# Combined Heat and X-ray Treatment of Experimental Tumours

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The usefulness of hyperthermia as an adjunct to radiotherapy depends upon achieving a greater thermal sensitization of tumours than of normal tissues. Thus quantitative studies of the thermal sensitization of both tumours and normal tissues treated under comparable conditions are necesaary pre-clinical studies.

$$\text{Thermal Enhancement Ratio (TER)} = \frac{\text{X-ray dose without heat}}{\text{X-ray dose with heat}}$$

to achieve the same level of damage.

$$\text{Therapeutic Gain Factor (TGF)} = \frac{\text{TER tumour}}{\text{TER normal tissue}}$$

In order to determine therapeutic gain factors we have assessed the response of skin and of a variety of transplantable mouse tumours to graded X-ray doses, given alone or in conjunction with a moderate heat treatment, e.g. $42.5^{\circ}C$ for 60 minutes.   Dose response curves have been constructed for the average early skin reaction (scored between 10 and 32 days), and for the induced delay in tumour regrowth to an arbitrary size (e.g. 4.5 mm larger diameter than at irradiation).

The details of the experimental procedures have been published elsewhere (Stewart & Denekamp, 1977;  Stewart & Denekamp, 1978;  Hill & Denekamp, 1979). Briefly, the mice are anaesthetised with sodium pentobarbital, irradiated with 240 kV X-rays and heated locally by immersion of the foot or the tumour in a waterbath maintained by a pump and thermostat at the desired temperature.

Several questions have been posed:

1)   Are tumours 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 tumour implant ?

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

5) Is there any influence of hyperthermia on 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 tumours ?

## Therapeutic gain

Figure 1 shows the dose response curves for skin treated with X-rays alone or with X-rays followed by heating at $42.5^\circ C$ for 60 minutes. Definite enhancement of radiation damage is observed with heat. The hatched areas represent envelopes drawn through the standard errors on the points and a significant TER is only observed where there is a clear space between the hatched areas.

Figure 2 shows the response of a transplantable mouse tumour treated in the same way. A significant sensitization is seen but it is smaller than the effect observed in the skin, and does seem to vary with dose level, being greatest at the higher dose levels.

Table 1 shows the TER values measured at equivalent dose levels for skin and for seven different transplantable mouse tumours, when the heat is given within minutes after irradiation. The tumour 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 $42.5^\circ C$ and $41.5^\circ C$ because the tumour may have regions that are significantly cooler than skin for the same waterbath temperature (see below). These tumour data are plotted in Figure 3 for comparison with all similar data from the literature. Our tumour data (solid symbols) are plotted as if $0.3^\circ C$

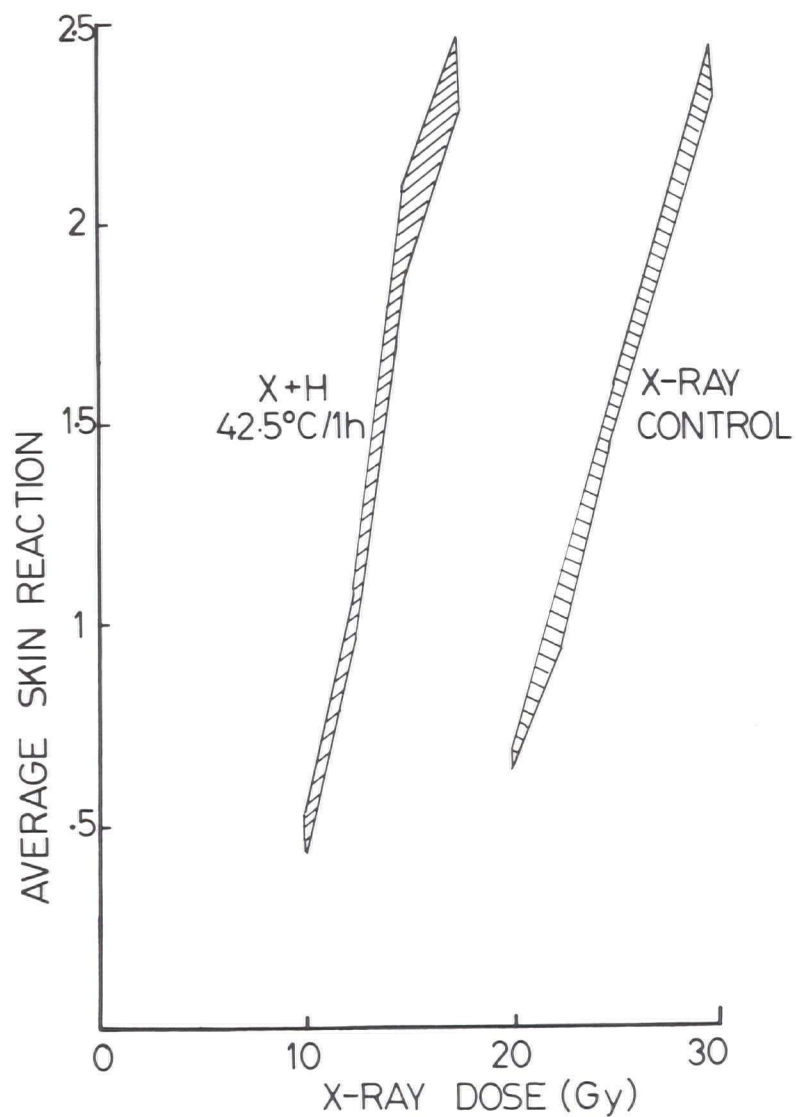Fig 1    Dose response curves for early skin reactions on mouse feet treated

