

Effects of Hyperthermia on Normal and Tumor Microenvironment¹

Haim I. Bicher, M.D., Ph.D., Fred W. Hetzel, Ph.D., Taljit S. Sandhu, Ph.D., Stanley Frinak, B.S.,
Peter Vaupel, M.D.,² Michael D. O'Hara, B.A., M.S., and Terrence O'Brien, B.A.

The effects of hyperthermia on pH, local blood flow (LBF) and tissue oxygen tension (TpO₂) in several normal and tumor tissues were studied. It was found that TpO₂, local blood flow and pH are inhomogeneous in tumor tissue. TpO₂ is very low in certain areas which also seem deprived of blood flow and are at very low pH. Hyperthermia has a dual effect: at temperatures below 41°C, it increases blood flow and TpO₂ while above 41°C, it causes a collapse in blood flow, lower TpO₂ and a shift of the tissue pH towards acidosis from the already low pH values found in tumors.

INDEX TERMS: Blood, flow dynamics • Hyperthermia • Oxygen

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THE revival of interest in hyperthermia as a possible adjunct to radiotherapy has led to increasing investigation of physiological alterations during the application of heat (2, 14, 16, 17, 19, 34, 40, 41, 44, 45, 48). Studies by both Eddy (16) and Reinhold *et al.* (33) employing "chamber systems" cite changes in the microvascular network as a function of temperature and exposure time. The apparent sensitivity of the neovasculature was observed by both authors. A knowledge of the effect of hyperthermia on tumor and normal tissue blood flow will enable us to understand its effects on hypoxic cells during radiotherapy and to decide on the optimal amount of heat for different neoplasms.

Studies with plethysmography show that elevation of normal tissue temperature to 41°C is accompanied by a considerable increase in blood flow (28). Cater and Silver (10) recorded changes in tumor oxygen tension with hyperthermia but not in temperature. They concluded that diathermy had not increased the oxygen tension in the tumor but, on the contrary, had decreased it.

Bicher *et al.* (1, 3) reported that tumor blood flow in a mouse leg tumor system increased up to 41°C and then decreased to 44°C. The oxygen tension in the tumor, as measured with a platinum electrode, generally followed the changes in tumor blood flow but the exact tumor temperature at which the oxygen tension decreased was not determined. Similar changes in brain tissue oxygenation had been reported earlier by the same author (4).

Although blood flow and the shifts induced in it by hyperthermia in both tumor and normal tissue are important, there are other significant factors. Several studies indicate that the pH of interstitial fluid in human and rodent solid tumors is 0.3 to 0.5 units lower than the normal tissue pH of about 7.4 (18, 26, 30, 31).

Reduced pH has also been shown to affect the trans-

plantability of tumor cells heated *in vitro* (32). In a recent paper, Gerweck (22) has shown that the influence of pH varies with temperature and that there is a critical point in the increased lethality of heat below pH 6.7. In addition, changes in glycolysis, respiration rate and lactic acid production will probably influence the response of the tissue.

Several other factors may change and subsequently influence the response of cells or tissues to supranormal temperatures. Paramount among those are the vascular changes, blood flow responses and the net result of this on tissue oxygenation that may change both the effect of hyperthermia or radiation therapy when used in combination in the treatment of tumors. Several authors have reported an increase of blood flow during hyperthermia. On the other hand, Dickson (15) has reported an increase in oxygen consumption during elevated local temperatures. The net result, therefore, could be either an increase or decrease in local oxygen tension.

Englund *et al.* (20) and Sutton (46) have demonstrated that during hyperthermia local blood flow increases in the tumor region and also in the organ hosting the tumor. While measuring the local oxygen levels as well as the local blood flow to the tumor, recent experiments demonstrate that a net result of this process is an increase in the local oxygen tension. This increase is quite remarkable and also leads to abolishing the local autoregulation processes that usually tend to keep oxygen levels constant in several organs as well as in tumors (1, 10, 11).

This article will address itself specifically to changes in the microenvironment of normal and tumor tissue as measured directly with ultramicroelectrodes. The patterns of change of oxygen partial pressure, pH and microflow between tissue types, and the possible clinical implications are also discussed.

¹ From the Department of Therapeutic Radiology, Division of Radiation Biology and Physics, Henry Ford Hospital, Detroit, MI 48202. Submitted for publication 5 April 1980; accepted after revision 12 August 1980.

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² Department of Physiology, University of Mainz, Saarstrasse 2, D-6500, Mainz, West Germany.

