Int. J. Heat Mass Transfer. Vol. 25, No. 9, pp. 1313–1320, 1982
Printed in Great Britain

0017–9310/82/091313–08 $03.00/0
Pergamon Press Ltd.

A UNIFORM THERMAL FIELD IN A HYPERTHERMIA CHAMBER FOR MICROVASCULAR STUDIES

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(*Received* 2 *April* 1982)

Abstract—Hyperthermia has been the subject of much research recently as a cancer treatment. Its greatest potential lies in its combination with radiotherapy and/or chemotherapy. Elevated temperatures have been shown to alter both tumor and normal tissue microcirculation. Changes in tissue perfusion could affect drug distribution and radiosensitivity via changes in hypoxic fraction. A thermal device using water as the heating fluid has been designed for the Arizona transparent access chamber to permit measurement of single vessel behavior *in vivo* at controlled temperatures. The device is made of lexan and encloses the chamber permitting fluid contact with the external window surface. The tissue temperature between the chamber's windows can be adjusted to within 0.1°C and the temperature over the surface of the tissue is uniform to ±0.1°C. A bypass for the heating fluid permits rapid changes in tissue temperature so that simultations of clinical hyperthermia treatments can be done. A numerical simulation model correctly predicts the temperature distributions in the chamber and the design is shown to provide rapid heating of the window chamber to the desired temperature.

NOMENCLATURE

- h, heat transfer coefficient;
- k, thermal conductivity;
- r, radial distance;
- t, temperature;
- z, axial distance.

INTRODUCTION

IT HAS been shown that there are significant differences in the biological effect of elevated temperatures on cells *in vitro* for temperatures varying by as little as ±0.1°C [1]. It has been possible to detect these differences because methods of heating cells *in vitro* can be temperature-controlled very precisely and accurately. However, it is usually not possible to heat tissues *in vivo* as uniformly. In rodents, for example, typical water bath heating of leg tumors can yield gradients as large as ±0.5°C or more within the tumor volume. Refinements such as the C-clip heating method [2] have effectively reduced the gradients to ±0.1°C with no detectable change in blood flow from the placement of the clip. In general, however, it is not known whether variations of the order of ±0.1°C yield significantly different biological effects *in vivo*. And, therefore, it is important to provide the basis for such experiments. We have been interested in studying the effect of hyperthermia on tumor and normal tissue microcirculation by using a transparent access chamber preparation. The purpose of the present paper is to report on the design of a system that can heat the preparation uniformly so that the observed biological effects can be more closely related to temperature. The design requirement for the chamber required a maximum tissue temperature range of ±0.1°C across the chamber for average tissue temperatures in the range of 38–48°C. The heating apparatus had to be optically clear so that it could be mounted on a microscope stage to permit video measurements of single vessel red-cell velocity and intraluminal diameter during the actual heating procedure. The following describes the system which was developed for this purpose. The design can easily be adapted for other similar window preparations.

BACKGROUND

Hyperthermia alone or in combination with radiation may become a useful tool for the treatment of cancer. The rationale for its use lies in some well-documented phenomena:

(1) Temperatures in the range of 42–48°C are cytotoxic, especially to chronically hypoxic cells at low pH [1, 3].

(2) Exposure to elevated temperatures reduces the relative radioresistance of hypoxic–acidotic cells and sensitizes all cells to radiation lethality [1].

(3) Elevated temperatures may result in preferential killing in tumors because of selective destruction of tumor microvasculature [4, 5].

Transparent chambers have been used successfully for long-term microscopic studies of tissue and circulation in the rabbit ear, the hamster cheek pouch and the mouse and rat dorsal skin folds. A review of the development of the transparent chamber for the rabbit ear starting with the early design [6, 7] has recently been given [8]. Sander and Shubick [9] and Goodall *et al.* [10] have also designed transparent chambers for the hamster cheek pouch and these were focused on studies of vascular patterns in living tumors. Algire [11, 12] adapted the early design to the mouse dorsal skin

