Potential for Hyperthermia and Radiation Therapy

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Abstract

The field of clinical application of hyperthermia to treatment of the cancer patient is developing rapidly both with respect to basic thermal biology, dose-effect relationships, technology for heating and monitoring temperature, efficacy of combination of heat plus radiation, and early clinical studies. The major attraction at this time in studies of hyperthermia is the convincing rationale that there is likely to be a differential cytotoxic effect of hyperthermia between tumor and normal tissue systems.

This is based on the increased effectiveness of hyperthermia to kill mammalian cells which are existing under conditions of reduced pH or metabolically deprived circumstances. Also, the temperature level in the tumor being treated by local or regional hyperthermia is expected to reach higher levels than that of the adjacent normal tissues. Combinations of hyperthermia and radiation are attractive because thermal killing is most effective against S-phase cells and the oxygen enhancement ratio (hyperthermia) is =1. That is, hyperthermia is effective against those cells which are least sensitive to radiation. The principal problems facing clinical application of hyperthermia are: only partially developed techniques for monitoring temperature in tissue, determining isothermal lines of surfaces in tissue, methodologies for achieving uniform heating of local regions of the human body, and information as to the frequency of distant metastasis after hyperthermia.

In addition, further studies are needed to determine the influence of the tumor and normal tissue microenvironment on thermal sensitivity, kinetics for repair of sublethal heat damage, and induction and decay of thermal tolerance.

Introduction

The potential for improving the efficacy of radiation therapy by combining it with hyperthermia is dependent upon the magnitude of the differential sensitization of the radiation effects or upon a differential cytotoxicity of the hyperthermic effects on the tumor and normal tissue cells.

There has been clear evidence from several laboratories that the radiation sensitivity of tumor cells and of normal cells studied both in vitro and in vivo is increased at moderate levels of hyperthermia, and the degree of sensitization is related to the temperature used and the time between the radiation and hyperthermia, the sequencing of the 2 procedures, and the radiation dose rate for concurrent heating and irradiation.

There have been, to our knowledge, no experimental data which demonstrate that the increase in radiation sensitivity by hyperthermia is greater for cells of tumor than for normal tissue, nor is there firm evidence that tumor cells are intrinsically more sensitive to hyperthermia than are the cells of the normal tissue of origin.

By contrast, there are strong indications that because of physiological factors the cytotoxicity of hyperthermia will be more damaging against tumor cells than to the cells of normal tissues. For example, because of the poor blood supply and blood flow through tumor tissue, the kinetics of heat loss during regional hyperthermia may be significantly slower and less efficient than in the well-vascularized surrounding normal tissues. This means that during local or regional hyperthermia, there might be a higher temperature in the tumor than in the adjacent normal tissue. Since the effectiveness of hyperthermia in inactivating mammalian cells increases very rapidly with small increments in temperature, this may be an extremely significant mechanism for a differential cytotoxicity of hyperthermia.

Further, hyperthermia would be predicted to be particularly toxic to cells distant from capillaries which are living under metabolically deprived and relatively acidic (low pH) conditions.

Of great potential significance for the combination of heat and radiation is that OER2 for hyperthermic cell kill is =1.0 (in contrast with 2.5 to 3.0 for radiation inactivation). Also an important factor is that the age response function for hyperthermic inactivation is characterized by a maximum effectiveness against cells in the S phase of the cell replication cycle. Thus there is a rational basis for expecting that the combination of heat with radiation will be a more effective treatment strategy than radiation alone.

This paper considers selected aspects of hyperthermic and radiation inactivation of mammalian cells with comments as to how they might influence the response of tissues to the combination of radiation and hyperthermia.

Hyperthermic Modification of Radiation Response

It is now well established from in vitro studies that the combination of hyperthermia and radiation kills cells in a synergistic manner.

Gerner et al. (18) found that the D0 of CHO cells heated for 1 hr immediately prior to irradiation decreased from 140 rads at 37°C to 130, 90, and 65 rads at 41, 42, and 43°C, respectively. Sensitization of cells heated after irradiation was also observed; however, the effect was less marked. The

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2 The abbreviations used are: OER, oxygen enhancement ratio; CHO, Chinese hamster ovary; RD50, 50% response dose; TCD50, 50% tumor control dose; TER, thermal enhancement ratio = radiation dose alone/radiation plus heat; TGF, therapeutic gain factor = thermal enhancement ratio = radiation dose alone/radiation dose plus heat for tumor/thermal enhancement ratio = radiation dose alone/radiation plus heat for normal tissue.

