Hyperthermia Treatment of Experimental Tumors

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The therapeutic advantage of combining hyperthermia with x-irradiation to treat tumors depends on whether or not it is possible to achieve greater thermal sensitization of tumors than of normal tissues. To determine such therapeutic gain factors (TGF), we assessed the response of mouse skin and seven transplantable mouse tumors to graded x-ray doses given alone or combined with moderate heat (42.5°C for 60 minutes). We constructed dose response curves for the average early skin reaction and for the induced delay in tumor regrowth to an arbitrarily chosen size.

We studied the following areas: 1) the therapeutic gain of combining heat with x-irradiation; 2) irradiation and heat sequencing; 3) vascular occlusion; 4) temperature uniformity; 5) hyperthermia and metastatic spread; 6) fractionated treatment; and 7) thermal tolerance. Our results are not as promising as those of other published studies.

We have shown that the time interval between heat and irradiation is important, and we believe that the separate cytotoxic action of heat and x-irradiation is likely to be more beneficial than the synergistic effect of combining the two in close sequence. We have also demonstrated the deficiencies of using hot water to achieve uniform heating, and the possible artefacts of vascular occlusion. We observed no significant effect on the spread of metastases when heat is used adjunctively with x-rays. We also induced thermal tolerance in a mouse tumor, which may account for the loss of therapeutic advantage seen with fractionated treatments.

The usefulness of hyperthermia as an adjunct to radiotherapy depends upon achieving a greater thermal sensitization of tumors than of normal tissues. Thus, quantitative studies of the thermal sensitization of both tumors and normal tissues treated under comparable conditions are needed before the technique can be adopted for clinical use.

Materials and Methods

In order to determine therapeutic gain factors,* we assessed the response of mouse skin and of seven transplantable mouse tumors to graded x-ray doses, given alone or combined with moderate heat (42.5°C for 60 minutes). We constructed dose response curves for the average early skin reaction (scored between 10 and 32 days) and for the induced delay in tumor regrowth to an arbitrarily chosen size (4.5 mm larger diameter than at irradiation).

The details of the experimental procedures have been published elsewhere (1-3). Briefly, the mice are anesthetized with sodium pentobarbital, irradiated with 240 kV x-rays, and heated locally by immersing the foot or the tumor in a water bath maintained by a pump and thermostat at the desired temperature.

Several questions have been posed:

1) Are tumors sensitized to x-rays more than skin if an equal heat treatment is applied to both?

2) Is the sequence of heat and x-irradiation important?

3) Are there experimental artefacts due to methods of restraint or the site of tumor implant?

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* Thermal Enhancement Ratio (TER) $= \dfrac{\text{x-ray dose without heat}}{\text{x-ray dose with heat}}$