with X-rays alone or X-rays followed immediately by 60 minutes heat

at 42.5°C.    The hatched area represents the envelope of the error
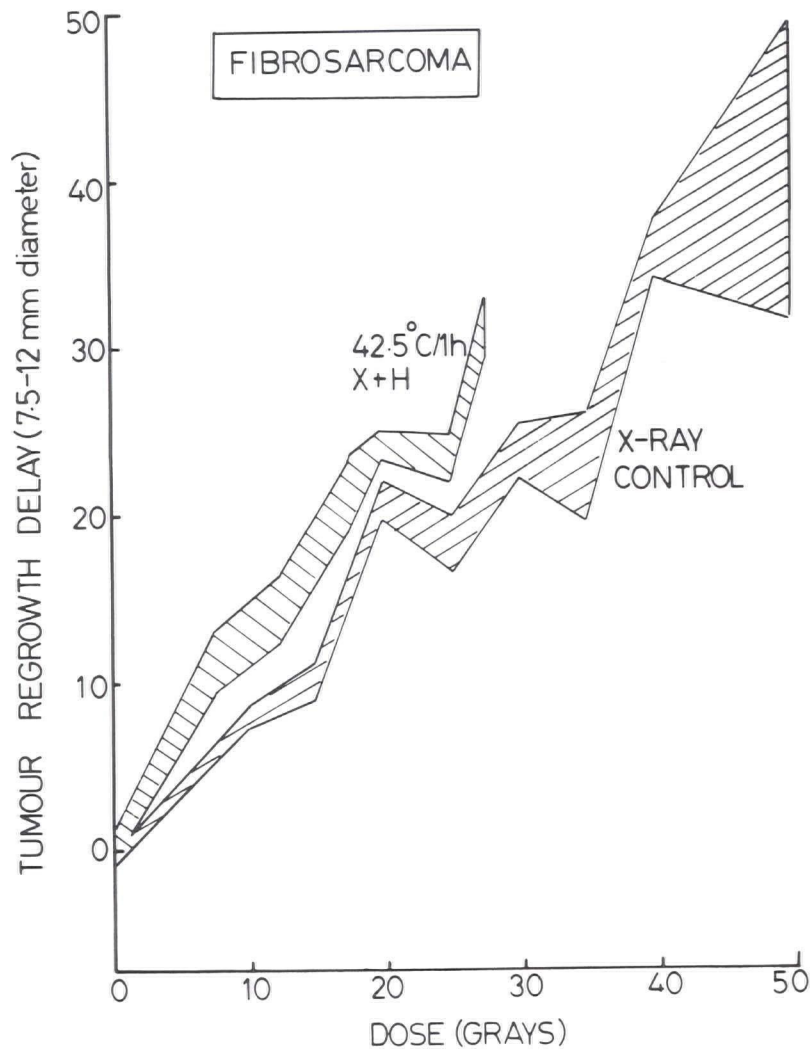
bars.

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 seen than in the skin.

TABLE 1

Thermal Enhancement & Therapeutic Gain for

X-rays and Heat in Close Sequence

|  |  | TER | $^a$TGF | $^b$TGF |
|---|---|---|---|---|
| skin | 42.5$^o$C | 1.7–1.8 | – | – |
| " | 41.5$^o$ | 1.5 | – | – |
| tumours | | | | |
| | 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 |

[a] TGF values calculated relative to the skin heated to 42.5$^o$C.

[b] TGF values calculated relative to the skin heated to 41.5$^o$C.

