To the Graduate Council:

I am submitting herewith a thesis written by Carlette Mathilde toe Water entitled "The Effect of Ultrasound-Induced Hyperthermia Combined with Local X-irradiation in Pre- and Posttransplantation Treatment of the Line 1 Lung Carcinoma." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Radiation Biology.

Robert L. Ullrich, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

B

THE EFFECT OF ULTRASOUND-INDUCED HYPERTHERMIA COMBINED WITH LOCAL X-IRRADIATION IN PRE- AND POSTTRANSPLANTATION TREATMENT OF THE LINE 1 LUNG CARCINOMA

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Carlette Mathilde toe Water

August 1980

To Harrie

ACKNOWLEDGMENTS

There are many people I need to thank for their help with the completion of this project.

I am grateful to the members of my advisory committee. To Dr. Robert L. Ullrich I am especially grateful for giving me the opportunity to work under his guidance. His support and encouragement have been invaluable. I also thank him for providing the animals and materials needed for the experiments.

My special thanks are due to Dr. M. A. Breazeale for the use of the ultrasound equipment and for his help and advice during the development of the heating technique in the preliminary experiments.

I would like to thank Dr. J. Gordon Carlson for providing the laboratory space and for his support. I especially thank him for his friendship and willingness to remain on my committee after his retirement. His replacement on the committee with such short notice by Dr. Solon Georghiou is greatly appreciated.

To Pat Benoit my thanks are due for her expert help with the statistical analysis of the data.

I thank Laurel Adams for her invaluable technical advice and help; I also thank Dr. Kathy Thomas for the fruitful discussions.

I would also like to thank Dr. H. E. Walburg for his support and for giving me the opportunity to finish this manuscript.

I thank Carole Byrd for editing and I thank Maxine Martin for the final preparation of the manuscript.

ABSTRACT

The purpose of this study was to investigate a possible enhancement of the effect of X-irradiation by ultrasound-induced hyperthermia on the tumor growth rate of the murine line 1 lung carcinoma and on host-tumor interactions as measured by the tumor bed effect (TBE). Initially experiments were designed to determine (1) the effect of ultrasound-induced hyperthermia for 60 minutes and 30 minutes on the tumor growth rate and (2) the effect of combined ultrasound and X-irradiation treatments on the tumor growth rate.

Pathogen-free Balb/c (14 to 16 weeks old) female mice were injected with 10^6 tumor cells in the left thigh. Preliminary experiments were done to determine the temperature rise and the thermal distribution within the tumor-bearing thigh. The temperature at the surface of the femur was assumed to be at least equal to the temperature measured by the thermocouple inserted at the center of the thigh. The skin temperature remained a few degrees lower than the temperature of the tumor.

The legs of each group of animals were treated on day seven or day eight after tumor transplantation with one of the five treatment modalities: ultrasound for 60 minutes, ultrasound for 30 minutes, 7000 rad X-irradiation, ultrasound for 60 minutes plus 7000 rad, ultrasound for 30 minutes plus 7000 rad. The ultrasound treatments were given immediately prior to the X-irradiation treatments. Ultrasound alone did not have an effect on tumor growth rate and did not enhance the effect of 7000 rad on tumor growth.

To determine the effects of ultrasound-induced hyperthermia and combined ultrasound-induced hyperthermia and X-irradiation on host-tumor interactions, experiments were designed to measure inhibition of tumor growth after pretransplantation treatment of the tumor bed.

The left thighs of the mice were treated with ultrasound for 30 minutes, 500 rad X-irradiation, or the combined treatments. The treatments were given one day, seven days or 30 days before tumor transplantation. A TBE was demonstrated with an X-irradiation dose of 500 rad, which persisted for at least 30 days. A TBE was also demonstrated after ultrasound exposure for 30 minutes. The effect of the ultrasound exposure was still observable after seven days, but not after 30 days. Enhancement by ultrasound of the TBE by 500 rad was only observed when the treatments were given one day before tumor transplant. The results indicate that the damage to the tissue produced by the ultrasound-induced hyperthermia is different from the damage produced by the X-irradiation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Rationale for Use of Hyperthermia in Cancer Therapy

During the last ten years, many radiotherapy centers have developed experimental treatment programs using hyperthermia combined with ionizing radiation in the management of advanced malignant diseases. The rationale for this treatment modality was based on the discovery that elevated body temperatures of 41.0°C to 42.0°C could have a destructive effect on cancer (1; 2; 3). Results from human clinical trials have been encouraging. Also, experimental studies on transplantable tumors in animals have resulted in significant increases in local tumor control and survival rates, and *in vitro* studies have demonstrated that hyperthermia could be an effective sensitizing agent when used in combination with radiotherapy (4; 5; 6).

Induction of Hyperthermia

The induction of whole-body or local hyperthermia has been achieved by the use of radiant heat, hot water bath immersion, hot air, exogenous pyrogens, microwaves, or ultrasound. All of the methods have limitations, but the most promising techniques at present are the use of microwaves or ultrasound beams because these techniques can induce temperature rises at a greater depth in tissue than any of the other methods (4).

Ultrasound has been used in physiotherapy for many years, and was first applied to cancer therapy in 1934 (4). The energy of an ultrasound wave is transferred from the wave to the tissue and is locally

dissipated as heat. The absorption and, therefore, the induced rise in temperature is determined by the absorption coefficient of the tissue, which is low in fat, high in muscle, and highest in bone. The reason for the current interest in the use of ultrasound for the induction of hyperthermia is that the absorption patterns in tissue can be modified by proper choice of frequency, which is not possible with microwaves. It is feasible to focus transducer patterns to deliver concentrated energy to volumes whose dimensions are of the order of one wavelength. The patterns of several transducers can thus be made to overlap in the tumor volume to improve the heat distribution locally. Also, the temperature measurements by thermocouples are not influenced by sound waves at the intensities that are required for heating, while microwaves interfere with such temperature measurements (7).

Little information is available regarding the biological effects of ultrasound on normal and neoplastic tissue and particularly the possible benefits in cancer therapy. Results from *in vitro* and *in vivo* studies, to be discussed in the following literature review, are conflicting. Therefore, this study was undertaken to determine the effects of ultrasound-induced hyperthermia and ultrasound-induced hyperthermia combined with radiotherapy as compared to the effects of radiotherapy alone on the growth rate of the line 1 lung carcinoma.

The Importance of the Tumor Bed to Radiotherapy

The mode of action of local X-irradiation on neoplasms *in vivo* is still a controversial issue. It is generally believed that the direct action of X-irradiation on the cancer cells is responsible for the

inhibition of tumor growth and sometimes the disappearance of the tumor, which is assumed to be dependent on the radiosensitivity of the tumor and the dose required to kill all tumor cells or the tumor cidal dose (TCD). However, the response of a given tumor to radiation varies from one individual to another. The difference in response may be due to a complex interplay of various factors, such as host-tumor interactions, and variations in cell populations within a tumor. These factors may influence the response of the entire organism, which in turn may modify the response of malignant disease to irradiation (8; 9; 10). Continued growth of a malignant tumor is dependent upon the characteristics of the abnormal cells as well as the environmental conditions compatible with survival (11, pp. 38-39). One is, therefore, not only concerned with the effects of radiation on the tumor but also with reactions in the immediate surrounding tissues that supply the stroma, sustain and nourish the malignant cells, and provide the metabolites for cell division and tumor growth. The capillary bed of tumors is known to be altered by irradiation, both in humans and in animals. Some of the lethal effects on the tumor cells may be indirectly mediated through the gradual narrowing and obstruction of the vasculature of the tumor bed and the resulting ischemia and anoxia. Successful destruction of the tumor may, therefore, depend mostly on obliteration of the vasculature of the tumor as a result of the radiation damage to the capillary bed when the tumor is relatively radioresistant. For a radiosensitive tumor, however, a preserved vasculature is essential to maintain adequate blood supply to the tumor cells, and obliteration of the vasculature may result in failure to destroy the tumor (12, pp. 894-913).

It is important for the management of cancer and clinical radiotherapy to obtain more information regarding the indirect influence from radiation of the tumor bed on the growth of the tumor. Preoperative irradiation is frequently applied in the management of breast cancer and cancers of the head and neck. However, this treatment method is still a controversial issue (11, p. 146). The biologic basis of preoperative irradiation is that, following radiation therapy, changes induced in the normal tissues might be expected to offset the survival of tumor cells spilled into the operative wound at subsequent surgery and thereby modify the number of cells that would be able to establish a focus of tumor growth (13; 14; 15, p. 209).

Therefore, the indirect effects from radiation of the tumor bed on the growth of the tumor have been investigated using transplantable experimental animal tumors. The effect of prior irradiation on the transplantability and growth of experimental animal tumors at the irradiated site and the net effect of host survival is known as the tumor bed effect or TBE (15, p. 323). To further investigate a possible synergism between ultrasound-induced hyperthermia and ionizing irradiation, experiments were designed to study the effect of prior treatment on the growth rate of the line 1 lung carcinoma.

CHAPTER II

LITERATURE REVIEW

A. HUMAN STUDIES

Effects of Hyperthermia

Many reports on the experimental and clinical aspects of hyperthermia have been published. These reports have been reviewed and summarized by Har-Kedar and Bleehen (4). Some of the original findings and the results of more recent studies will be reviewed here.

The observation that febrile temperatures occasionally resulted in tumor regression was first made by the German physician Busch. His observations and those that were made later by others, were reported by Bruns in 1887 (1). In all cases reported, the febrile reaction had been caused by an erysipelas infection, and Bruns emphasized that, although the dramatic tumor regressions appeared to be due to the high temperatures, "other factors" might have been involved. Coley (2) treated inoperable tumors by repeated inoculations of erysipelas, "Coley's toxins," and found that significant tumor regression occurred when a fever lasting several days had been induced. Andrews (15, p. 206) used the same "Coley's toxins" and "watched patients become febrile without knowing whether we were practicing immunology, hyperthermia, or black magic. Some patients got better." It is possible that these inoculations induced an immune response in the patients resulting in stimulation of production of non-specific antibodies.

In 1935, Warren (3) reported on 32 cases with advanced malignant disease that he treated by inducing an artificial fever of 41.5°C. The elevated temperatures were induced locally or whole-body by diathermy or by radiant heat from carbon filament lamps. No results approaching cure were obtained, although siginificant tumor regression was observed.

Effects of Hyperthermia and Radiation

A greater interest in hyperthermia for cancer therapy developed during the 1960s and early 1970s when it was found from animal and *in vitro* studies that heat sensitized cells and tissues to ionizing radiations. These data provided the rationale for later clinical trials in cancer therapy using combined hyperthermia and radiotherapy.

Hartman and Crile (16) treated five patients suffering from osteogenic sarcoma of the extremities with combined microwave and X-ray irradiation. Two patients appeared to be tumor-free after five years. Using heat (hot air or microwave) and X-rays, Brenner and Yerushalmi (17) treated six patients who had metastatic disease. They found that four of the six patients were free of metastases at the end of three years. Kim and co-workers (18) treated 36 patients who had malignant cutaneous lesions, including mycosis fungoides, Kaposi sarcoma, malignant melanoma, lymphoma cutis and other metastatic skin lesions. The heating methods used were temperature-regulated water bath immersion, and microwaves of frequency 27.12 MHz. The initial tumor regression rates were faster in patients treated with X-irradiation plus hyperthermia than in patients treated with X-irradiation alone, particularly in patients with Kaposi sarcoma and lymphoma cutis. All of these patients showed significant prolonged benefits achieved by the combined treatments.

Hornback and co-workers (19) applied diathermy of frequency of 433 MHz because of the increased penetration ability. Patients having advanced malignancies were treated with shortwave and X-irradiation. Complete regression of all localized tumors resulted in 80 percent of the patients who completed the full course of treatments.

Although these clinical reports were very encouraging, most human studies either lacked controls or had unacceptable controls, and optimum temperatures, time of exposure, and radiation dose relationships are yet to be determined. It is known that the temperature range available is very limited. The temperature needed to obtain a significant therapeutic effect exceeds 41.0°C; however, whole-body hyperthermia is limited to 42.0°C in man and probably most animals (4). The magnitude of the therapeutic effect of heat is directly related to the duration of exposure to that temperature. Also, the upper limit of tolerance of normal tissue is about 45.0°C for a few hours of exposure, which would indicate that better results might be obtained with localized hyperthermia. A major problem, however, is to achieve uniform heating of the tumor volume and to continuously monitor the temperature.

Effects of Ultrasound

The first report on the application of ultrasound in the treatment of human cancer was published in 1944 by Horvath (20), who treated multiple skin metastases of sarcomas by ultrasound alone. The rationale for the treatment was based on results from animal experiments obtained by Japanese investigators in 1931 and 1938, and from histological findings obtained by Auler and Woite (reviewed in 20). The Japanese

investigators found that the growth rate of tumors in animals could sometimes be retarded, occasional complete regression could be observed after ultrasound exposure, or the growth rate could be accelerated. In addition, ascites tumors in mice treated with ultrasound revealed histological evidence of heat damage. These results led Horvath to conclude that the effect of ultrasound on the growth rate of tumors was mainly dependent upon the intensities of the sound waves. He treated patients with skin metastases by ultrasound of frequency 800 kHz at an intensity of 15 watt for 15 minutes. The doses applied varied from patient to patient because they reacted differently to the pain caused by the heat. All tumors treated had completely regressed six to eight weeks later, even though the immediate reaction to the treatment showed severe burns and edema.

Woeber (21) later concluded from experimental animal studies that ultrasound alone was ineffective in cancer therapy. However, he was able to demonstrate that ultrasound of intensities of 0.2-0.3 W/cm^2 and frequency 1 MHz enhanced the effect of X-irradiation on tumor regression. He applied the combined ultrasound and radiation treatment to 50 patients with primary skin cancers. After a six-year follow-up period, only one case had recurred. Woeber attributed the success of the combined treatments to the induction of heat by ultrasound. Temperature measurements were not done, but the exposure time of the ultrasound was about five minutes. The radiation dose used was 66 percent of the dose necessary to achieve complete tumor regression with X-irradiation alone, demonstrating the enhancement of radiation therapy by ultrasound.