Therapeutic Gain Factor (TGF) $= \dfrac{\text{TER tumor}}{\text{TER normal tissues}}$
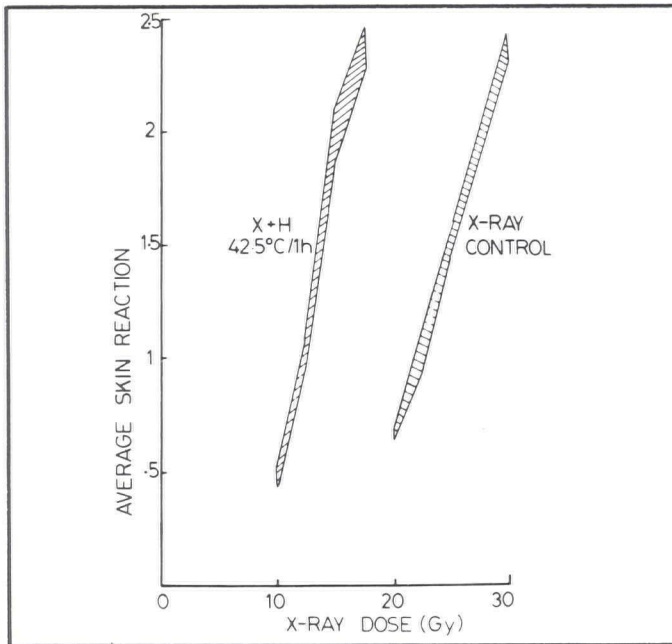
Fig. 1
Dose response curves for early skin reactions on mouse feet treated with x-rays alone or x-rays followed immediately by heat for 60 minutes at 42.5°C. Hatched areas represent envelopes drawn through the standard errors on the points; a significant TER is observed only in the clear space between the hatched areas.
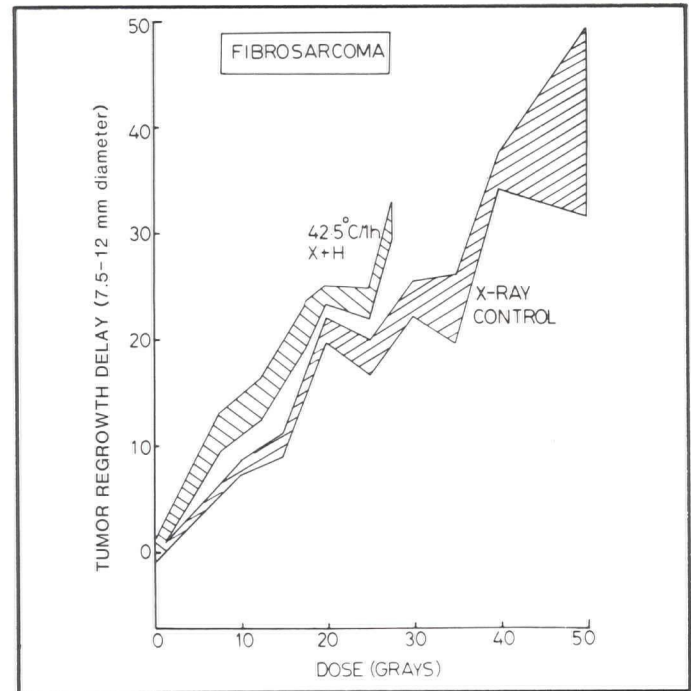

Fig. 2
Dose response curves for regrowth delay of the fibrosarcoma SA FA treated with x-rays alone or combined with heat. Less thermal sensitization is evident than in the skin (Fig. 1).

4) How non-uniform is the heating of tissues with hot water?

5) Does hyperthermia influence the incidence or time of appearance of metastases?

6) Is the same therapeutic gain observed with single doses and with fractionated treatments?

7) Is thermal tolerance induced in both skin and tumors?

Results

Therapeutic gain

Fig. 1 shows the dose response curves for skin treated with x-rays alone or with x-rays followed by heating at 42.5°C for 60 minutes. We found that heat definitely enhanced the effect of radiation. Fig. 2 shows the response of a transplantable mouse tumor treated in the same way. The amount of sensitization is significant but less than the effect observed in the skin. Sensitization does seem to vary with dose level and seems greatest at the higher levels.

Table I shows the TER values measured at equivalent dose levels for skin and for seven different transplantable mouse tumors, when the heat is given within minutes after irradia-

tion. The tumor TER values are similar to or less than those observed in skin, indicating no therapeutic gain relative to treatment with x-rays alone. Skin TER values are shown for heat treatments at temperatures of both 42.5°C and 41.5°C, because the tumor may have regions significantly cooler than skin for the same water bath temperature (see below). These tumor data are plotted in Fig. 3 for comparison with all similar data from the literature. Our tumor data (solid symbols) are plotted as if 0.3°C below the waterbath temperature. The Gray Laboratory tumor data clearly give a more pessimistic picture than many other published tumor results, which may be artificially high because of inadvertent vascular occlusion (see below).

Sequencing of irradiation and heat

TER values have been measured for both skin and the seven types of transplantable tumor with intervals ranging from 0-24 hours and with heat given either before or after irradiation (1,3). Fig. 4 shows the data for one tumor (SA FA) compared with the results for skin heated at 42.5°C for one hour. The thermal sensitization of skin (solid line) is rapidly lost as the intervals increase, particularly when the heat follows irradiation, but an effect is still observed in the tumor at six hours. Thus, although the absolute thermal sensitization of tumors is greatest with consecutive treat-

TABLE I

Thermal Enhancement and Therapeutic Gain for X-rays and Heat

	TER	TGF*	TGF**
skin			
42.5°C	1.7-1.8	—	—
41.5°C	1.5	—	—
tumors			
SA.FA	1.5-1.7	0.9	1.1
CA.SQ.D	1.5-1.7	0.9	1.1
CA.NTa	1.2-1.4	0.7	0.9
SA.S	1.1-1.3	0.7	0.8
SA.F	1.2-1.4	0.7	0.9
CA.MT	1.5-1.7	0.9	1.1
CA.RH	1.0-1.3	0.6	0.8

* TGF values calculated relative to the skin heated to 42.5°C.

