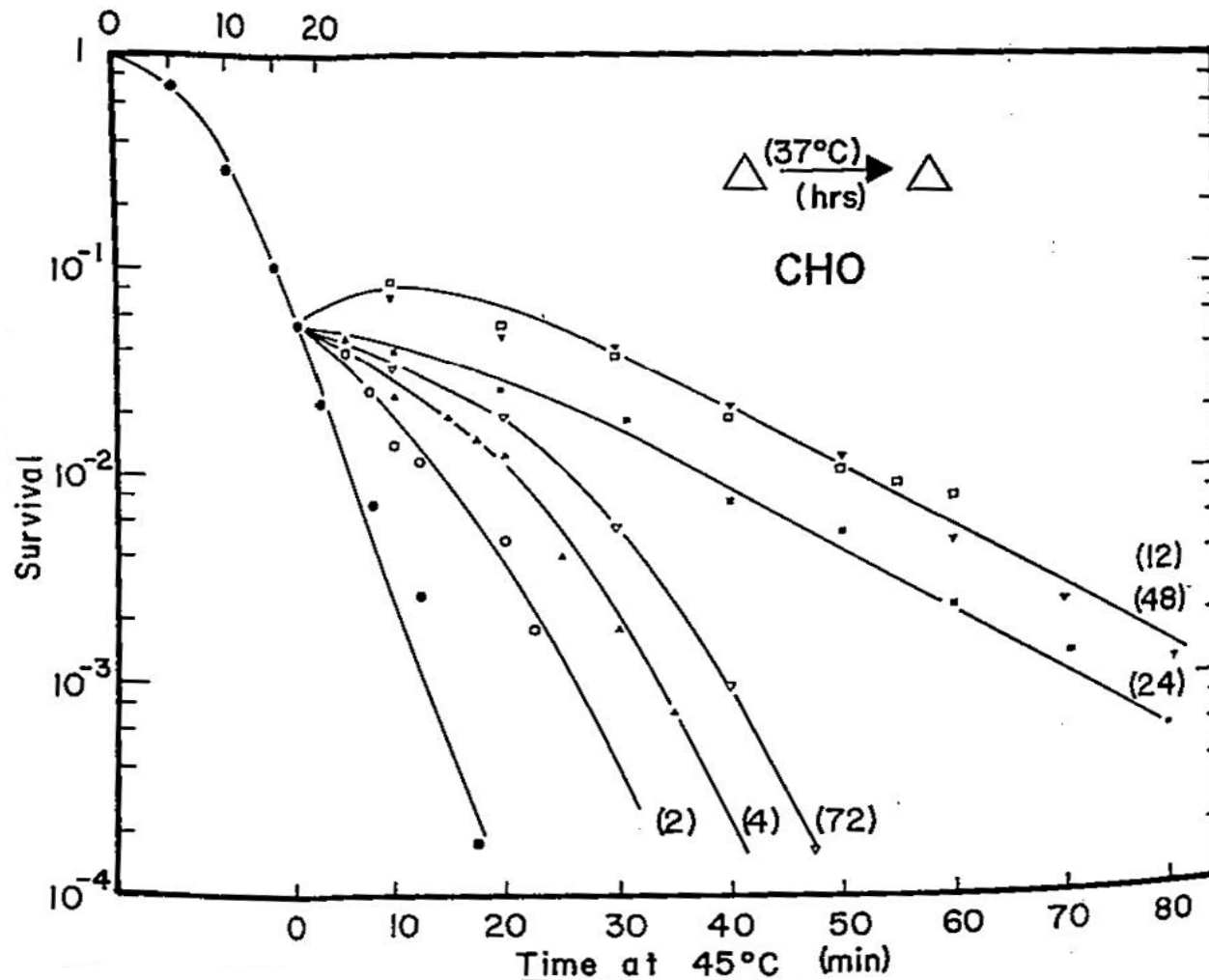


The dose survival response for asynchronous Chinese hamster ovary cells at various temperatures plotted as a function of equivalent-minutes at 43°C . Error bars have been omitted for clarity. The data at 41.5 , 42.0 , and 42.5 deviate from a single line, as shown by the dashed lines, due to the development of thermotolerance. Actual data are taken from reference 29 and replotted.

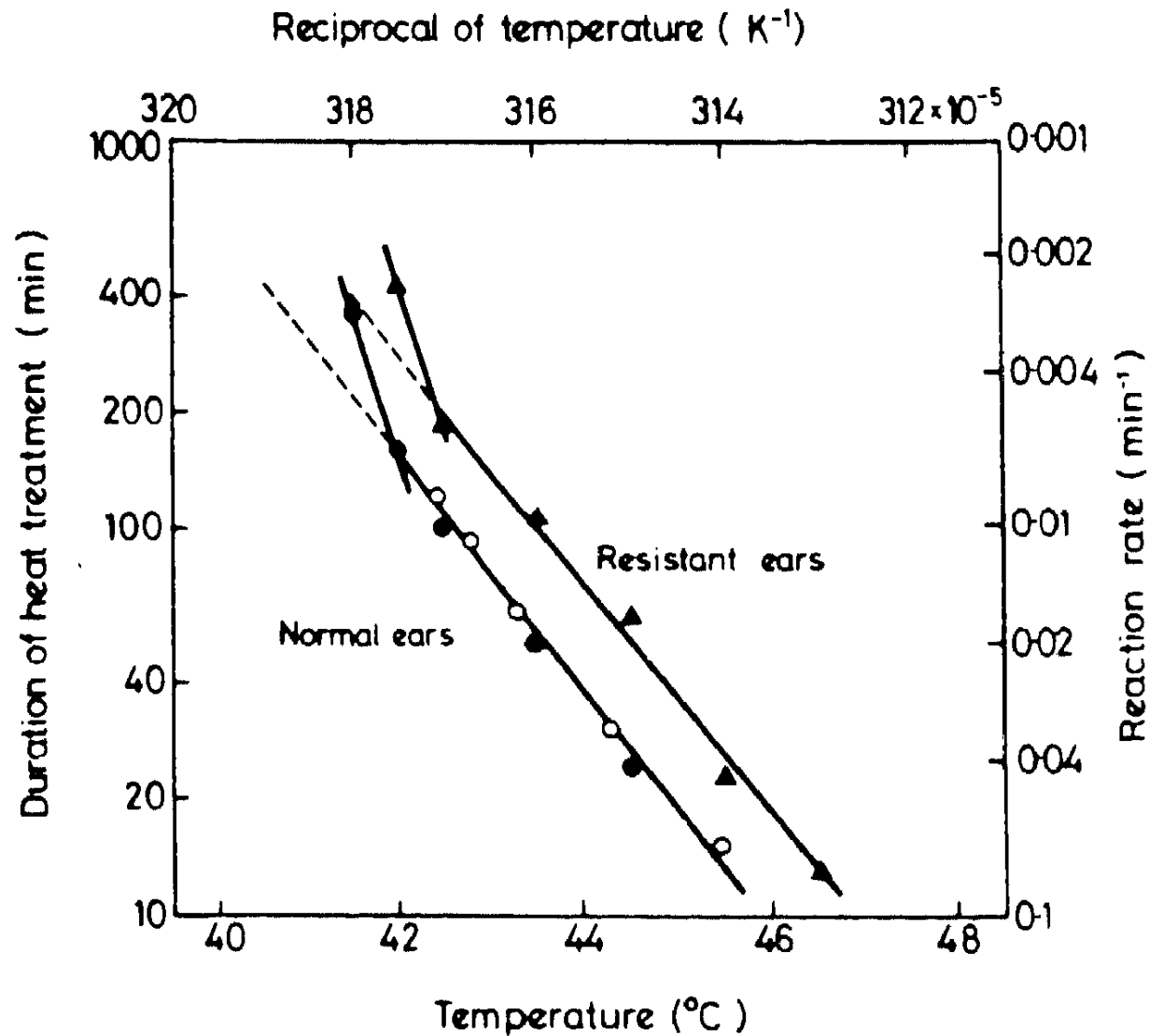
CUTANEOUS EXPOSURE TEMPERATURE



Data were taken from Henriques (1947). 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. $R=2.1$, $\Delta H=150\text{kcal/mol}$.