3 See Ref. 11 for review of in vitro studies.
importance of sequencing on the sensitizing effect was studied in detail by Sapareto et al. (53) with CHO cells at 42.5 or 45.5°C. When relatively moderate heat doses were applied either before, during, or after irradiation, the maximum reduction in survival occurred when the 2 treatments were applied simultaneously (Chart 1). Radioresistant S-phase cells which are relatively sensitive to hyperthermia are also preferentially sensitized by hyperthermia to radiation [Gerweck et al. (22) and Sapareto et al. (53)]. Relatively few studies have examined the combination of hyperthermia with low-dose-rate irradiation, an approach which may have clinical applicability. Ben-Hur et al. (3) simultaneously heated and irradiated Chinese hamster V79 cells at 3.3 rads/min. The decrease in D0 from 378 rads at 37°C to 60 rads at 42°C was far more pronounced than was observed at relatively high dose rates (360 rads/min). Presumably, irradiated cells were unable to repair sublethal radiation damage at 42°C.

The influence of hyperthermia on the radiation OER of mammalian cells has been examined by a number of investigators with variable results. For example, Robinson et al. (50) used mouse bone marrow treated in vitro and assayed viability by the spleen colony technique in their determination of OER. For their studies, gas containing <10 ppm of oxygen was passed over the cells in test tubes. Their OER values were 2.47, 1.69, and 1.38 for temperatures before, during, and following irradiation of 37.5, 42.5, and 43°C. A similar reduction in the OER was observed by Kim et al. (35) when HeLa cells were heated to 42°C for 2 hr postirradiation. However, Power and Harris (47) determined OER values for exponential-phase V79 cells exposed to 43°C for 45 min immediately before or after X-irradiation; the results were 3.9 as compared with 3.0 for unheated cells. In similar studies on EMT-6 cells, the OER values were 3.0 and 3.2 for heated and unheated cells. In experiments by Myers and Field4 using stunting of growth of the rat tail and skin reactions of the rat tail, OER values were the same at increased and at normal temperatures. In a study using survival of stationary-phase yeast (Saccharomyces cerevisiae), Kiefer et al. (33) determined OER at room temperatures, 30, 43, 47, and 53°C; the results were 2.10, 2.00, 1.94, 1.78, and 2.00, respectively. There is, therefore, uncertainty regarding the OER at hyperthermia, and it cannot be assumed that the OER for most tissue systems is reduced at hyperthermia.

In summary, radiation and hyperthermia kill cells synergistically, and this effect appears to be most pronounced in S-phase cells. There are no data from in vitro studies, however, to indicate that the degree of sensitization is greater in cancer cells as opposed to normal tissue cells of origin. Some preferential killing of tumor cells may be expected, however, if the tumor contained a relatively large fraction of S-phase cells compared to the treated normal tissue component. Recent studies indicate that treatment by hyperthermia does not decrease the radiation OER; this important question is not satisfactorily resolved.

**Hyperthermic Sensitivity of Mammalian Cells**

Biological end points used to assess hyperthermic damage include: staining with vital dyes; respiration inhibition; loss of various synthetic activities; and, less often, loss of reproductive capacity. This latter end point is most relevant to the concern of the cancer therapist.

The lethal response of cells to hyperthermia is dependent on the time and temperature of treatment, as shown in Chart 2 for CHO cells (11). At relatively low temperatures (41–42°C), survival initially decreases with increasing treatment time and then plateaus as cells develop and exhibit thermal tolerance or resistance during treatment. Between 42 and 43°C, survival decreases markedly, and the plateau in response develops at a much lower survival level. At 43°C and higher, the shape of the heat response is similar to that observed for ionizing radiation, i.e., following an initial shoulder region, survival decreases exponentially. Above 43°C, a 1°C increase in temperature doubles the rate of cell killing (slope) on the exponential portion of the survival curve. At a specific temperature, however, the sensitivity between cell lines varies considerably (9). For example, at 42°C, heat treatment for 3 hr reduces the surviving
fraction of cultured human glioblastoma cells to 0.8, whereas the surviving fraction of CHO cells is 0.1. At 44°C, the D50's differ by a factor of ~7: 5 min for CHO cells, and ~35 min for glial cells (20). This extreme variation in cellular thermal sensitivity is much broader than is the variation in cellular radionecrotogenicity. This suggests that in some normal tumor tissue situations there may be striking differences in the thermal sensitivity between the cells of the tumor and critical surrounding normal tissue.