** TGF values calculated relative to the skin heated to 41.5°C.

TABLE II

Thermal Enhancement Ratios with Different Intervals Between Heat and X-rays

Hours	Heat + X-ray						X-ray + Heat					
	24	6	3	2	1	0	0	1	2	3	6	24
Skin	—	1.2	1.2	1.3	1.4	1.7	1.8	1.3	1.2	1.0	1.0	—
SA FA	—	1.0	1.8	1.3	1.1	1.4	1.7	1.3	1.4	1.3	1.2	—
CA SQ D	1.3	1.4	1.4	1.6	1.3	1.7	1.6	1.3	1.5	1.2	1.2	1.2
CA NTa	1.2	1.2	1.2	—	1.3	1.3	1.3	1.4	—	1.3	1.3	1.2
CA MT	0.9	—	—	—	—	—	1.6	1.5	—	~1.8	1.5	1.1
SA F	1.3	—	—	—	—	—	1.3	1.4	—	1.4	1.3	1.3
SA S	1.0	—	—	—	1.3	—	1.2	1.3	—	1.0	1.1	1.0

ments, a therapeutic advantage is seen *only* with the longer intervals. For consecutive heat and irradiation, there is often a therapeutic *loss,* whereas for heat before irradiation the response of both skin and tumor is more unpredictable, showing sensitization at some intervals and not at others (4).

Table II shows the TGF for six tumors compared with skin for the different time intervals tested. Because the sensitizing effect on skin diminishes with time, an interval between x-rays and heat of three to six hours has the advantage that no reduction in radiation dose is needed to prevent excessive injury to normal tissue. For shorter intervals the radiation dose would have to be reduced to stay within the limits of normal tissue tolerance. This separation of x-rays and heat probably utilizes the independent cytotoxic action of the two agents rather than their synergistic interaction. Results consistent with ours have been reported for normal tissues by the Hammersmith group (5) and for tumors by Jansen, et al (6) and by Overgaard (7).

Vascular occlusion

Hypoxia, nutrient deficiency, and low pH are all factors known to influence the sensitivity to direct heat killing. We have shown that occluding the blood supply with a clamp can result in tumor cures with immersion at 44.8°C for 15 minutes, whereas no cures are achieved with this heat dose in unobstructed tumors (8). These results of two different types of tumor (previously unpublished for SA F) are shown in Figs. 5 and 6 (9). The fact that prolonged clamping is necessary to achieve the full effect suggests that neither hypoxia nor the loss of the cooling effect of flowing blood are major factors, as both of these would occur very rapidly after vascular occlusion.

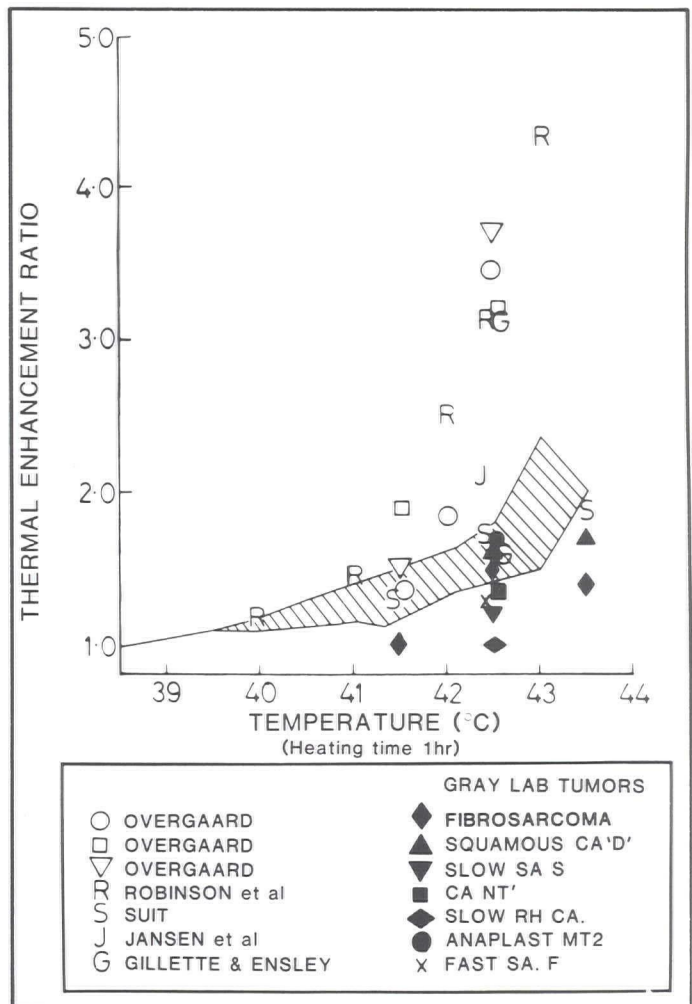If a clamp is applied for a heat treatment of 42.8°C for one