Repair of heat damage in asynchronous CHO cells is illustrated by survival curves determined for split doses with 37°C between the two heat treatments. The first heat treatment was at 45°C for 17.5 minutes. The second heat treatment was as indicated on the lower abscissa. The survival curve for a single heat treatment is indicated by the curve (●), with the time axis indicated by the upper abscissa. The other values on the curves indicate the number of hours between the two heat treatments (date from Ref. 36). □ = 12 hours and ▼ = 48 hours.



An Arrhenius-like plot of the relationship between time and temperature to cause 50% necrosis in mouse ears. Resistant ears received a treatment of 43.5°C for 20 min., 24 hours prior to the test treatment. Reprinted with permission from reference 20.

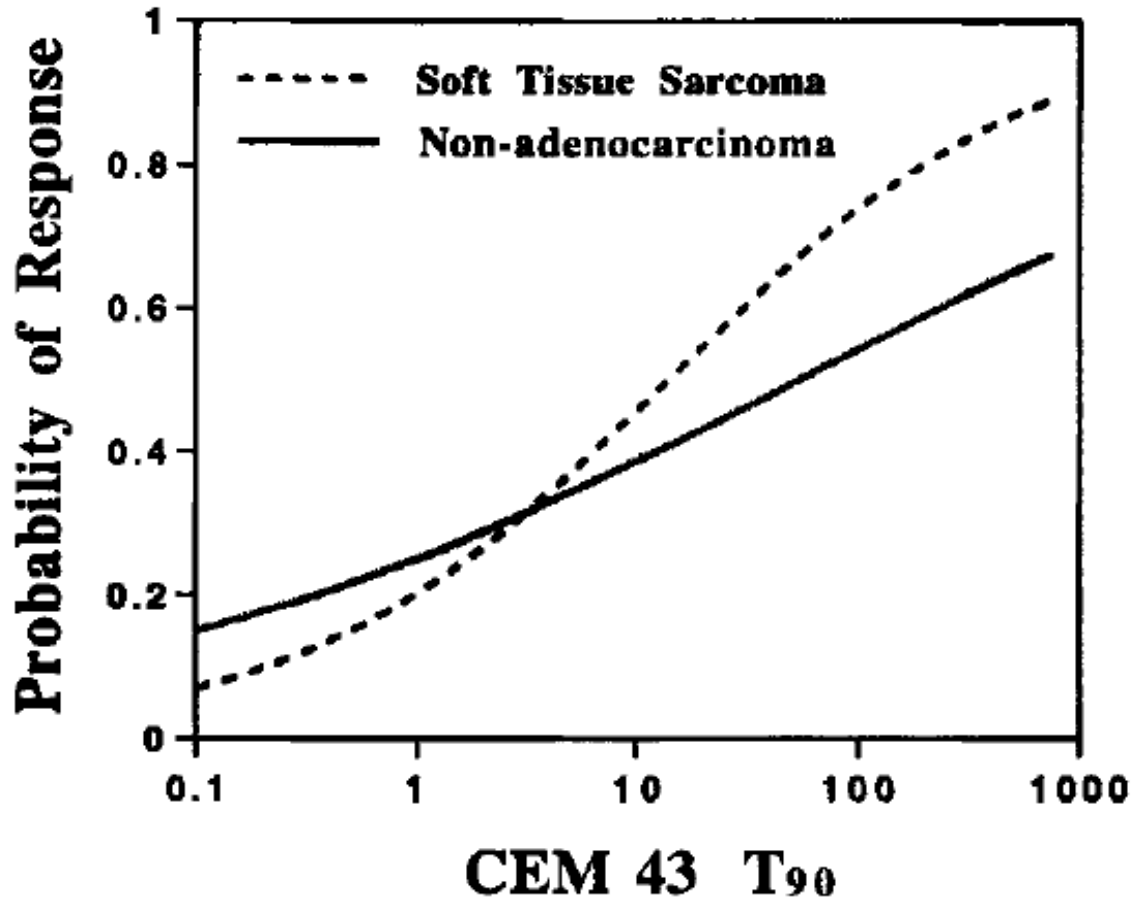
$$\text{equiv min } 43^{\circ}\text{C} = EM_{43} = \sum_{t=0}^{t=\text{final}} \Delta t \times R^{(T_t - 43)}$$

Cummulative equiv min 43°C for $T_{90} = CEM_{43} T_{90}$

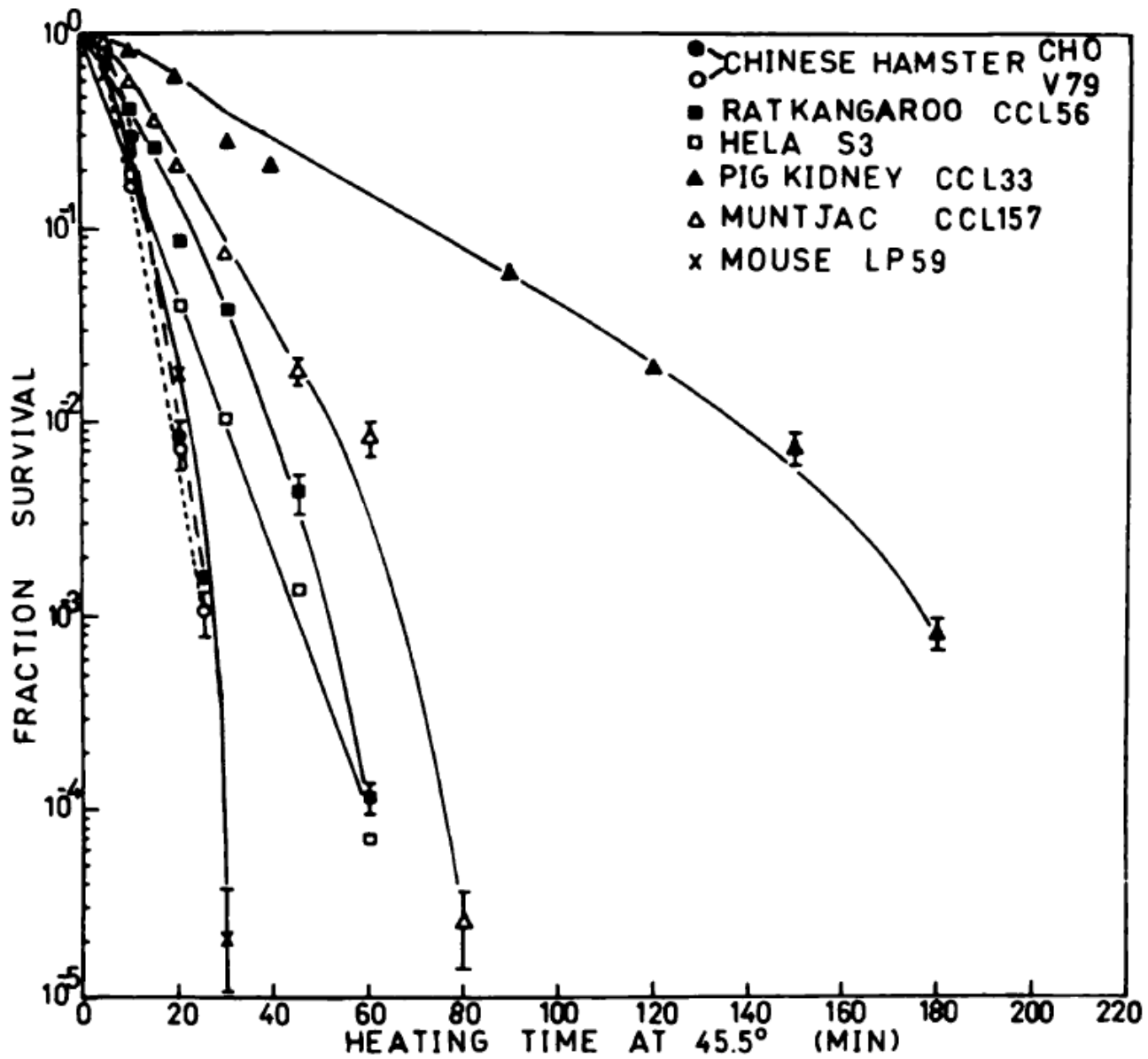
Cummulative equiv min 43°C for $T_{50} = CEM_{43} T_{50}$