Of considerable interest is the possibility that transformed cells are intrinsically more heat sensitive than are the normal cells from which the transformed cells originated. This question has been subjected to extensive study. When cells are heated in vitro under identical nutritional and growth conditions and response is assessed as loss of reproductive capacity, the published results are not consistent (8, 29, 31, 32, 43).

In summary, these studies show that thermal sensitivity varies considerably between cell lines which can result in substantial differences in cell survival level when temperatures exceed ~42°C. This suggests that in some normal tumor tissue situations there may be striking differences in the thermal response of cells in the tumor and critical surrounding normal tissues. Experimental data do not convincingly demonstrate a greater intrinsic heat sensitivity of transformed cells compared to normal cells.

Influence of the Cellular Micrometabolic Environment on the Lethal Response of Mammalian Cells to Hyperthermia

Extracellular Metabolites in Normal and Tumor Interstitial Fluid

The micrometabolic environment of cells appears to be a critical determinant of the response of tissues to hyperthermia. During the past several years, evidence has accumulated which shows that rodent and human tumors contain nutritionally deprived foci, namely regions of necrosis are seen in most malignant tumors.

Hypoxic Regions in Tumor. Gray et al. (23) in 1953 proposed that some of the failures of radiation therapy might be due to the presence of hypoxic and hence relatively radiation-resistant tumor cells. These were presumed to be present at intermediate positions between the vessel and the necrotic regions. This work was expanded by Thomlinson and Gray (65), in 1955 who found that necrosis appeared in human bronchogenic carcinomas at distances from capillaries which would be predicted to anoxic on the basis of oxygen diffusion coefficient, rates of utilization of oxygen by cells, PO2 of arterial and venous blood, and rates of blood flow through the capillaries. Tannock (64), did further work on this subject and concluded that necrosis was found at distances from capillaries corresponding to the diffusion length of oxygen; the critical metabolite for tumor cells appeared to be oxygen. Additionally, results from polargraphic electrode measurements on human as well as the radiobiologic "oxygen effect" in rodent tumors (59) provide support for the proposal that hypoxic and viable cells are present in some tumors.

Reduced pH in Tumor Tissue. A common feature of neoplastic tissue is the elevated rate of glucose consumption and lactic acid production under oxygenated conditions (5). Hypoxia further increases the rate of lactate production commonly by a factor of 2 to 3 or more (10). Gullino et al. (24) compared the composition of serum, tumor, and s.c. interstitial fluid in 3 rat hepatomas, a fibrosarcoma, and a carcinoma. The only consistent differences obtained between normal and tumor tissue fluid were decreased glucose content, elevated lactate, and elevated carbon dioxide content in the tumor fluid. No significant differences were observed between the tumor and normal tissue fluid protein, amino acid, cholesterol, or lipid phosphorous content. Comparable results were obtained by Burgess and Sylven (6) who demonstrated a 2- to 3-fold increase in lactic acid and a 2- to 10-fold decrease in glucose in several solid and ascitic mouse tumors.

Interstitial fluid pH has been studied extensively by Gullino et al. (26) in a variety of rat tumors. The tumor interstitial fluid pH ranged from 7.19 to 6.95 which was 0.2 to 0.4 units lower than the blood and s.c. fluid pH. This pH difference was due to the elevated levels of lactate and carbon dioxide. The abnormally low pH in tumor interstitial fluid has also been demonstrated by Eden et al. (15) in rats and by Naeslund and Swenson (42) in mice. Similar observations were made in several varieties of human tumors by Naeslund and Swenson (42) and Ashby (1); these data are summarized in Table 1.