Fig. 3

Thermal enhancement ratio (TER) for consecutive x-rays and heat to skin (hatched area) and to tumors reported in the literature (see key) or from the Gray Laboratory. Much less sensitization is seen in our tumors than in many published studies. There is no therapeutic gain.
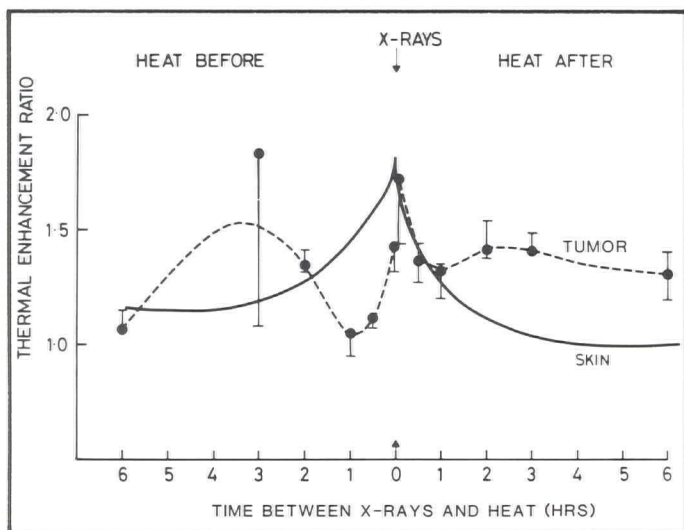
Fig. 4

Thermal Enhancement Ratio (TER) for skin and for fibrosarcoma SA FA as a function of the sequence and intervals between x-rays and heat.

Fig. 5

Tumor control for heat treatment applied at various times relative to an 80-minute period during which the blood supply is occluded (8).

hour combined with graded x-ray doses, thermal sensitization is much greater than in unclamped tumors. Similarly high TER values occurred for regrowth delay of unclamped tumors when they were implanted subcutaneously on the tail. While the tail is a popular site for hyperthermia experiments because it is easy to heat without raising the body core temperature, the extreme constriction imposed by the skin on the tail may also act as a natural means of vascular occlusion (10). When TER values from clamped tumors or from tumors growing on the tail are compared with those in Fig. 3, they are among the higher values recorded in some other published studies (10). If these latter TER values result from inadvertent vascular occlusion, they will not be relevant to most human tumors. Deliberate vascular occlusion is not likely to be useful for clinical therapy because it has been observed that the effectiveness of heat is also increased in normal tissues if the blood supply is occluded (11).