For $T > 43\text{C}$ $R = 0.5$

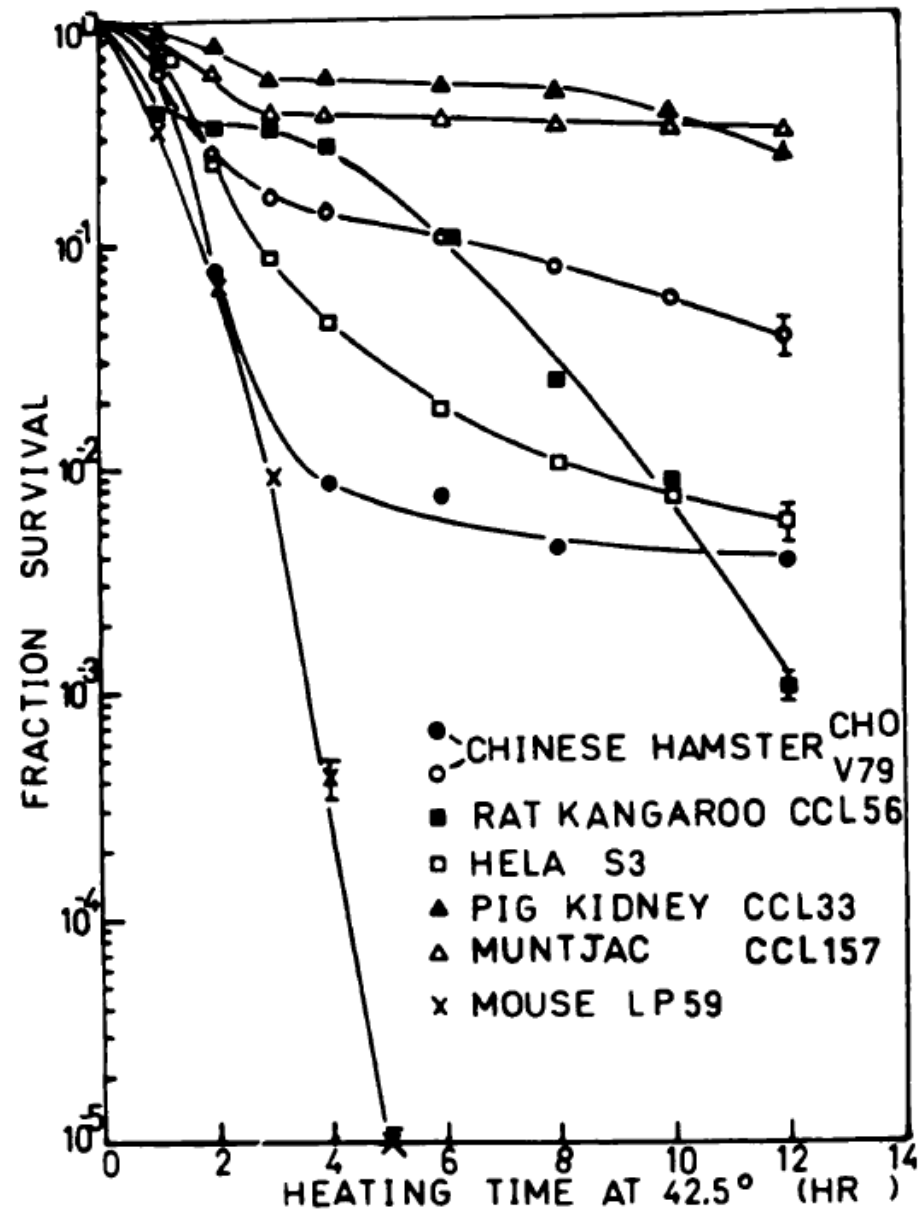
For $T < 43\text{C}$ $R = 0.25$



Data (Oleson *et al.* 1933, M. Dewhirst, personal communication) for probability of complete response of 57 superficial non-adenocarcinomas and probability of necrosis ($\geq 80\%$ 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 min 43°C T_{90} ($\text{CEM}_{43} T_{90}$) was $5 \cdot 4$ for soft tissue sarcomas and $2 \cdot 1$ min for superficial tumours. The curves were derived from empirical equations obtained from the clinical data base. See text and Oleson *et al.* (1933) for details.



Survival curves of 7 cell lines exposed to 45.5° for various times are shown.

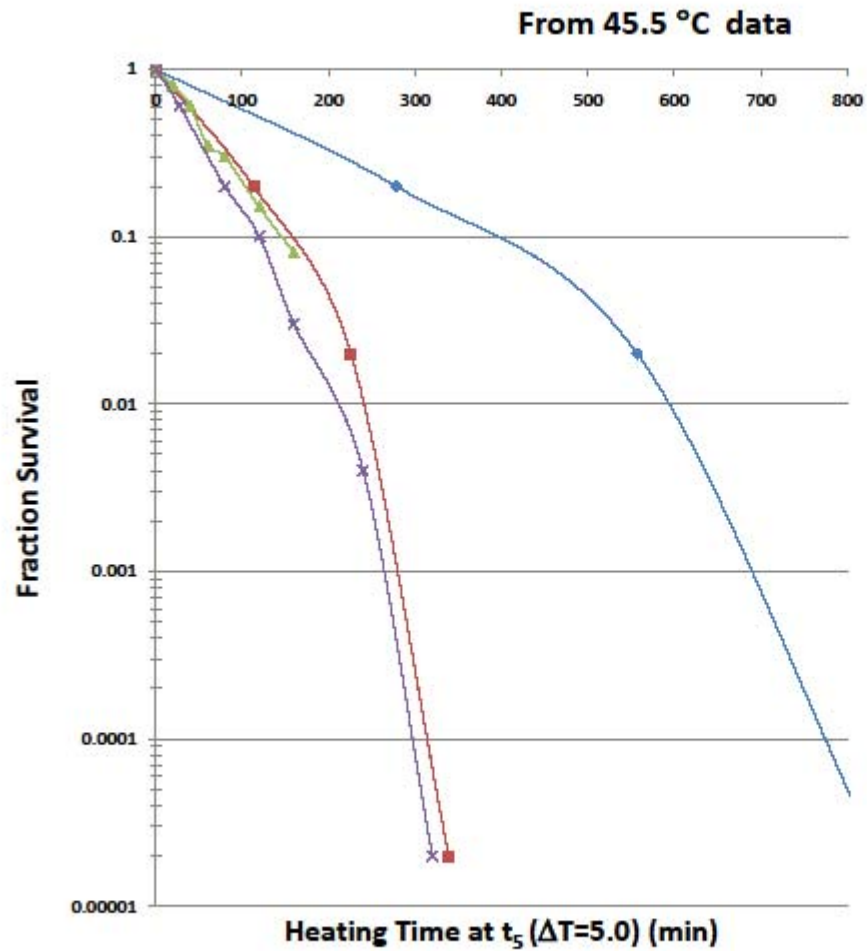


Survival curves of 7 cell lines exposed to 42.5° for various times are shown.