EFFECT OF HYPERTHERMIA ON T_pO_2

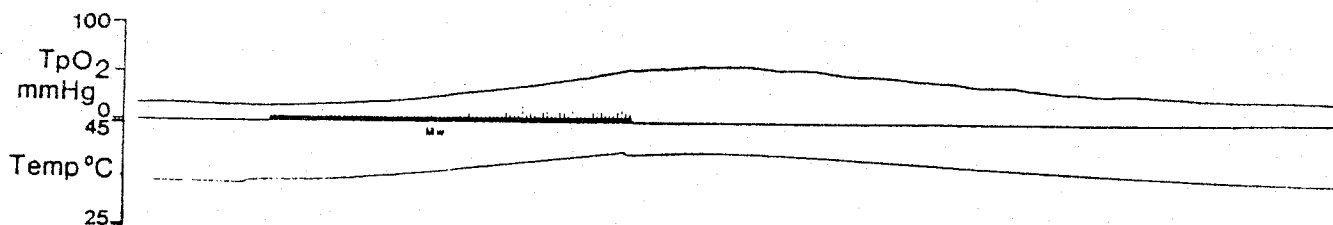


Fig. 1. Effect of microwave hyperthermia on T_pO_2 in a representative mouse tumor. The upper channel records T_pO_2 and the lower channel temperature in degrees Celsius. Microwaves are on when indicated by the timing pulse. One minute is indicated by the space between two large peaks on the microwave tracing (center). Cooling and declining pO_2 can be seen when the microwaves are turned off.

MATERIALS AND METHODS

1: Tissue Systems

Measurements were performed in both mouse and human tumors as well as in two normal tissues as follows:

(a) *C₃H mouse mammary adenocarcinoma*: *In situ* studies were carried out in fourth generation transplants of *C₃H* mammary adenocarcinoma implanted in the hind leg of *C₃H* SED-BH mice. The tumors were obtained from the Radiobiology Division, Massachusetts General Hospital (43). This is a syngenic implantable tumor that is kept at our facility using solid tissue transplants inoculated subcutaneously into recipient mice. Tumors used for experimentation were approximately 10 mm in diameter. The mice were anesthetized during microelectrode introduction with a combination of ketamine 40 μ g/kg i.m. and Thiozazine, 50 mg/kg i.m.

(b) *Human tumors*: Determinations were made in subcutaneous metastases in a group of 15 patients. Tumors represented different histologies and locations, but are grouped together as the responses were homogeneous. There were four melanomas, six chest wall recurrences of mammary adenocarcinoma and five peripheral metastases of squamous cell carcinoma of the lung. The patients were not anesthetized. Oxygen was administered through a facial mask when required (see below: *Oxygen Ultramicroelectrodes*).

2. Normal Tissues

(a) *C₃H mouse muscle*: Employing the same animal system as in 1(a) measurements were made in the muscle tissue of the hind leg. Determinations were obtained in both controls and in animals bearing an implanted tumor in the opposite leg. Since no difference was observed, no distinction is made in the results.

(b) *Cat brain*: All brain studies were performed in cats. In each case the animal was anesthetized with Nembutal (30 mg/kg) prior to and during the procedure. After opening the scalp, a small opening (5 mm) is made

in the skull with a dental hand drill and the dura is carefully opened. Throughout the entire procedure, including the microelectrode introduction and measurements, the opening is kept moist with isotonic saline.

3. Physiological Determinations

(a) *Oxygen ultramicroelectrodes*: The O_2 ultramicroelectrodes were "gold-in-glass" types as described by Cater and colleagues (12). They were made by pulling a glass tube (KG-33, ID 1.5mm, OD 2.0mm, Garner Glass Co., Claremont, California), encasing a 20 μ gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) in a David Kopf Model 700C vertical pipette puller. The exposed gold tip is about 10 μ in diameter, and coated with a Rhoplex (Rhom Haas, Philadelphia, Pennsylvania) membrane as previously described (4). This probe is used as an "external reference" O_2 microelectrode.

The operative characteristics of O_2 microelectrodes in solutions have been well defined prior to their use for measuring *in vivo* tissue responses, their more common present application (5, 39).

Their performance has proven to be in good agreement with what could be expected according to the theories of polarographic recording of partial pressures of oxygen in tissue. The adoption of industrial technology ensures good reproducibility between different electrodes, and different electrodes in different experiments, and makes a hitherto complicated and sophisticated technique into a simple determination, potentially useful in a variety of physiological and *in vitro* experiments.

The electromagnetic force to activate the oxygen cathode and its current output are provided and measured with a Transidyne Model 1210 microsensor amplifier (Transidyne General Corp., Ann Arbor, Michigan) and recorded with an appropriate chart recorder.

