IMPORTANCE OF PREHEATING TEMPERATURE AND TIME FOR THE INDUCTION OF THERMOTOLERANCE IN A SOLID TUMOUR IN VIVO

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Summary.—The importance of the priming heat treatment temperature and heating time for the degree and kinetics of thermotolerance was investigated in a C3H mammary carcinoma inoculated into the feet of CDF1 mice. A single heat treatment in the range 41.5-44.5°C resulted in a linear relationship between heating time and tumour growth time (i.e. the time for tumours to reach a volume five times that of the first treatment day). An Arrhenius plot showed an inflection point at 42.5°C with activation energies of 635 and 1508 kJ/mol, respectively, above and below 42.5°C. The degree and kinetics of thermotolerance were independent of the preheating temperature, if the heating time was adjusted to give the same level of heat damage. A pre-treatment at these temperatures with a tumour growth time of approximately 10 days, equivalent to 30 min at 43.5°C, resulted in maximal thermotolerance at a 16-h interval with a thermotolerance ratio (TTR\text{max}) of approximately 5-2. Preheating of the tumours at 43.5°C for 3-5, 7-5, 15, 30, or 45 min, showed that if the preheating time was increased, both the TTR\text{max} and the time interval necessary to develop TTR\text{max} increased, both being linear functions of the duration of the preheating time. Maximal thermotolerance was obtained at intervals of 2, 4, 8, 16, and 28 h with TTR\text{max} of 1-6, 2-2, 3-7, 5-2, and 7-7, respectively.

Thermotolerance, indicated by an increased resistance to hyperthermia resulting from a prior exposure to heat, seems to be a general phenomenon applying to all biological tissues (Henle & Dethlefsen, 1978; Nielsen & Overgaard, 1979; Field & Anderson, in press: Kamura et al., 1982; Spiro et al., 1982). Therefore, quantitative investigations on the factors which may affect thermotolerance are of biological and clinical importance. One such factor is the priming heat dose (time and temperature).

The results from studies on cell cultures and normal tissues indicate that both the degree and kinetics of thermotolerance are related to the magnitude of the priming heat treatment (Henle & Dethlefsen, 1978; Field & Anderson, in press). Using either a constant preheating time at different temperatures (Hume & Marigold, 1980; Li & Hahn, 1980) or different preheating times at a constant temperature (Gerner et al., 1976; Henle et al., 1978; Law et al., 1979; Rice et al., 1982), these studies suggest that the higher the degree of damage induced by preheating, the larger the induced thermotolerance, and the later the maximum tolerance is expressed. However, from these studies it is not clear whether it is the level of heat damage after preheating or the preheating temperature itself which is most important for thermotolerance. A recent in vitro study (Nielsen & Overgaard, in press) showed the same degree and kinetics of thermotolerance irrespective of the preheating temperature if the preheating times were adjusted to

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givetheidenticalsurvivallevels.Similarly,in
astudyonmousepinna(ears)in vivo, Law
(1981)foundnosignificantdifference
between either the degree or the kinetics of
thermotolerance induced by preheating at
temperatures between 41·5 and 45·5°C if
the pretreatments were adjusted to give
thesamedegeofear necrosis.Unfortunately,
a paucity of information exists about the dependence of thermotolerance
on the priming heat dose in solid tumours
in vivo. Recently, Urano and co-workers
(Maher et al., 1981; Urano et al., 1982)
haveshownthatthedegreeofthermo-
tolerance in a solid tumour increased with
prolonged preheating time at 45·5°C.
However, as these studies were performed
at a single temperature with only one
fractionation interval (24 h), they did not
provide information about the time course
ofthermotolerancein solids tumours.

In the present study, the importance of
the primary heat treatment temperature
and heating time for the degree and
kinetics of thermotolerance was investi-
gated in a solid mammary carcinoma.
the investigations were based on an experi-
mental tumour model, which we have
recently established for quantitative
studies on the development of thermotol-
tolerance in solid tumours (Kamura et al.,
1982). In addition, studies were performed
todetermine there lationship between heat
effect and temperature on tumours given
single heat treatment.