Influence of Micrometabolic Environment on Hyperthermic Lethality

OER for Hyperthermic Cell Killing. Evidence has been presented which suggests that tumor tissues are poorly oxygenated compared to normal tissue. These hypoxic foci may reduce the effectiveness of radiation therapy. A survival curve comparing the lethal response of aerobic and of hypoxic cells to hyperthermia is shown in Chart 3 (21). CHO cells were heated to 45.5°C under oxygenated or acute hypoxic conditions. The thermal OER was 1 for survival values down to 0.037. At survival levels below 0.01, hypoxic cells appeared to be slightly more sensitive than were oxygenated cells. The authors concluded that hypoxic CHO cells were at least as sensitive to hyperthermia as were oxygenated cells. A number

Table 1

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Tumor types</th>
<th>Normal Mean ± S.D.</th>
<th>Tumor Mean ± S.D.</th>
<th>Assay conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gullino et al. (26)</td>
<td>Rat, n = 5</td>
<td>7.33 ± 0.00</td>
<td>7.05 ± 0.09</td>
<td>In vivo</td>
</tr>
<tr>
<td>Eden et al. (45)</td>
<td>Rat, n = 8</td>
<td>7.39 ± 0.12</td>
<td>7.03 ± 0.12</td>
<td>In vivo</td>
</tr>
<tr>
<td>Ashby (1)</td>
<td>Human melanomas</td>
<td>7.43 ± 0.08</td>
<td>6.78 ± 0.09</td>
<td>In vivo</td>
</tr>
<tr>
<td>Naeslund and Swenson (42)</td>
<td>Human gynecological, n = 5</td>
<td>7.57 ± 0.25</td>
<td>6.89 ± 0.28</td>
<td>In vivo</td>
</tr>
</tbody>
</table>

* Mean, ± S.D.
Effects of pH and temperature on solid tumor frequency.

Tumor cells were cultured in vitro for 1 hr. Each mouse was given an injection of $5 \times 10^6$ cells (44). The results are summarized in Table 2.

<table>
<thead>
<tr>
<th>pH</th>
<th>Controls at 37°C</th>
<th>Heat-treated at 42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7.0</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>6.8</td>
<td>100</td>
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<td>6.6</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>6.4</td>
<td>100</td>
<td>0</td>
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</table>

In recent experiments, we compared the lethal responses of oxygenated and acutely or chronically hypoxic cells under well-defined and relatively constant nutritional conditions. Representative results are shown in Chart 4. Oxygenated and acutely hypoxic cells were equally sensitive to 42°C treatment for 3 hr; however, as culturing time under hypoxia increased beyond 12 hr, the surviving fraction decreased to a minimum after 30 hr hypoxia. Similar observations at 42 to 43.5°C have been made by Born and Troll (4); after 14 hr gassing under hypoxia, the shoulder of heat survival curves was eliminated. Thus, at this time the OER for hyperthermic cell killing appears to be $=1.0$ for acutely hypoxic cells and slightly less for chronically hypoxic cells provided they are treated at normal glucose, pH, etc., levels.

