A System for Developing Microwave-Induced Hyperthermia in Small Animals

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Abstract—**A system has been developed at the University of California, San Francisco, for producing microwave-induced hyperthermia in small laboratory animals by irradiating an entire transverse section of defined height, for the purpose of assessing the effects of heat alone and heat plus ionizing radiation on normal tissue and tumors. A thermometry system, consisting of a thermistor array, a low-voltage scanner, and a single channel recorder is described. Thermographic studies on sacrificed animals and thermistor studies in a phantom reveal a pattern of deep heating in a well-defined region, corroborated by thermistor measurements *in vivo*. Long-term animal survival at elevated temperatures ($\simeq 45°C$) throughout a large volume is not readily obtained. Seven out of 17 animals heated to 44.8°C throughout the entire abdominal region survived seven days post-heating, and histologic findings on this group of animals are briefly described. Various aspects of the biological techniques and the endpoints for current and planned experiments are discussed.**

I. Introduction

THE EARLIEST recorded attempt to use heat to cure tumors is found in the Edwin Smith Papyrus [1] which is believed to have been written about 3000 B.C. This involved the use of a so-called fire drill which was a smoldering stick of wood inserted in the tumor. More modern methods, i.e., the use of electrostatically generated heat, were suggested soon after Hertz [2] in 1886 first produced oscillating currents in a sustained manner. Within a few years, d'Arsonval, a physician–physiologist in France, suggested their use in medicine. deKeating–Hart applied a modified d'Arsonval circuit capable of producing a spark to destroy tumor tissue and demonstrated the procedure at the International Congress of Electrology at Milan in 1906. In the early 1900's, what we would now call long-wave diathermy was used to treat a variety of conditions. In about 1928, short-wave diathermy came into being and many exhorbitant claims were made concerning therapeutic benefits for a variety of conditions with numerous machines that were being marketed. Denier [3] in France (1939) used microwaves combined with X-rays against tumors (80-cm wavelength). By this time (1937), more than 750 articles and 18 books had been devoted to short-wave diathermy. Following the intense technological development of microwave generating devices during World War II, numerous reports [4], [5] on the ability of microwaves to heat living tissue appeared in the literature. Considerable activity in this area has been carried out more recently in the 1960's and 1970's in this country. The several reports of Crile [6], [7] predate the more recent quantitative *in vitro* cell culture studies, but these articles are intriguing for their provocative insights. Crile reported on systematic studies involving tumors in dogs and in patients, and determined some empirical dose–time relationships for the heat treatment. He observed that if the tumor cells of the transplant are heated previously *in vitro*, or heated *in vivo* and then transplanted to another animal, the tumor grows as if it had not been heated at all, even though subjected to a normally lethal dose of heat (determined empirically *in vivo*).

In 1971, Westra and Dewey [8] published a systematic, quantitative study of *in vitro* cell survival curves. It is not appropriate to review these data thoroughly here, but some general features of the results will be mentioned. When the logarithm of the surviving fraction is plotted against time of treatment, a family of curves (reflecting temperature dependency) is obtained, with higher temperatures producing a steeper slope. These curves are qualitatively similar to ionizing-radiation cell-survival curves with shoulder and an exponential portion and demonstrate the cells' capacity for sublethal repair, as the shoulder reappears in a fractionated treatment. From their data, they calculate an activation energy of 141 kcal/mole, consistent with heat denaturation of several proteins and enzymes, which they postulated. (Activation energies for heat-induced damage to DNA are much lower, of the order of 25 kcal/mole.) These studies involved an asynchronous population of cells. For synchronous cells, they found a cell-cycle dependence essentially the reverse of that for x irradiation. Palzer and Heidelberger [9] reported a seven-fold dependence upon cell cycle phase (HeLa cells), and that hyperthermia inhibited various processes of macromolecular synthesis.

The *in vivo* studies, particularly those involving the effects of heat on normal tissues, are less numerous. Robinson [10], in studies involving the enhancement effect of heat with ionizing radiation, reported the provocative

Manuscript received July 15, 1977; revised March 16, 1978. This work was supported in part by National Cancer Institute Research under Grant CA 17227.