MATERIALS AND METHODS

Animal tumour system.—Ten-to-12-week-
old male and female C3D2F1/Bom (C3H/Tif
♀ × BDA/2 ♂) mice were challenged with a
spontaneously C3H/Tif mammary carcinoma,
which was propagated by serial transplanta-
tion. Tumour material for inoculation was
obtained by sterile dissection of large flank
tumours. Macroscopically viable tumour tis-
ue was minced with a pair of scissors, and
5–10 μl of this minced tumour was injected
into the foot on the right hind limb of the
experimental animals. The transplant take
was 95%. Tumours reaching a volume of
~ 200 mm³ (determined by the π/6 × D1 ×
D2 × D3 formula in which the D’s are 3
orthogonal diameters) within 12–24 days after
inoculation were used for treatment.

Hyperthermic treatment.—The mice were
randomly allocated into the different treat-
ment groups. All treatments were admin-
istered to unanaesthetized mice placed in
lucite jigs with the tumour-bearing legs
loosely fixed with tape without impairing the
blood flow to the feet (Overgaard, 1981).
Local hyperthermia was performed with the
tumour-bearing leg immersed into a circu-
lating water bath stabilized to ± 0·05°C of
the adjusted temperature. The intratumoral
temperature stabilized within a few minutes
to approximately 0·2°C below the water-bath
temperature. The water bath was therefore
adjusted to 0·2°C above the desired tumour
temperature, and all temperatures mentioned
in this paper refer to the intratumoral
temperature. Further details of the tempera-
ture measurements and the treatment pro-
cedures are described elsewhere (Overgaard,
1980a, b).

Evaluation of results.—After treatment,
tumour volume was measured daily. The
tumour response was evaluated as tumour
growth time, i.e. the time required for a
tumour to reach a volume 5 times that of the
first treatment day. As previously described
(Kamura et al., 1982), at a given temperature
tumour growth time depends only on heating
time but is independent of sex, batch of mice,
and initial tumour volume (within 150–257
mm³). Dose–response curves for tumour
growth versus heating time were plotted by
means of linear regression calculations.
Student’s t-test or analysis of variances
were used for statistical analysis.

RESULTS

Single heating

The effect of a single heat treatment at
41·5–44·5°C for various periods is shown in
Fig. 1. Tumour growth time was depend-
ent on both temperature and heating time,
and at all temperatures, there was a linear
dose–response relationship between tum-
our growth time and heating time. Table I
shows the calculated characteristics of
these dose–response curves. The heat
sensitivity, based on the slope value,
gradually increased with higher tempera-
tures, whereas the calculated intercept
heat inactivation and temperature, the slope values from Table I were plotted as a function of the reciprocal temperature (Fig. 2). The slope of the resulting Arrhenius plot is a measure of the activation energy $\mu$. This Arrhenius plot showed an inflection point at 42.5°C. The calculated activation energy was 635 kJ/mol (152 kcal/mol) and 1508 kJ/mol (361 kcal/mol), respectively, above and below the inflection point. For the remainder of our studies heating was only performed at temperatures above the inflection point (i.e. $\geq 42.5^\circ$C).

**Effect of preheating temperature on thermotolerance**

Based on the Arrhenius curve, the heating times at 42.5 and 44.5°C, resulting in the same tumour growth time as that of 43.5°C for 30 min, were calculated. Experiments were then made to determine the dependence of thermotolerance on preheating temperature when the heating times were adjusted to give the same level of heat damage.

Recovery from hyperthermic damage at
various temperatures was evaluated by application of 2 separate hyperthermic treatments at different intervals. The first heating was for 60 min at 42.5°C, 30 min at 43.5°C and 15 min at 44.5°C, respectively, the second for 60 min at 43.5°C, respectively, the second for 60 min at 43.5°C (Fig. 3). To ensure that the warm-up time was the same for all treatment groups, irrespective of pretreatment temperature, the 2 treatments were separated by 5 min at the 0-h interval. At all 3 temperatures the tumour growth time decreased with increasing interval to reach its minimum at a 16-h interval, and there was no marked difference between the 3 recovery curves. These recovery curves may illustrate the kinetics of thermotolerance, and thus, the data in Fig. 3 may indicate that thermotolerance developed identically at all 3 temperatures with a maximum at a 16-h interval. This was determined quantitatively at the time of maximum recovery by giving graded second doses at 43.5°C (Fig. 4). Thermotolerance developed, as demonstrated by a lesser slope of the curves for tumours preheated at a 16-h interval as compared to that for tumours treated at a 0-h interval. Table II shows the dose–response curve characteristics and the values of the "thermotolerance ratio" (TTR). As previously discussed (Kamura et al., 1982), this ratio is a measure of the degree of thermotolerance induced by a single hyperthermic treatment and developed during a postheating interval. There was no difference between the intercept and TTR values at the 3 temperatures. So, for a given level of heat damage, the degree of the subsequent thermotolerance was almost independent of temperature, i.e. the relationship between heating time and temperature for the induction of thermotolerance was the same as that found for cell killing by hyperthermia. Therefore, the experiments on the influence of heating time were carried out at the same temperature, i.e. 43.5°C.