Electrodes are calibrated as described by Silver (38) in buffered saline solutions of known pO_2 values. The electrodes are conditioned by placing them in buffered saline and applying 0.8 V potential for 2 hrs. After this treatment they are usually very stable. The current reading at zero

TpO₂ IN MOUSE TUMOR 46° MICROWAVE HYPERTHERMIA

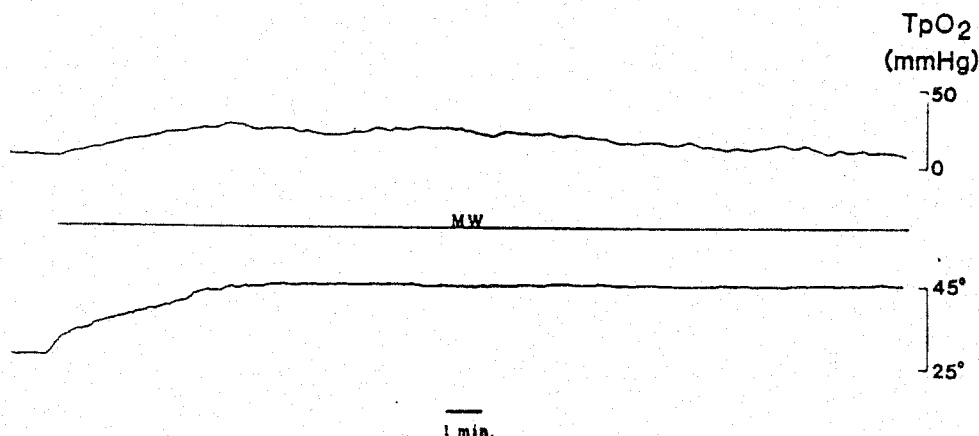


Fig. 2. In this figure, microwaves (MW) are indicated by the solid center line. Note that the temperature is constant at 46°C except for the first 5 minutes. The drop in TpO₂ is clear after only 10 minutes at 46°C.

oxygen tension is very low (residual current) and the response of the microelectrode to changes of oxygen tension is very rapid.

The current voltage polarogram of these electrodes shows a quasi-plateau between 0.3 and 0.7 V. The plateau, however, is not absolute and small differences can be detected at the extreme values. This type of plateau can be expected when using "open tip" electrodes [Davies and Brink (13)]. In these experiments a polarizing voltage of 0.6 V has been used. The relationship between current output and oxygen tension is linear, the current per mm Hg being of the order of magnitude of 0.6×10^{-11} A.

In human experiments, a platinum-iridium Teflon-coated wire, 120 μ in diameter, was used as the O₂ electrode. Although the calibration was not as reliable for determining actual TpO₂ values, it was found in determining transients (response to oxygen breathing or hyperthermia) that the values correlated well with those obtained using microelectrodes. The responses to O₂ breathing were determined by administering pure oxygen to the mouse or patient for one minute. The height of the tissue oxygen response provided an indication of the ability of the circulation to transport oxygen, probably dependent on the blood flow. The temperature artifact of both types of oxygen electrodes was determined and found to be 5% per degree Celsius. All results were corrected by taking this artifact into account.

(b) *Other microelectrodes* are now available to measure K⁺, Cl⁻, pH, etc. An antimony electrode has long been used in the measurement of pH, but investigators have found that the electrode potential is linear with increasing pH only to pH 7.0. Consequently, these electrodes require frequent calibration. Bicher and Ohki (6)

successfully used an antimony pH microelectrode. Basically, it consists of a very finely drawn (1 micron or less tip diameter) glass micropipette which has a thin film overcoating of antimony. It is then coated with two layers of insulating epoxy resin leaving an exposed tip approximately 2 microns in length. A microcalomel electrode inserted into the same cell, tissue or solution serves as a reference. Results obtained in the giant squid axon were very satisfactory (6).

Designs for glass pH microelectrodes have been developed, most notably by Hinke (27) and Thomas (47). The Thomas electrode consists of a Pyrex glass micropipette drawn to a fine point into which is inserted and fused a second pipette made of pH-sensitive glass. The tip of the pH-sensitive glass pipette is recessed in the tip of the Pyrex glass pipette and the electrode is filled with KCl electrolyte. The Hinke-type electrode also consists of a pH-sensitive glass micropipette inside a Pyrex glass pipette, the major difference being that the tip of the pH-sensitive micropipette is not recessed but extruded from the Pyrex glass pipette. A silver/silver chloride electrode is inserted into the electrode stem which is filled with 0.1 N HCl. The Hinke microelectrode then, has an exposed tip and its response time is instantaneous. This is an advantage over the Thomas microelectrode in which the recessed tip may result in a response time of up to several minutes.