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0018-9480/78/0800-0541$00.75

TABLE I
LIST OF CONVENTIONAL RECTANGULAR WAVEGUIDES INDICATING DIMENSIONS, OPERATING FREQUENCIES, CUTOFF FREQUENCIES, CUTOFF WAVELENGTHS AND DEPTHS OF PENETRATION FOR PLANE-WAVE IRRADIATION OF MUSCLE TISSUE

EIA Designation	Common Designator	Dimensions a(cm)	b(cm)	Recommended Operating Range (GHz)	Cut off frequency (GHz)	Cut off wavelength (cm in air)	Depth of Penetrating at λc (cm)*
WR-650	L	16.51	8.255	1.12 - 1.70	0.908	33.0	3.05
WR-430	LS.R	10.92	5.461	1.70 - 2.60	1.375	21.8	2.55
WR-340	-	8.636	4.318	2.20 - 3.30	1.686	17.3	2.30
WR-137	XN, C, G	3.48	1.58	5.85 - 8.20	4.301	7.0	1.00
WR-112		2.85	1.26	7.05 - 10.0	5.260	5.7	0.61

*Defined as depth at which E decreases by a factor of e^{-1} or power density decreases by a factor e^{-2}. Values interpolated from Table I of Johnson and Guy [17] and pertain to a plane-wave irradiation of a homogeneous muscle equivalent phantom or tissue.

result that tumors (C3H mouse mammary) were characterized by greater thermal enhancement ratios (TER's) than normal mouse skin, thus producing a favorable therapeutic ratio. In this report, the ratio of the TER for the tumor to that of normal mouse skin (therapeutic ratio) increased with increasing temperature to a value of 2.10 at 43.0°C.

Zimmer [11] reported the selective heating of tumors in small laboratory animals, and although the goal (drug uptake by a tumor in a hypothermic animal) was different from ours, it is an interesting technique in that he was able to maintain a 25°C differential between the tumor and whole-body temperature (body in deep hypothermia and tumor at normal temperature). Mendecki [12] has reported dramatic cure rates with all treated animals (mammary adenocarcinoma implanted in C3H mice) cured after two applications of microwave-induced local hyperthermia, without any other effects from the heat, while all nontreated controls died. Marmour, Hahn, and Hahn [13] reported similarly dramatic cures for the EMT-6 sarcoma tumor in mice and only somewhat more resistance to cure for the KHJJ carcinoma in Balb/Cka mice. They used radio-frequency heating (13.56 MHz) *in vivo*, and in an effort to explain the remarkable effectiveness of the techniques, performed studies of tumor-cell survival and concluded that direct cell killing by RF heating alone could not explain the dramatic cures. One possible mechanism suggested by them involves host immune response, and they point out that this postulate is consistent with the relatively higher thermal resistance to cure of the KHJJ, which is less immunogenic than EMT-6. Thus some of the much earlier results and suggestions of Crile are now being confirmed.

Robinson [14] describes a system for heating an extruded, transplanted tumor using 2450 MHz, which he describes as marginally effective (excessive temperature gradient in tumor), and consequently the free-field microwave heating was augmented using warm air. The tumors were 1 cm in diameter, and this result illustrates the inherent problem of depositing the microwave energy deeply in tissue.

The apparatus described in this report was designed to achieve deep heating, e.g., in the abdomen of a mouse, to assess thermal effects on normal tissue and a variety of tumors. It appears that the immune response may play a pivotal role, and it was desired to be able to assess effects at various sites without being restricted to extruded and transplanted tumors.

II. METHOD

For deep, noninvasive heating of living organisms, ultrasonic and electromagnetic techniques suggest themselves. The modality of heating using microwaves was chosen for this study, primarily because 1) this modality will be employed extensively in clinical trials (currently underway) and the expertise acquired will be valuable and 2) there is a considerably more extensive body of literature concerning this technology, as compared to ultrasonic heating of living organisms.

Table I gives an abbreviated list of conventional rectangular waveguides available, and a glance at this table reveals an inherent dilemma: to choose a frequency providing good depth of penetration results in too large an applicator if it is to be air filled.

Thus numerous reports in the literature (e.g., Guy [15], [16]) employ dielectric loading, allowing the designer greater range of applicator sizes, while still achieving good depth of penetration. A system using this principle, while attempting to provide maximum flexibility in the timing of microwave heating and x irradiation, has been described elsewhere [18].

For our specific biological application, it was felt that the endpoints in the first phase of the experiments had been chosen such that it was desirable to heat an entire transverse section of the experimental animal (mouse). Difficulties were envisioned with the contact applicator approach, e.g., lack of reproducibility due to variations in positioning, curvature, air gaps, etc. These considerations gave rise to the applicator described in this report.