Comparison of heat sensitivity with body temperature of donor mammal

Order of thermal resistance

	42.5C	45.5C	Body temperature
Pig kidney	1	1	39.4C
Muntjac	2	2	38.5C
HeLa S3	4	4	37C
Chinese hamster CHO	5	5	36.1-38.3C
Chinese hamster V79	3	5	36.1-38.3C
Kangaroo rat		3	35-36C
Mouse LP 59	6	5	35.7-37.7C



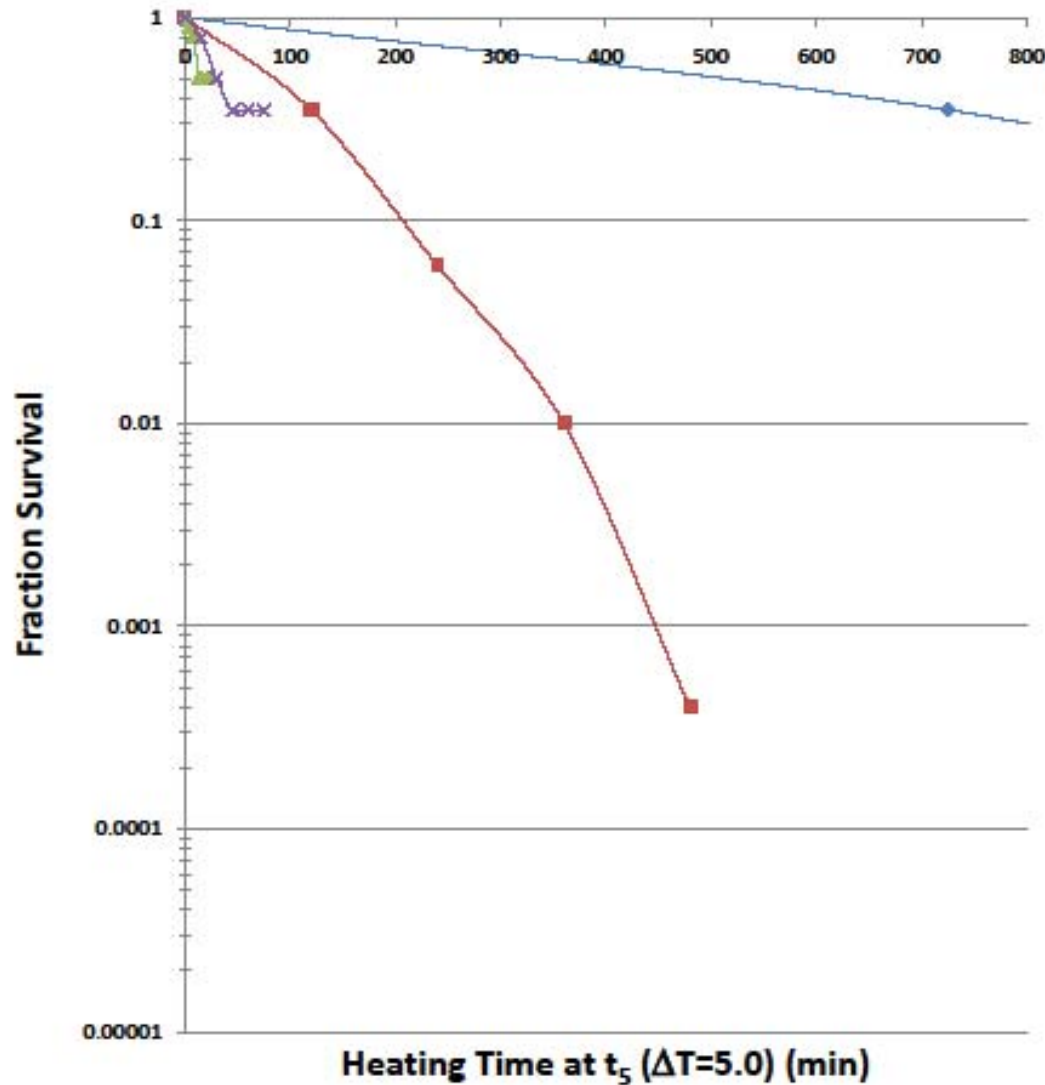
$$t_5 = t_{45.5} \times R$$

$$R = 2^{\Delta T - 5.0}$$

$$\Delta T = 45.5^\circ\text{C} - \text{BT}$$

- ◆ Mouse LP-59
 (BT=35.7; $\Delta T=9.8$)
- Mouse LP-59
 (BT=37.0; $\Delta T=8.5$)
- ▲ Pig Kidney
 (BT=39.4; $\Delta T=6.1$)
- × Muntjac
 (BT=38.5; $\Delta T=7.0$)

From 42.5 °C Data



$$t_5 = t_{42.5} \times R$$

$$R = 4^{\Delta T - 5.0}$$

$$\Delta T = 42.5^\circ\text{C} - \text{BT}$$

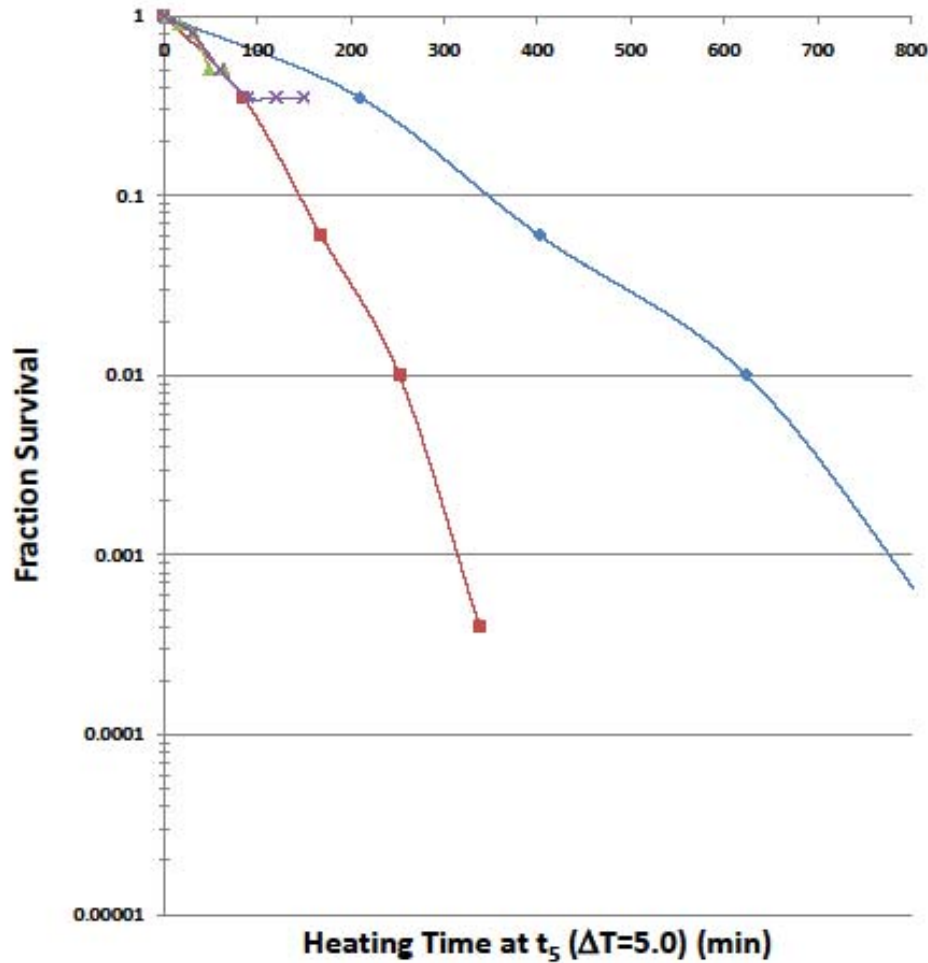
—●— Mouse LP-59
(BT=35.7; $\Delta T = 6.8$)