**The effect on thermotolerance of varying the preheating time at 43.5°C**

The kinetics of thermotolerance as a function of preheating time is illustrated by the recovery curves in Fig. 5 which shows the tumour response to 43.5°C for 60 min at various intervals following preheating at 43.5°C for 3.5, 7.5, 15, 30, or 45 min, respectively. It is seen that the longer the preheating time, the longer the fraction interval necessary to obtain maximum thermotolerance and the longer the time for complete decay of thermotolerance. A quantitative evaluation of the development of thermotolerance at the time of maximum recovery (Fig. 6 and Table III) showed also that the degree of thermotolerance clearly depended on the duration of the priming 43.5°C-treatment. The longer the preheating time at 43.5°C, the higher the maximum thermotolerance ratio (TTRmax).

This dependence on the primary heating time is seen clearly in Figs 7 and 8, which show that both TTRmax (Fig. 7) and the interval required to obtain TTRmax (Fig. 8) were linear functions of the preheating time.
Table II.—Dose–response curve characteristics of tumours heated at 43.5°C 16 h after preheating at different temperatures

| Temperature (°C) | Preheat time (min) | No. of mice | Tumour growth time after preheat (days) | Intercept<sup>a</sup> (days) | Slope (/min) | Heat treatment at 43.5°C | Intercept<sup>a</sup> (days) | Slope (/min) | TTR<sub>16</sub><sup>b</sup> |
|------------------|--------------------|-------------|---------------------------------------|----------------------------|-------------|------------------------|----------------------------|-------------|-----------------|
| 42.5             | 60                 | 139         | 9·9 (9·3–10·5)<sup>c</sup>             | 10·0                       | 0·154       | 9·9                    | 0·030                       | 5·1         |
| 43.5             | 30                 | 152         | 10·2 (9·6–10·8)                        | 10·3                       | 0·145       | 10·2                   | 0·028                       | 5·2         |
| 44.5             | 15                 | 93          | 10·3 (9·1–11·5)                        | 10·0                       | 0·157       | 9·7                    | 0·029                       | 5·4         |

<sup>a</sup> Not significantly different from the tumour growth time after preheat (P > 0·60).

<sup>b</sup> Thermotolerance ratio (TTR) = slope (0h interval)/slope (16h interval); no significant difference (P > 0·60).

<sup>c</sup> 95% confidence limits in parentheses.
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Fig. 4.—Development of thermotolerance in solid tumours treated at 43.5°C 16 h after preheating for 60 min at 42.5°C (Δ), 30 min at 43.5°C (●) and 15 min at 44.5°C (○) respectively. Curves at 0 h represent tumours treated at 43.5°C at a 0 h interval after preheating. Curves were plotted by linear regression calculations based on the individual mouse tumour growth times (calculated slope values, all significantly different from 0 (P < 0.001)). Each point represents the mean of a group of mice (at least 5), the vertical bars representing ± 1 s.e. See Table II for curve characteristics.