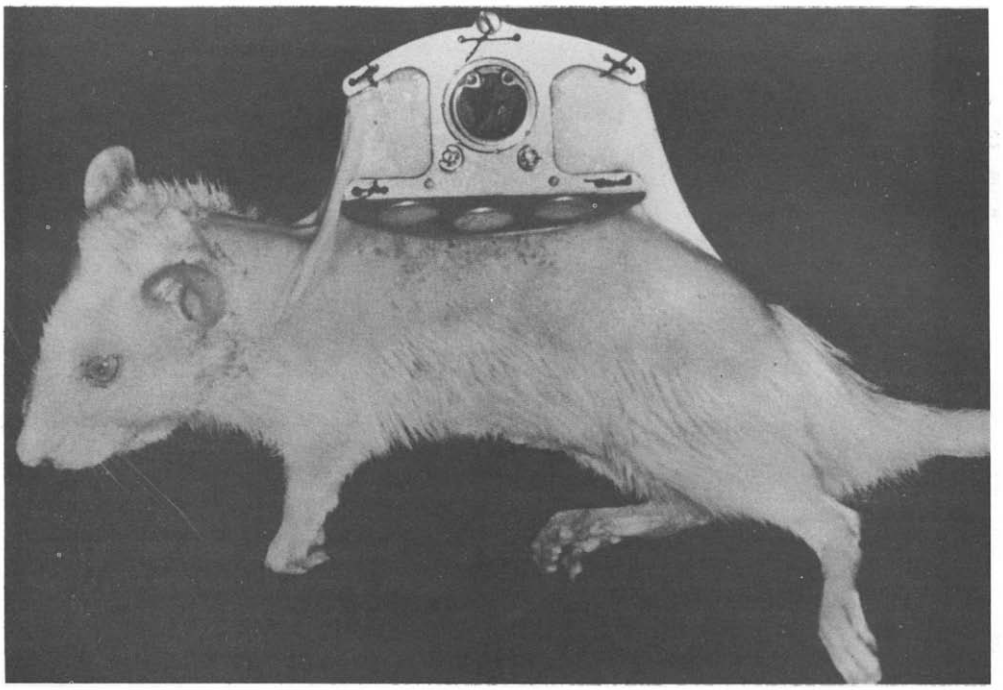

Fig. 1. The transparent access Arizona window chamber

fold and Yamaura *et al.* [13, 14] modified the mouse chamber and adapted it to the skin folds of rats for the purpose of studying cancer metastasis. Finally, a new chamber design to observe the microcirculation of tumor growth *in vivo* over extended periods was developed by Gross and co-workers (Fig. 1). The chamber was constructed of aluminum (Alloy 6061-T6 Bare Spec. QQ-A-3276 T) and contained a removable window to permit invasive measurements of intraluminal hydrostatic pressure and fluid transport. The details of the chamber design are given in Papenfuss *et al.* [15].

DESIGN OF HYPERTHERMIA CHAMBER

The design of the Arizona hyperthermia chamber (AHC) was carried out to accommodate the basic requirements of the experimental procedures. The heating system must regulate the temperature level of the current transparent access chamber, i.e. the Arizona Window Chamber (AWC), and provide for access to the tissue by experimental probes or micromanipulators. A major requirement for such experiments is the precise control of the temperature of the tissue in the chamber. This is particularly important because the effects of hyperthermia are quite nonlinear and it has been observed that the most significant vascular effects can occur in a rather restricted and critical temperature interval. In order to record these changes quantitatively, a uniform, controllable temperature distribution must be achieved in the tissue. Therefore, the temperature must be predictable to $\pm 0.1^{\circ}$C and maintained for periods up to 1 h. To avoid physiological complications, it is also necessary that the heated region be limited to the tumor and surrounding tissue, and not the areas adjacent to the chamber.

The equipment used in this system is shown in Fig. 2. The water for the thermal chamber is provided by a thermal calibration bath which can regulate the temperature to $\pm 0.05^{\circ}$C. The heated water is pumped to the thermal device through a system of insulated tubes which are so arranged with a bypass that the flow in the thermal device can be switched on when the water has reached the required temperature. The thermal chamber then achieves that temperature in about 10 min.