Thermal Sensitivity and pH. Recent studies indicate that exposure of cells to hyperthermia at below normal pH conditions (<7.4) enhances cell lethality. Overgaard (44) provided quantitative evidence for this effect by heating L1A2 ascites cells in Eagle’s medium without serum for 1 hr at 42.5°C. The cells were subsequently inoculated in the axilla of mice, and the frequency of tumor occurrence was determined. The results (Table 3) show that reduction of pH from 7.2 to 6.4 reduced tumor transplantation frequency from 100 to 0%. In Chart 5, heat survival curves of CHO cells at various temperatures and pH conditions are shown (19). At all temperatures, a pH-sensitizing effect was demonstrated in CHO cells. This effect was most prominent below pH 7.0 and at temperatures which are well tolerated in vivo, i.e., 42°C. For example, after 240 min at 42°C, the ratio of survival fractions for exposure at pH 7.4/pH 6.7 is $=500 (8 \times 10^{-2}/1.5 \times 10^{-4})$. This big effect is in part due to the induction of thermal tolerance at pH 7.4 after $\approx 180$ min at 42°C which does not develop at pH 6.7. By contrast, at 43°C thermal tolerance does not develop at any pH, and the ratio of survival fraction for 90-min treatment for pH 7.4/pH 6.7 is $=40 (8 \times 10^{-2}/2 \times 10^{-3})$. Further, at 41°C the ratio is also much less because tolerance develops even at a pH of 6.7; the ratio of survival fractions at 300 min for pH 7.4/pH 6.7 is $=18 (5.5 \times 10^{-1}/3 \times 10^{-2})$. These data are of the greatest interest because they point out a potential for greatly...
The relationship between hypoxia, pH, and the gas phase at the time of heat treatment is illustrated in Chart 6. When \( \approx 6 \times 10^6 \) CHO cells in 1 ml of medium are cultured under hypoxic conditions, metabolic acidification (lactate production) decreases extracellular pH in a manner shown at the bottom of Chart 6. When these cells are heated at the specified pH under hypoxia (closed circles) or immediately following reoxygenation (open circles), survival begins to decrease after \( \approx 12 \) hr hypoxia as the pH drops. The upper dashed curves represent separately the effect of the 2 factors accounting for the increased heat sensitivity, i.e., the reduced pH and chronic hypoxia.

**Glucose Level and Thermal Sensitivity.** In addition to reduced O\(_2\) and pH, the tumor interstitial fluid glucose content is apparently reduced at sites distant from capillaries because of the high rate of glucose consumption and limited blood supply. Hahn (28) investigated the importance of glucose on heat sensitivity. Exponential-phase cells were cultured in medium plus glucose (1 mg/ml) with or without an inhibitor of glucose transport. The results obtained indicated that glucose concentration (in the range studied) did not play a detectable role in cellular response to 43°C hyperthermia under oxygenated conditions. Similar results were recently reported by Kim et al. (37); however, when cells were deprived of both oxygen and glucose, sensitivity was markedly increased between 40.5 and 42.5°C.

In summary, the OER for radiation lethality does not appear to vary with temperature. The OER for hyperthermic lethality of increasing the differential thermal sensitivity of tumor and normal tissue cells by very small decreases in systemic pH. Namely, a lowering of pH by 0.2 to 0.3 units would result in a pH level for normal tissue of \( \approx 7.1 \) to 7.2 but cause the pH of tumor tissue to fall to perhaps 6.8 to 6.9. If this was achieved, there would be a great differential cell kill between tumor and normal tissue. The exact differential will apparently depend on the relationship between time, temperature, and pH at which tolerance develops. Our data indicate that: (a) at any one temperature, tolerance develops at a much lower survival fraction for low as opposed to normal pH values; and (b) the temperature at which tolerance develops during exposure is lower for acidic than for normal cells. This is an important basis for the prospect of a big differential cell kill between cells of normal and tumor tissues.

The magnitude of the pH-sensitizing effects at a particular pH and temperature probably varies between cell lines. For example, a large pH-sensitizing effect is not evident in cultured human glioblastoma cells unless extracellular pH is decreased below 6.6 to 6.8.\(^6\)

\(^6\) L. E. Gerweck, unpublished data.
acutely hypoxic cells is approximately 1 and is less than 1 for chronically hypoxic cells. Low pH substantially increases thermal sensitivity, and the sensitizing effect of reduced pH appears to be most prominent at moderate temperatures (namely, 41.5–42.5°C). This is the consequence of a suppression of development of tolerance by cells at low pH. Additional studies will be required to determine the effect of reduced O2 tension and pH on cells exposed to fractionated hyperthermia. However, the data from completed studies suggest that moderate hyperthermia, e.g., 42°C for 3 hr, would be selectively toxic to tumor cells which are relatively unaffected or resistant to radiation. These microenvironmentally related effects may provide for a differential between tumor and normal tissue whether heat is used alone or in combination with radiation.

Variation in the Heating Pattern of Normal and Tumor Tissue

Local or regional hyperthermia is unusual as a treatment modality in that the physiological characteristics of tumor tissue may result in a greater concentration of the lethal agent in the tumor because dissipation or loss of heat from tumor is less rapid than for normal tissue. The principal factors controlling heat loss from tissue are surface cooling, thermal conduction, and convection. Convection of heat out of a volume of tissue is primarily dependent on the rate and volume of blood moving through the tissue. Assuming an equal rate of heat input to tumor and normal tissue, differences in temperature between normal and tumor tissue do develop where there are differences in conduction and convection characteristics between the 2 tissues.