GRAY LAB TUMOURS

○ OVERGAARD   ◆ FIBROSARCOMA
□ OVERGAARD   ▲ SQUAMOUS CA.'D'
▽ OVERGAARD   ▼ SLOW SA. S
R ROBINSON et al.   ■ CA. NT'
S SUIT   ◆ SLOW RH. CA.
J JANSEN et al.   ● ANAPLAST. MT2
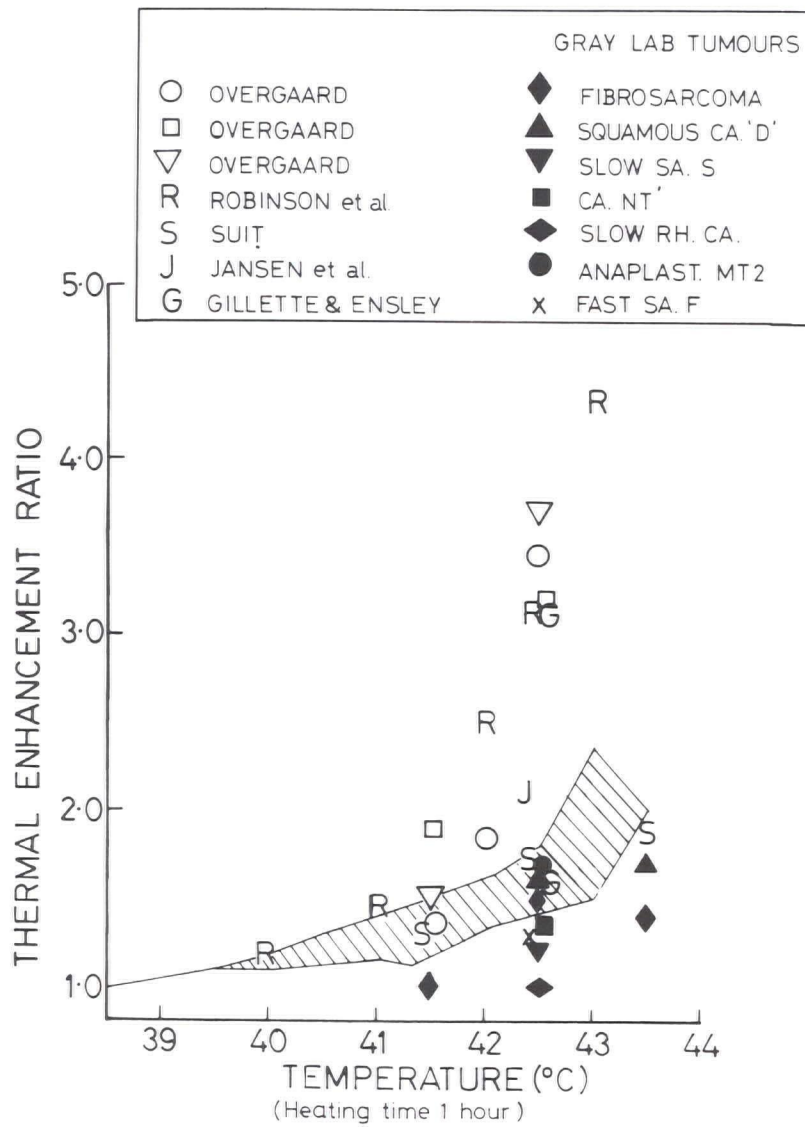G GILLETTE & ENSLEY   X FAST SA. F

Fig 3.  Thermal Enhancement Ratios for consecutive X-rays and heat to skin (hatched area) and to tumours from the literature (see key) or from the Gray Laboratory.  Much less sensitization is seen in our tumours than in many published studies.  There is no therapeutic gain.

below the waterbath temperature.  It is clear that the Gray Laboratory tumours

give a more pessimistic picture than many of the other sets of published

tumour results.  We believe that some of the published TER values may be

artificially high as a result of inadvertent vascular occlusion (see below).

Sequencing of irradiation and heat

TER values have been measured for both skin and the 7 types of transplant-

able tumour with intervals ranging from 0-24 hrs and, with heat given either

before or after irradiation (Stewart & Denekamp, 1977; Hill & Denekamp, 1979).

Fig 4 shows the data for one tumour (SA FA), compared with the results for

skin heated at 42.5$^\circ$C for 1 hour.  The thermal sensitization of skin (solid

line) is rapidly lost with increasing intervals, particularly when the heat

follows irradiation, but an effect is still observed in the tumour at 6 hours.

Thus although the absolute thermal sensitization of tumours is greatest with

consecutive treatments, a therapeutic advantage is only seen with the longer

intervals.  For consecutive heat and irradiation there is often a therapeutic

loss, and for heat before irradiation the response of both skin and tumour

is more unpredictable, showing sensitization at some intervals and not at

others (Law et al.,1978).

The TGF for six tumours compared with skin are shown in Table 2 for the

different time intervals tested.  Because the sensitizing effect on skin

diminishes with time, an interval between X-rays and heat of 3-6 hours has

the advantage that no reduction in radiation dose is necessary to avoid

excessive normal tissue injury.  Any shorter interval, where normal tissue

sensitization is observed, would require a reduction in the radiation dose

to stay within normal tissue tolerance limits.  This separation of X-rays

and heat is probably utilizing 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 (see Field &