Typically, an investigator chooses an ISM frequency, e.g., 915 or 2450 MHz, and then attempts to optimize an applicator for that frequency. In these animal studies, an alternate approach was chosen, that is, a general-purpose laboratory quality RF power supply with broad frequency range capability was selected, and then an applicator was designed to achieve the desired goal, i.e., that of heating

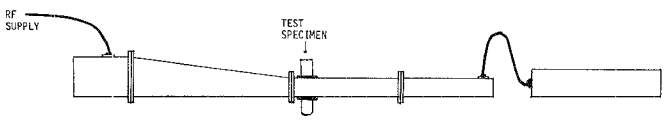

Fig. 1. Lateral view of microwave applicator system. Waveguide components, left to right, are a conventional WR-650 coax-to-waveguide adapter, an electrically compensated one dimensional transition section, tapering to a reduced height test section, a reduced height coax-to-waveguide adapter, followed by a coaxial dummy load.

the entire transverse section of a mouse. Then the applicator need not be optimized for that frequency but, rather, the generator can simply be tuned by the turn of a dial to achieve optimum results with that applicator.

The unit selected is a model 15022 RF power supply (MCL, Inc., La Grange, IL), which can excite any one of six available planar triode cavity-oscillator plug-in units, covering a total frequency range from 10 to 2500 MHz. Power available depends upon the frequency ranging from 30 to 90 W and is adequate at 915 MHz for application to human patients. Two cavity-oscillator plug-in units are on hand, models 6050 and 6051, providing a continuous frequency range from 500 to 2000 MHz at the turn of a dial.

The basic philosophy underlying the design of our applicator can be stated simply. In the TE_{10} mode, the a dimension (width) determines the cutoff frequency, and the b dimension (height) is irrelevant. Thus a relatively large waveguide (WR-650) is used allowing use of a low frequency to ensure good depth of penetration, but of reduced height to limit the region of heating. The mouse, in a plastic test tube, is inserted in a 2.5-cm diameter hole centered in the broad face of the waveguide which, due to its small diameter, is nonradiating (see Fig. 1).

To test the viability of this approach, a prototype (using WR-430 components) of the system shown in Fig. 1 was constructed in our laboratory. Thermographic studies were performed on a sectioned mouse (cleaved along the saggital plane after freezing in liquid nitrogen). These two halves were then reunited and heated (from room temperature) in the applicator. A clinical thermographic camera was previously set up by focusing on a beaker of water at 43°C in a 23°C ambient background. The mouse was removed, situated in place of the beaker, and photographed, as rapidly as possible to preclude substantial thermal diffusion. These thermograms consistently revealed not only deep heating, but preferential heating in the center of the animal, and qualitatively, a reasonable degree of symmetry in the heat deposition pattern. Subsequently, custom components were fabricated compatible with a WR-650 coax-to-waveguide adapter by Microwave Techniques, Inc., Raymond, ME.

III. Thermometry and Recording System

Thermistors encapsulated in a Teflon sheath (0.61 mm) were chosen for the temperature monitoring ($\alpha = 4$ percent/°C). Unfortunately, in this small diameter clinical series of probes, the thermistors are not specified as being

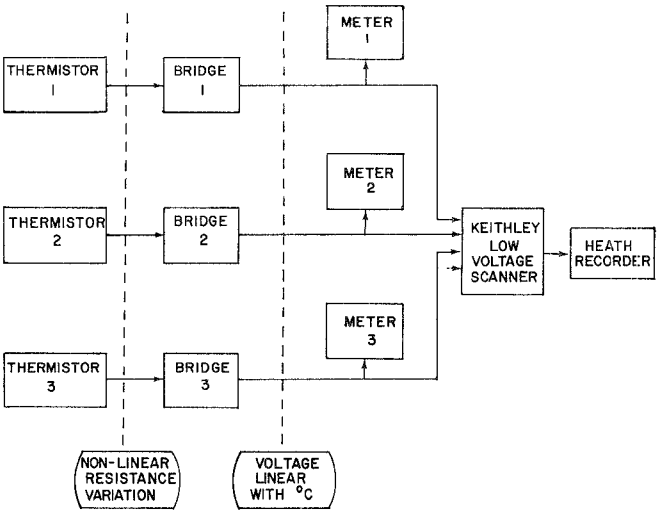

Fig. 2. Thermometry and recording system, consisting of thermistor array, individually calibrated linear-output deflection bridges, digital meters, scanner, and chart recorder.

interchangeable, which presents a problem when using an array of thermistors in a scanning mode, such as in our application. Initially, a Keithley low-voltage scanner was used to sequentially connect each thermistor to a linearizing bridge circuit and display (Yellow Springs Instrument Company) which provided an output signal for a Heath multirange potentiometric amplifier and single channel 1-V chart recorder. The thermistors exhibited high sensitivity with relatively small artifacts due to RF interference. For example, with 20 W of forward power at 1210 MHz, the indicated temperature was approximately 0.35°C higher than with no RF power. Some failures (discontinuities) of the thermistor occurred. The main drawback with this instrumentation was the inconvenience of using nonmatching thermistors with a single readout. This deficiency has now been corrected by using individually calibrated deflection bridge circuits for each thermistor which give linear outputs of 0.01 V/°C over a limited temperature range. This arrangement is shown schematically in Fig. 2.