—■— Mouse LP-59
(BT=37.0; $\Delta T = 5.5$)

—▲— Pig Kidney
(BT=39.4; $\Delta T = 3.1$)

—×— Muntjac
(BT=38.5; $\Delta T = 4.0$)

From 42.5 °C Data



$$t_5 = t_{42.5} \times R$$

$$R = 2^{\Delta T - 5.0}$$

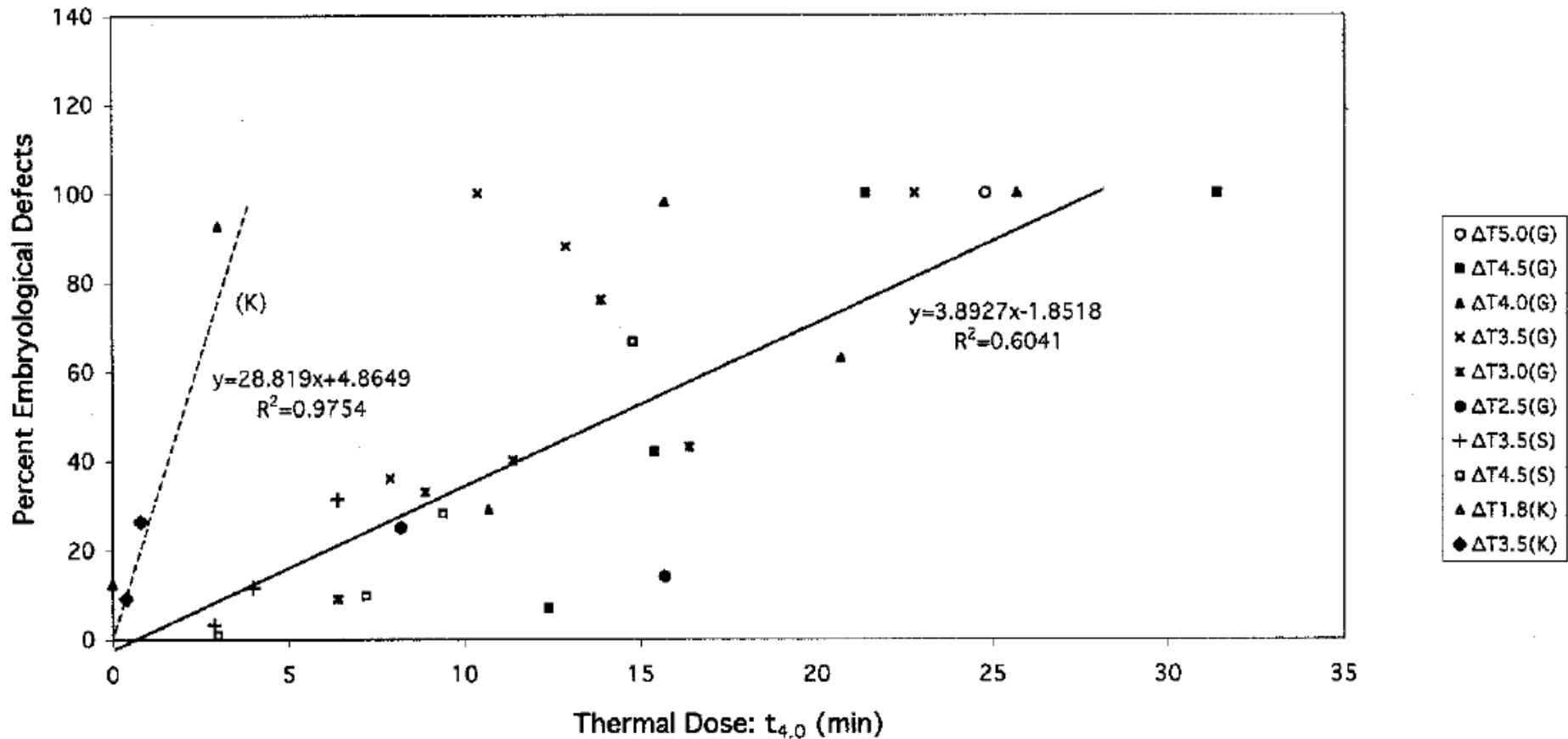
$$\Delta T = 42.5^\circ\text{C} - \text{BT}$$

—●— Mouse LP-59
(BT=35.7; $\Delta T = 6.8$)