The thermal chamber itself is shown in Figs. 3 and 4. It consists of two hollow sections through which the heated water flows. The sections are constructed of lexan which is transparent yet has a low thermal conductivity, and they are designed so that the AWC can be placed between the two halves. The upper section has an open area which permits direct visualization of the tissue or access to it by probes or micromanipulators. Both sections were designed to be compact in order to allow the complete assembly to be readily mounted on a microscope stage. The chamber is attached to the thermal device through O-rings which prevent heating water from filtering into the chamber itself and contacting the tissue directly. The only portions of the AWC in direct contact with the flowing water are the glass window surfaces and the adjacent aluminum ring. The flow is regulated by

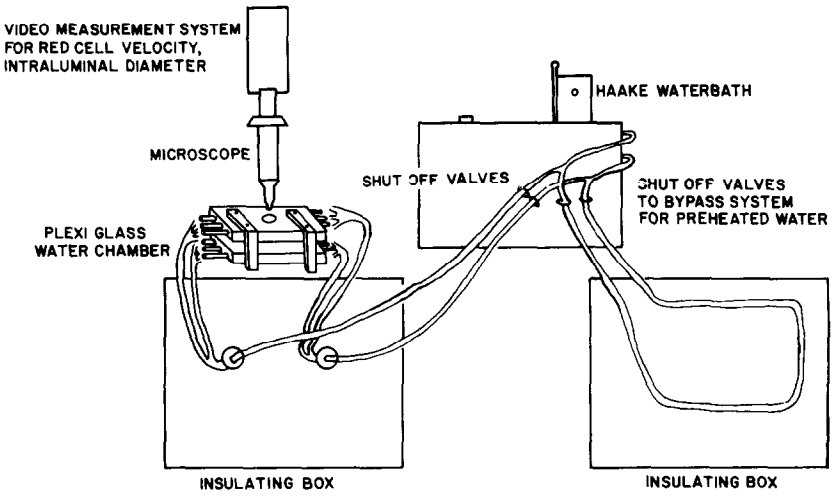

FIG. 2. The water bath system for the hyperthermia chamber.

adjustable clips in order to provide a convective velocity which gives a constant temperature on the window surfaces. This goal was also achieved by providing manifolds at the inlets and outlets of the upper and lower sections. This was done to give a uniform velocity across the window surface, and prevent the development of large recirculating eddies inside the sections. It will be shown later that the tissue temperatures across the window surface can be regulated to within $\pm 0.1°C$.

EXPERIMENTAL PROCEDURE

The animals with chamber preparations were anesthetized with pentobarbitol sodium (40 mg kg^{-1}). The heating chambers were fitted onto both sides of the window preparation as shown in Fig. 5. The rat and waterbath were mounted on the microscope stage. The rats were wrapped in a sheet of thin styrofoam to minimize heat losses. All water lines leading to and from the heating chamber were insulated.

Intrachamber temperature measurements were made by inserting calibrated 20 gauge needle thermistor probes (Yellow Springs Instruments) within the preparation [16]. The thermistors are calibrated to an accuracy of $\pm 0.01°C$. Measurements were taken in the 3, 6, 9 and 12 o'clock and center positions. In addition,