Bleehen 1979 for review) and for tumours by Jansen et al. (1978) and by
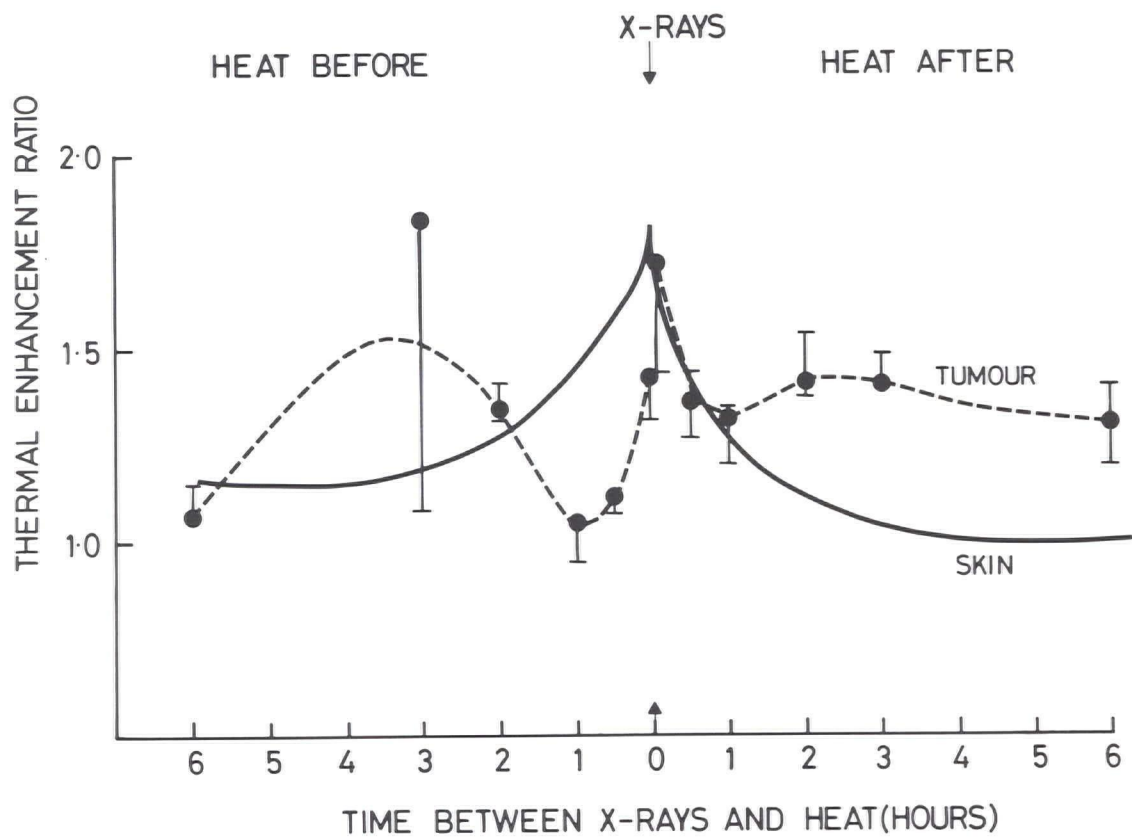
Overgaard (1978).

Fig 4. Thermal Enhancement Ratios for skin and for fibrosarcoma SA FA as a function of the sequence and intervals between X-rays and heat.

TABLE II

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

| | HEAT + X | | | | | | X + HEAT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24hr | 6hr | 3hr | 2hr | 1hr | 0hr | 0hr | 1hr | 2hr | 3hr | 6hr | 24hr |
| 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 |

### Vascular occlusion.

Hypoxia, nutrient deficiency and low pH are all factors that are known to influence the sensitivity to direct heat killing. We have shown that application of a clamp to occlude the blood supply can result in tumour cures with immersion at $44.8^\circ$C for 15 minutes, whereas no cures are achieved with this heat dose in unobstructed tumours (Hill & Denekamp, 1978). These results for two different types of tumour (previously unpublished for SA F) are shown in Figure 5. 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; these would both occur very rapidly after vascular occlusion.

If a clamp is applied for a heat treatment of $42.8^\circ$C for 1 hour combined with graded X-ray doses, much more thermal sensitization is observed than in unclamped tumours. Similar high TER values were observed for regrowth delay of unclamped tumours when they were implanted subcutaneously on the tail. This is a popular site for hyperthermia experiments because of the ease of heating without raising the body core temperature. However the extreme constriction imposed by the skin of the tail may also be acting as a natural means of vascular occlusion (Hill et al. 1980). When TER values obtained from clamped tumours or from tumours growing on the tail are compared with those in Figure 3, they fall among the high values recorded in some other published studies (Hill et al. 1980). If the published high TER values result from inadvertent vascular occlusion they will not be relevant to most human tumours. Deliberate vascular occlusion for clinical therapy is unlikely to be useful because an increased effectiveness of heat has also been observed in normal tissues if the blood supply is occluded (Morris et al. 1977).