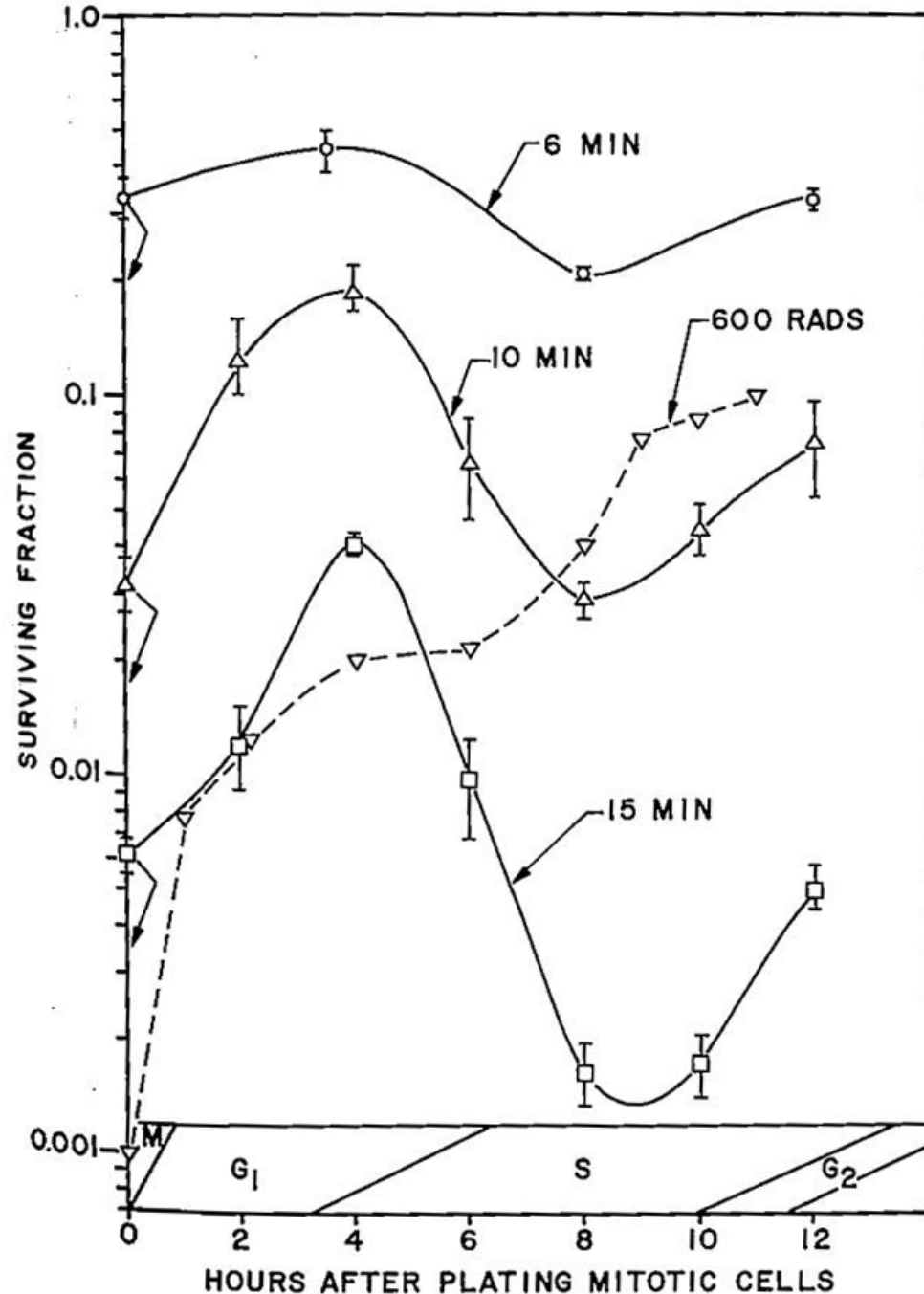
—■— Mouse LP-59
(BT=37.0; $\Delta T = 5.5$)

—▲— Pig Kidney
(BT=39.4; $\Delta T = 3.1$)

—×— Muntjac
(BT=38.5; $\Delta T = 4.0$)



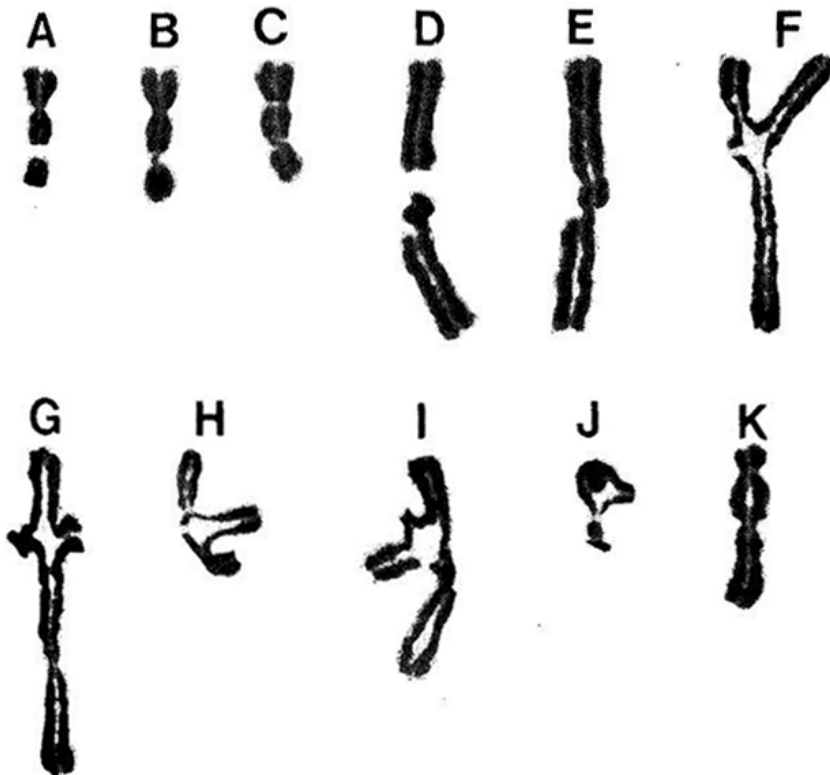
Percent embryological defects vs $t_{4.0}$ based on the results for Kimmel (K) *et al.*²², Germain (G) *et al.*²⁰, and Shiota (S)³¹. These were obtained from temperature:time combinations represented by some of the points plotted in figure 2, using the relationship represented by equation (2). These data were used to establish dose-response relations for heat-induced teratogenic effects in laboratory animals (rats, rats, and mice, respectively). The keyed, author-specific designations refer to the maximum temperature increase (ΔT) above core value for a specific experimental protocol. Equivalent $t_{4.0}$ values were determined by integrating at 0.5°C intervals during the heating:cooling periods (see figure 1 and table 1 for an example) and using the temperature profiles given in the original reports. Equation (2) with $R=0.25$ was used. The solid line is a best fit to the data of Germain *et al.* and Shiota, the dashed line a best fit to the data for Kimmel *et al.* Both line plots essentially intersect at the origin. The control and sham-exposed levels of foetal malformations were 12.3 and 9.1%, respectively, in Kimmel *et al.*²², and the sham-exposed levels for foetal malformations in Germain *et al.*²⁰ and Shiota³¹ were 0%; see text.