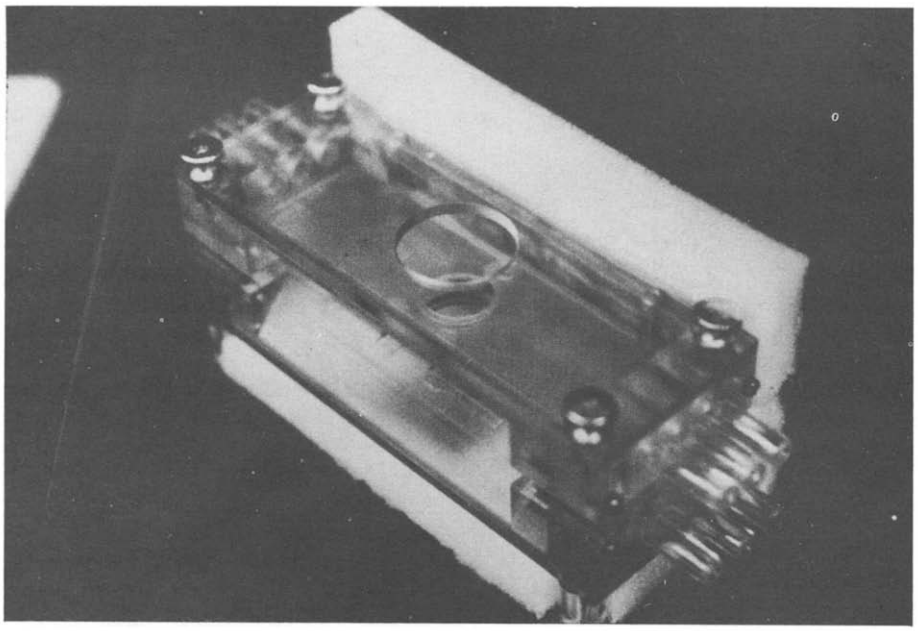

FIG. 3. Top view of the Arizona Hyperthermia Chamber (AHC).

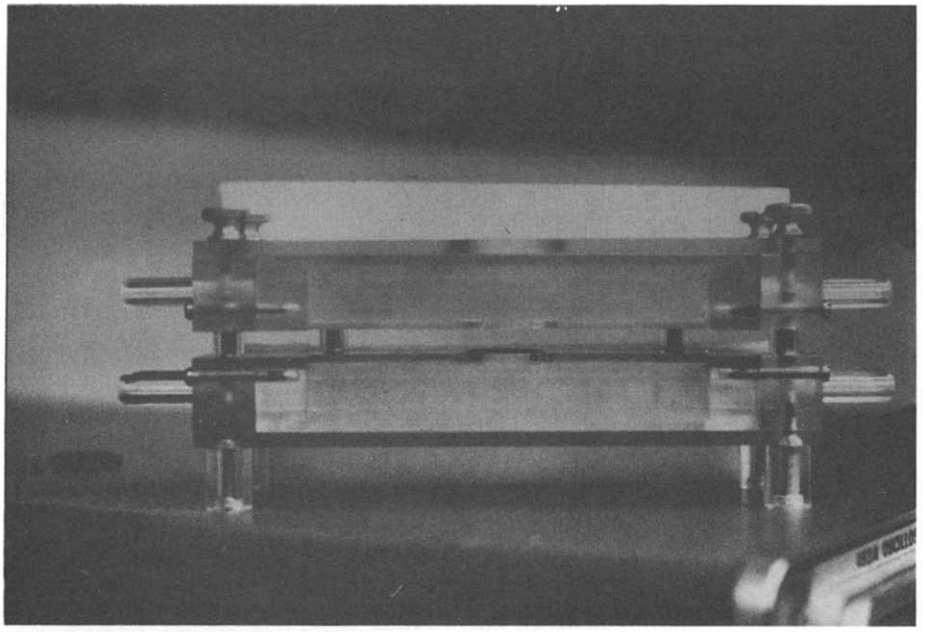

FIG. 4. Front view of the Arizona Hyperthermia Chamber (AHC).

water temperatures at the inlet of the heating chamber and in the circulating water bath were taken. Core temperature of the rat was monitored with a rectal probe. Thermistor readings were taken every 20–30 s using a microcomputer-based data-acquisition system.

The water was preheated to desired temperatures by using the bypass system. Once the desired temperature was achieved, water was shunted into the AHC system and measurements were begun. Measurements were taken until steady state was achieved.

SIMULATION OF ARIZONA CHAMBERS

A mathematical model to simulate the tissue temperature distribution in the tissue preparation in the Arizona window chamber and the access window was deemed necessary in order to predict the steady-state thermal distributions and their relationship to the key