(c) *Microflow*: Flow in microareas of tumor tissue was determined using the hydrogen diffusion method as described by Stosseck *et al.* (42). The method is based on the polarographic determination of the amount of hydrogen gas reaching a platinum electrode (hydrogen detector) from a hydrogen-generating electrode located at a fixed dis-

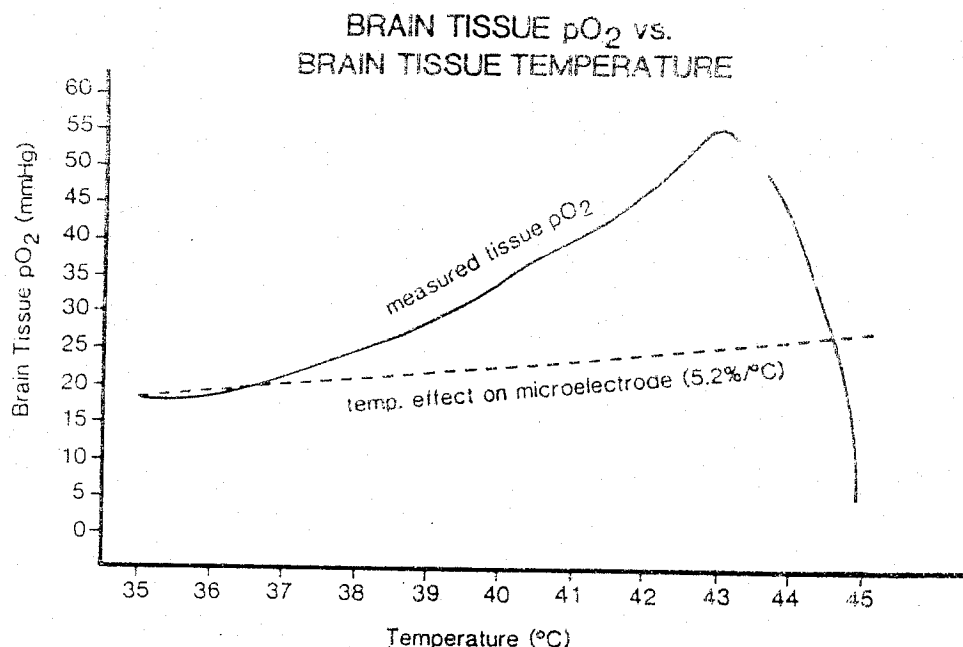


Fig. 3. Brain tissue pO₂ (equilibrium, steady state values) is plotted as a function of brain tissue temperature in this composite figure obtained in a representative rabbit brain. A breaking point is clearly seen at approximately 43°C. The temperature artifact induced in the microelectrode (5.2%/°C) is plotted on this graph to clearly show that the observed curve is not artifactual in nature.

tance. The amount of hydrogen reaching the reading electrode depends on the generation and diffusion rates, which are constant, and the blood flow clearance of hydrogen which can be thus determined. In the present experiments, two platinum in Teflon 100 μ wires placed 100 μ apart were used. The reading device was applied to the surface of the tumor. In the present experiments only relative changes in the rate of blood flow were determined. This method was used in the experiments on mouse tumors *in situ* [Methods 1a].

(d) *Temperature determinations:* Tumor and mouse core temperatures were recorded with Copper-Constantan microthermocouples (tip diameter 30–100 μ) inserted into the tumoral tissue in close proximity to the O₂ microelectrode or in the animal's rectum for core measurements. An Omega Engineering Model 250 Digital Voltmeter amplifier was used as a link between the microthermocouple and the polygraph. Microwaves of a frequency 2450 MHz were produced by a Raytheon Magnetron and delivered through a specially designed 2.5-cm side square TE₁₀ waveguide applicator loaded with low-loss dielectric material with a dielectric constant of 6 (36).

RESULTS

As seen for a representative mouse tumor in Figure 1, there is a rise in TpO₂ that parallels the application of the microwaves and closely follows changes in tissue temperature. The response is very fast, with TpO₂ increasing shortly after the rise in temperature, and then decreasing as the tumor cools off. This effect was always present

when heating was carried out up to 41°C. At higher temperature (Fig. 2), there was an initial increase in TpO₂ which was followed by a decrease to very low levels as the temperature was held constant at 46°C.