Temperature uniformity

Our initial studies were published on the basis that tumors achieved a temperature 0.3°C below water bath temperature, within three to five minutes of immersion. This statement was based on readings with a Bailey 29G needle thermocouple in two tumor types, with the probe placed at various depths in each tumor. Although we observed very little variability, subsequent measurements on a larger number of tumors of varying histological types have failed to confirm this early observation. As others have reported (12), there are considerable temperature gradients across tumors and considerable variation from one tumor to another, even within the same histological type. Fig. 7 shows
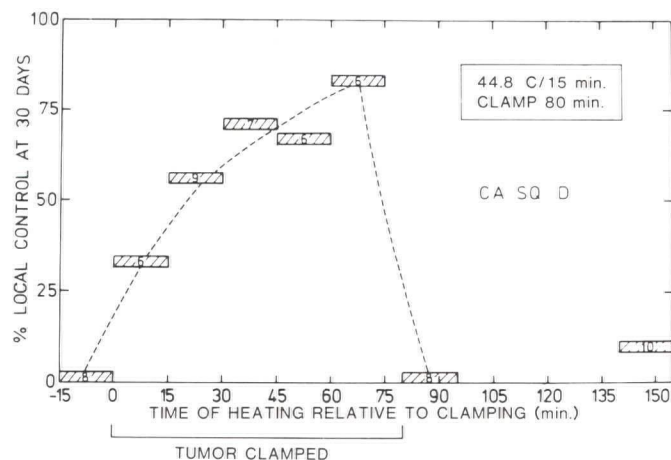
the probe measurements on many samples of four different types of tumor, with readings taken simultaneously with three probes at different positions within each tumor (5.5-6.5 mm diameter). The temperature near the skin surface sometimes reaches 0.3-0.1°C below the water temperature, but at deeper levels adjacent to the underlying muscle much lower temperatures are recorded. A similar variation in temperature in relation to the main blood vessels has been reported for normal tissue (the intestine) by Hume, et al (13).

Our observations of temperature non-uniformity prompted us to attempt to quantitate thermal damage at different positions in the tumor by histological assessment of tumors
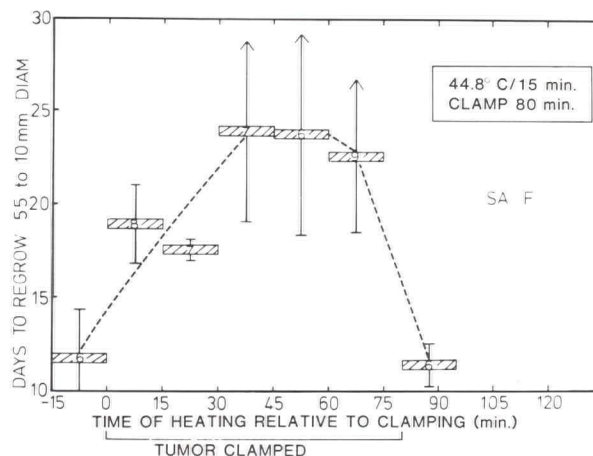

Fig. 6

Regrowth delay for heat treatment applied relative to the time of clamping. Increased delay is seen in clamped tumors (9).
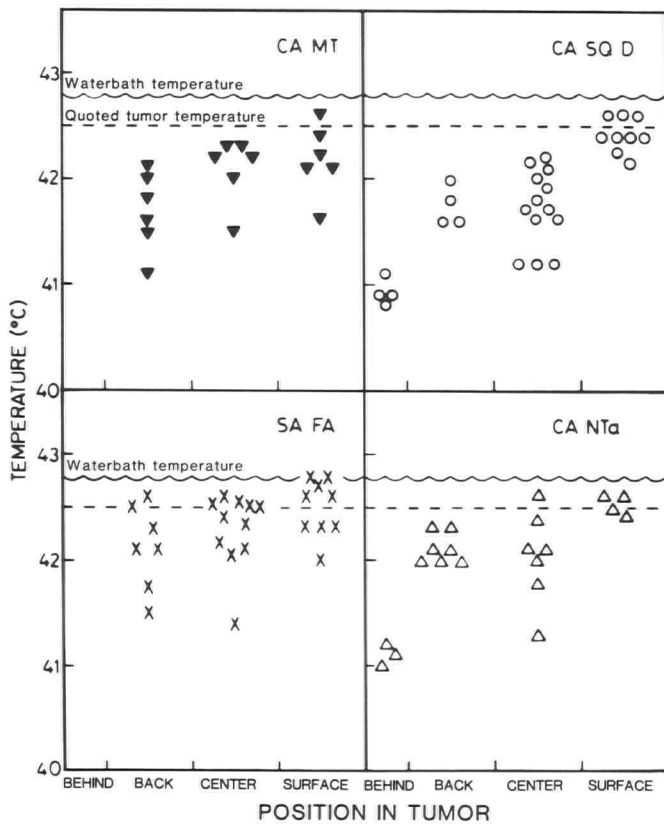
Fig. 7
Thermocouple determinations of the temperature at various positions within mouse tumors of four different types. There is a wide variation in the measured values, both between tumors and at different points within each one (9).