DISCUSSION

The kinetics of thermal inactivation of most cell lines differ above and below 42.5–43.0°C, as indicated by an inflection on an Arrhenius curve (Dewey et al., 1977; Bhuyan, 1979; Henle, 1982; Nielsen et al. (in press). In the present study, the Arrhenius curve (Fig. 2) also showed an inflection point at 42.5°C below which a 2–3-fold increase in the activation energy was observed. A similar biphasic pattern was also obtained in other studies of normal tissues and tumours heated in vivo (Overgaard & Suit, 1979; Henle, 1982). However, in these in vivo studies the measurement of heat effects was based on fixed end-points. The Arrhenius analysis requires the measurement of a rate under at least quasi-steady-state conditions, and thus these in vivo data cannot be represented on an Arrhenius plot without assuming that the accumulation of heat

Fig. 5.—Tumour growth time of solid tumours determined at various intervals after an initial exposure at 43.5°C for 3.5 (Δ), 7.5 (■), 16 (□), 30 (●), and 45 (○) min, respectively. The second treatment was at 43.5°C for 60 min. The tumour growth time after preheating is indicated in Table III. Each point represents the mean of a group of mice (at least 5), the vertical bars representing ± 1 s.e.
damage in vivo is a purely exponential function of heating time (Myers et al., 1980; Henle in press). This assumption is unnecessary in the present study, since the use of tumour growth time as an end-point provided a graded quantitative response to hyperthermia. It should be noted that this assay of tumour response was based on the existence of a linear relationship between tumour growth time and heating time (Fig. 1), and on the fact that the regrowth rate of tumours subjected to hyperthermia did not differ from that of untreated tumours (Kamura et al., 1982). The significance of this independence of the regrowth rate on heating time has recently been discussed by Wheldon & Hingston (1982).

The present experiments showed that thermotolerance could be induced by a prior heating at temperatures ranging from 42.5 to 44.5°C, and the relationship between heating time and temperature for this induced thermotolerance was the same as that found for cell killing by hyperthermia. In other words, both the degree and kinetics of thermotolerance were independent of the preheating tem-
perature (in the range 42·5-44·5°C) if the heating times were adjusted to give the same degree of heat damage. This agrees with data from L1A2 cells in vitro (Nielsen & Overgaard, in press) and mouse pinna (ears) in vivo (Law, 1981).

On the other hand, at a given temperature the development of thermotolerance in the tumours clearly depended on the duration of the primary heat treatment (Figs 5 and 6). Both the fractionation interval necessary to obtain maximum thermotolerance increased as the preheating time was increased. This agrees with earlier observations on cell cultures in vitro (Gerner et al., 1976; Henle et al., 1978; Li et al., 1982; Nielsen & Overgaard, in press, and on different normal tissues in vivo (Law et al., 1979; Hume & Marigold, 1980; Urano et al., 1980; Rice et al., 1982; Field & Anderson, in press). Recently, studies on solid tumours have also shown that at a given interval the degree of thermotolerance increased as the preheating time was increased (Maher et al., 1981; Urano et al., 1982). However, these studies did not provide information about the time course of thermotolerance.

It has been demonstrated on cell cultures (Henle et al., 1978; Li & Hahn, 1980; Li et al., 1982; Nielsen & Overgaard, in press) that the rate of both development and decay of thermotolerance are independent of preheating time. The data in Fig. 5 may also indicate that the rate of decay was independent of the preheating time, although these data provide less detailed information on the decay rate than the in vivo studies. In contrast, the rate of development seemed to be faster following short pretreatments (Fig. 5), as also suggested by Urano et al. (1982). In addition, a recent in vitro study (Nielsen & Overgaard, in press) has shown that preheating also induces a delay in onset of thermotolerance, and that this lag period increases with longer priming treatment periods. Such a lag period was not observed in the present study.

The time for loss of thermotolerance clearly depended on the preheating time as also demonstrated on normal tissues in vivo (Law et al., 1979; Hume & Marigold, 1980). After preheating for 15 min or longer this time for loss of thermotolerance coincided with the occurrence of renewed tumour growth after prior heating. With shorter pretreatments the tumour regrowth was observed before the time for complete decay of thermotolerance. How-