### Temperature Uniformity.

Our initial studies were published on the basis that tumours achieved a temperature $0.3^\circ$C below waterbath temperature, within 3-5 minutes of immersion.
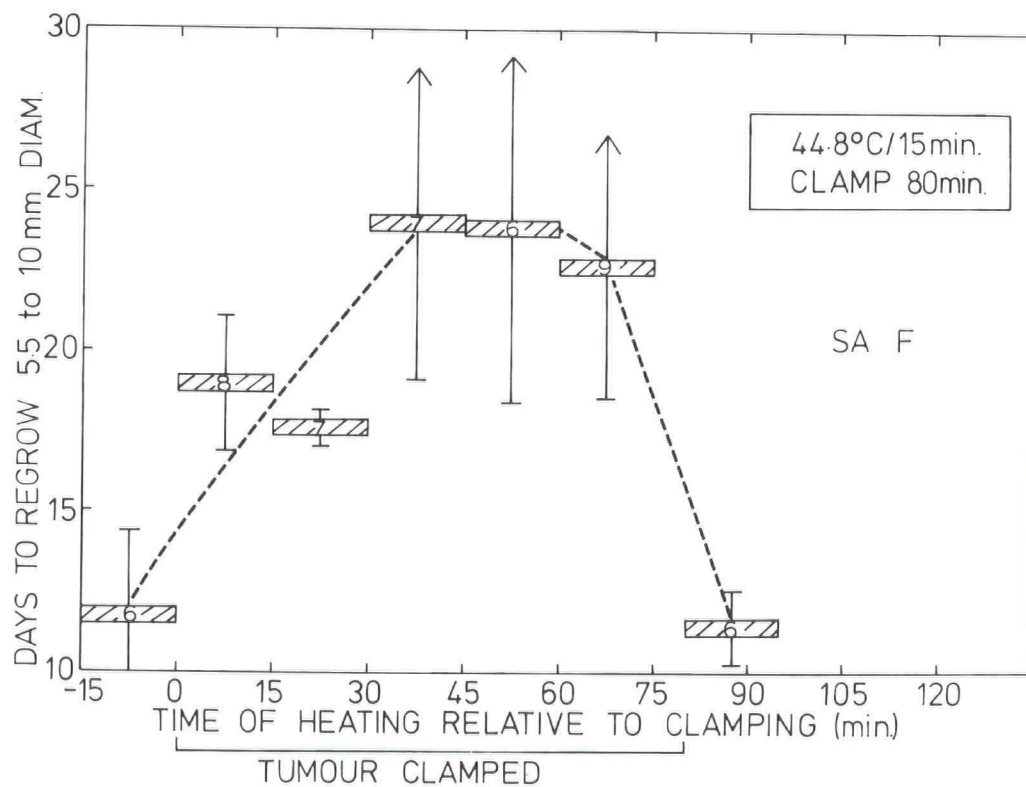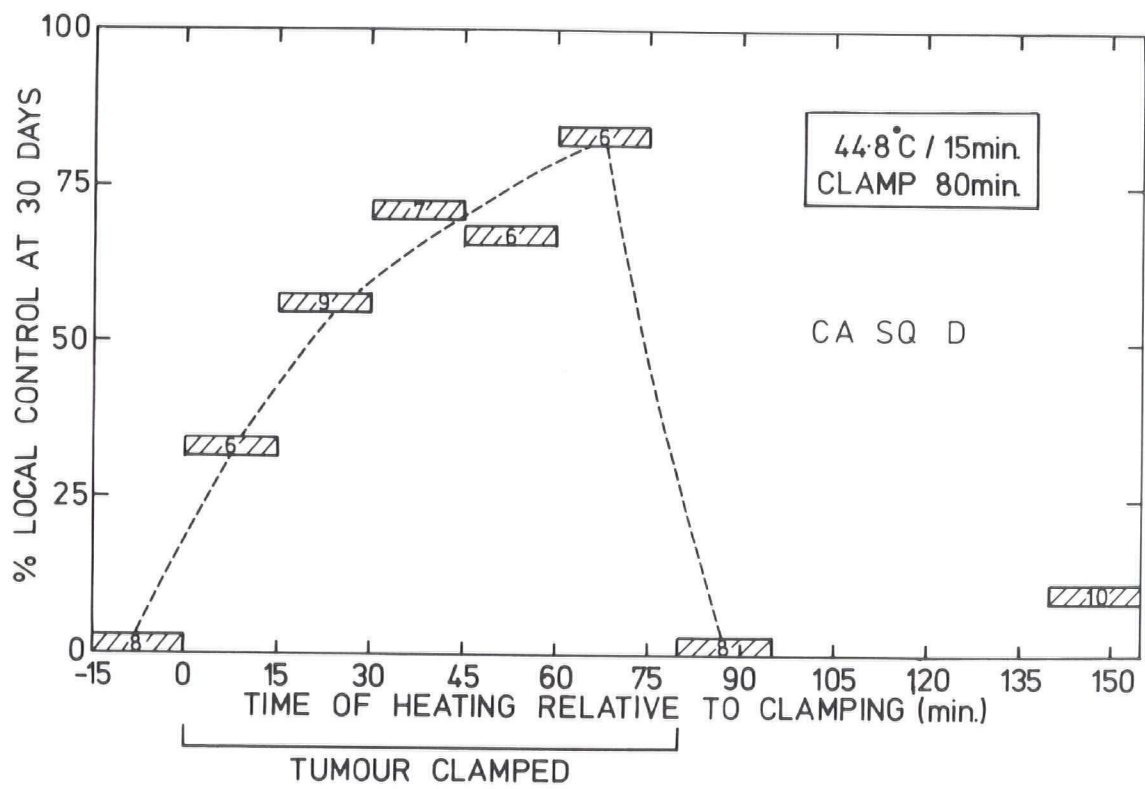
Fig 5.  a)  Tumour control for heat treatment applied at various times
relative to an 80 minute period during which the blood supply
is occluded (Hill & Denekamp, 1978).

b)  Regrowth delay for a heat treatment applied relative to the
time of clamping.  Increased delay is seen in clamped tumours
(Hill, 1980).

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This statement was based on Bailey 29G needle thermocouple readings in two tumour types, with the probe placed at various depths in each tumour. Very little variability was observed. Subsequent measurments on a larger number of tumours, of varying histological types have failed to confirm this early observation. As was reported by others (Bleehen et al, 1978) we now observe considerable temperature gradients across tumours and a considerable variation from 1 tumour to another, even within the same histological type. Fig 6 shows the probe measurements on many samples of four different types of tumour, with readings taken simultaneously (with 3 probes) at different positions within each tumour (size 5.5 - 6.5 mm diameter). The temperature near the skin surface sometimes reaches $0.3 - 0.1^{\circ}C$ below the water temperature, but at depth, i.e. 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., (1979).