obtained at sequential intervals after heating for one hour at 42.8°C or 44.8°C (10). The results for one type of tumor are shown in Fig. 8. Dead cells were apparent within 24 hours of heating. At the lower temperature the pattern was not clear, with pyknotic and viable cells being seen at all positions across the tumor diameter. At the higher temperature (44.8°C), only a few viable cells were seen in the tumor, most of them as a thin rim adjacent to the underlying muscle. On successive days we observed that this rim expanded as the thermally protected cells proliferated.

Thus, it is clear that water bath heating is inadequate as a means of elevating the temperature, even through 5-6 mm of tissue. For tumors, the critical temperature will be in the cold spots, since these will result in surviving tumor foci from which the tumor can grow again. Such foci may occur adjacent to a heat sink (as in the subcutaneous muscle), or more locally around large blood vessels, where the heat can be dissipated by blood flow. In normal tissues, by contrast, the critical temperatures will be those in the hot spots, since even a tenth of a degree can transform an acceptable normal tissue response into necrosis (14).

Metastases and hyperthermia

We have attempted to study the effect of heat on metastatic spread in both retrospective and prospective studies. In the retrospective analysis of animals in regrowth delay studies, the analysis is complicated by the duration of the regrowth delay and hence the time available for latent metastases to grow to an observable size. Fig. 9 compares the percentage of animals with metastases that died within certain time intervals (because of a regrowing primary tumor or because of sickness due to metastases) to the percentage of animals treated with x-rays alone or with x-rays plus heat. The combined treatments have been separated into those given in close sequence and those given with an interval longer than one hour between the x-rays and heat. The tendency noted toward more metastases in the heat-treated groups than in those treated with x-rays alone is not significant. It may result from the more effective treatment of the primary tumor so that a longer time is available for latent metastases to appear. In the SA FA, metastases tended to occur earlier, although the same high proportion developed after x-rays or the combined treatment. The results from five retrospective analyses of metastases are summarized in Table III (9).

Fractionated treatments

On the basis of our single dose data, we concluded that heat given three hours after irradiation was more likely to be beneficial than heat given immediately after x-rays. We extended this study to two and five daily fractions of x-rays, with heat (42.5°C/60 minutes) given immediately or three hours after *each* fraction. Dose response curves were obtained for both skin and tumor (SA FA) as before (15). The results are summarized in Table IV. In the fractionated experiment, the therapeutic gain observed with single

TABLE III
Incidence of Metastases After
X-rays Alone or X-rays plus Heat

Tumor	X-rays + Heat		X-rays alone	Heat + X-rays	
	long interval	consec		consec	long interval
CA SQ D	29%	34%	28%	45%	34%
CA NTa	78%	65%	68%	65%	78%
SA F	94%	89%	84%	—	78%
CA MT	20%	68%	50%	—	36%
SA S	21%	22%	15%	—	15%

None of these tumors shows a significant increase in the incidence of metastases after the combined treatment.
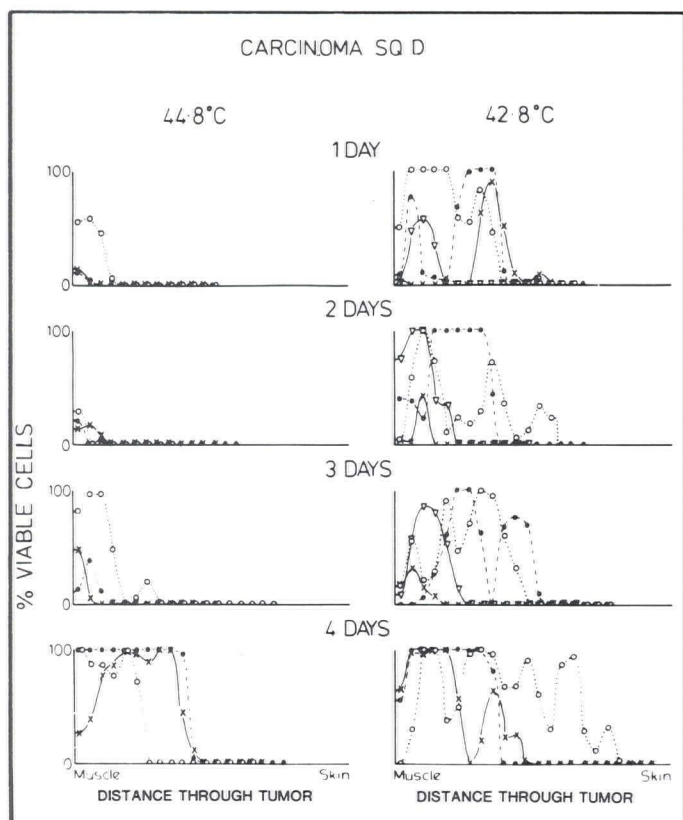
Fig. 8