More recently, Marmor and co-workers (22) used ultrasound-induced hyperthermia to treat 25 patients with recurrent or metastatic skin cancers refractory to conventional treatments. The temperatures were measured during each treatment and ranged from 43.0-45.0°C. Complete tumor regression was observed in two patients and partial regression was observed in 11 of the 25 patients who completed a course of six treatments over a period of two weeks. However, the follow-up period was only six weeks. Therefore, the authors stressed that the results should be interpreted with caution since the partial response of most of the tumors could be a transitory effect. Hopefully, more information regarding the results of this first clinical trial will become available later.

It is clear from the above that ultrasound may be applied effectively for the induction of hyperthermia in superficial tumors. However, the applicability of the thermal effect of ultrasound as a source of hyperthermia needs further study. The construction of hyperthermia equipment utilizing ultrasound for the heating of deep-seated tumors is still in very early stages. Furthermore, possible immediate and long-term toxic effects on normal tissues of the ultrasound treatments need to be further investigated.

B. ANIMAL STUDIES

Effects of Hyperthermia

Much information has been obtained from studies on transplantable tumors in animals. Due to the smaller volumes involved, uniform heating is greatly facilitated. Heat can selectively destroy certain cancers

without causing significant damage to surrounding tissues. This was shown by Crile (23) in 1962. Tumors implanted on the feet of mice were heated by immersion in a hot water bath at 44°C for 30 minutes. Crile found that the destructive effects of heat began at 42°C and that for each degree rise up to 45°C, the time required to produce the same biological effect was halved. Selective destruction of tumor cells was also shown by Overgaard and Overgaard (24). Using microwaves of frequency of 27.12 MHz for the treatment of the mouse mammary carcinoma, a cure rate of 25 percent of the animals treated was achieved without damage to normal tissue. The temperature ranges in the tumors were 41.5 to 43.5°C.

Thrall, Gillette and Bauman (25) evaluated the effect of heat on the mammary carcinoma implanted in the hind leg of C_3H mice. Immersion of the tumor in a 44.5°C water bath for 15 to 60 minutes did not result in any cures. It was suggested that this could be due to insufficient heating of the tumors. Intratumor temperatures stabilized in three minutes at 44.1°C. Delay in tumor growth was found to be dependent upon the duration of the heat treatment. Longer heat treatments resulted in longer delays in tumor growth. Fractionated heat doses were less effective in producing tumor growth delay than an equivalent single heat dose. It was suggested that part of the heat damage was repaired during the interval between the treatments.

Effects of Hyperthermia and Radiation

Robinson, Wizenberg, and Cready (26) also used the *in vivo* C_3H mammary tumor system. The tumor was implanted subcutaneously in the flank of the mouse so that it could be retracted slightly from the body

and submerged below the water surface for the hyperthermic treatment. Heat at 41.0°C was applied to the tumor using a water bath for 30 minutes before, during, and following X-irradiation. Both skin response using a scoring system and tumor response using regrowth and tumor cidal dose (TCD) values were obtained for control and heated animals. The thermal enhancement ratios (TER) (the ratio between the effect of combined hyperthermia and radiotherapy and the effect of radiotherapy alone) were obtained for skin and tumor. At 41.0°C a TER of 1.18 was determined for skin, and a TER of 1.42 was determined for the tumor. This resulted in a therapeutic ratio of 1.2 for the 41.0°C temperature, indicating that the tumor was 1.2 times more sensitive than the skin to the combined treatments. The sequence of application of heat and radiation showed no difference in the results.

Thrall, Gillette, and Dewey (27) also evaluated the effects of hyperthermia and X-irradiation on normal tissue (skin) and the C_3H mammary tumor. However, radiation was given under various conditions of tissue oxygenation and heat was applied either before or after irradiation. The tissue response was measured in terms of $DD_{50/21}$ (the dose required to produce full moist desquamation in 50 percent of the legs in 21 days) and $TCD_{50/120}$ (the dose needed to control tumors in 50 percent of the animals as observed at 120 days). For the tumor, the order of application was found to be significant only when radiation was given under hypoxic and hyperbaric oxygen conditions, but not under ambient conditions. Under these conditions, hyperthermia was found to be more effective after irradiation. In skin, the order of application was significant only when irradiation was given under ambient conditions,

in which case hyperthermia was more effective prior to irradiation. These results would suggest that for the treatment of tumors, application of heat after irradiation would be more beneficial because of the improved therapeutic ratio.

Using the same C_3H mouse tumor system and tissue response measurements of $DD_{50/21}$ and $TCD_{50/120}$, Gillette and Ensley (28) further examined the effect of heating order on radiation response of tumor and skin. From the TER for tumor and skin, a therapeutic gain factor (TGF) was calculated. It was found that the largest TGF of 1.3 was obtained for simultaneous heat treatment for 30 minutes at 42.5°C and X-irradiation. Also of interest is the finding that TGFs of 1.3 were obtained with 15 minutes of heating two hours prior to irradiation. The greater TGFs for the shorter heating were attributed to the fact that there was little or no effect on skin response, while there was a significant effect on TCD_{50}s.

From the above, it can be seen that the sequence for applying hyperthermia and X-irradiation, optimum temperature, and duration of heat exposure need to be further investigated.

Effects of Ultrasound

All of the human clinical trials with ultrasound were undertaken because of the results obtained from animal experiments. As already mentioned, Horvath based his rationale for ultrasound treatment on the findings of Japanese investigators (20).

Woeber (21) treated rats inoculated with Walker carcinoma with 350 R X-irradiation and five minutes ultrasound (1 W/cm^2) either alone

or in combination. The combined therapy controlled all the tumors and the effect was equivalent to the effect of 600 R X-irradiation, while 350 R or ultrasound alone did not have a significant effect on tumor growth. Temperature measurements were not reported.

Southam, Beyer, and Allen (29) also determined that ultrasound alone did not have an effect on tumor growth. These investigators designed a study to determine the relative susceptibility of various tissues, both neoplastic and normal, to the destructive effects of ultrasound. Five groups of mice each bearing different tumors were treated with ultrasound of frequencies of 0.5 MHz and 3.8 MHz at 1-10 W/cm^2. The solid tumors were bilateral and one of each pair was shielded during treatment. No changes in rate of growth or histological appearance were detected that could be attributed to the ultrasonic treatment. The authors concluded that their studies gave no reason to believe that ultrasonic energy alone might be useful in the treatment of inoperable neoplastic disease in man.

Other investigators, however, obtained more encouraging results with ultrasonic treatments of animal tumors. Longo and his group (30) irradiated a subcutaneously implated rat Wilm's tumor in Wistar-Furth rats with ultrasonic energy of 1 MHz and 1.5 W/cm^2 for five minutes. The experiments demonstrated that ultrasound could inhibit the growth of the tumor and that the inhibition resulted in prolonged survival of the host animals. Histological assessment exhibited a sharp line of demarcation between the necrosed, sonicated portion of the tumor and the viable intact nonsonicated area of the tumor. Temperature measurements were not done, and the authors questioned whether the effect was due to

hyperthermia because of the short sonication time of five minutes. Utilizing an ultrasound transducer operating at 2.7 MHz and 1-3 W/cm^2 to raise the intratumor temperatures to 43.0-44.5°C in transplantable murine tumors, Marmor and co-workers (31) observed tumor regression and cures with increasing frequency at longer exposures and at higher temperatures. Cell survival studies on tumors treated in situ showed that cell inactivation by ultrasound heating was similar to that for heating by water bath. However, results from experiments using a tissue culture system showed that direct in vitro cell killing by ultrasound was considerably less than that expected based on tumor cure rates achieved. That is, more ultrasonic energy was needed to inactivate tumor cells in vitro. Following these findings, Marmor (32) treated spontaneous tumors in cats and dogs applying the same temperature range of 43.0-44.5°C induced by ultrasound with reasonable success. Squamous cell carcinomas appeared to be most responsive, but there was no apparent correlation between temperature or duration of treatment and response rate. From their results it was concluded that ultrasonic irradiation could have an effect on the tumor growth rate of only certain types of tumors.

To determine a possible synergism between ultrasound and X-irradiation, Clarke and Hill (33) designed a study based on Woeber's work. In the hind leg of Marshall rats, BICR/M1 transplanted tumors were treated by ultrasound only, X-irradiation only, or the combined treatments. The results indicated that ultrasound had no significant effect on the growth of the tumors, either alone or by altering their response of X-irradiation. However, intratumor temperatures of only

38.0-39.0°C were reported, and it is known that the temperature required to obtain a therapeutic effect exceeds 41.0°C (5).

Witcofski and Kremkau (34) found that ultrasound treatment (1.5 W/cm^2, 1.9 MHz) for 15 minutes either before or after X-irradiation reduced the TCD 50 of the radiosensitive sarcoma-180 by approximately 40 percent, while similar ultrasound treatment for up to 30 minutes did not reduce the TCD 50 of the C_3HBA mammary carcinoma, a radioresistant tumor. Water bath heating (44.5°C for 15 minutes) after X-irradiation reduced the TCD 50 of both tumors. Ultrasound alone for up to 30 minutes had no effect on growth or cure rate of either tumor.

In summary, experiments with animal tumors have not shown definitely that treatment by ultrasound alone is effective in cancer therapy. In addition, the enhancement of radiotherapy by ultrasound varies with the frequencies employed and the duration of the ultrasound treatment as well as the type of tumor treated. It appears that enhancement with ultrasound occurs with radiation sensitive tumors but not with radioresistant tumors, while water bath heating definitely shows a radiation enhancement effect in a variety of animal tumors.

C. *IN VITRO* STUDIES

In vitro studies using a variety of cell lines during the last decade have demonstrated that individual cell lines show different thermal responses. A discussion on the cellular aspects of hyperthermia and X-irradiation is beyond the scope of this thesis, but the results of the *in vitro* studies have been reviewed by Thrall and co-workers (5) and

Dewey and co-workers (35). The cellular effects and its significance to cancer therapy have also been discussed in detail by Bronk (6). Suffice it here to mention the two most important aspects of the combined treatment: the reduction of the oxygen enhancement ratio and the difference in sensitivity of cycling cells to the two agents. Hypoxic cells are more sensitive to heat than oxygenated cells. Since hypoxic cells are protected from the effects of radiation, the dramatic response of tumors to combined heat and radiation treatment could be due to the increased sensitivity of the hypoxic cells within the tumor to hyperthermia. Cells appear to be most sensitive to hyperthermia during the S phase, which is normally radioresistant. The differences in the cell-age response to hyperthermia and radiation may also in part explain the observed synergistic effect of the two agents.

Very few in vitro studies on the application of ultrasound in cancer therapy have been done. Perhaps one of the reasons for this is the variety and complexity of effects achieved by interaction of ultrasound with cells. Cells in suspension can be severely damaged by rupture of the cell membrane due to a phenomenon called cavitation in which dissolved gases in fluid grow to bubbles that may behave as resonant cavities and vibrate at much higher amplitudes than the incident ultrasonic wave. This results in disruption of cell membranes. Cavitation supposedly does not occur in organized tissues. When exposing cells in culture, cavitation can be avoided by placing the cells in a gel suspension as suggested by Clarke and Hill (33). These investigators found that when both heating and cavitation effects were deliberately avoided

ultrasound had no effect on the radiosensitivity of mouse leukemia cells.

A radiation enhancement effect by ultrasound using an *in vitro* system was demonstrated by Todd and Schroy (36). Ultrasound was applied to Chinese hamster cells after various doses of X-irradiation. A temperature of 42.0°C was obtained. Ultrasound alone had no effect on the colony-forming ability of the cells, but enhanced the radiation effect by a factor of 1.3. A small degree of synergism between ultrasound and X-irradiation was also observed by Martins, Raju, and Hayes (37) using Chinese hamster bone-marrow cells. The extent of synergism depended on the X-irradiation dose applied, the time interval between ultrasound and X-ray treatment and the order in which the treatments were given. The synergism increased when low doses of X-irradiation were used, when the time interval between treatments was 30 minutes and when ultrasound treatment followed X-irradiation. The cell cultures were maintained at 37.0°C, and ultrasound exposure induced a temperature rise of only 1.0°C. Therefore, the effect could not be attributed to hyperthermia. It was suggested that the synergism might be due to an interaction between nuclear damage caused by X-rays and the damage to the cell membrane caused by ultrasound. Ultrasound alone showed a cell-killing effect that was dose dependent. Lethal effects of ultrasound were also demonstrated by other investigators (4; 38; 39). The mechanisms involved, however, are unknown and need further investigation.

D. TUMOR BED STUDIES

As previously mentioned, several investigators (8; 9; 10) suggested that the variation in response of a given tumor to radiation is due to the effect of radiation on host-tumor interactions. The experimental studies of tumor radiobiology using transplantable animal tumors have provided unique material because they have allowed investigation of the direct effects on the tumor cells and the indirect effects of the tumor bed both separately and concomitantly.

The question whether "X- and -radiations have a direct effect on tumor growth" or whether their effect "on blood supply and other cells play a determining role, so that the effect is more indirect," was first raised by Wasserman in 1944 (40). Using a transplantable mammary tumor in mice of unknown genetic background, Wasserman demonstrated that the tumor cells were killed by the direct action of the radiation on the cells. Tumor homogenates were irradiated before subcutaneous inoculation into the dorsal region of the mice. The irradiated inoculates failed to develop into tumors, while equivalent nonirradiated inoculates produced rapidly growing tumors that killed the animals within one month. Although these experiments demonstrated a "direct effect" on tumor growth, Wasserman emphasized that since no information regarding the role of the surrounding tissue in supporting tumor growth could be obtained, different experiments should be designed to investigate a possible "indirect effect." Wasserman's publication stimulated other investigators to examine the effect of irradiation on host-tumor interactions. From these studies information was obtained indicating an

effect of pretransplantation irradiation on tumor growth. Thus the importance of host-tumor interactions on tumor growth and the influence from radiation on these host-tumor interactions was suggested. The effect of prior irradiation on the transplantability and growth of tumors at the irradiated site is known as the tumor bed effect (TBE).