A thermocouple system (Bailey Scientific Company) with probes comparable to the above in physical dimensions (clinical series) was also investigated. Convenience is assured by electronic compensation (no temperature reference junction required), and interchangeability of probes to within 0.1°C is specified. RF interference was a problem, and although the manufacturer claims that at 2450 MHz this can be eliminated by shielding and filtering the signal, we found that at 1210 MHz this effect could not be eliminated by these techniques. For example, with 20 W of forward power, the indicated temperature was approximately 12°C higher than with no RF power.

IV. Applicator and Results

An applicator was fabricated and is shown in Fig. 1. It consists of a conventional WR-650 coax-to-waveguide adapter, followed by the electrically compensated transi-

tion section (tapered in the b dimension only) to adapt the larger component to the reduced height waveguide test section. The remaining waveguide component is a reduced height coax-to-waveguide adapter, enabling the energy to be absorbed by and dissipated in the dummy load. Three test sections are on hand, with the b dimensions 3.18, 2.54, and 1.91 cm. These may be adapted to the two contiguous components by quarter-wave transformer sections. Measurements indicate that with the test port empty, >99 percent of the energy delivered to the WR-650 component is delivered to the dummy load. With a mouse in the test port, it is found that about 20–50 percent of the energy incident upon the mouse is reflected back to the generator and up to 20 percent of the incident energy reaches the dummy load (dependent upon the choice of many parameters, such as frequency, etc.), with the remainder being absorbed by the mouse.

To test how effectively the system limits the microwave heating to the region defined by the interior of the waveguide, the data shown in Fig. 3 were obtained, using thermistors in the center of the waveguide, and in the center of the "guard rings." Fig. 4 shows a preliminary experimental run with the three thermistors placed as indicated in an Agar and NaCl phantom. (This simplified phantom material will not adequately simulate the loss tangent of tissue, but it was felt it would be suitable to gain an idea of the relative heat distribution pattern. These results are still under investigation, but appear qualitatively similar to those obtained using thermographic techniques in a sacrificed animal which showed preferential heating in the center.) To test the validity of thermistor readings in the microwave field, comparisons of the thermistor with an alcohol thermometer, both in the phantom in the interior of the waveguide, were made.

The frequency of 1210 MHz is somewhat arbitrary, but was chosen on the basis of measurements that indicated maximum energy absorption in the test animal at this frequency. Higher frequencies result in altered thermal-distribution patterns (less symmetrical, with higher temperatures nearer the generator). For use at 915 MHz, larger components would be required.

Biological results to date are still preliminary, and the techniques are currently under review. In an initial trial, 15 mice were anesthetized with 70–90 mg/kg of Diabutol (a barbiturate anesthetic) and heated to 41.2°C for 25 min or 44.8°C for 15 min at 1210 MHz (4 to 6 W), using the 1.91-cm high microwave section. Temperature in the heated region was monitored by a thermistor intubated via the esophagus and centrally located in the microwave field. Another thermistor was positioned 1.5 cm into the rectum to monitor whole-body temperature outside the field. Typical results are shown in Fig. 5. Long-term animal survival was not obtained in this trial. There was an indication that LAF mice tolerated the heating procedure better than BALB/C mice which showed poorer survival times.

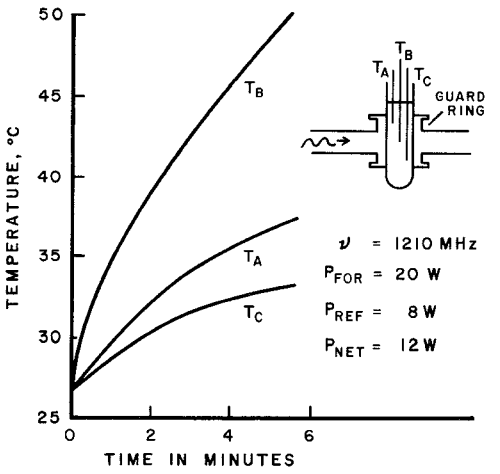

Fig. 3. Heating developed in thermistors located 0.6 cm outside of the waveguide (T_A, T_C) compared to thermistor located at center of waveguide (T_B).