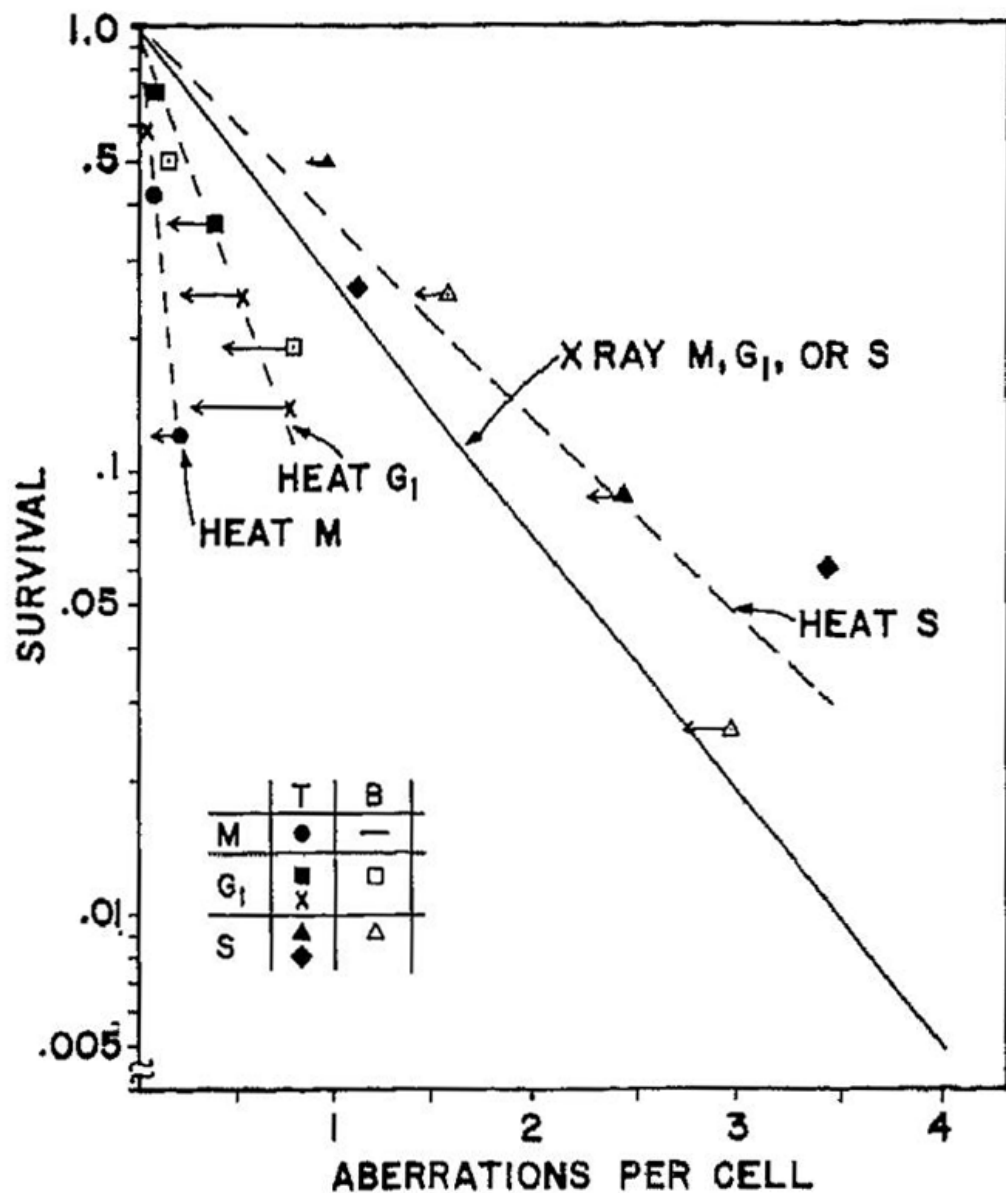


Variation in the fraction of CHO cells surviving heat or x-irradiation delivered during various phases of the cell cycle. The cells were heated for 6, 10, or 15 minutes at 45.5°C, or were x-irradiated with 600 rads. Except for cells heated in mitosis, all survival points have been corrected for a cellular multiplicity of 1.8; a multiplicity correction of 1.8 for mitotic cells would lower the survival values as indicated by the arrows. The positions of the cells in the cycle at the time of heating (as indicated on the abscissa) were obtained from the percentage of cells incorporating tritiated thymidine during a 10-15 minute period (data from Ref. 13).

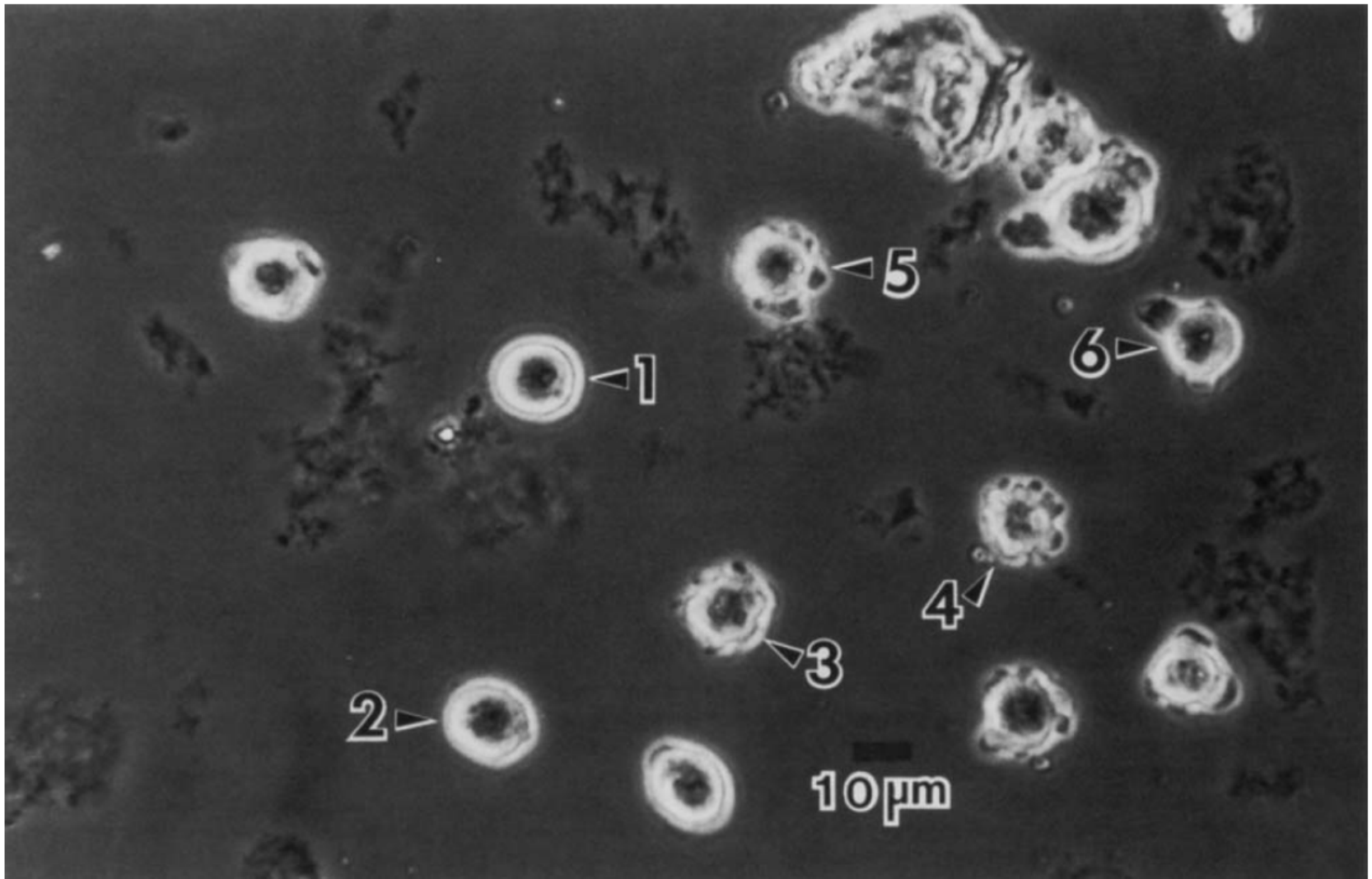


Top: Complement of 21 chromosomes observed in about 95% of the untreated cells. The arrow indicates the secondary constriction in the X chromosome. Bottom: Examples of aberrations observed. A-C=Deletions at the secondary constriction of the X chromosome. D=Chromatid and isolocus deletions. E=Chromatid deletion. F-I=Chromatid interchanges. J=Chromatid intrachange. K=Chromosome interchange (dicentric).