Our observations of temperature non-uniformity prompted us to attempt to quantitate thermal damage at different positions in the tumour by histological assessment of tumours obtained at sequential intervals after heating for 1 hour at $42.8^{\circ}C$ or $44.8^{\circ}C$ (Hill et al., 1980). The results for 1 type of tumour are shown in Figure 7. Dead cells were apparent within 24 hours of heating. At the lower temperature the pattern of cell kill was not clear, with pyknotic and viable cells being seen at all positions across the tumour diameter. At the higher temperature ($44.8^{\circ}C$), a very few viable cells were seen in the tumour, and most of these were seen as a thin rim adjacent to the underlying muscle. On successive days this rim could be seen to be expanding as the thermally protected cells proliferated.

Thus it is clear that waterbath heating is inadequate as a means of elevating the temperature, even through 5-6 mm of tissue. For tumours the
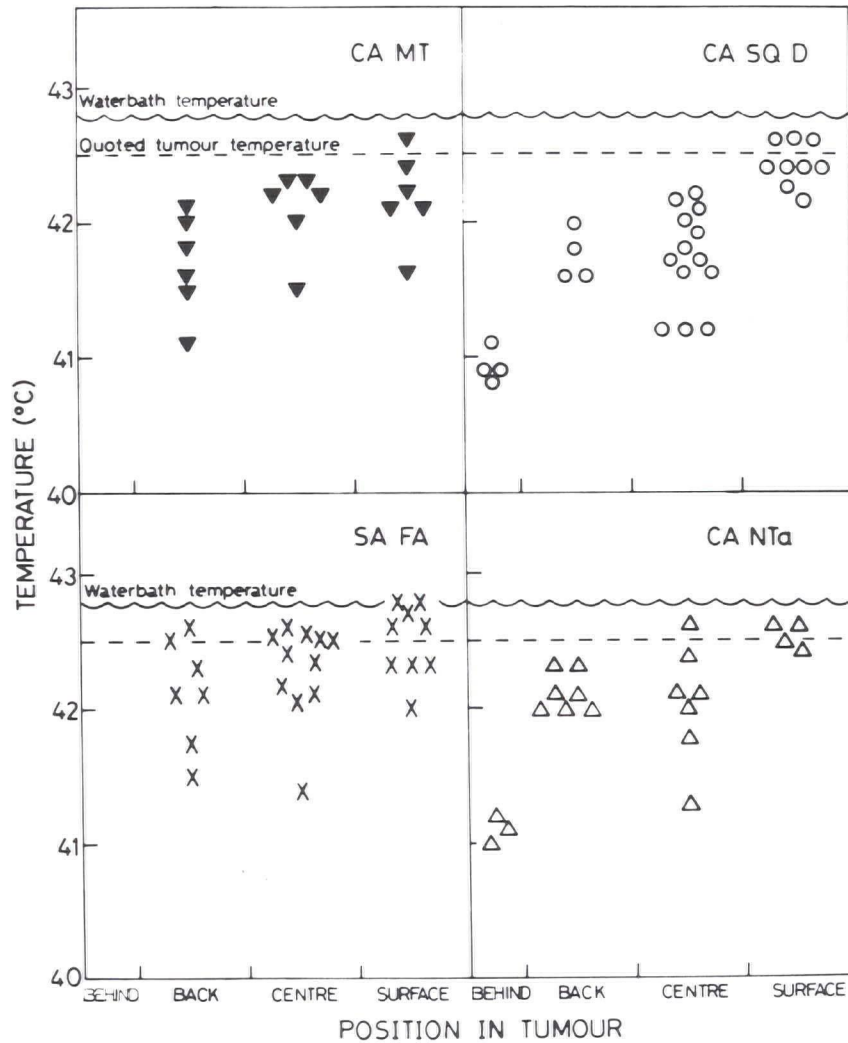
Fig 6.    Thermocouple determinations of the temperature at various positions within mouse tumours of four different types.    There is a wide variation in the measured values, both between tumours and at different points within each tumour (Hill, 1980).