A TBE was first demonstrated by Frankl and Kimball (41) who obtained the transplantable mammary carcinoma from Wasserman and were able to demonstrate a "powerful influence from irradiation of the tumor bed, resulting in an indirect influence on the growth of the tumor, indirect because the tumor transplant was never hit directly by the radiation." Two typical experiments were reported in detail. In each experiment six mice were given an epilation dose expressed as "50X" to the dorsal region and 0.2 ml of the tumor cell suspension was then injected into the irradiated area. The same volume of tumor cells was injected into six nonirradiated mice and all of them developed rapidly growing tumors that killed the animals within one month. In the first experiment described, three of the six irradiated animals failed to develop tumors. Small tumors appeared in two animals but later regressed and became necrotic. The sixth mouse developed a tumor in the irradiated area, but it grew slowly until the animal died after two months. In the second experiment reported, the six nonirradiated animals died also within one month after tumor transplantation. Five of the irradiated mice developed very small tumors which became necrotic. The mice survived much longer than the nonirradiated animals. The sixth irradiated mouse failed to develop a tumor altogether. Five experiments were done on a total of 60 mice,

and each produced similar results. The authors concluded that besides a direct effect of radiation on the tumor cells, the effect on the tumor bed also plays a significant role in tumor growth inhibition. They suggested that these findings could be important for the treatment of human cancer since it would justify preoperative and postoperative irradiation.

The importance of host-tumor interaction also has been shown by many other investigators. Murphy and co-workers (42) conducted studies extending over several years, the results of which they interpreted as suggesting the close relation between lymphocytes and resistance or susceptibility to cancer growth. One of Murphy's co-workers, Nakahara (43), demonstrated that small doses of X-irradiation induced an inflammatory response that resulted in resistance to the tumor transplant that was effective up to seven days after treatment. Later Nakahara (44) examined histologically the series of degenerative changes that took place in the cancer cells implanted in areas of skin previously exposed to radiation and compared these findings to those that had been reported previously as the typical changes taking place in cancer cells following radiation. He concluded that since the changes observed were the same whether the cancer cells had been directly exposed in situ or merely implanted in previously irradiated skin, it could be established that a direct injury from radiation was not the principal factor in the therapeutic action of X-rays on cancer.

To further examine the theory of the "indirect action" of X-rays on tumor growth, Murphy and co-workers (45) replanted autografts from spontaneous cancers of mice into previously irradiated skin areas of

these mice. The autografts failed to grow in irradiated areas, while similar grafts inoculated into nonirradiated areas grew actively. Murphy also showed that autografts of spontaneous cancers, established and growing in the skin, disappeared in 76 percent of the animals after the tumor and the surrounding tissues had been irradiated, whereas other autografts of similar derivation that had been given an equivalent dose of radiation outside of the body and had been implanted in the same animals grew progressively in 96 percent of cases. This result was not due to a greater susceptibility of the cancer cells to radiation when treated in situ. To demonstrate this, tumors were irradiated in situ without irradiating the surrounding tissue. However, these tumors grew actively when replanted into the nonirradiated skin of the same animal.

The importance of the effect of irradiation of the tissues surrounding a tumor on tumor growth was further emphasized by the experimental results of Russ and Scott (46). Young rats were irradiated with an epilation dose through a 2-mm-thick lead shield with a cut-out of two opposing segmental areas each covering about 45° of a circular disc. The remaining areas of the disc, including the center, were shielded by the lead. At varying time periods, small pieces of Jensen's rat sarcoma were inoculated into the nonirradiated center. The tumors grew invariably into the shielded tissues, indicating that malignant tumors tended to grow more easily in the normal nonirradiated tissues than in the irradiated tissues. In 1940 Russ and Scott (47) designed experiments to find out how far it might be possible to check an actively growing Jensen's sarcoma by irradiating merely the tissue surrounding the tumor, while the tumor itself was shielded from radiation. They found that

tumor growth was considerably slowed down, and 37 percent of the tumors disappeared. These results again demonstrated the influence of the irradiated tumor bed on tumor growth.

Lasnitzky (48) determined quantitatively the direct and indirect action of radiation by comparing the incidence of mitotic and degenerate cells, *in vitro* and *in vivo*, following a larger dose sufficient to involve the circulatory system. Tumors and tissue cultures of mouse adenocarcinoma 63 were subjected to a dose of 2000 R of X-irradiation. The changes observed for a period of ten days were qualitatively similar *in vivo* and *in vitro*. While the incidence of degeneration on the first day was of the same order *in vivo* and *in vitro*, on the second day and later a striking rise in the number of degenerate cells *in vivo* was observed accompanied by a marked vascular reaction. It was concluded that during the first day after exposure the effect of radiation is mainly a direct one, that from the second day onwards the additional indirect effect via a damaged blood circulation comes into play. Cell counts showed that about one-third of the radiation effect was due to the direct action and two thirds to the indirect action of radiation on the tumor bed. Lasnitzky's results clearly demonstrated the importance of the effect of radiation on host-tumor interactions.

The tumor bed effect has also been studied extensively in mice of known genetic background using isologous transplants of mammary adenocarcinoma by Vermund and co-workers (49; 50; 51). The effects of pretransplantation irradiation were evaluated in terms of delay in tumor growth, reduction of rate of tumor growth, and increase in survival time of the tumor-bearing animals. Pretransplantation irradiation of

1500 rad delivered locally to one leg 48 hours before subcutaneous transplant of tumor cells, was found to significantly inhibit the development and the growth rate of the mammary carcinoma (49). Quantitative differences in responses were shown between different tumor lines of the same type of mammary carcinoma. An optimal dose of 3000 rad was found. No further inhibition of growth rate was observed when higher doses were used. All animals eventually developed tumors and succumbed (50).

The effects of pre- and posttransplantation irradiation at different time intervals on the average survival time and tumor growth rate were also compared (51). Tumors were found to become very radioresistant when irradiated more than three weeks after transplantation. But tumor growth could be completely suppressed or greatly delayed if the treatment was given two weeks after transplantation. Pretransplantation irradiation of the tumor bed did not prevent tumor growth completely, but did retard tumor development and increased the survival time of the animals. It was also found that tumor cells that had been transferred by serial transplantation over many generations appeared more radioresistant to posttransplantation irradiation than tumor cells transferred only a few times. No explanation was given for this phenomenon.

The tumor bed effect was further studied by Summers and Vermund (52) using several transplantable tumors, namely gastric adenocarcinoma 328, mammary adenocarcinoma 755, the melanoma B-16 in BDF_1 mice and the squamous cell carcinoma G8755 in C_3H mice. It was found that in all animals the increase in survival time was proportional to the dose of local irradiation up to a maximum of 3000 rad. The increase in survival was also found to be related to the growth rate of the tumor.

That is, preirradiated hosts with slowly growing tumors showed a smaller survival increment than preirradiated hosts inoculated with rapidly growing tumors. Results from later experiments (53) using the same tumor systems showed that a TBE persisted for at least nine months. No TBE could be demonstrated when tumors were implanted into irradiated brain tissue. Dose response curves were obtained for most of the tumors, and inhibition of tumor growth was found to increase linearly with increased dose to a maximum at 3000 rad. However, no TBE could be demonstrated at doses lower than 500 rad. It was suggested that there could exist a threshold dose for a TBE. This finding was confirmed by Hewitt and Blake (54). However, these investigators found no difference in the magnitude of the TBE over the dose range 1500-4000 rad, for the CBA/Ht Sarcoma F and the CBA/Ht Sarcoma S in CBA/Ht mice. Experimental evidence strongly suggested that preirradiation of the transplantation site had no influence on the latent phase of subsequent tumor growth. The limitation of the effect to later phases of growth was considered to be associated with an ability of microtumors to grow independently of vascularization, and the authors concluded that from the results of their experiments no evidence was provided to support the assertion that "indirect" effects of radiation on the stroma of tumors are of importance to their radiotherapeutic eradication. Urano and Suit (55) supported this conclusion based on the results of their experiments. They evaluated the TBE by determining the TD_{50}, the number of cells expected to transplant the tumor into half of the recipients, for the C_3H mouse mammary carcinoma, and the C_3H fibrosarcoma in preirradiated and normal tissues of C_3H mice. Although tumor growth was

significantly slowed down, and host survival increased, the TD_{50} was found to be independent of the prior radiation history of the transplantation site. The authors concluded that there was no apparent effect of the changes induced in the irradiated tissue to inactivate or prevent proliferation of injected tumor cells.

A different point of view was presented by O'Brien and co-workers (56). Irradiation with 988 rad to the surface of the abdomen of New Zealand rabbits prior to implantation of V-2 carcinoma into the colon of the animals resulted in a demonstrable inhibition of tumor growth and increased the survival time of the animals significantly as compared to the non-irradiated animals. It was suggested that the TBE could be organ specific since no TBE could be demonstrated for tumors transplanted into preirradiated brain as was previously shown by Summers and Vermund (53). These findings may support the theory that the connective tissue reaction is a necessary component in the complex mechanisms responsible for the tumor bed response.

The vascular changes following irradiation have been described in great detail. The literature on this subject has been reviewed and summarized by Rubin and Casarett (12, pp. 1-38), Vermund (8), and more recently by Clifton and Jirtle (57), and Andrews (15, pp. 51-55). In summary, irradiation of normal tissue before transplantation not only "kills" endothelial cells, but also causes morphological changes in the vessels. The vascular changes caused by preirradiation of the normal tissue in which a tumor grows may reduce the bloodsupply to the tumor, which could thus reduce the tumor growth rate.

It is clear that preirradiation of the transplantation site inhibits the growth of a subsequent tumor transplant of many experimental animal tumors. Although pretransplantation irradiation also may delay development of a tumor after transplantation into the irradiated tissue and even prolong the host survival time, it does not prevent eventual development of a tumor that subsequently kills the animal.

It has also been shown that hyperthermic treatment at moderate temperatures can result in local tumor control and that hyperthermia can be an effective sensitizing agent when applied in combination with radiotherapy. However, little information is available regarding the effects of hyperthermia on host-tumor interactions. Information may be obtained by investigating the indirect influence from hyperthermia of the tumor bed on the growth of the tumor. It is reasonable to expect that a TRE with local hyperthermia, as well as an enhancement of the TRE of X-irradiation by hyperthermia, can be demonstrated.

Yerushalmi (58) found an enhanced resistance to tumor development in animals pretreated by combined simultaneous local heat and X-irradiation, as compared to animals only preirradiated, only preheated, or to control untreated tumor-bearing animals. Two tumor systems were used: the Lewis Lung carcinoma in $(C_3H/eB/BL)F_1$ mice and the SV-40 fibrosarcoma in $(Balb/c \times C57BL/6)F_1$ mice. The method of heating was not described. The stimulated resistance was found in animals inoculated in the pretreated leg as well as in animals inoculated in the contralateral untreated leg. These results led the author to suggest that the combined treatments trigger or increase an immune response in the host and support the model of indirect activity of the

treatments. To further investigate the mechanisms involved, Yerushalmi and Weinstein (59) tested the contribution of T-lymphocytes to the stimulation of resistance against local tumor growth. For this purpose athymic nude mice were selected as hosts. Pretreatment by combined local heat and X-irradiation resulted in a significant increase in survival time of the animals. It was concluded that T-lymphocytes are not involved in the protection against tumor growth. The results demonstrated a small TBE for X-irradiation but failed to demonstrate a TBE after hyperthermia. Similar results were obtained by Urano and Cunningham (60). Isotransplants of a fibrosarcoma in female C_3Hf/Sed mice were used. The tumor was designated FSa-II. Hyperthermia was applied to the feet by immersion into a hot water bath at 43.5°C. Pretransplantation hyperthermia resulted in no retardation of tumor growth. A dose of 3500 rad -irradiation resulted into a significant TBE. The effect of the combined treatments was not tested.

CHAPTER III

MATERIALS AND METHODS

A. MATERIALS

Ultrasound Equipment

The ultrasound equipment was kindly provided by Dr. M. A. Breazeale, Department of Physics, The University of Tennessee. The equipment consisted of a quartz crystal transducer 2.5 cm in diameter driven by a radio frequency generator. Initial experiments showed variations in tissue temperature from animal to animal. This was traced to fluctuations in the line voltage. Therefore, the line voltage to the ultrasonic generator was stabilized by a voltage regulator during all experiments. The ultrasonic power output was determined by measuring the radio frequency voltage at the transducer and using the equation:

$$I=F^2U^2_{eff},$$

where I is the intensity in watts/cm^2, F is the frequency in MHz, and U_{eff} is the effective voltage. For the experiments to be described, the frequency was 4 MHz and the ultrasonic intensity was determined to be 1 watt/cm^2. Water was used as the coupling agent. To avoid cavitation the water was degassed by boiling for 30 minutes and stored in sealed flasks while cooling. Fresh water was used for each experiment, which was maintained at 37.5°C ± 0.3°C in a thermostatically controlled water bath.

The temperature measurements were done with two copper-constantan thermocouples using two Leeds and Northrop potentiometers. The

thermocouple readings were calibrated against a National Bureau of Standards certified thermometer.

X-Ray Equipment

The X-ray equipment was a General Electric Maxitron 300 operated at 250 kVp and 20 mA. The Half Value Layer was 0.5 cm Cu and the dose rate at a distance of 40 cm was 480 rad/min. Dosimetry was performed using a Victoreen condenser r-meter.

Animals

Fourteen- to sixteen-week-old pathogen-free Balb/c female mice were used in all experiments. The animals were obtained from the breeding colony at the Oak Ridge National Laboratory, Biology Division, Oak Ridge, Tennessee, and were maintained at least two weeks in the animal facilities at The University of Tennessee, Knoxville, prior to experimental use. The mice were housed in plastic cages in groups of eight. Woodshavings were used for bedding and Purina Laboratory Chow and chlorinated water were given *ad libitum*.