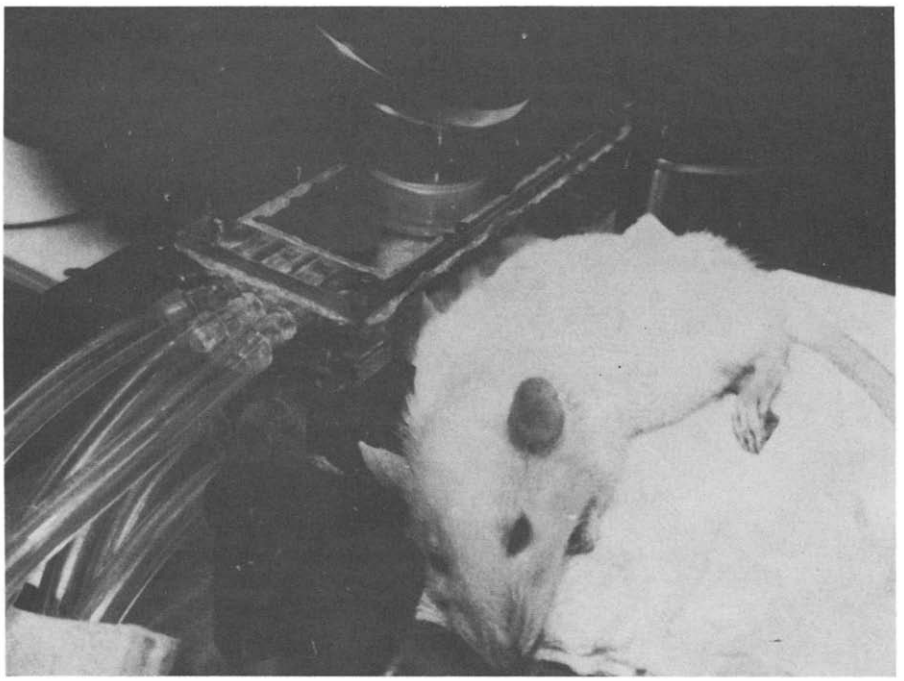

FIG. 5. Experimental set up for microvascular hyperthermia measurements.

Table 1. Temperature distribution map in the chamber (see Fig. 6). Right hand column is the water temperature. Top row corresponds to the centerline of the window and bottom row to the radius outside of the window. The first five rows of the last two columns are the tissue temperatures. $t_{fluid} = 42°C$, $t_{air} = 22°C$, and $t_{animal\,core} = 37°C$

		$h = 50$			
37.02605	37.02610	37.83186	39.44526	41.06313	42.00000
37.02581	37.02586	37.08049	39.44260	41.06152	42.00000
37.02439	37.02444	37.81927	39.41905	40.04648	42.00000
37.02202	37.02207	37.77207	39.29020	40.95203	42.00000
37.01080	37.01884	37.62632	38.68587	40.22899	42.00000
37.01499	37.01504	37.81806	37.02273	37.03216	42.00000
		$h = 10\,000$			
41.26852	41.27407	41.31769	41.40091	41.52725	42.00000
41.23810	41.24466	41.28887	41.37511	41.50655	42.00000
41.05350	41.06112	41.11200	41.21629	41.37016	42.00000
40.70872	40.71675	40.77225	40.09684	41.10976	42.00000
40.19902	40.19957	40.20362	40.30451	40.54708	42.00000
39.70478	39.77747	39.74608	39.74560	39.74306	42.00000

parameters of the system such as the thermal conductivity of the chamber elements and the convective velocity of the heating fluid. The complex configuration of the chamber made an analytical solution impossible and a finite difference numerical procedure was implemented. The area modelled is shown in Fig. 6. Energy is delivered to the window area of the chamber by the heating fluid and is lost through conduction along the aluminum structure and into the animal skin surface.

The steady-state 2-dim. temperature distribution for the window, support structure and tissue preparation is given in cylindrical coordinates by

$$\frac{\partial}{\partial r}\left(rk\frac{\partial t}{\partial r}\right) + r\frac{\partial}{\partial z}\left(k\frac{\partial t}{\partial z}\right) = 0.$$

This equation was solved in finite difference form using Gaussian elimination. The nodal network and equations were arranged to account for the variable thermal conductivities of the materials present: glass, window, aluminum support structure, the tissue preparation and the animal's normal tissue. The boundary conditions used were: forced convection to water for the surface of the window and the annular aluminum ring in contact with the water; insulated surface for the remainder of the interface between the AWC structure and the AHC; symmetry about the centerline of the window and the plane of symmetry of the preparation; $t = t_{animal\,core}$ for the plane of symmetry of the animal and for the animal core; and a convective boundary condition to the ambient air for the exposed metallic tip of the chamber. Several conservative assumptions were made in the simulation to ensure that the temperature difference across the preparation would be over predicted. These included using a very large air heat transfer coefficient for the end of the aluminum support structure and neglecting conduction through the wall of the AHC to the aluminum support structure that it is in contact with.