CARCINOMA SQ D

44·8°C            42·8°C

1 DAY

2 DAYS

% VIABLE CELLS

3 DAYS

4 DAYS

Muscle           Skin           Muscle           Skin
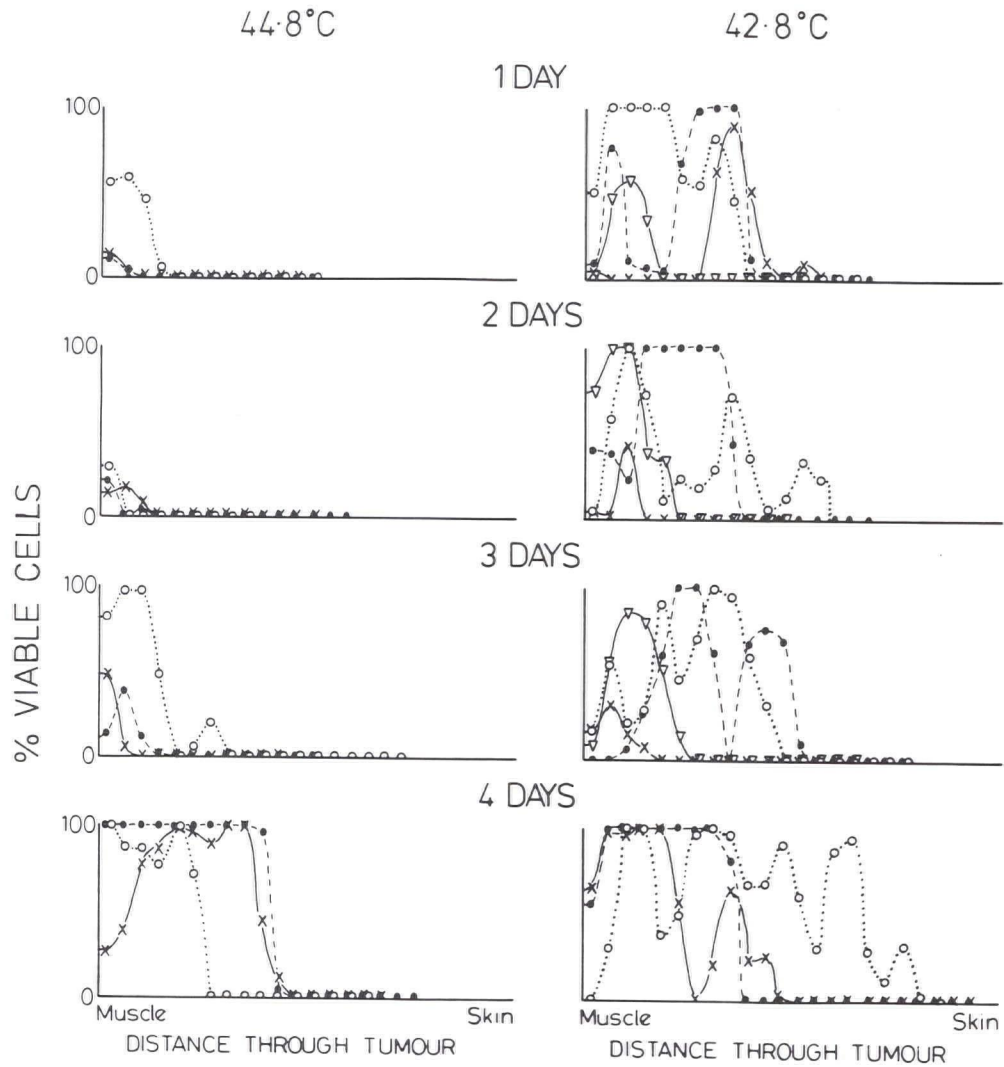DISTANCE THROUGH TUMOUR           DISTANCE THROUGH TUMOUR

Fig 7.     Histological assessment of the cells surviving and repopulating

tumours after two different heat treatments (duration 60 minutes).

Each symbol represents a tumour.    After the higher temperature cells

survived only adjacent to the body and regrowth occurred from this

region.

critical temperature will be the cold spots, since these will result in surviving tumour foci that can regrow the tumour. 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 tenths of a degree can transform an acceptable normal tissue response into necrosis (Law et al., 1978).

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. Figure 8 shows the method we have used in our retrospective analysis. The percentage of animals with metastases killed within certain time intervals (because of a regrowing primary tumour or because of sickness due to metastases) is compared in histogram format for 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 1 hour between the X-rays and heat. Although there sometimes appears to be a tendency towards more metastases in the heat treated groups than in those treated with X-rays alone, this is not significant. It may result from the more effective treatment of the primary so that a longer time is available for latent metastases to appear. In the SA FA there was a tendency for the metastases to occur earlier, although the same high proportion of metastases developed after X-rays or the combined treatment. The results from five retrospective metastases analyses are summarised in Table 3 (Hill, 1980).

Fractionated treatments.

On the basis of our single dose data it was concluded that heat given
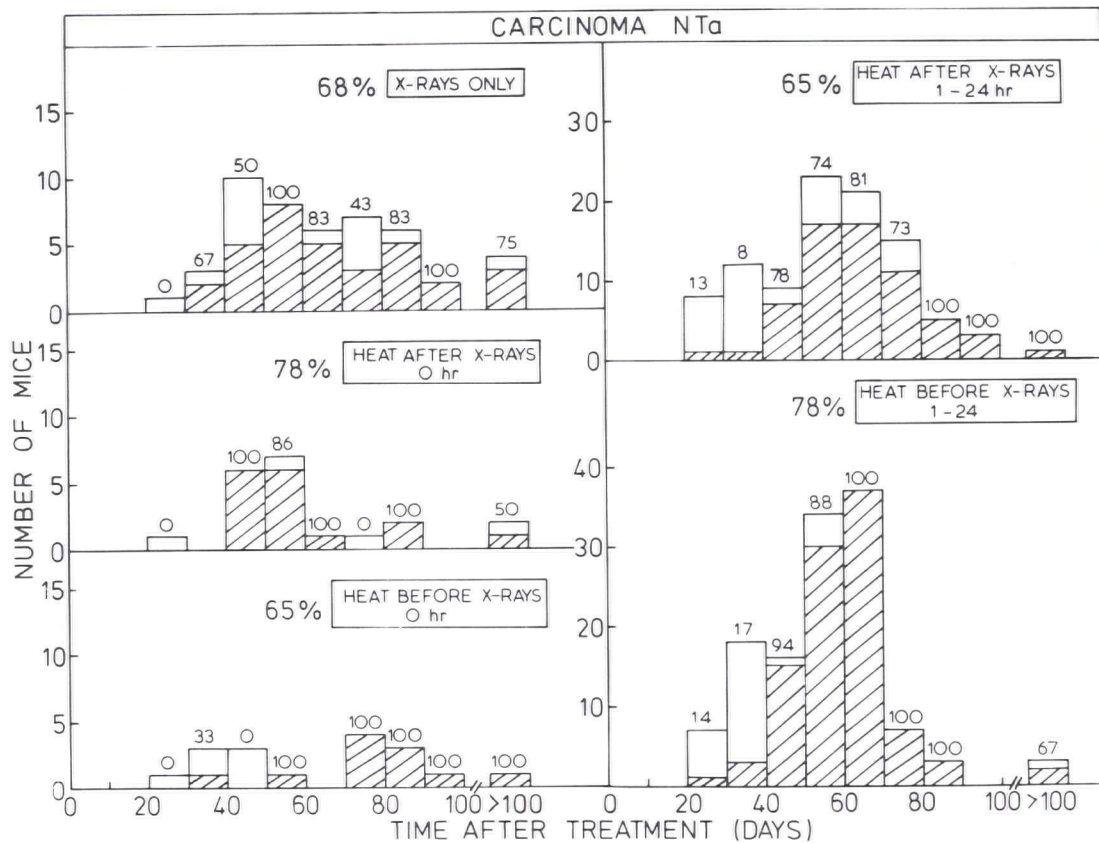
Fig 8. Metastases incidence as a function of the time at which the animal was sacrificed because of local recurrence of a treated tumour or because of sickness due to metastases. No significant change in the incidence or time of appearance is seen for the different treatments.