Tumor

The line 1 lung carcinoma was originally isolated from a 12-month old untreated Balb/c female mouse, and was subsequently maintained *in vivo* and *in vitro* for several years by Yuhas and co-workers (61). On histologic examination, the tumor was classified as an alveolar cell carcinoma. The tumor is weakly immunogenic and highly metastatic.

For these experiments, the tumor was maintained by serial subcutaneous transplantation of crude homogenates into the dorsal region of female Balb/c mice. For experimental use, single-cell suspensions of

the tumor were prepared by forcing the tissue mince through a fine wire mesh. The cells were then washed twice by centrifugation in saline solution, resuspended, counted, diluted to a concentration of 20×10^6 cells/ml, and 0.05 ml of the suspension (i.e. 10^6 cells) was injected into the left thigh. Tumor growth rates were assessed by measuring the tumors in two dimensions and determining the diameter every two or three days after injection.

B. METHODS

Induction of Hyperthermia by Ultrasound

For all ultrasound treatments the mice were restrained in a small plastic container with the left leg exposed. The container was clamped in a three-prong water bath clamp in such a way that the leg was immersed while the body remained above the water surface. To immobilize the leg during the ultrasound exposure, the foot was taped to a string that was suspended from the container and held taut by a lead weight. The transducer was placed posteriorly to the leg with the center aimed at the center of the thigh. A photograph of a mouse leg suspended in an ultrasonic beam is shown in Figure 1. In this schlieren photograph, the ultrasonic beam shows up as a light band. Figure 2 shows a mouse in position before the ultrasonic beam is turned on.

The temperature measurements were done in tumors of 8.0-9.0 mm in diameter and in normal thighs. To determine the temperature, thermocouple tips were inserted directly into the thigh at different locations. After the ultrasound exposure, the mice were sacrificed and the position of the thermocouple tips was determined. It was not possible

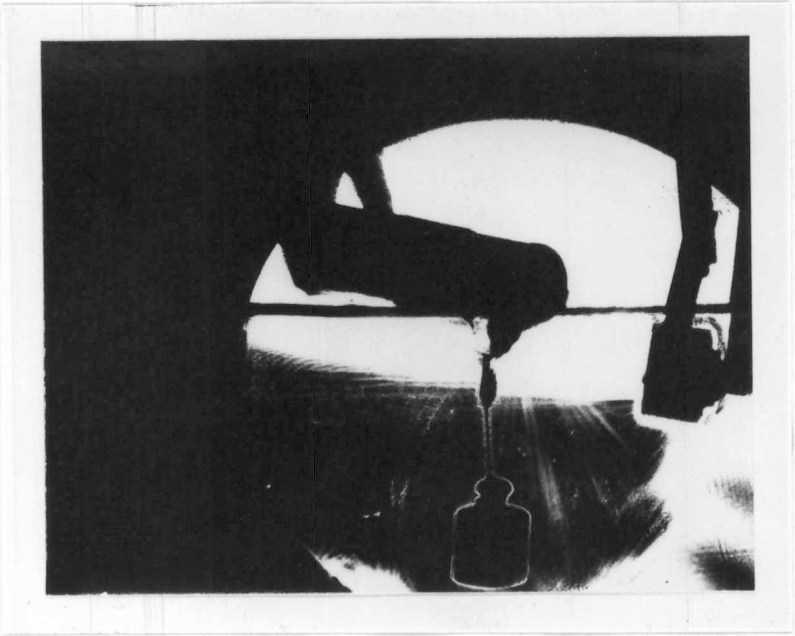

Figure 1. Schlieren Photograph of a Mouse Leg Suspended in the Ultrasonic Beam.

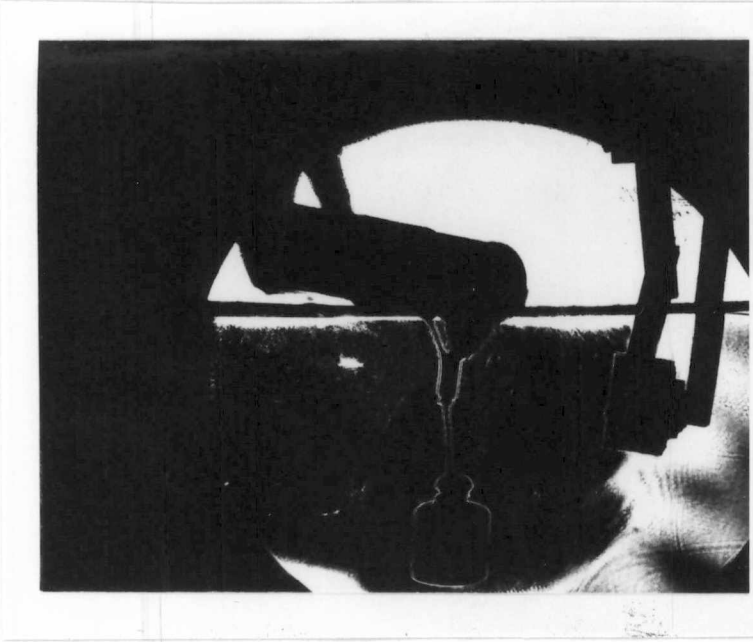

Figure 2. Mouse Leg in Position Relative to the Transducer.

to measure the temperature at the surface of the femur due to the fact that bone as well as the thermocouple have a very high absorption coefficient and the interaction of the two would result in exaggerated temperature readings.

X-irradiation

For all X-irradiation treatments the mice were restrained in the same plastic container as was used for the ultrasound treatments. While the thigh remained exposed, the body of the mouse was shielded with sheets of lead that were moulded to fit the contour of the plastic container. The total thickness of the lead shield was 1.2 cm. The dose at the center of the container was then measured under treatment conditions and was found to be less than 0.3 percent of the dose in the center of the treatment field. It was assumed that under these conditions the dose to the body is less than 0.3 percent of the dose delivered to the thigh. Mice were irradiated singly since only one mouse at a time could be treated with ultrasound.

C. EXPERIMENTAL DESIGN

Effect of Posttransplantation Treatment on Mean Survival Time (MST) and on Tumor Growth Rate

On the seventh or eighth day after tumor cell transplantation the animals were divided into groups of four or five, depending on the number of animals that had a tumor diameter of 8-9 mm. Animals having a tumor larger or smaller were not included in the experiments. The average tumor diameter of each group was 8.5 ± 0.5 mm at the time

of treatment. Because of the length of time required to deliver the ultrasonic treatments, the number of animals per group was kept small and multiple replicates were done.

Each group of animals was treated with either ultrasound for 60 minutes, ultrasound for 30 minutes, 7000 rad X-irradiation, or with ultrasound for 60 minutes plus 7000 rad, or ultrasound for 30 minutes plus 7000 rad. The ultrasound treatments were given immediately prior to the X-irradiation and the time needed to transfer the animals from water bath to the X-ray table was 4-5 minutes.

Mean survival times were determined by maintaining daily records of the death of the animals within each experiment. Complete postmortem examinations were performed on all animals to verify cause of death and presence or absence of tumor in the thigh.

Tumor growth rates were assessed by measuring the tumors in two dimensions and determining the diameters every two or three days after treatment.

Effect of Pretransplantation Treatment on Tumor Growth Rate

A preliminary experiment to study the tumor bed effect was done to determine at which dose of X-irradiation a TBE could be demonstrated using the line 1 lung carcinoma. Groups of four mice received no treatment, 500 rad, 1000 rad, 2000 rad, and 7000 rad, respectively, to the left thigh one day before injection of 10^6 tumor cells. Two groups of mice were included in this experiment that were treated with ultrasound only for 60 and 30 minutes. Tumor growth rates were assessed by determining the average diameter of the tumors within each group every two or three days after injection of the tumor cells. Based on

the results obtained from this experiment, further experiments were designed to determine the effect on tumor growth rate of pretransplantation treatment with ultrasound, X-irradiation and combined ultrasound and X-irradiation. Groups of five or six mice were treated with either 500 rad, ultrasound for 30 minutes, or ultrasound for 30 minutes immediately prior to 500 rad X-irradiation one day before injection of 10^6 tumor cells. To examine the duration of possible effects, groups of animals were treated respectively seven days and 30 days prior to tumor transplantation. In each experiment a group of control animals was injected with the same tumor cell suspension on the same day as the experimental groups.

Mean survival times were determined by maintaining daily records of the death of the animals within each experiment and complete postmortem examinations were performed on all animals to verify cause of death and presence or absence of tumor in the thigh. Tumor growth rates were determined by obtaining the average tumor diameter within each group every two or three days after tumor cell transplant.

Statistical Analysis

The effect of both pre- and posttransplantation treatments on the MST was determined by calculating the mean life span in days after the day of tumor transplant of the groups of animals exposed to the same treatment and comparing these to the MST of the group of animals not treated.

The effectiveness of the different treatment modalities on tumor growth rate was assessed by a multiple regression analysis, the methodology of which has been described by Draper and Smith (62) and

Searle (63). The analysis of the model was performed using the statistical package as described by Helwig (64).

The equation applied was:

$$\text{Tumor diameter} = (\beta_0 + \beta_{1i}\ \text{trmt}) + (\beta_2 \text{day} + \beta_{3i} \text{trmt} * \text{day})$$

in which the parameters are interpreted as follows:

β_0 = mean tumor diameter for control at time zero (intercept)

β_{1i} = difference between mean tumor diameter for treatment (trmt_i) and control intercepts.

β_2 = mean rate of growth per unit time for control (slope of the regression line for control).

β_{3i} = difference between mean rate of growth for treatment and control (difference between slopes).

Differences between the mean rate of growth of the groups of animals treated and the group of animals not treated were determined by testing the hypothesis:

$$H_0: \beta_{3i} = 0 \text{ (for all i) vs } H_a: \beta_{3i} \neq 0 \text{ (for some i)}$$

To examine whether ultrasound enhanced the effect of X-irradiation, the mean rate of growth of the groups of animals that received the combined treatments was compared to the mean rate of growth of the group that received X-irradiation only. That is, the above hypothesis was tested when

β_{3i} = the difference between mean rate of growth for combined treatments and X-irradiation only.

The results were considered to be significant at the $\alpha \leq 0.02$ level ($p \leq 0.02$).

The increases in tumor size became nonlinear 20 days after tumor transplant. Therefore, to determine a treatment effect, the growth rates were calculated over the period of time in which the tumor diameter increased linearly.

CHAPTER IV

RESULTS

Induction of Hyperthermia by Ultrasound

Preliminary experiments were done to determine the temperature rise at the center of the thigh tissue, the length of time required to reach that temperature, and to establish the amount of variation during the exposure times of 30 and 60 minutes. It was also of interest to determine how much of a temperature gradient existed within the tissue. The results of these measurements are shown in Figure 3. A typical temperature reading is demonstrated for tumor-bearing limbs and normal limbs. In a tumor-bearing limb, a temperature of 42.5°C was obtained within 3 minutes from the beginning of the ultrasound exposure as measured with a thermocouple inserted into the tumor at a distance of 1.0 mm from the femur. During an exposure of 60 minutes, the temperature varied slightly between a maximum of 42.8°C and a minimum of 42.2°C. The skin temperature remained fairly constant at 39.9°C. This lower temperature was presumably due to cooling by the water. The temperature profiles were similar when one thermocouple was inserted at distances of 2.0-3.0 mm from the femur. The distribution of the temperature induced by the ultrasonic irradiation was found to be similar in normal thighs; however, the maximum temperature obtained never exceeded 42.25°C. Positioning of the leg of the animal with respect to the ultrasound beam was extremely critical. A slight variation in position could produce a very different temperature profile. However, the exact positioning of the mouse leg and the transducer were found to be reproducible after some

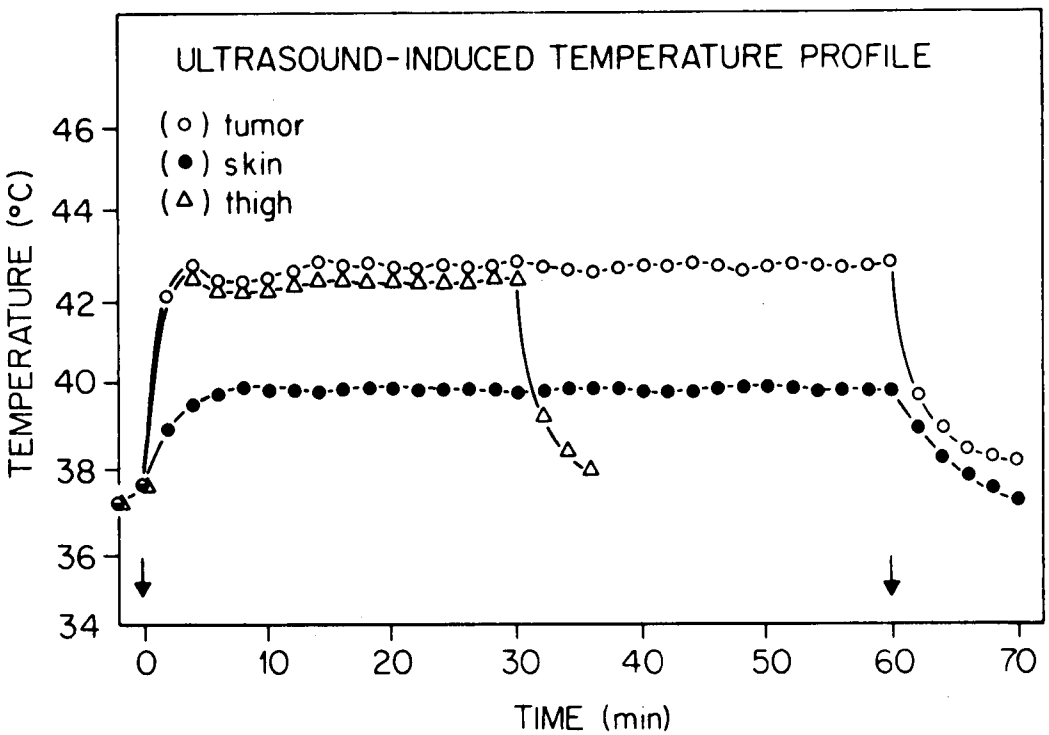

Figure 3. Temperature Profiles Measured Simultaneously at the Center of the Tumor and on the Skin During Ultrasound Exposure for 60 Minutes and at the Center of the Normal Thigh During Ultrasound Exposure for 30 Minutes.

practice, and the results of the temperature measurements remained consistent.