Gullino and Grantham (25) have quantitated tumor blood flow by measuring venous outflow of organs totally replaced by tumor and rubidium-86 or potassium-42 clearance techniques in s.c. tumor implants. Blood flow per g of tissue was 15- to 20-fold lower in tumor than in normal tissues. The principal factors controlling heat loss from tissue are surface cooling, thermal conduction, and convection. Convection of heat out of a volume of tissue is primarily dependent on the rate and volume of blood moving through the tissue. Assuming an equal rate of heat input to tumor and normal tissue, differences in temperature between normal and tumor tissue do develop where there are differences in conduction and convection characteristics between the 2 tissues.

Fractionation of Hyperthermia and Radiation

In the clinical application of hyperthermia combined with functional vascular volume and permeability in muscle and skin but not in the Walker carcinoma 256 (8-mm diameter). Gullino et al. (27) found that warming of tumors produced changes in blood supply and O2; these were neither conspicuous or predictable. These observations suggest that local heating of tumors does not substantially influence blood flow, but does, in fact, enhance blood flow in normal tissue. The result is a preferential heating of tumor tissue. Further studies on this effect as a function of temperature, duration of heating, tumor size, etc., are needed.

Carefully recorded measurements of tumor and normal tissue temperature, especially in deep tumors, have been infrequently recorded and have generally been handicapped by problems associated with thermometry. In addition, the input of energy may commonly vary considerably between tumor and normal tissue (e.g., skin).

LeVeen et al. (39) heated deep human tumors by radio-frequency (13.56 MHz) therapy and reported that the tumor temperature was up to 10°C higher than in adjacent normal tissue. Kim et al. (34) observed a 5°C temperature differential occurred in some patients also treated by radio-frequency therapy. Dickson et al. (14) treated rabbit carcinoma VX2 of up to 22 ml volume in the hind limb with a single exposure to radio-frequency heating; the temperature of the skin and normal muscle was 3–4°C below the minimum intratumoral temperature. There were variations of 2–3°C throughout the tumor. In contrast, Marmor et al. (40) treated 100-cu mm tumors in mice by localized radio-frequency heating and did not observe a substantial difference in temperature between the tumor and immediately adjacent muscle.