The program uses the following parameters:

(1) Water heat transfer coefficient on window surface (forced convection).

(2) Air heat transfer coefficient (free convection).

(3) Water temperature.

(4) Ambient air temperature.

(5) Animal core temperature.

(6) Thermal conductivity (a) tissue, (b) glass, (c) aluminum.

The temperatures at all nodes are calculated and, using a Ramtech transformation, are reproduced in a color display. Table 1 shows the configuration of the numerical display with the water temperatures representing the hyperthermia bath on the right vertical column and the tissue temperature in the left vertical column. Runs were made with

$t_{fluid} = 42°C$,
$t_{air} = 22°C$,
$t_{animal\,core} = 37.16°C$,
$h = 50$ and $10\,000$ [W m^{-2} °C^{-1}],
$h_{free} = 50$ [W m^{-2} °C^{-1}].

These heat transfer coefficients were chosen to give a large free convection effect and a large range of forced convection coefficients foi illustrative purposes. Since it was important to reduce thermal gradients in the preparation, a numerical sensitivity study was performed to determine the effect of the system parameters. These calculations led to several conclusions. Higher forced convection coefficients yielded smaller gradients in the tissue as did lower free convection coefficients. It was also found that the core temperature of the rat was an important variable and that for best results, the rat's core temperature should not be

Table 2.

Mean Intrawindow Temperature	Maximum Temperature Range
38	±0.04
40	±0.06
42	±0.07
44	±0.10
46	±0.10

allowed to drop significantly. The latter generally occurs during anesthesia.

An example of the results are given in Table 1. Air temperature, animal core temperature and thermal conductivities of the tissue, glass and aluminum were held constant. The values were

$t_{air} = 22°C$,
$t_{core} = 37.16°C$,
$k_{tissue} = 0.54\ [W\,m^{-1}\,°C^{-1}]$,
$k_{glass} = 0.78\ [W\,m^{-1}\,°C^{-1}]$,
$k_{aluminum} = 164\ [W\,m^{-1}\,°C^{-1}]$.

The forced convective heat transfer coefficient was taken to be 50 and the free convective coefficient was also 50 for case 1. The fluid temperature was taken to be 42°C. In case 2, the heat transfer coefficient was raised to 10 000. The importance of maintaining a high value of the forced convective coefficient is evident. The calculations demonstrated that for the case of low forced convection, there were substantial differences between the fluid temperature and the temperature of the preparation. In fact, almost no elevation of tissue temperature occurred. When the high forced convection coefficient was introduced, the tissue temperature was raised close to the fluid temperature and only small radial gradients existed.

EXPERIMENTAL RESULTS

Thermal gradients within chamber preparations were within the required conditions for temperatures ranging from 38 to 46°C (Table 2). The gradients had a tendency to be larger for higher temperatures but did not exceed the designated limitation of ±0.1°C.

The mean intrachamber temperature was found to be linearly related to the inlet temperature, thus providing a means of non-invasive temperature calibration (Fig. 7). Core rectal temperatures did not rise more than 0.5°C for up to 1 h of heating. Steady state was achieved in less than 10 min for all temperatures tested (Fig. 8).

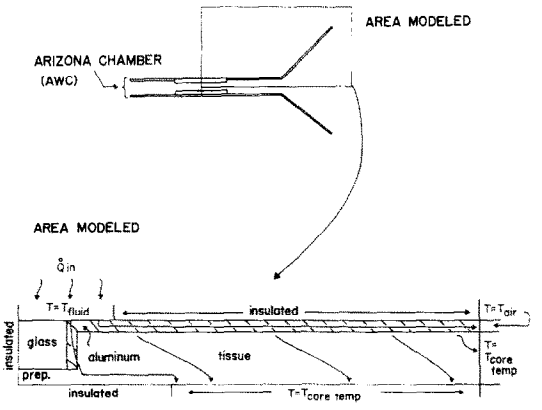

Fig. 6. Model area of chambers.