Effect of Posttransplantation Treatment on MST

The effectiveness of the different treatment modalities was first assessed by comparing the MST of the groups of animals exposed to the same treatment. Since no differences were observed within the replicate groups, the data were pooled. These results are shown in Table I. Surprisingly, no differences in MST among the groups exposed to the different treatment modalities were observed. Even treatment of 7000 rad did not have an effect on the MST, and no cures were observed.

TABLE I

EFFECT OF POSTTRANSPLANTATION TREATMENT ON MEAN SURVIVAL TIME

Treatment	Total number of animals	MST ± S.E.*
Control	44	27.70 ± 0.43
Ultrasound - 60 minutes	18	27.40 ± 0.54
Ultrasound - 30 minutes	20	27.90 ± 1.30
7000 rad	38	28.20 ± 0.92
Ultrasound - 60 minutes + 7000 rad	12	26.90 ± 1.50
Ultrasound - 30 minutes + 7000 rad	12	28.50 ± 1.40

*Mean Survival Time in days ± Standard Error.

About three weeks after tumor transplantation the animals showed signs of increasing dyspnea. Postmortem examination revealed that all animals had extensive pulmonary metastases, and many also had liver metastases. It was assumed that the animals died due to the pulmonary metastases that caused the dyspnea. Local tumor control was difficult to assess since at the time of death most animals had lost the tumor-bearing leg due to cannibalism.

Effect of Posttransplantation Treatment on Tumor Growth Rate

As before, all data from this series of experiments were pooled since no significant differences within the replicate groups of animals exposed to the same treatment were observed. The effect of the different treatment modalities on tumor growth rate is demonstrated in Figure 4. These data on growth represent the calculated mean increase in tumor diameter in mm per day for each group within a treatment.

Regression analysis revealed significant differences in growth rate for some of the groups treated. These results are shown in Table II. Ultrasound treatment for 60 minutes alone enhanced the tumor growth rate; that is, the mean increase in tumor diameter per day was significantly different from the mean increase of the control group at the $\alpha = 0.02$ level. X-irradiation with 7000 rad alone, as well as the combined treatments of ultrasound and X-irradiation, decreased tumor growth rate. The growth rates of these groups were significantly different from the growth rate of the control group at the $\alpha = 0.01$ level. The growth rate of the group treated with ultrasound for 30 minutes alone was not significantly different from that of the control group.

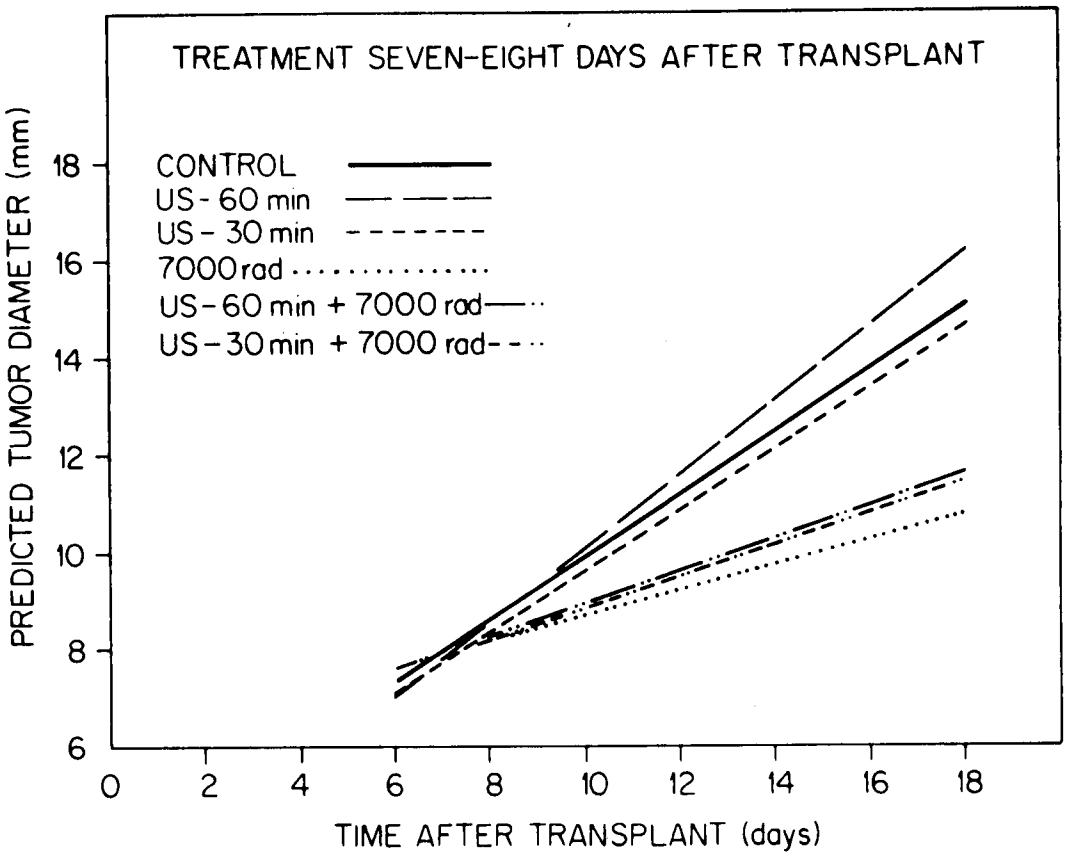

Figure 4. Effect of Posttransplantation Treatment on Tumor Growth Rate.

TABLE II

EFFECT OF POSTTRANSPLANTATION TREATMENT ON TUMOR GROWTH RATE

Treatment	Mean increase in tumor diameter in mm/day ± S.E.
Control	0.66 ± 0.03[a]
Ultrasound - 60 minutes	0.77 ± 0.07[a,b]
Ultrasound - 30 minutes	0.65 ± 0.07[a]
7000 rad	0.26 ± 0.06[b]
Ultrasound - 60 minutes + 7000 rad	0.33 ± 0.07[b]
Ultrasound - 30 minutes + 7000 rad	0.35 ± 0.07[b]

[a]Significantly different from 7000 rad at $\alpha \leq 0.02$ level.

[b]Significantly different from controls at $\alpha \leq 0.02$ level.

To examine a possible enhancement or diminution of X-irradiation effect by ultrasound, the rates of growth of all groups were compared to the growth rate of the group treated with 7000 rad. The growth rate of the control and both groups treated with ultrasound alone were significantly different. The combined ultrasound and X-irradiation treatments did not alter the effect of 7000 rad alone.

Effect of Pretransplantation Treatment on Mean Survival Time

The effect of pretransplantation treatment on MST was evaluated as previously described. The MSTs of all groups of animals were between 27 and 28 days, which indicated that there is no difference between

pre- and posttransplantation treatment on MST. Postmortem examination was performed on all animals and revealed extensive pulmonary metastases often invading the mediastinum and pleura. Most of the animals also had many large metastases in the liver. Presence or absence of tumor in the thigh could not be determined since most animals had lost the leg at the time of death.

Effect of Pretransplantation Treatment on Tumor Growth Rate

Results of the preliminary TBE study. As discussed in Chapter III, a preliminary study on the TBE was performed to determine the appropriate doses. The results of this study are shown in Figure 5. The growth curves represent the mean increase in tumor diameter in mm per day over the period of time in which tumor sizes increased linearly. The curves were obtained by calculating a linear regression using

$$\bar{Y} = mx + b$$

and clearly demonstrate the effect of pretransplantation treatments on the tumor growth rate. A TBE was demonstrated with a dose as low as 500 rad. Higher X-ray doses showed a greater effect; that is, tumor growth rates were further diminished with increasing dose as compared to the rate of tumor growth in the control group. However, the radiation doses of 1000 rad, 2000 rad and particularly a dose of 7000 rad, resulted in radiation reactions that caused the legs of the animals to be so sensitive to touch, that it was not possible to inject the tumor cells. Ultrasound exposure of 60 minutes caused severe edema, which lasted more than a week and therefore made evaluation of tumor growth

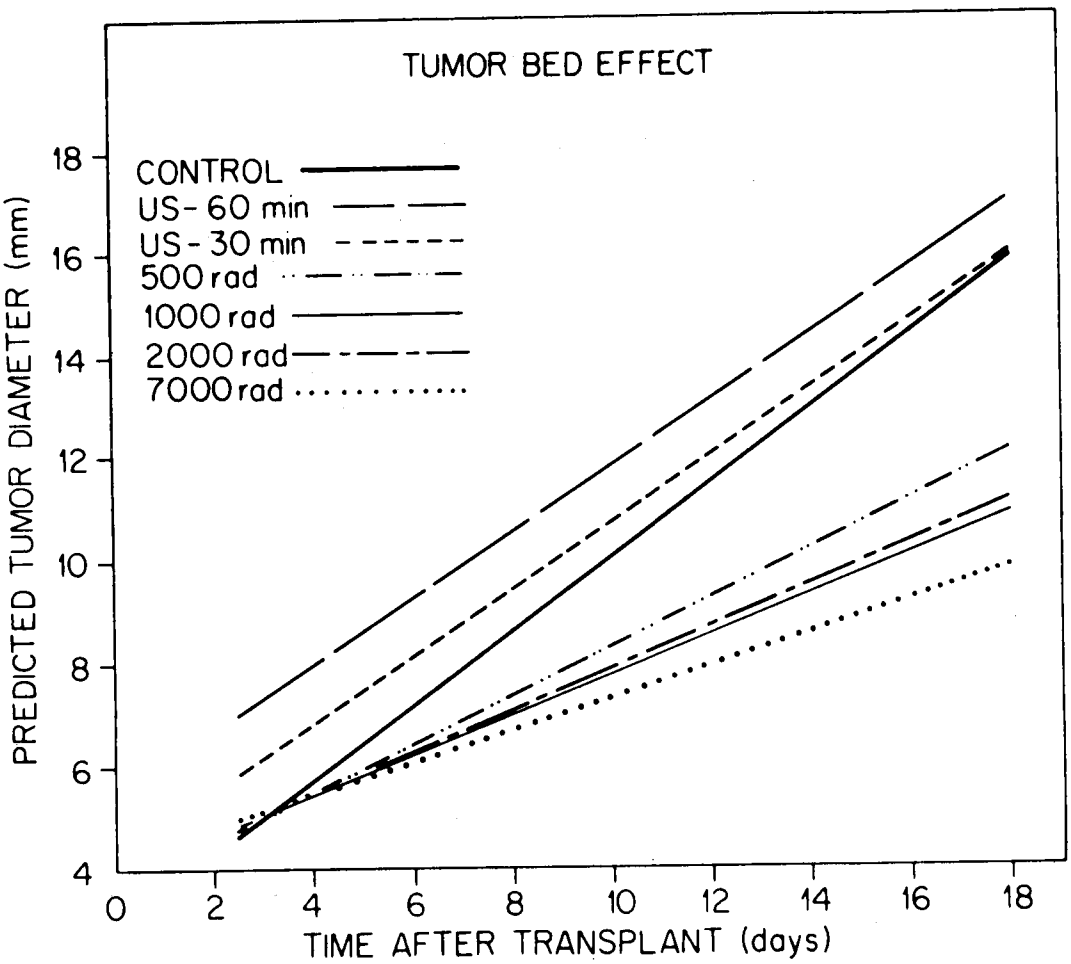

Figure 5. Effect of Pretransplantation Treatment of the Thigh with Increasing Ultrasound Exposure and X-irradiation Dose on Tumor Growth Rate.

rate difficult to assess. A 30-minute exposure to ultrasound still resulted in some edema, but this had mostly disappeared within 24 hours. The difference in the slopes of the lines for the group of animals treated with ultrasound for 30 minutes and the control group suggested that this treatment also has an inhibitory effect on tumor growth rate.

Since the purpose of the following experiments was to investigate a possible synergism between ultrasound and X-irradiation, it was felt that this could best be demonstrated by using 500 rad and ultrasound for 30 minutes, thus eliminating complications of edema and radiation reactions.

In all further TBE experiments, the effectiveness of the different treatment modalities on tumor growth rate was evaluated by a regression analysis using the general linear model as previously discussed. Since no differences were observed between the replicate groups of animals exposed to the same treatment on the same day before transplantation of the tumor, the data of each series of experiments were pooled.

Effect of treatment one day before transplant. When the treatment was given one day before the tumor transplant, the tumor growth rates for all groups of animals treated were retarded. As shown in Table III and Figure 6, the growth rate of all groups of animals treated was significantly different from the untreated group of animals at the $\alpha = 0.01$ level, clearly demonstrating an enhancement of the X-irradiation effect by ultrasound. The average size of the normal thigh was 4.5-5.0 mm, and tumor growth was not observable by measuring the diameter until after the fifth day following the transplantation of the tumor. Therefore, differences between the intercept of the curves for the

treated groups of animals as demonstrated in Figure 6 could be due to residual edema that was induced by the ultrasound treatment, or other factors that will be discussed later. To illustrate graphically that ultrasound enhanced the effect of X-irradiation, it was necessary to normalize the growth curves (Figure 6) (that is, the mean tumor diameter for each group was assumed to be equal to the mean tumor diameter of the control group on the fifth day after transplant).