In summary, these various data presented above suggest that during local hyperthermia the actual temperature of tumor tissue may be higher than in the adjacent normal tissue, principally as a consequence of differences in blood flow characteristics of the 2 tissues. These observations should be confirmed and studied in greater detail because they may serve as an important basis for expecting an increased therapeutic ratio in thermotherapy. Even a small differential in heating could be the critical factor in determining the success of hyperthermia. This is supported by the experimental finding that a quite small change in temperature can result in truly fantastic differences in cell survival. This is illustrated by the work of Sapareto et al. (54), who determined survival curves for asynchronous CHO cells treated by 42.0, 42.2, 42.3, and 42.5°C. For that system, the survival fractions for 5 hr of hyperthermia were 3 × 10^-2 and ≈10^-3 for temperature of 42.2 and 42.5°C; that is, an increase in temperature by 0.3°C resulted in a 30-fold decrease in survival. These very large survival differences occur in the temperature range where the transition is being made from that at which thermotolerance does and at which thermotolerance does not develop during the heat treatment. Provided one could know that range beforehand and work in that range clinically, the really small temperature differences between tumor and normal tissue could result in the most important differential cell kill (tumor versus normal). As mentioned earlier, this effect would be expected to be even greater where the tumor cells were living at a reduced pH.
radiation therapy, the most likely protocol will be multiple fractions of both the hyperthermia and radiation. Planning of such treatments will be aided by: (a) an understanding of the kinetics and magnitude of repair of the radiation and of the heat damage; (b) modification by hyperthermia of the repair of radiation damage; (c) modification by radiation of the repair of hyperthermic damage; and (d) the time course and magnitude of any induced thermal tolerance under these conditions. The total heat dose to elicit a specified response increases as the hyperthermia is fractionated. This has been shown by split dose and multiple dose studies on normal and tumor tissue responses to hyperthermia. For example, Suit (60) observed that the RD50 for loss of feet of C3H mice increased from a control value of 173 min at 43.5°C for a single exposure by factors of 3.1 and 5.3 for 5 and 10 fractions (1 day between fractions), respectively. For the TCD50 of a methylcholanthrene-induced fibrosarcoma (FSa I), the single dose TCD50 value was 83 min at 43.5°C; this increased by factors of 2.8 and 4.4 for 5 or 10 fractions, respectively. This "fractionation effect" is greater than that observed for response to radiation. For example, RD50 = 10/RD50 = 1 for acute moist reaction of mouse skin was 2.0 (61) and TCD50 = 10/TCD50 = 1 for the FSa I was 2.4 (63). Accordingly, the fractionation effect for hyperthermia was in these systems greater on tumor and normal tissue than the radiation effect; further, the fractionation effect on hyperthermic response was larger for normal than for tumor tissue. This suggests that in this tumor system the therapeutic efficacy would be enhanced by the use of fractionated hyperthermia. There is now demonstrated a marked difference in the kinetics of repair of thermal as opposed to radiation damage as evaluated by the split-dose study. In our laboratory using loss of the normal foot and the TCD50 of F5a I as end points, there was no increase in the total heat dose (time at 43.5°C) using single or split treatments with time intervals between Doses 1 and 2 up to 6 hr (60). More recently, this same phenomenon has been observed by using temperatures of 41.5 and 42.5°C as well as 43.5°C, and the same results were obtained (45). Law et al.† reported that the total heating time to produce necrosis of one-half of the treated ears of mice was constant for heat given in a single dose or 2 equal doses with intertreatment periods of up to 4 hr but increased for intervals of 8 hr. In comparison, for radiation damage the total dose increases rapidly with time between treatments; a clear increase is seen at 2 hr with some increase between 2 and 6 hr and only slight increase thereafter (66).

Another aspect to the subject of "repair" of thermal damage is the induction of tolerance. Henle and Dethlefsen (30) have reviewed this subject comprehensively; nearly all of the quantitative data are from in vitro systems. Law et al.† have examined the response to heat of the mouse ear when pretreated by 40 min at 43.5°C. They interpreted their data as evidence for induction of a modest level of thermoderatude.

Field and Law (17) determined thermal enhancement ratios of radiation effects by using moist desquamation of < 0.5 of the outer surface of the pin as the end point. Their result was 1.66 for single dose radiation and heat (the heat being 42.5°C for 30 min with radiation given within 6 min of the completion of the heating). However, TER decreased with increasing numbers of fractions; it was 1.25 for 10 equal treatments (17). This thermal enhancement ratio was relatively similar to that obtained in another study by that group using inhibition of growth of the baby rat tail as the end point (41).