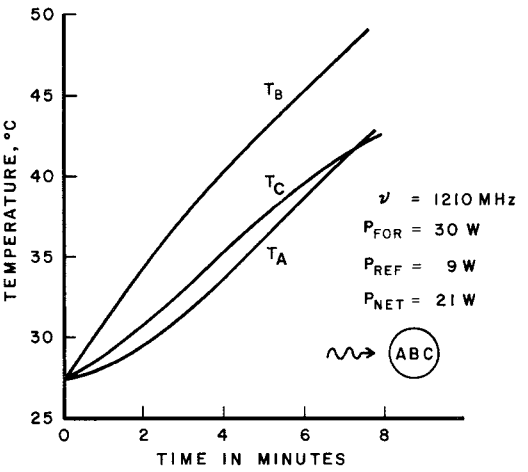

Fig. 4. Thermistor measurements at positions A, B, C, showing slightly preferential heating rate at the center of simulated mouse phantom (consisting of 0.90 percent NaCl and 1.25 percent Agar in water, about 50 ml total phantom size).

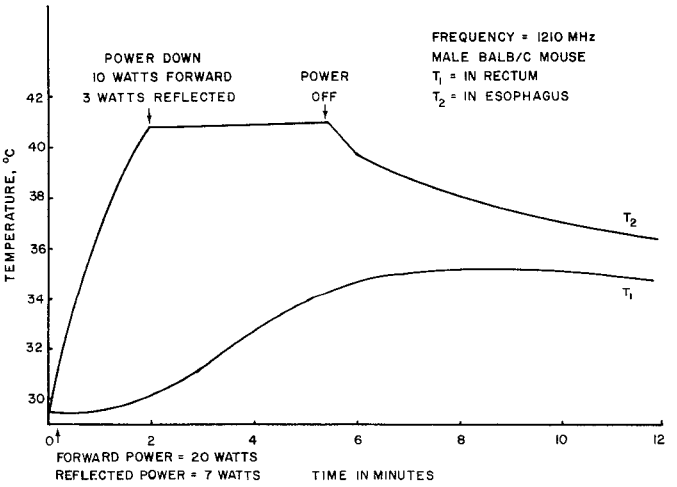

Fig. 5. Thermistor measurements in a live mouse, with T_1 located in the rectum (outside of the microwave field, monitoring whole body temperature) and T_2 in the esophagus (lungs exposed to the microwave field).

In a second trial experiment, the anesthetic dose was reduced to 60 mg/kg. Diabutol and two thermistors were used to monitor temperature in the microwave field. One thermistor was inserted 2.5 cm into the rectum locating it at mid-abdomen, while the second thermistor was implanted subcutaneously in the ventral mid-abdomen using a trocar which was subsequently withdrawn. Both thermistors were placed at mid-height in the waveguide.

Uniform heating (within 0.25°C) resulted when the test tube containing the mouse was rotated 180° at four times per minute during the heating period. Seven out of 17 animals heated to 44.8°C, as indicated by the subcutaneous thermistor, for 5 or 10 min survived to 7 days post-heating.

Histological data taken after three days from animals heated to 44.8°C for 15 min exhibited damage of the crypt cells and villi of the jejunum only. The kidney, lung, liver, and spleen were all normal. Gross examination of animals one hour after heating revealed edematous swelling of the heated portion of the thorax, and erythemateous appearance of the gastrointestine, especially the distal portion. Because of the possibility of a heat differential between different parts of the intestine dependent upon food and moisture content, animals were deprived of food for 48 hours and water for 24 hours, but showed no significant difference in survival after heating. The level of anesthesia, which affects respiration rate and heat loss, is also important.

Diabutol has a long-lived effect and is difficult to control at small dosages. A test of the efficacy of interperitoneal injection of saline (to dilute the barbiturate) immediately post-heating; or of injecting 10 mg/kg of Dopran (a cardiac stimulant) to improve survival, was inconclusive. Numerous other anesthetics are currently being evaluated.

On the basis of these preliminary studies with this method of producing hyperthermia in mice, we feel it is possible to assess cellular and tissue damage in tumor and normal tissue. Future experiments being considered are concomitant heat and ionizing radiation treatment *in vivo* of EMT-6 mammary carcinoma, grown on the flank and assayed for cell survival in tissue culture, LD-50 determinations (dose required to produce death in 50 percent of sample animals) using irradiation of normal tissues (i.e., jejunum, kidney, esophagus, bone marrow, and lung), and combination drug-hyperthermia-ionizing radiation treatment of these tumor and normal tissues.

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