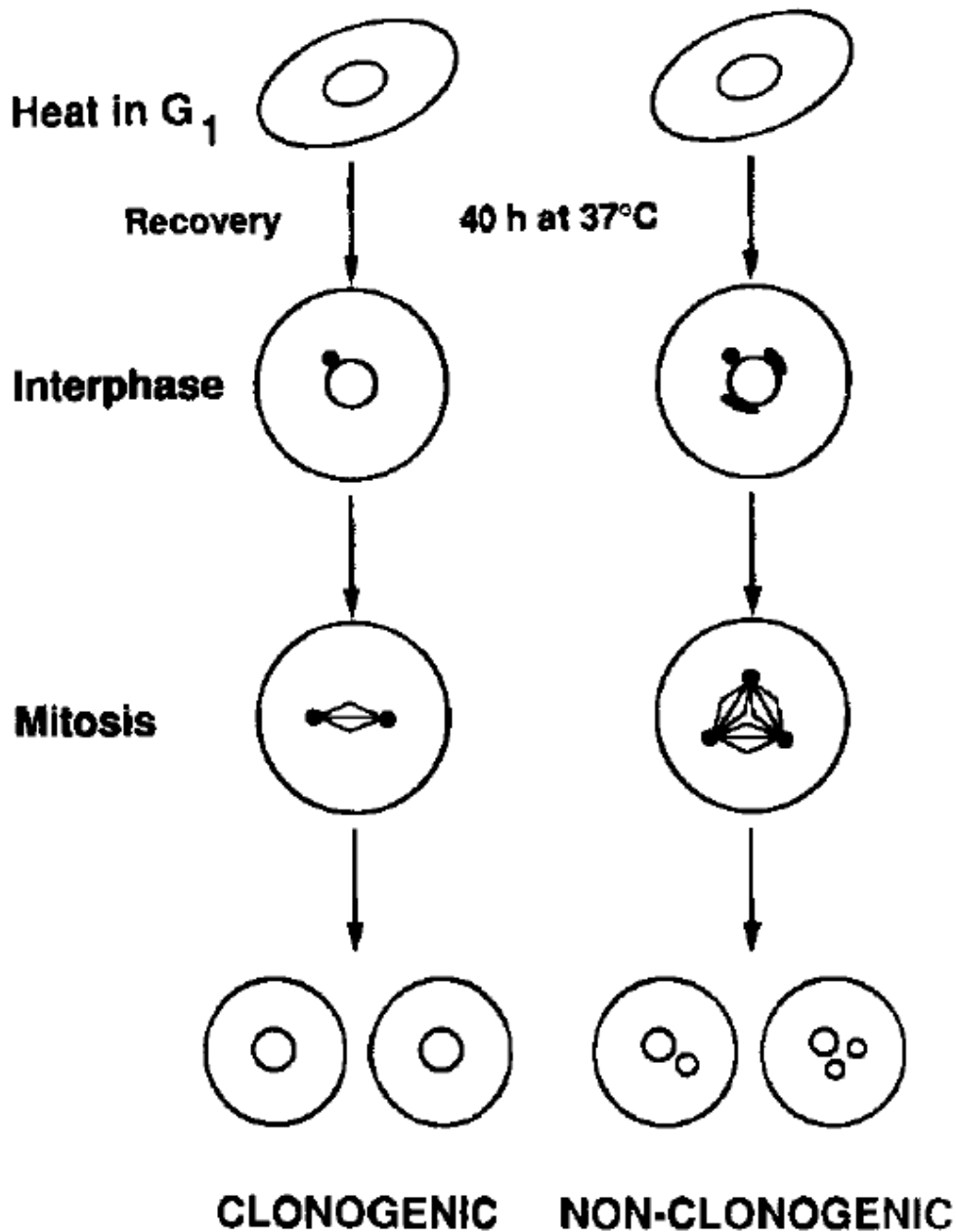




Survival versus aberrations per cell for TdR-control (T) cells and BUdR-treated (B) cells heated or x-irradiated in mitosis (M), G₁, or S. The relationship plotted for x-irradiation (data points not shown) was reported (Dewey *et al.* 1971b) for both TdR-control and BUdR-treated cells. The relationships plotted for heat treatments (■, □, ▲, △) were obtained by relating the survival data in figure 4 with the aberration data in figure 6 (the control frequency for unheated cells was subtracted from each point); the points (●, x, ◆) were obtained from another set of experiments (figure 1 and Westra and Dewey 1971). The tips of the horizontal arrows indicate the aberration frequencies when the deletions occurring at the secondary constriction of the X chromosome were subtracted.



Phase contrast micrograph of G₁ cells heated for 10 min at 45.5°C. Some cells retained shapes indistinguishable from control cells (state 1), while others exhibited various degrees of membrane surface blebbing (states 2-6). x700.



A schematic of results (Vidair *et al.* 1993) illustrating that the centrosome is a critical target for the killing of CHO cells heated (EM₄₃ of 52) in G₁ to result in survival levels >10%. The lack of staining of centrosomes with antisera is observed in all G₁ 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.

Factors that alter heat sensitivity

- **Replace one amino acid by another – for denaturation of a specific protein, Arrhenius curve can be shifted by 3 degrees; by 9 degrees for 3 amino acids.**
- **Acute thermal tolerance for second heat treatment a few hours after an initial heat treatment decreases sensitivity (shifts Arr. Curve to higher temperature with no change in slope) – Also seen for chronic thermal tolerance observed after a few hours at a relatively low temperature. Decays in 2-3 days.**
- **Step-down heating – relative low temperature immediately after a higher temperature, sensitizes cells and eliminates break in Arrhenius curve**
- **Heat sensitivity is different for different tissues in the same animal**

Factors that alter heat sensitivity

- **For different mammals, heat sensitivities appear to relate more closely with elevation above base line temperatures of mammals instead of absolute hyperthermic temperature**
- **Lower pH – increase sensitivity – shift Arrhenius curve to lower temperature, with no change in slope**
- **Heating cells in presence of glycerol – stabilizes proteins – decrease sensitivity – shifts Arrhenius curve to higher temperature, with no change in slope**
- **Heat sensitivity varies during cell cycle – S-phase and mitosis more sensitive than G₁**

Summary

- To obtain same lethal effect, i.e., isoeffect, decrease temperature by 1°C and increase duration of heating about two-fold (about 4-fold below 43°C)
 - (For activation energy, ΔH , of about 140 kcal/mole)
- This relationship holds between 43°C and 60°C or higher
- Protein denaturation and/or aggregation is critical
- For different biological systems and different cell types, absolute heat sensitivity varies (dependent on entropy term, ΔS), but ΔH is relatively constant.

Contributors to Concepts Presented

A Westra, S Sapareto, H Nagasawa,
D Leeper, M Freeman, J Oleson,
M Dewhirst, C Diederich, P Stauffer,
P Raaphorst, M Miller, M Borrelli, R Wong,
R Coss, J Dynlacht, C Vidair, C Landon,
M Dewey

References

- Westra and Dewey Int. J. Radiat. Biol. Vol 19, pp 467-477, 1971
- Dewey et al Int. J. Radiat. Biol. Vol 20, pp 505-520, 1971
- Dewey et al Radiology Vol 123, pp 463-474, 1977 (citation classic)
- Raaphorst et al Cancer Res. Vol 39, pp 396-401, 1979
- Sapareto and Dewey Int. J. Radiat. Oncology Biol. Phys. Vol 10, pp 787-800, 1984
- Borrelli, Wong, and Dewey J. Cellular Physiol. Vol 126, pp181-190, 1986
- Dewey Radiat. Res. Vol 120, pp 191-204, 1989
- Dewey Int. J. Hyperthermia Vol 10, pp 457-483, 1994
- Miller et al Int. J. Hyperthermia Vol 18, pp 361-384, 2002
- William C. Dewey, Chris J. Diederich, and Mark. W. Dewhurst. Hyperthermia classic commentary: 'Arrhenius relationships from the molecule and cell to the clinic. Int. J. Hyperthermia, **10**, 457-483, 1994. Publ. Int. J. Hyperthermia, Feb. (2009); 25 (1) 21-24.