TABLE III

EFFECT OF TUMOR BED TREATMENT ONE DAY BEFORE TRANSPLANTATION

Treatment	Mean increase in tumor diameter in mm/day ± S.E.
Control	0.86 ± 0.02^{a}
Ultrasound - 30 minutes	$0.70 \pm 0.05^{a,b}$
Ultrasound - 30 minutes + 500 rad	$0.43 \pm 0.05^{a,b}$
500 rad	0.64 ± 0.05^{b}

[a]Significantly different from 500 rad at $\alpha \leq 0.02$ level.

[b]Significantly different from controls at $\alpha \leq 0.02$ level.

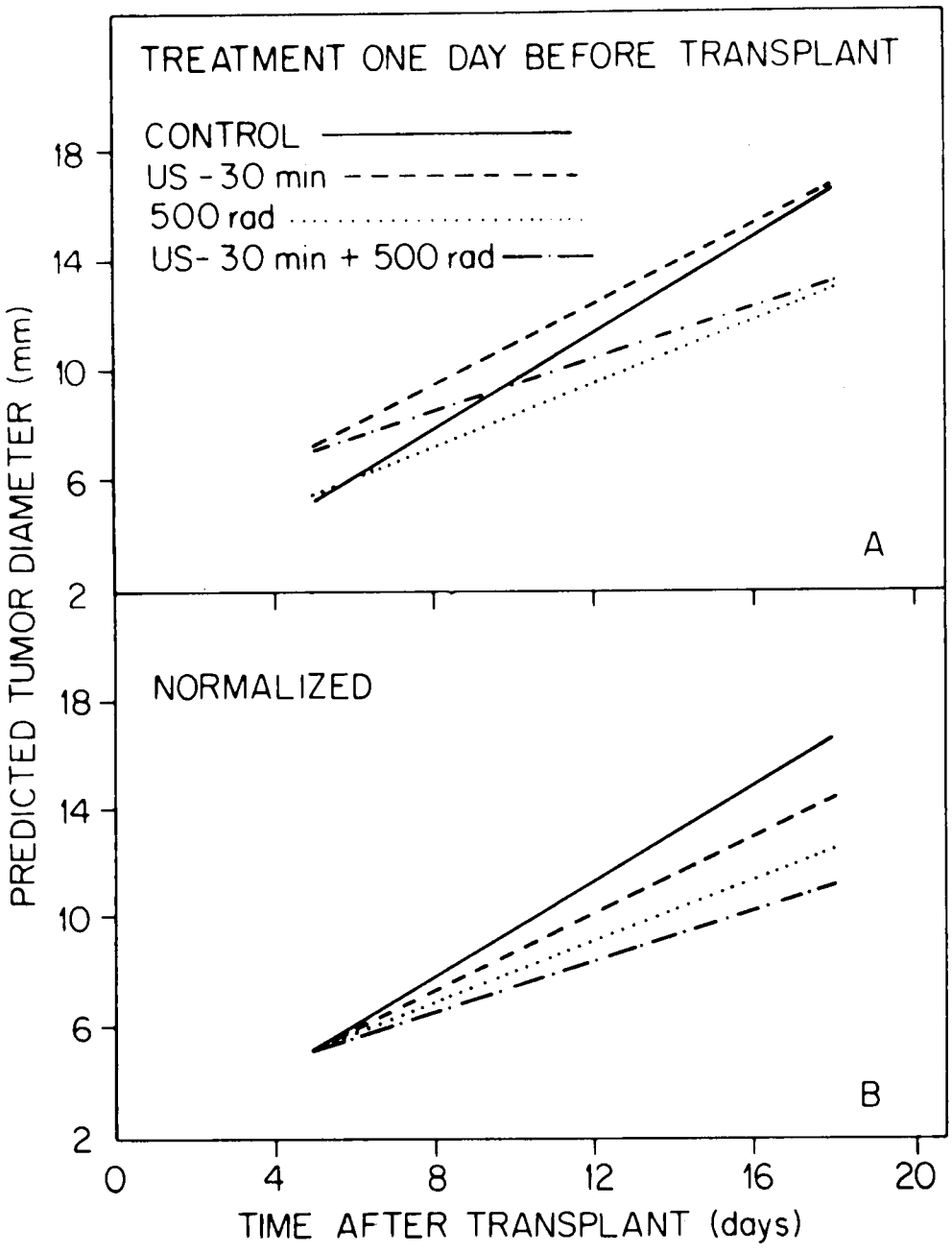

Figure 6. Growth Curves of Tumors Transplanted One Day After Treatment of the Tumor Bed with Ultrasound, Ultrasound Plus X-irradiation and X-irradiation Only.

Effect of treatment seven days before transplant. When the treatments were given seven days before tumor transplantation, the tumor growth rates were still decreased in all treated groups as shown in Table IV and Figure 7. Three experiments were conducted on a total of 18 mice per treatment group. The rate of growth for the animals treated by ultrasound only was still significantly different from that for the untreated group at the $\alpha = 0.02$ level. The growth rates for the groups treated with 500 rad and the combined ultrasound and radiation treatments were also still different from the control group at the $\alpha = 0.01$ significance level. As before, the growth curves were normalized to illustrate the difference.

TABLE IV

EFFECT OF TUMOR BED TREATMENT SEVEN DAYS BEFORE TRANSPLANTATION

Treatment	Mean increase in tumor diameter in mm/day ± S.E.
Control	0.90 ± 0.02[a]
Ultrasound - 30 minutes	0.80 ± 0.05[a,b]
Ultrasound - 30 minutes + 500 rad	0.61 ± 0.06[b]
500 rad	0.67 ± 0.06[b]

[a]Significantly different from 500 rad at $\alpha \leq 0.02$ level.

[b]Significantly different from controls at $\alpha \leq 0.02$ level.

A comparison between the growth rate of the tumors in the animals treated with the combined treatment and those of the animals treated

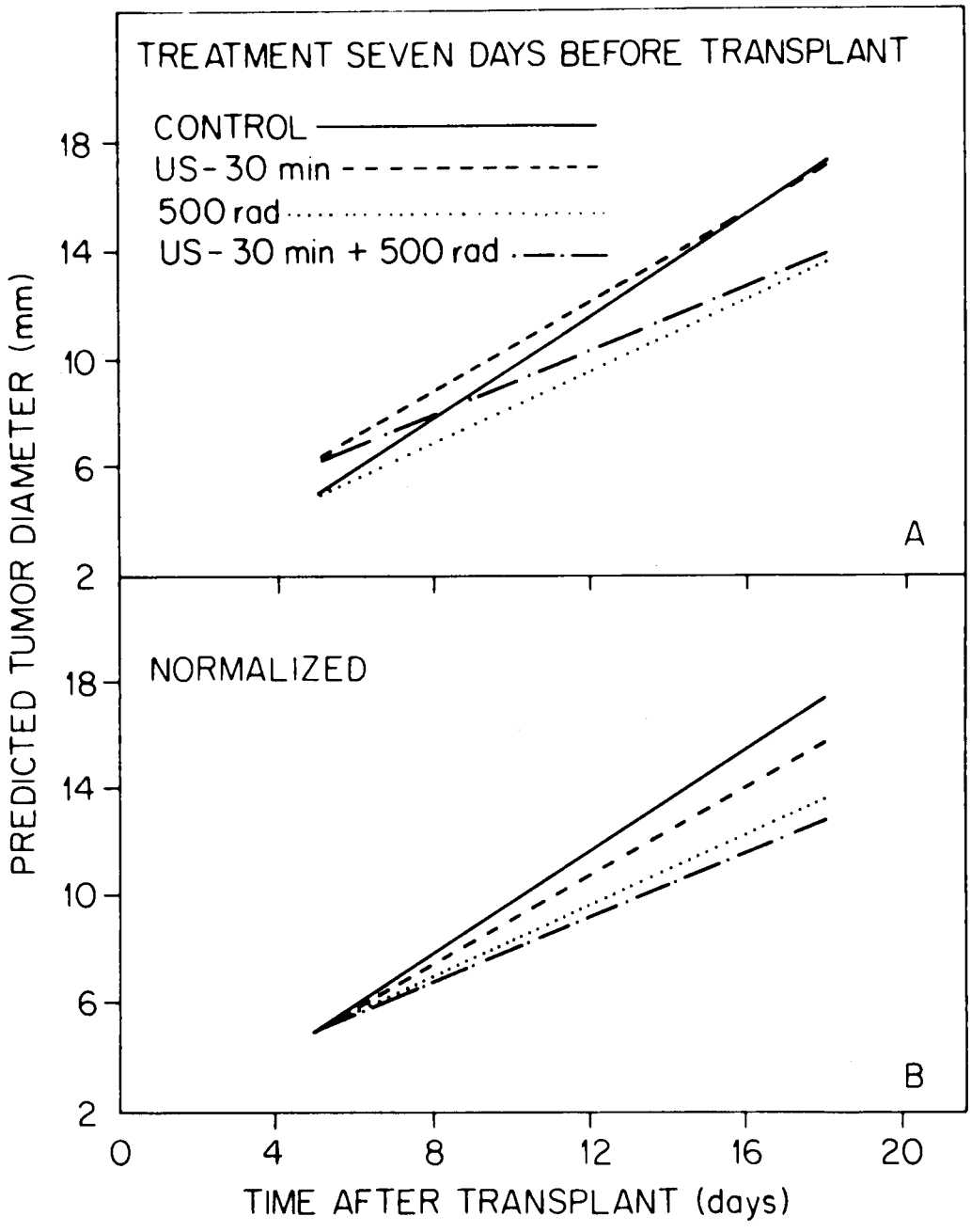

Figure 7. Growth Curves of Tumors Transplanted Seven Days After Treatment of the Tumor Bed.

with only 500 rad proved no longer significant. Although the differences between the intercepts of the growth curves for the groups treated with ultrasound and the control and X-irradiated groups appeared to become smaller, a significant difference was still observed as demonstrated in Figure 7. Those results indicate that after seven days ultrasound by itself was still effective, but did not enhance the effect of X-irradiation.

Effect of treatment 30 days before transplant. The persistence of the TBE was tested in one experiment with eight mice in each group. As shown in Table V and Figure 8, a TBE could still be demonstrated by 500 rad when animals were treated 30 days before tumor transplantation. Regression analysis revealed a significant difference only for the groups of animals treated with 500 rad and the combined treatments. Ultrasound exposure for 30 minutes had no effect on tumor growth rate. However, a TBE could be observed for the combined ultrasound and X-irradiation treatments. Differences between the intercepts of the curves for the treated groups and the intercept of the curve for the control group were no longer discernable as demonstrated in Figure 8. The slopes of the regression lines are equal for the ultrasound treated and the control group and for the X-irradiated group and the combined ultrasound and X-irradiated groups. These results indicate that for this treatment the TBE is solely due to the X-irradiation and that the effect of ultrasound is no longer observable.

TABLE V

EFFECT OF TUMOR BED TREATMENT 30 DAYS BEFORE TRANSPLANTATION

Treatment	Mean increase in tumor diameter in mm/day ± S.E.
Control	0.88 ± 0.02[a]
Ultrasound - 30 minutes	0.85 ± 0.05[a]
Ultrasound - 30 minutes + 500 rad	0.66 ± 0.05[b]
500 rad	0.67 ± 0.05[b]

[a]Significantly different from 500 rad at $\alpha \leq 0.02$ level.

[b]Significantly different from controls at $\alpha \leq 0.02$ level.

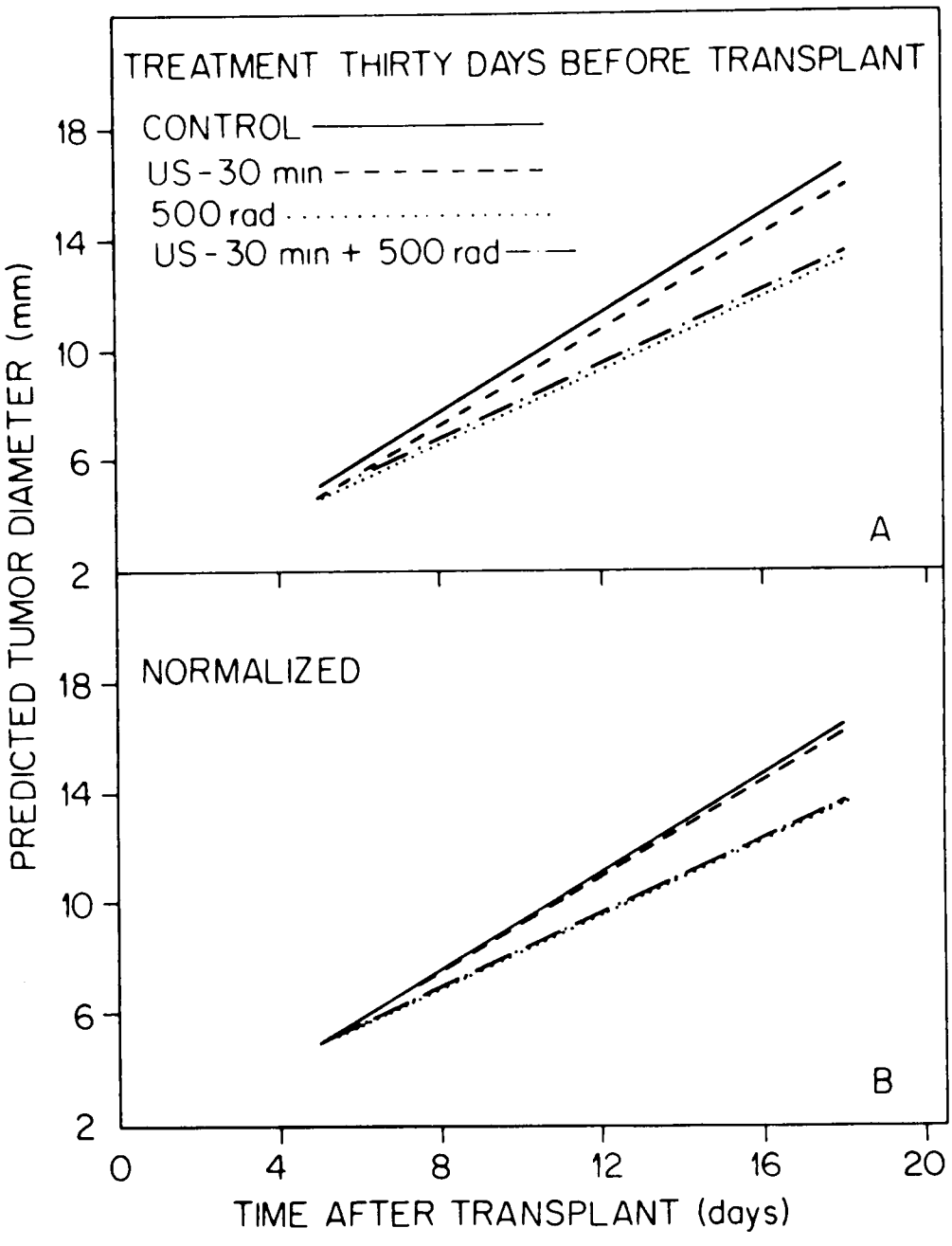

Figure 8. Growth Curves of Tumors When Treatment to the Tumor Bed was Given 30 Days Before Tumor Transplant.