Histological assessment of the surviving cells and repopulating tumors after two different heat treatments (duration: 60 minutes). Each symbol represents a tumor. After the higher temperature, cells survived only adjacent to the body, and regrowth occurred from this region.
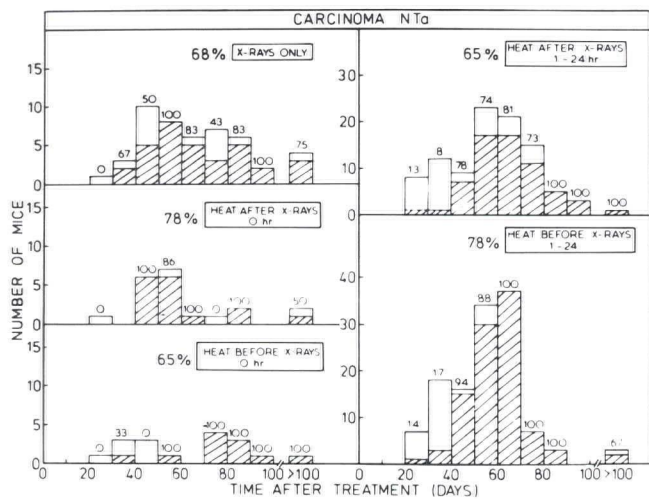

Fig. 9

Incidence of metastases as a function of the time at which the animal was sacrificed because of local recurrence of a treated tumor or because of sickness due to metastases. No significant change in the incidence or time of appearance occurred for the different treatments.

doses with an interval of three hours was completely lost. This pessimistic result needs to be tested in other tumor types and with intervals other than 24 hours between successive doses. Longer intervals are not possible in this rapidly growing fibrosarcoma, but a 24-hour interval means that each heat treatment is given 21 hours *before* the next x-ray fraction as well as three hours *after* the last. The loss of therapeutic advantage could result from heat-induced thermal tolerance, reoxygenation and recruitment, or increased blood flow.

Thermal tolerance

Thermal tolerance has been demonstrated both in vitro and in vivo. Joshi, et al (16) showed that quite low heat treatment (38°C) could induce a tolerance to subsequent thermal cell killing. Law, et al (14) showed that thermal tolerance to direct heat damage was greater, and lasted longer, than tolerance to heat sensitization of x-ray damage. If thermal tolerance could be induced in normal tissues but not in tumors, then the therapeutic gain of fractionated treatments would be expected to be much greater than that seen with single doses. Unfortunately, for the tumor and the normal tissue on which we have tested this idea, the reverse seems to be true, i.e., induced thermal tolerance in the fibrosarcoma is greater than in the skin. We used a priming temperature of 42.5°C, and pretreatments with four daily heat treatments, each lasting 60 minutes, or a single heat treatment were followed 24 hours later by graded x-ray doses and heating at 42.5°C for 60 minutes (Table V). The thermal sensitization produced in the fibrosarcoma by x-rays and heat given in close sequence (TER = 1.4) was completely lost if the tumor was preheated with either one or four doses of heat. Thus, thermal tolerance was readily induced in this tumor, a result which could explain the loss of therapeutic gain with fractionated treatments. By contrast, the thermal sensitization of skin was the same (TER = 1.6) whether it was preheated or not, so that no induced thermal tolerance was observed.