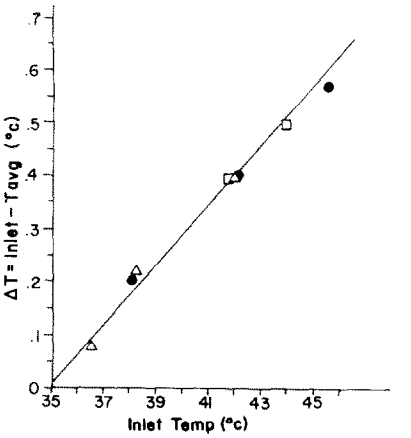

Fig. 7. Calibration chart-relationship between water inlet temperature and average window temperature.

DISCUSSION

There were three major thermal design goals for the hyperthermia chamber. The major requirement was to provide an intrawindow temperature to close tolerance. The experimental results given in Table 2 summarize a series of experiments in which the mean intrawindow tissue temperatures and the maximum

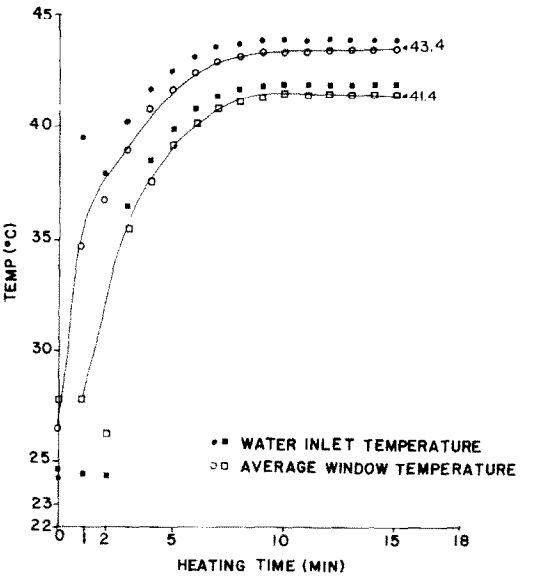

Fig. 8. Time to reach steady state for two average window temperatures.

deviations from that temperature are shown. In all cases, the variation across the window was within the specified temperature limits to provide a uniform tissue temperature within the window area.

Direct temperature monitoring of the window is not possible for the *in vivo* hyperthermia experiments because it would destroy the preparation. Therefore, it is necessary to have an accurate correlate of the mean preparation temperature. To obtain this we have calibrated the temperature difference between the mean intrawindow tissue temperature and the water inlet temperature. Figure 7 shows the results of this study, which shows that the temperature difference increases linearly with the inlet water temperature. A temperature monitor at the inlet thus provides an accurate estimate of the mean intrawindow tissue temperature.

Finally, the time for the window to reach an equilibrium temperature should be short because of the difficulties associated with the animal preparation and because it will better simulate heat up times observed clinically. This will also minimize complications from development of thermotolerance (i.e. decreased cytotoxicity for a given temperature [1]. Typical temperature–time curves of the window for equilibrium temperatures of 42°C and 44°C are shown. The time to reach equilibrium is 10 min and well within the time to carry out the experiment.

An hyperthermia chamber has been designed which provides very accurate temperature regulation of the Arizona Window Chamber. This design has been simulated using a numerical analysis which predicts the temperature field in the tissue. The design can easily be altered to accommodate other types of chambers used to study the microcirculation.

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SIMULATION DU CHAMP THERMIQUE DANS UNE CHAMBRE HYPERTHERMIQUE POUR DES ETUDES MICROVASCULAIRES