CHAPTER V

DISCUSSION

Induction of Hyperthermia by Ultrasound

The results of this study have shown that ultrasound is an effective method for producing local hyperthermia in normal and in tumor-bearing thighs of mice. The temperature profiles were similar to those published by Marmor and co-workers (22). These investigators treated superficial human neoplasms with higher temperatures (43-45°C) than those used for this study (42.5°C). Marmor considered 43°C a satisfactory treatment temperature, but this could not always be achieved as many patients experienced significant pain during the ultrasound exposure. Most of these patients had tumors located near a bone, and the pain was probably caused by excessive heat created by the reflection of the ultrasound by the bone. The purpose of this study was to investigate a possible enhancement of the X-irradiation effect by ultrasound on tumor growth. Significant enhancement has been demonstrated using temperatures of 41.0-42.5°C (26; 27; 28). Therefore, a temperature of 42.5°C was considered sufficient for this study. The thermal distribution within the thigh tissue was almost uniform. Because of the high absorption coefficient of bone, the temperature at the surface of the femur was assumed to be at least equal to the temperature measured by the thermocouple inserted at the center of the thigh. Marmor's studies showed that heating was not uniform in most tumors. Frequencies of 1-3 MHz and transducers of 2 cm and 4 cm in diameter were used, and the incident power required to give the desired temperatures varied from

0.5-1.2 W/cm^2. The reason for these variations is that the conditions for the induction of hyperthermia vary with the requirements of the experimental system. That is, the radiofrequency, the intensity, and the size of the transducer used are dependent upon the type of tissue, anatomical location, and the volume of tissue as well as the desired temperature increase. Many of the variables were eliminated in this study by using a transplantable murine tumor. The tissue volume to be heated was constant, as were the size, shape, and position of bone within the volume. The proper frequency, intensity, and the temperature of the water bath used to obtain uniform heating within the volume of the thigh (approximately 1 cm^3 for tumor-bearing legs) were determined experimentally by trial and error. For the normal thighs the conditions for ultrasound heating were found to be the same except that the distance between transducer and the mouse leg was slightly increased. The skin temperature remained several degrees lower than the tumor temperature during ultrasound exposure. This is a major advantage in clinical radiotherapy when ultrasound heating is combined with X-irradiation to treat deep-seated tumors because the lower skin temperature increases the therapeutic ratio. Obviously, the trial-and-error method to determine the proper conditions for ultrasound heating cannot be applied to humans. Also the volume and shape of a mouse leg does not resemble any part of the human anatomy, and the heating conditions are probably not applicable. It is essential, therefore, that further studies be done using an experimental system in which conditions resembling those in clinical radiotherapy can be simulated. This could be done by using larger experimental animals --for example, pigs.

Effect of Treatment on MST

None of the treatment modalities had an effect on the mean survival time of the animals. Surprisingly, even treatment with 7000 rad at seven days after tumor transplantation had no effect, while Ullrich and Adams (65) previously found 37.5 percent of the animals to be tumor-free after 120 days. No cures were observed in this study. The different animal environments in the two studies may partly explain the different results. However, Ullrich and Adams later also observed no cures (personal communication). It is possible that after serial transplantation over several generations the line 1 lung carcinoma became more malignant and more radioresistant. Vermund and co-workers (51) found that the mammary carcinoma that had been transferred by serial transplantation over many generations became more resistant to posttransplantation irradiation than tumor cells transferred only a few times. The results of these studies indicate that the antigenicity of tumor cells may be altered after serial transplantation.

There was no difference between pre- and posttransplantation treatments on MST. Other investigators have found that pretransplantation X-irradiation increased the survival time of the hosts (41; 49; 50; 51; 52). Most of the tumors, however, were transplanted subcutaneously, while the line 1 lung carcinoma was transplanted into the thigh muscle. Differences in type of tissue and vascularization of the transplantation site may account for the different results obtained in this study. Because all the animals died from pulmonary metastases, it is clear that prior irradiation of the thigh did not prevent the tumor cells from entering the blood stream.

Effect of Posttransplantation Treatment on Tumor Growth Rate

Ultrasound treatment for 60 minutes significantly enhanced tumor growth rate, but treatment by ultrasound for 30 minutes did not show an effect. The apparent enhancement by the 60-minute treatment could be an artifact. This treatment was later found to produce severe edema that lasted more than a week in normal mouse legs, and it is probable that ultrasound for 60 minutes produced a long-lasting edema within the tumors resulting in the apparent increase in tumor growth. However, ultrasound for 30 minutes produced an edema which was no longer apparent after 24 hours.

In conclusion, ultrasound alone does not appear to have an effect on the growth rate of the line 1 lung carcinoma. That ultrasound alone does not inhibit tumor growth has been reported previously by Woeber (21) and Southam, Beyer, and Allen (29). As was expected, treatment with 7000 rad significantly decreased the growth rate of the tumor; however, ultrasound did not enhance the effect of the X-irradiation. These results are similar to those reported by Clarke and Hill (33). These investigators, however, reported intratumor temperatures of only 38.0-39.0°C. In vitro and in vivo studies have shown that, to obtain a significant enhancement of the effect of X-irradiation by hyperthermia, a temperature of at least 41.0°C is required (5). It is, therefore, not surprising that an enhancement by ultrasound was not observed by Clarke and Hill. In contrast, Woeber (21) found significant enhancement by ultrasound. Temperature measurements were not reported in Woeber's study, and it is possible that higher temperatures were induced. It is interesting to note that the tumor used in Woeber's study was curable by

radiation alone. Witcofski and Kremkau (34) observed enhancement of the effect of X-irradiation by ultrasound for the sarcoma-180, also a radiosensitive tumor. No enhancement was observed for the radioresistant C_3HBA mammary carcinoma, while water bath heating after X-irradiation was effective for both tumors. The line 1 lung carcinoma is very radioresistant and no enhancement of the effect of X-irradiation by ultrasound was observed. These findings may indicate that enhancement by ultrasound occurs with radiosensitive tumors but not with radioresistant tumors.

Effect of Pretransplantation Treatment on Tumor Growth Rate

The results from the TBE studies are important for clinical radiotherapy. Current cancer therapy includes pre- and postoperative treatment with radiotherapy. The rationale for preoperative irradiation is that, following radiation therapy, changes induced in the normal tissues surrounding the tumor reduce the survival of tumor cells spilled into the operative wound at subsequent surgery. The purpose of postoperative irradiation is to destroy any tumor cells that might have been left and to reduce the possibility for survival by irradiating the tumor bed, and thus avoid local recurrence. The value of pre- and postoperative irradiation has been the subject of controversy for many years. Limited clinical trials have shown that pre- and postoperative irradiation sometimes improved local tumor control; however, survival rates did not significantly increase (11, pp. 1109-1113; 14; 66; 67). Similarly, results from pretransplantation irradiation studies have shown that, although tumor growth was significantly delayed, dissemination of the tumor cells into the bloodstream was not prevented because all animals died of metastases. This indicates that X-irradiation

of the tumor bed suppresses rather than stimulates an immune response in the host as was previously suggested (58; 59).

In this study, pretransplantation irradiation with 500 rad significantly decreased the growth rate of the line 1 lung carcinoma. The effect persisted for at least 30 days. A TBE at 500 rad was also demonstrated with other tumors by Summers and Vermund (53) and Hewitt and Blake (54). However, Hewitt and Blake found no difference in magnitude of the TBE over the dose range of 1500-4000 rad, while Summers and Vermund found that inhibition of tumor growth increased linearly with increased dose to a maximum of 3000 rad. A linear relationship in growth inhibition could not be demonstrated for the line 1 lung carcinoma over the dose range of 500-2000 rad. The reason for the differences in the results of these studies is unknown.

Application of pretransplantation hyperthermia using hot air or hot water bath immersion have so far failed to demonstrate a TBE (58; 59; 60). In this study a significant TBE was demonstrated when a tissue temperature of 42.2°C was induced by ultrasound for 30 minutes. The TBE persisted for at least seven days, but for a treatment given 30 days before transplantation the effect was no longer observable. This study appears to be the first one in which a significant TBE has been observed after hyperthermia alone. Urano and Cunningham (60) were unable to demonstrate a TBE with a temperature of 43.5°C for 120 minutes. These investigators suggested that if hyperthermia induced a TBE, it is dose dependent and possibly exhibits a large threshold dose. However, the results obtained in this study have shown that a smaller temperature dose is capable of producing a hyperthermia TBE. The magnitude of a

threshold for the production of a TBE cannot be determined from the present study. It is possible that a different threshold dose exists for different methods of induction of hyperthermia. Experiments should be designed to determine and compare thresholds for the different methods of producing hyperthermia in a particular experimental animal system. No TBE could be detected after ultrasound-induced hyperthermia at 42.2°C for 60 minutes, and in the studies of Urano and Cunningham no TBE was observed after higher temperatures. The findings suggest that a TBE induced by hyperthermia does not exhibit a large threshold dose. It is possible that any TBE could go undetected at temperature doses that produce severe damage, edema and swelling.

It has also been shown in the present study that ultrasound significantly enhanced the TBE after 500 rad, but after seven days the TBE was no longer enhanced. Yerushalmi and Weinstein (59) also demonstrated enhancement of the X-irradiation effect on the tumor bed by hyperthermia, but the persistence of the effect was not tested. The data obtained from this study suggest that a different type of damage results from the ultrasound-induced hyperthermia and the X-irradiation. The damage due to the ultrasound treatment was completely repaired after 30 days, but not the damage by 500 rad. The initial response of the normal tissue to the ultrasound treatment might be an inflammatory response accompanied by edema that is observable for 24 hours. It is possible that some residual edema and inflammation were present much longer, which could explain the differences in the intercepts of the growth curves in Figure 6 (page 47) and Figure 7 (page 49). Inflammatory reactions to hyperthermia have not been reported. The response of normal tissues to moderate heat

(42.0-42.5°C) need to be further investigated, particularly when the method of heating is by ultrasound. Possible mechanical damage, such as rupture of cell membranes and the development of edema could be studied by the application of ultrasound-induced hyperthermia over a range of temperatures and for various durations of time. Changes in normal tissue could be evaluated by examining early and late responses using histological and, perhaps, biochemical techniques.

Yerushalmi (58) has also suggested that combined hyperthermia and X-irradiation treatments increase an immune response in the host. However, some observations were made during this study that indicate a possible suppression of an immune response in the host by the hyperthermia and the combined hyperthermia and X-irradiation treatments. The postmortem examinations showed that the groups of animals exposed to the different treatment modalities had larger and more metastases as compared to the animals not treated. Particularly the groups of animals treated with ultrasound alone and the groups treated with ultrasound and X-irradiation appeared to have more metastases in the liver. Obviously, much more information about the mechanisms involved in host-tumor interactions and the effect of various treatments on the local immune response is required. There may be a critical threshold dose that could trigger or suppress a local immune response for X-irradiation as well as for hyperthermia. Further studies on the TBE using transplantable animal tumors may provide such information. For example, experiments could be designed to determine the effects of pretransplantation treatments on the rate of dissemination of tumor cells. Using a quickly metastasizing tumor such as the line 1 lung carcinoma, the influence of

pretransplantation treatments could be studied by determining both number and size of lung and liver metastases in the animals at different times after tumor transplantation.

The major problem with the management of highly malignant tumors is how to prevent these tumors from metastasizing. X-irradiation of the tumor bed may stimulate and hyperthermia may increase an immune response in the host, but the response is apparently only local and temporary. Local hyperthermia in combination with X-irradiation has been used only in the treatment of advanced malignancies when all conventional treatments have failed (17; 18; 19). While the clinical reports are encouraging, they do not provide information regarding the effect of the treatments on host-tumor interactions. A better understanding of the host-tumor interactions is essential so that methods can be devised to prevent malignant tumors from metastasizing. This information can only be obtained with transplantable animal tumors.

Some superficial human cancers have been treated with ultrasound alone (22). The major problems were nonuniform heating of the tumor volume, blisters at the site of the thermocouple, and pain. Clearly, techniques of ultrasound heating should be properly developed and tested on animals before application to humans. Also, the effects on normal tissues of mild hyperthermia (42.2°C) induced by ultrasound are not understood and should be further investigated.

Conclusions

1. Ultrasound can be used effectively to induce constant and uniform heating in the thigh tissue of normal and tumor-bearing mouse legs.
2. Posttransplantation hyperthermia of 42.5°C induced by ultrasound had no effect on the growth rate of the line 1 lung carcinoma implanted into the thigh of Balb/c mice.
3. Posttransplantation X-irradiation of 7000 rad significantly reduced the growth rate of the tumor.
4. Ultrasound-induced hyperthermia did not alter the effect of X-irradiation on the tumor growth rate.
5. Pretransplantation treatment with 500 rad X-irradiation resulted in a significant TBE, which persisted for at least 30 days.
6. Pretransplantation treatment with ultrasound-induced hyperthermia (42.2°C) for 30 minutes resulted in a significant TBE, which persisted for seven days.
7. Ultrasound exposure for 30 minutes enhanced the TBE after 500 rad only for one day.
8. The results indicate that a different type of damage to the normal tissue is produced by the ultrasound and the X-irradiation.