TABLE IV
Thermal Enhancement and Therapeutic Gain
with Fractionated Treatments

	Tumor TER	Skin TER	TGF
Heat given immediately after each fraction			
Single doses	1.5-1.7	1.7-1.8	0.8-1.0
2F/24 hrs	1.0	1.9	0.5
5F/4 days	1.1-1.3	1.7	0.6-0.8
Heat given three hours after each fraction			
Single doses	1.2-1.5	1.0	1.2-1.5
2F/24 hrs	1.0-1.1	1.1	0.9-1.0
5F/4 days	1.0-1.3	1.0-1.1	0.9-1.3

TABLE V
Thermal Tolerance in Skin and Tumor (SA FA)

	TER tumor	TER skin	TGF
No preheating	1.4	1.6	0.9
1 pretreatment (42.5°C/1 hr)	0.9	1.6	0.6
4 pretreatments (each 42.5°C/1 hr)	1.0	1.6	0.6

is likely to be more beneficial than the synergistic effect of combining the two in close sequence. We have also demonstrated the deficiencies of using hot water to achieve uniform heating, and the possible artefacts of vascular occlusion. We observed no significant effect on the spread of metastases when heat was used adjunctively with x-rays, although the metastases may appear earlier. We also induced thermal tolerance in a mouse tumor, but not in mouse skin, which may account for the loss of therapeutic advantage seen with fractionated treatments.

Summary

The results using water bath heat combined with 240 kV x-rays to look at the therapeutic benefit of the combined modality are not as promising in our seven transplantable mouse tumors relative to skin as in many of the previously published studies. We have shown that the time interval between heat and irradiation is important, and we believe that the *separate* cytotoxic action of heat and x-irradiation

References

1. Stewart FA, Denekamp J. Sensitization of mouse skin to x-irradiation by moderate heating. Radiology 1977;123:195-200.

2. Stewart FA, Denekamp J. The therapeutic advantage of combined heat and x-rays on a mouse fibrosarcoma. Br J Radiol 1978; 51:307-16.

3. Hill SA, Denekamp J. The response of six mouse tumors to combined heat and x-rays: Implications for therapy. Br J Radiol 1979;52:209-18.

4. Law MP, Ahier RG, Field SB. The response of the mouse ear to heat applied alone or combined with x-rays. Br J Radiol 1978;51:132-38.

5. Field SB, Bleehen NM. Hyperthermia in the treatment of cancer. Cancer Treat Rev 1979;6:63-94.

6. Jansen W, Van Der Schueren E, Breur K. Thermal enhancement of the radiation response of the skin and a mammary carcinoma in mice. In: Streffer, et al, eds. Cancer therapy by hyperthermia and radiation. Munich: Urban and Schwarzenberg, 1978:253-54.

7. Overgaard J. The response to combined hyperthermia and radiation of an experimental mammary carcinoma and its surrounding normal tissue. Br J Radiol 1978;51:935-36.

8. Hill SA, Denekamp J. The effect of vascular occlusion on the thermal sensitization of a mouse tumor. Br J Radiol 1978;51:997-1002.

9. Hill SA. Factors influencing the response of six types of mouse tumors to combined heat and X-rays. Ph.D. thesis. London University, 1980.

10. Hill SA, Denekamp J, Travis EL. Temperature non-uniformity in water-bath-heated tumors. Eur J Radiotherapy 1980 (in press).

11. Morris CC, Myers RG, Field SB. The response of the rat tail to hyperthermia. Br J Radiol 1977;50:576-80.

12. Bleehen NM, Honess DJ, Morgan JE. The combined effects of hyperthermia and hypoxic cell sensitizers. In: Streffer, et al, eds. Cancer therapy by hyperthermia and radiation. Munich: Urban and Schwarzenberg, 1978:62-71.

13. Hume SP, Robinson JE, Hand JW. The influence of blood flow on temperature distribution in the exteriorized mouse intestine during treatment by hyperthermia. Br J Radiol 1979;52:219-22.

14. Law MP, Coultas PC, Field SB. Induced thermal resistance in the mouse ear. Br J Radiol 1979;52:308-14.

15. Stewart FA, Denekamp J. Fractionation studies with combined x-rays and hyperthermia in vivo. Br J Radiol 1980;52:346-56.

16. Joshi DS, Jung H. Thermotolerance and sensitization induced in CHO cells by fractionated hyperthermia treatments at 38°-45°C. Eur J Cancer 1979;15:345-50.