### Table III.—Dose-response characteristics of tumours treated at 43·5°C at the time interval of maximum recoverya after preheating for different times at 43·5°C

| Preheat at 43·5°C (min) | No. of mice | Tumour growth time after preheat (days) | Timea interval of TTRmax (h) | Interceptb (days) | Slope (/min) | TTRmaxc |
|-------------------------|-------------|---------------------------------------|-----------------------------|------------------|-------------|---------|
| 0                       | 149         | —                                     | —                           | 5·9              | 0·147       | 1·0     |
| 3·5                     | 47          | 6·0                                   | 2                           | (5·5-6·3)        | (0·138-0·156) | 1·6     |
| 7·5                     | 74          | 6·4                                   | 4                           | 5·7              | 0·094       | 2·2     |
| 15                      | 59          | 7·5                                   | 8                           | 6·1              | 0·066       | 3·7     |
| 30                      | 91          | 10·2                                  | 16                          | 7·1              | 0·040       | 5·2     |
| 45                      | 80          | 12·8                                  | 28                          | 9·9-10·5         | (0·022-0·034) | (4·2-6·2) |

a Obtained from Fig. 5.
b Not significantly different from the tumour growth after preheat (P > 0·60).
c Maximum thermotolerance ratio (TTRmax) = slope (no preheat)/slope (after preheat).
d 95% confidence limits in parentheses.
ever, as this regrowth appeared late in the decay period, it may not have influenced the results.

Both the TTR$_{\text{max}}$ and the time interval required for its development were linear functions of the priming treatment time (Figs 7 and 8). A similar relationship has also been demonstrated for cell cultures (Henle et al., 1979; Nielsen & Overgaard, in press). However, in these studies the TTR$_{\text{max}}$ and the time required to obtain TTR$_{\text{max}}$ were also linear functions of the logarithm of the relative survival following preheating. Also the data from Law et al. (1979) may indicate a linear relationship between the maximum degree of thermotolerance in mouse ears and the duration of the prior heat treatment at 43.5°C, at least after pretreatments up to 20 min. With pretreatments longer than 20 min, the maximum degree of thermotolerance did not increase further. Such a plateau was not observed in the present study which may be due to a difference in the experimental design. However, in concordance with the present studies, Law et al. (1979) observed that even pretreatments as brief as a few minutes at 43.5°C, which had no detectable heat effects per se, induced thermotolerance.

If the observation of the degree and the kinetics of thermotolerance as linear functions of the level of heat damage following preheating is a general phenomenon, it would have clinical implications. As previously discussed in detail (Nielsen & Overgaard, in press), the degree and kinetics of thermotolerance in different tissues induced by equal pretreatments show great variation and therefore information on thermotolerance in one tissue may not predict the degree and kinetics of tolerance in others. Despite this variation, if the tumour suffers greater primary heat damage than the normal tissues, the tumour may develop greater thermal resistance to a subsequent treatment than the normal tissues, thus cancelling any therapeutic gain. On the other hand, this will depend on the heat sensitivities of the 2 tissues and on the interval between the treatments. This may be further complicated by heterogeneous tumour heating. Due to either vascular cooling or a technically heterogeneous heat distribution, the development of thermotolerance in one part of a tumour may differ from that of other areas within the same tumour. So, given these complications, the problems related to the development of thermotolerance may pose such difficulties that clinical hyperthermia should be administered with sufficiently long fractionation intervals to ensure complete disappearance of thermotolerance (Nielsen & Overgaard, in press; Urano et al., 1982).

In conclusion, the present data indicate that in the temperature range 42.5-44.5°C, both the degree and kinetics of thermotolerance in a solid tumour depend on the level of heat damage following preheating irrespectively of the treatment temperature and heating time used to obtain this level of heat damage.

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REFERENCES

Bluyan, B. K. (1979) Kinetics of cell kill by hyperthermia. Cancer Res., 39, 2277.

Dewey, W. C., Hopwood, L. E., Sapareto, S. A. & Gerweck, L. E. (1977) Cellular responses to combinations of hyperthermia and radiation. Radiology, 123, 463.

Field, S. B. & Anderson, R. L. (1982) Thermotolerance: a review of observations and possible mechanisms (in press).

Gerner, E. W., Boone, R., Connor, W. G., Hicks, J. A. & Boone, M. L. M. (1976) A transient thremotolerant survival response produced by single thermal doses in HeLa cells. Cancer Res., 36, 1035.

Henle, K. J. (1982) Arhenius analysis of thermal responses. In Hyperthermia in cancer therapy (Ed. Storm et al.). Boston: Hall & Co. (in press).