LIST OF REFERENCES

LIST OF REFERENCES

1. Bruns, P. 1887. Die Heilwirkung des Erysipels auf Geschwülste. Beitrage Klin. Chir. 3:443-466.

2. Coley, W. B. 1893. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. Amer. J. Med. Sci. 105:48-511.

3. Warren, S. L. 1935. Preliminary study of the effects of artificial fever upon hopeless tumor cases. Amer. J.Roentgen., 33:75-87.

4. Har-Kedar, I. and N. M. Bleehen. 1976. Experimental and clinical aspects of hyperthermia applied to the treatment of cancer with special reference to the role of ultrasonic and microwave heating. In: Advances in Radiation Biology, 6:229-266, Academic Press, New York.

5. Thrall, D. E., L. E. Gerweck, E. L. Gillette, and W. C. Dewey. 1976. Response of cells in vitro and tissues in vivo to hyperthermia and X-irradiation. In: Advances in Radiation Biology, 6:211-228, Academic Press, New York.

6. Bronk, B. V. 1976. Thermal Potentiation of mammalian cell killing: Clues for understanding and potential for tumor therapy. In: Advances in Radiation Biology, 6:267-324, Academic Press, New York.

7. Hahn, G. M. 1978. Ultrasound for the induction of localized hyperthermia. Int. J. Radiation Oncol. Biol. Phys. 4:1117-1118.

8. Vermund, H. 1966. Experimental approaches to radiation therapy. In: The Biological Basis of Radiation Therapy, E. E. Schwartz Ed. pp. 518-572, J. B. Lippincott Company, Philadelphia.

9. Johns, H. E. 1966. The Physics of Radiology, 2nd edition, p. 681. Charles C. Thomas, Springfield, Ill.

10. Fisher, E. R. and B. Fisher. 1966. Host influence on tumor growth and dissemination. In: The Biological Basis of Radiation Therapy, pp. 484-508, E. E. Schwartz Ed., J. B. Lippincott Company, Philadelphia.

11. Ackerman, L. V. and J. A. del Regato. 1962. Cancer, 3rd Edition, pp. 38-39, 146, 1109-1113, The C. V. Mosby Company, St. Louis.

12. Rubin, P. and G. W. Caserett. 1968. Clinical Radiation Pathology, Vol. I, pp. 1-38 and Vol. II, pp. 894-913, W. B. Saunders Company, Philadelphia.

13. Fletcher, G. H. 1973. Textbook of Radiotherapy, 2nd Edition, pp. 166-169 , 470, Lea and Febiger, Philadelphia.

14. Scott, O. 1973. Pre-operative and post-operative irradiation Brit. Med. Bull. 29:59-62.

15. Andrews, J. R. 1978. The Radiobiology of Human Cancer Radiotherapy, p. 51-55, 206, 209, 323, University Park Press, Baltimore.

16. Hartman, J. J. and G. Crile. 1968. Heat treatment of osteogenic sarcoma. Clin. Orthop. and Related Res. 61:269-276.

17. Brenner, H. and A. Yerushalmi. 1975. Combined local hyperthermia and X-irradiation in the treatment of metastatic tumours. Brit. J. Cancer 33:91-95.

18. Kim, J. H., E. W. Hahn, N. Tokita and L. Z. Nisce. 1977. Local tumor hyperthermia in combination with radiation therapy. Cancer 40:161-169.

19. Hornback, N. B., R. E. Shupe, H. Shidnia, B. T. Joe, E. Sayoc, and C. Marshall. 1977. Preliminary clinical results of combined 433 megahertz microwave therapy and radiation therapy on patients with advanced cancer. Cancer 40:2854-2863.

20. Horvath, J. 1944. Ultraschallwirkung beim menschlichen Sarkom. Strahlentherapie 75:119-125.

21. Woeber, K. 1965. The effect of ultrasound in the treatment of cancer. In: Ultrasonic Energy, E. Kelly Ed., pp. 137-149, University of Illinois Press, Urbana.

22. Marmor, J. B., D. Pounds, T. B. Postic, and G. W. Hahn. 1979. Treatment of superficial human neoplasms by local hyperthermia induced by ultrasound. Cancer 43:188-197.

23. Crile, G., Jr. 1963. The effects of heat and radiation on cancers implanted on the feet of mice. Cancer Res. 23:372-380.

24. Overgaard, K. and J. Overgaard. 1972. Investigations on the possibility of a thermic tumor therapy: Shortwave treatment of a transplantable isologous mouse mammary carcinoma. Europ. J. Cancer 8:65-78.

25. Thrall, D. E., E. L. Gillette, and C. L. Bauman. 1973. Effect of heat on the C_3H mouse mammary adenocarcinoma evaluated in terms of tumor growth. Eurp. J. Cancer 9:871-875.

26. Robinson, J. E., M. J. Wizenberg, and W. A. McCready. 1974. Radiation and hyperthermal response of normal tissue in situ. Radiology 113:195-198.

27. Thrall, D. E., E. L. Gillette, and W. C. Dewey. 1975. Effect of heat and ionizing radiation on normal and neoplastic tissue of the C_3H mouse. Rad. Res. 63:363-377.

28. Gillette, E. L., and B. A. Ensley. 1979. Effect of heating order on radiation response of mouse tumor and skin. Int. J. Radiat. Oncol. Biol. Phys. 5:209-213.

29. Southam, C. M., H. Beyer and A. C. Allen. 1953. The effects of ultrasonic irradiation upon normal and neoplastic tissues in the intact mouse. Cancer 6:390-396.

30. Longo, F. W., P. Tomashefsky, B. D. Rivin, W. Longo, J. K. Lattimer and M. Tannenbaum. 1975. Interaction of ultrasound with neoplastic tissue. Local effect on subcutaneously implanted Furth-Columbia rat Wilms' tumor. Urology 6(5):631-634.

31. Marmor, J. B., C. Nager and G. W. Hahn. 1977. (Abstract) Tumor regression and immune recognitiion after localized ultrasound heating. Rad. Res. 70:633.

32. Marmor, J. B., D. Pounds, N. Hahn and G. W. Hahn. 1979. Treating spontaneous tumors in dogs and cats by ultrasound-induced hyperthermia. Int. J. Radiat. Oncol. Biol. Phys. 4:967-973.

33. Clarke, P. R., C. R. Hill and K. Adams. 1970. Synergism between ultrasound and X-rays in tumor therapy. Br. J. Radiol. 43:97-99.

34. Witcofski, R. L. and F. W. Kremkau. 1978. Ultrasonic enhancement of cancer radiotherapy. Radiology 127:793-797.

35. Dewey, W. C., L. E. Hopwood, S. A. Sapareto and L. E. Gerweck. 1977. Cellular responses to combinations of hyperthermia and radiation. Radiology 123:463-474.

36. Todd, P. and C. B. Schroy. 1974. X-ray inactivation of cultured mammalian cells: Enhancement by ultrasound. Radiology 113:445-447.

37. Martins, B. I., M. R. Raju, T. L. Hayes and C. A. Tobias. 1970. Survival of cultured mammalian cells exposed to ultrasound. Rad. Environ. Biophys. 14:243-250.

38. Moore, J. L. and W. T. Coakley. 1977. Ultrasonic treatment of Chinese hamster cells at high intensities and long exposure times. Br. J. Radiol. 50:46-50.

39. Harkányi, Z., J. Szollár, and Z. Vigvári. 1978. A search for an effect of ultrasound alone and in combination with X-rays on chromosomes in vivo. Brit. J. Rad. 51:46-49.

40. Wasserman, A. V. 1914. Analyse der Wirkung radioactiver Substanzen auf Maüsekrebs. Deut. Med. Wschr. 11:524-528.

41. Frankl, O., and C. P. Kimball. 1914. Ueber die Beinflussung von Maüsetumoren durch Röntgenstrahlen. Wien. Klin. Wschr. 27:1448-1450. 1914.

42. Murphy, J. B., Hussey, R. G., Nakahara, W., and Sturm, E. 1921. Studies on X-ray effects. VI Effect of the cellular reaction induced by X-rays on cancer grafts. J. Exper. Med. 33:299-313.

43. Nakahara, W., and J. B. Murphy. 1921. Studies on X-ray effects. VII Effects of small doses of X-rays of low penetration on the resistance of mice to transplanted cancer. J. Exper. Med. 33:429-432.

44. Nakahara, W. 1923. Studies on X-ray effects. XIII Histological study of the fate of cancer grafts inoculated into an X-rayed area. J. Exper. Med. 38:309-314. 1923.

45. Murphy, J. B., J. Maisin and E. Sturm. 1923. Local resistance to spontaneous mouse cancer induced by X-rays. J. Exper. Med. 38:645-653.

46. Russ, S. and G. M. Scott. 1940. The growth of tumour in tissues exposed to X-rays and radium. Br. J. Radiol. 32:289-295.

47. Russ, S. and G. M. Scott. 1940. The effect of X-rays upon tumour growth when the tumour itself is not irradiated. Br. J. Radiol. 13:267-272.

48. Lasnitsky, I. 1947. A quantitative analysis of the direct and indirect action of X-radiation on malignant cells. Brit. J. Radiol. 20:240-247.

49. Stenstrom, K. W., H. Vermund, D. G. Mosser, and J. F. Marvin. 1955. Effects of roentgen irradiation on the tumor bed. I. The inhibiting action of local pretransplantation roentgen irradiation (1500 r_a) on the growth of mouse mammary carcinoma. Rad. Res. 2:180-190.

50. Vermund, H., K. W. Stenstrom, D. G. Mosser, and E. A. Johnson. 1956. Effects of roentgen irradiation on the tumor bed. II. The inhibiting action of different dose levels of local pretransplantation roentgen irradiation on the growth of mouse mammary carcinoma. Rad. Res. 5:354-364.

51. Vermund, H., K. W. Stenstrom, D. G. Mosser, and M. K. Loken. 1958. Effects of roentgen irradiation on the tumor bed. III. The different inhibiting action on the growth of mouse mammary carcinoma resulting from pre- or posttransplantation irradiation. Rad. Res. 8:22-31.

52. Summers, W. C. and H. Vermund. 1963. Studies on the response of the tumor bed to irradiation (Abstract). Rad. Res. 19:240.

53. Summers, W. C. and H. Vermund. 1964. X-irradiation of the tumor Bed. I. A study of the indirect actions of radiation on transplantable tumors. Radiology 82:691-703.

54. Hewitt, H. B. and E. R. Blake. 1968. The growth of transplanted murine tumours in pre-irradiated sites. Br. J. Cancer 22:808-824.

55. Urano, M. and H. Suit. 1971. Experimental evaluations of tumor bed effect for C3H mouse mammary carcinoma and for C3H mouse fibrosarcoma. Rad. Res. 45:41-49.

56. O'Brien, P. H., C. Sweitzer, J. O. Sherman, and W. T. Moss. 1969. Tumor bed effect of ionizing irradiation in experimental cancer of the colon. Cancer 23:451-454.

57. Clifton, K. H. and R. Jirtle. 1975. Mammary carcinoma cell population growth in preirradiated and unirradiated transplant sites. Radiology 117:459-465.

58. Yerushalmi, A. 1978. Stimulation of resistance against local tumor growth of host pretreated by combined local hyperthermia and X-irradiation. Bull. Cancer (Paris) 65.4:475-478.

59. Yerushalmi, A. and Y. Weinstein. 1979. Stimulation of resistance to tumor growth of athymic nude mice pretreated by combined local hyperthermia and X-irradiation. Cancer Res. 39:1126-1128.

60. Urano, M. and M. Cunningham. 1980. Insignificant tumor bed effect after pretransplantation hyperthermia. Cancer Res. 40:26-28.

61. Yuhas, J. M., N. H. Parmino, J. O. Proctor, and R. E. Taya. 1974. A direct relationship between immune competence and the sub-cutaneous growth rate of a malignant murine tumor. Cancer Res. 34:722-728.

62. Draper, N. R. and H. Smith. 1966. Applied Regression Analysis, John Wiley and Sons, Inc., New York.

63. Searle, S. R. 1971. Linear Models, John Wiley and Sons, Inc., New York.

64. Helwig, J. T., K. A. Council. 1979. SAS User's Guide, 1979 Edition, SAS Institute Inc., Raleigh, N.C.

65. Ullrich, R. L. and L. M. Adams. 1978. The combined use of local irradiation and Corynebacterium parvum in the treatment of the murine line 1 lung carcinoma. Rad. Res. 73:267-273.

66. Paterson, R. 1963. The treatment of malignant desease by radiotherapy, Second ed., p. 312, The Williams α Wilkins Company, Baltimore.

67. Stearns, M. W., Jr., M. R. Deddish and S. H. Q. Quan. 1959. Preoperative reontgen therapy for cancer of the rectum. Surgery, Gynecology and Obstetricts 109:225-229.

VITA

Carlette Mathilde toe Water was born in Malang, Indonesia, on November 11, 1935.

She graduated from the Goois Lyceum in Bussum, Holland, in June 1953. She was certified by the Nederlandse Vereniging voor Radiologische Laboranten in Radiologic Technology in June 1959 and in Radiotherapy Technology in June 1960. She worked as a Radiotherapy Technologist in treatment planning, dosimetric research, and radiotherapy in Holland and England before coming to New York in 1965. She received a Bachelor of Science degree in Biology from Columbia University, New York, in May 1973.

Her working experience in the United States includes training of Radiologic Technologists and Radiotherapeutic Technologists, development of training programs, and teaching radiotherapy technique courses, physics, and biology.

She held a teaching assistantship for two years while at The University of Tennessee, Knoxville. She is apresently working as Senior Research Assistant at the Comparative Animal Research Laboratory in Oak Ridge, Tennessee.