TABLE III

Metastases Incidence After

X-rays Alone or X-rays plus Heat

Retrospective:

| Tumour | 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 tumours shows a significant increase in
the incidence of metastases after the combined treatment.

3 hours after irradiation was more likely to be beneficial than heat given immediately after X-rays. This study was extended to 2 and 5 daily fractions of X-rays, with heat ($42.5^{\circ}$C/60 min) given immediately or 3 hours after each fraction. Dose response curves were obtained for both skin and tumour (SA FA) as before (Stewart et al., 1980). The results were summarised in Table 4. The therapeutic gain observed with single doses, with an interval of 3 hours was completely lost in the fractionated experiment. This pessimistic result needs to be tested in other tumour 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 3 hours after the last. The loss of therapeutic advantage could result from heat induced thermal tolerance, reoxygenation and recruitment, or increased blood flow. The possible influence of induced thermal tolerance has been studied as a factor in this loss of therapeutic advantage.

Thermal tolerance

Thermal tolerance has been demonstrated both in vitro and in vivo. Joshi et al (1979) showed that quite low heat treatments ($38^{\circ}$C) could induce a tolerance to subsequent thermal cell killing. Law et al (1979) 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 tumours then the therapeutic gain of fractionated treatments would be expected to be much greater than that seen with single doses. Unfortunately for the tumour and normal tissue in which we have tested this idea, the reverse seems to be true, i.e. there is more induced thermal tolerance in the fibrosarcoma than in the skin. A priming temperature of $42.5^{\circ}$C was used and pre-treatments with 4 daily heat treatments, each lasting 60 minutes, or a single heat treatment, were followed 24 hours later by graded X-ray doses and heating for $42.5^{\circ}$C/60 min. The

## TABLE IV

### Thermal Enhancement & Therapeutic
### Gain with Fractionated Treatments

|  | Tumour TER | Skin TER | TGF |
|---|---|---|---|
| [a] Single doses | 1.5-1.7 | 1.7-1.8 | 0.8-1.0 |
| [a]2F/24 hrs | 1.0 | 1.9 | 0.5 |
| [a]5F/4 days | 1.1-1.3 | 1.7 | 0.6-0.8 |
| [b] Single doses | 1.2-1.5 | 1.0 | 1.2-1.5 |
| [b]2F/24 hrs | 1.0-1.1 | 1.1 | 0.9-1.0 |
| [b]5F/4 days | 1.0-1.3 | 1.0-1.1 | 0.9-1.3 |

a = heat given immediately after each fraction

b = heat given 3 hours after each fraction

## TABLE V

Thermal Tolerance in Skin and Tumour (SA.FA)

|  | TER tumour | 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 |

results are summarised in Table 5. The thermal sensitization seen in the fibrosarcoma if X-rays and heat were given in close sequence (TER=1.4) was completely lost if the tumour was preheated with either 1 or 4 doses of heat. Thus thermal tolerance was readily induced in this tumour and could explain the loss of therapeutic gain with fractionated treatments. By contrast, the thermal sensitization of skin was the same (TER = 1.6) whether the skin was pre-heated or not. Thus for this sequence no induced thermal tolerance was observed in skin.

## Summary

The results using waterbath heat combined with 240 kV X-rays to look at the therapeutic benefit of the combined modality are not as optimistic in our seven transplantable mouse tumours relative to skin as are many of the previously published studies. We have shown the time interval between heat and irradiation to be important and feel that the separate cytotoxic action of heat and X-irradiation are likely to be of more benefit than the synergistic effect of using the two in close sequence. The deficiencies of using hot water to achieve uniform heating and the possible artefacts of vascular occlusion have been demonstrated. No significant effect on the spread of metastases has been observed when heat is used adjunctively with X-rays, although the metastases may appear earlier. Thermal tolerance was induced in a mouse tumour, but not in mouse skin and this may account for the loss of therapeutic advantage that we have seen with fractionated treatments.

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