Experimental Hyperthermia and Radiation Therapy

From the above analysis, we conclude that the potential of hyperthermia for radiation therapy depends essentially on the development of effective strategy for combining 2 modalities which are cell lethal. The attraction of combining these 2 modalities is due to the facts that heat is relatively more damaging to S-phase cells, to hypoxic cells, and to acidic cells than is low linear energy transfer radiation and that tumor tissue especially the central regions of large tumor masses may be heated to higher levels than adjacent normal tissues principally because of differences in blood flow. There is no strong data basis as of this date for proposing hyperthermia alone as an effective treatment method for the common and deeply sited tumors in humans, although there clearly have been some impressive regressions of solid tumors following hyperthermia alone. Thus, the current effort is directed at combining the 2 approaches. One of the major problems attending the planning of the combined modality treatment approach is that of deciding the temporal relationship between the heat and radiation. There are data which suggest that the "therapeutic gain" from combining the 2 modalities is affected by sequencing of the treatments. Thermal enhancement ratios or TER have been determined experimentally for several tumor and normal tissues. For treatment which elicits a specified tissue response, the TER is the ratio of dose of radiation alone to dose of radiation combined with heat. TER's have been found to be maximum for radiation and heat administered concurrently both for cells in vitro and for tissue systems observed in vivo. However, the decay of TER with time between radiation and heating is slower for tumor tissue than for normal tissue for the 6 tissue systems studied [see review by Field (16)]. In those studies, the effect of the heat dose on normal tissue was not seen if time between radiation and heat was greater than 4 hr. That is, the TER had decreased to 1. For tumor tissues, there was a prominent elevation in TER even after 4 hr. Accordingly, for the systems they studied there would be positive therapeutic gain for certain timing protocols but not for others. In a study of a mouse fibrosarcoma growing as a transplant in the s.c. tissue of the ventral thorax, Stewart and Denekamp (58) reported that a TGF of 1.3 was obtained for radiation followed 2 to 6 hr later by hyperthermia; no gain was seen for shorter periods between the treatments. In studies where heat preceded radiation, a positive TGF was obtained at 3 hr but not at 0.5, 1.2, or 6 hr. Robinson et al. (51) observed TGF values of 1.2 to 2.1 when radiation and heat were concurrent in the treatment of C5H mammary carcinoma and for temperatures of 41, 42, 42.5, and 43°C. Stewart and Denekamp (58) have tabulated the reported TGF values, which for many test systems are 1.0. This raises the real prospect that the combined treatment may not be beneficial, and that a positive TGF may be critically dependent upon the level of heat and the sequencing used. A concern raised by the studies of Stewart and Denekamp (58) and Field (16) is that TER and TGF might be smaller for small radiation doses, i.e., fractionated treatments. Clinical applica-

† M. Law et al., unpublished data.
tions are likely to feature fractionated treatment rather than single doses which have been investigated so intensively in the laboratory to date. Attention should be given to understanding the mechanism of dependence of TGF on the sequencing, e.g., substantial hyperthermia once/week plus radiation 5 days/week. Also, more effort should be directed towards animal tumor models in addition to murine models.

Problems in Clinical Application of Hyperthermia

The most serious and possibly absolute constraint in the clinical use of hyperthermia is the question which was raised by Dickson and Muckle (13) that hyperthermia was in fact associated with an increase in frequency of distant metastasis in the VX2 rabbit tumor system (13) and in the Yoshida tumor system in the rat (12). Studies in other tumor systems have not confirmed an increased frequency of metastasis. Yerushalmi (67) investigated the Lewis lung carcinoma in the mouse, and Schecter et al. (55) investigated the Me-H mammary carcinoma of the rat. In neither of these systems was an increased incidence of metastases seen following local hyperthermia. In our laboratory, Uruno9 has studied a spontaneous fibrosarcoma (FSa II) growing as early generation isointransplants in the foot pad. These tumors were treated by local hyperthermia, whole-body hyperthermia, or amputation. There was no increase in frequency of distant metastases following local hyperthermia or amputation, and only a slight suggestion of an increase following treatment with whole-body hyperthermia. Further studies are in progress on this subject using other spontaneous tumors. At present, no definitive statement can be made regarding the impact of local or whole-body hyperthermia on the incidence of distant metastasis from spontaneous tumors either treated by hyperthermia alone or combined with radiation. Clearly, there is a need for substantial further data on this subject which is of potential critical importance to clinical applications of hyperthermia. Our review does not indicate that for spontaneous tumor or nonimmunogenic ones that there will be an increase in distant metastasis frequency following hyperthermia.

Another problem is that of the development of means of achieving hyperthermia regionally and to deeply sited tumors and providing a method for measuring the temperature achieved in the tumor and adjacent normal tissues. Obviously, the application of hyperthermia at a clinical level requires the technical means of doing so. This technical development has not been achieved except for superficial lesions, and even there, temperature measurement throughout the tumor is not entirely satisfactory. Along with the technical effort to develop means of heating regionally will be research into strategies for increasing the differences in heat levels between tumor and normal tissues. Superficial tissues may simply be cooled. A potentially important avenue of study is that of the temperature and pH levels at which tolerance would be induced during the treatment. Planning the hyperthermia strategy around the differential in thermotolerance induction could yield an enormous differential in thermal damage; the potential here for therapeutic gain appears to be really great.

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9 M. Uruno, unpublished data.
Potential for Hyperthermia and Radiation Therapy

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