Résumé—L'hyperthermie a fait le sujet de beaucoup d'études récentes pour le traitement du cancer. Son plus grand potentiel tient dans sa combinaison avec la radiothérapie et/ou la chimiothérapie. Des températures élevées altèrent la microcirculation à la fois dans la tumeur et dans le tissu normal. Des changements dans la perfusion à l'intérieur de tissu peut affecter la distribution de la drogue et la radiosensibilité par des changements dans la fraction hypoxique. Un modèle thermique utilisant l'eau comme fluide chauffant à été réalisé pour permettre la mesure du comportement *in vivo* d'un seul vaisseau à des températures controlées. Le modèle est en lexan et enferme la cellule en permettant le contact du fluide avec la surface de la fenêtre. La température du tissu entre les fenêtres de la chambre peut être ajustée à mieux que 0,1°C et la température à la surface du tissue est uniforme à + 0,1°C. Un bypass pour le fluide chauffant permet des changements rapides de la température du tissue de façon à simuler les traitements cliniques hyperthermiques. Un modèle numérique prédit correctement les distributions de température dans la cellule et le montage réalisé montre qu'il fournit un chauffage rapide à la température voulue de la chambre avec fenêtres.

SIMULATION DES THERMISCHEN FELDES IN EINER HYPERTHERMISCHEN KAMMER FÜR MIKROVASKULÄRE UNTERSUCHUNGEN

Zusammenfassung—Hyperthermie als Krebsbehandlung ist in jüngster Zeit Gegenstand vieler Forschungsarbeiten gewesen. Ihr größtes Potential liegt in ihrer in ihrer Kombination mit der Radio- und/oder der Chemotherapie. Es ist gezeigt worden, daß erhöhte Temperaturen sowohl in Tumorgeweben als auch in normalen Geweben die Mikrozirkulation verändern. Änderungen der Gewebedurchströmung können die Verteilung von Medikamenten und über Änderungen des Blutsauerstoffgehalts die Strahlungsempfindlichkeit beeinflussen. Es wurde eine thermische Apparatur entworfen, die Wasser als Wärmeträgerfluid für die durchsichtige Arizona-Versuchskammer verwendet, um Messungen des Einzelgefäßverhaltens am lebenden Objekt bei geregelten Temperaturen zu ermöglichen. Der Apparat ist aus Lexan hergestellt und schließt die Kammer ein, wobei das Fluid in Kontakt mit der äußeren Glasscheibe ist. Die Gewebetemperaturen zwischen den Fenstern der Kammer können innerhalb von 0,1°C geregelt werden. Die Temperaturgleichförmigkeit über der Gewebeoberfläche liegt bei $\pm 0{,}1$°C. Ein Bypass für das Heizfluid erlaubt schnelle Wechsel der Gewebetemperaturen, so daß Simulationen klinischer Hyperthermiebehandlungen durchgeführt werden können. Ein numerisches Simulationsmodell berechnet die Temperaturverteilungen in der Kammer sehr genau, und es wird gezeigt, daß mit diesem Entwurf schnelles Aufheizen der Kammer auf geforderte Temperaturen erreicht werden kann.

МОДЕЛИРОВАНИЕ ТЕПЛОВОГО ПОЛЯ В ГИПЕРТЕРМИЧЕСКОЙ КАМЕРЕ ДЛЯ МИКРОСОСУДИСТЫХ ИССЛЕДОВАНИЙ

Аннотация—В последнее время многочисленные исследования гипертермии связаны с проблемой лечения рака. Самый существенный аспект метода состоит в возможности его использования совместно с радио- и(или) химиотерапией. Показано, что повышение температуры изменяет микроциркуляцию как в опухоли, так и в нормальной ткани. Изменения в перфузии ткани могут влиять на распределение лекарства и чувствительность к облучению за счет изменений гипоксической фракции. С целью исследования поведения отдельного сосуда в естественных условиях при контролируемой температуре для Аризонской прозрачной камеры был спроектирован термостат, в котором в качестве нагревающей жидкости используется вода. Прибор изготовлен из лексана. В него помещается камера, так что жидкость может контактировать с внешней поверхностью окна камеры. Температуру ткани между окнами камеры можно устанавливать с точностью до 0,1 °С, а температура на поверхности ткани изменяется не более чем на $\pm 0{,}1$ °С. Обводной патрубок для нагревающей жидкости позволяет быстро изменять температуру ткани, так что можно проводить моделирование процесса клинического гипертермического лечения. С помощью численной модели точно рассчитывается распределение температуры в камере. Показано, что конструкция прибора дает возможность производить быстрый нагрев камеры до требуемых температур.