Henle, K. J. & Dethlefsen, L. A. (1978) Heat fractionation and thermotolerance: a review. Cancer Res., 38, 1843.

Henle, K. J., Bittner, A. F. & Dethlefsen, L. A. (1979) Induction of thermotolerance by multiple heat fractions in Chinese hamster ovary cells. Cancer Res., 39, 2486.
Hume, S. P. & Marigold, J. C. L. (1980) Transient, heat-induced thermal resistance in the small intestine of mouse. Radiat. Res., 82, 526.

Kamura, T., Nielsen, O. S., Overgaard, J. & Andersen, A. H. (1982) Development of thermotolerance during fractionated hyperthermia in a solid tumour in vivo. Cancer Res., 42, 1744.

Law, M. P. (1981) The induction of thermal resistance in the ear of the mouse by heating at temperatures ranging from 41-5 to 45-5°C. Radiat. Res., 85, 126.

Law, M. P., Coultas, P. G. & Field, S. B. (1979) Induced thermal resistance in the mouse ear. Br. J. Radiol., 52, 308.

Li, G. C. & Hahn, G. M. (1980) A proposed operational model of thermotolerance based on effects of nutrients and the initial treatment temperature. Cancer Res., 40, 4501.

Li, G. C., Fisher, G. A. & Hahn, G. M. (1982) Induction of thermotolerance and evidence for a well-defined thermotropic cooperative process. Radiat. Res., 89, 381.

Maher, J., Urano, M., Rice, L. & Sutt, H. D. (1981) Thermal resistance in a spontaneous murine tumour. Br. J. Radiol., 54, 1086.

Myers, R., Robinson, J. E. & Field, S. B. (1980) The relationship between heating time and temperature for inhibition of growth in baby rat cartilage by combined hyperthermia and x-rays. Int. J. Radiat. Biol., 38, 373.

Nielsen, O. S. & Overgaard, J. (1979) Effect of extracellular pH on thermotolerance and recovery of hyperthermic damage in vitro. Cancer Res., 39, 2772.

Nielsen, O. S. & Overgaard, J. (1982) Influence of time and temperature on the kinetics of thermotolerance in L1A2 cells in vitro. Cancer Res. (in press).

Nielsen, O. S., Hume, K. J. & Overgaard, J. (1982) Arrhenius analysis of survival curves from thermotolerant and step-down heated L1A2 cells in vitro. Radiat. Res. (in press).

Overgaard, J. (1980a) Simultaneous and sequential hyperthermia and radiation treatment of an experimental tumor and its surrounding normal tissue in vivo. Int. J. Radiat. Oncol. Biol. Phys., 6, 1507.

Overgaard, J. (1980b) Effect of misonidazole and hyperthermia on the radiosensitivity of a C3H mouse mammary carcinoma and its surrounding normal tissue. Br. J. Cancer, 41, 10.

Overgaard, J. (1981) Effect of hyperthermia on the hypoxic fraction in an experimental mammary carcinoma in vivo. Br. J. Radiol., 54, 245.

Overgaard, J. & Sutt, H. D. (1979) Time-temperature relationship in hyperthermic treatment of malignant and normal tissue in vivo. Cancer Res., 39, 3248.

Rice, L. C., Urano, M. & Maher, J. (1982) The kinetics of thermotolerance in the mouse foot. Radiat. Res., 89, 291.

Spiro, I. J., Safareto, S. A., Raaphorst, G. P. & Dewey, W. C. (1982) The effect of chronic and acute heat conditioning on the development of thermal tolerance. Int. J. Radiat. Oncol. Biol. Phys., 8, 53.

Urano, M., Rice, L. C. & Montoya, V. (1982) Studies on fractionated hyperthermia in experimental animal systems. II. Response of murine tumors to two or more doses. Int. J. Radiat. Oncol. Biol. Phys., 8, 227.

Urano, M., Rice, L., Kahn, J. & Sedlack, R. S. (1980) Studies on fractionated hyperthermia in experimental animal systems. I. The foot reaction after equal doses: heat resistance and repopulation. Wieldon, T. E. & Hingston, E. C. (1982) Differential effect of hyperthermia and x-irradiation on regrowth rate and tumour-bed effect for a rat sarcoma. Br. J. Cancer, 45, 260.