Similar effects are also seen in both normal tissues studied (muscle and brain) with one major difference, this being the temperature at which the TpO₂ begins to fall or the "breaking point." In Figure 3, the rise in oxygen tension in brain continues up to 43°C but declines sharply at higher temperatures. A composite of representative results obtained for both normal and tumor tissues is presented in Figure 4. It is evident that the breaking point (in the pO₂ vs. temperature curve) exists for each tissue, and at least for the tissues studied in these experiments (at least 15 determinations per tissue type), the breaking point temperature is significantly lower for tumor.

Figures 5 and 6 show examples of the effect of hyperthermia (low and complete range hyperthermia, respectively) on local blood flow in mouse tumors. In both cases it is clear that blood flow increased significantly up to approximately 41°C. In addition, examination of the data in Figure 6 shows the strong correlation between decreases in TpO₂ and blood flow as the temperature is increased up to 45°C.

In 8 different animals with implanted tumors, a large number of single-point pH determinations were made both prior to and following hyperthermia. The results are plotted in histogram form showing frequency of values at different pH levels (Fig. 7). This method of representing microenvironment tissue distribution was introduced by Stossek *et al.* (42) for O₂ tissue levels.

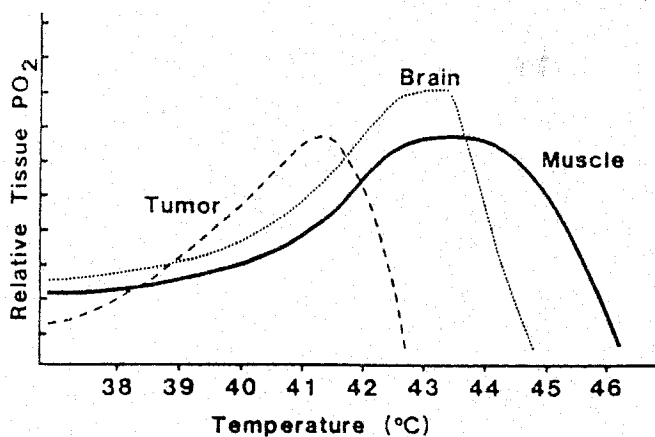


Fig. 4. Relative tissue pO_2 plotted as a function of temperature similar to the plot in Figure 3. In this case representative curves obtained for tumor, brain and muscle are superimposed so that the differential effect and the different position of the breaking point can be seen more clearly. Note that relative TpO_2 has been used to aid in clarification.

The mean value of tissue pH was found to be 6.8 pH units in mouse tumors. Upon heating for one hour at $43^\circ C$, there was a pH decrease of 0.5 to 1 pH unit to an average of 6.2. The individual variations from point to point within a single tumor are seen more clearly in Figure 8. For each value of pH obtained at normal temperatures, the pH microelectrode was left in place throughout the one hour of 43° hyperthermia and a new value obtained. In each case, related data points are joined by a solid line.

Breathing pure O_2 for one minute usually caused a very small rise in TpO_2 indicative of the autoregulation of the microcirculation. Local hyperthermia caused an increase in this response proportional to the local tumor tissue temperature. The threshold was about $37.5^\circ C$ and a 40–50 mm Hg increase could be recorded at approximately $41^\circ C$ (Fig. 9).

The effect was reversed when the tumor was heated to $45^\circ C$ (for example, see Fig. 6).

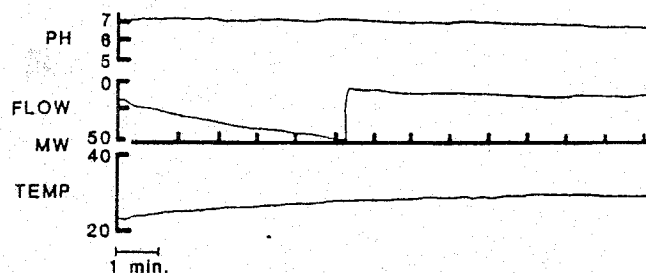


Fig. 5. In this graph, the effect of low-level microwave hyperthermia on pH and blood flow on a mouse tumor is shown. The upper tracing indicates a decline in pH values as hyperthermia is continued, even though the temperature never reaches $40^\circ C$. The effect on blood flow is shown in the second tracing with an increase in flow being in the down direction. The dramatic increase in blood flow required a recalibration after 5 minutes which is the reason for the vertical shift in the curve at that point.

DISCUSSION

There is considerable controversy as to whether the blood perfusion of tumors is greater than that of normal tissues. LeVeen *et al.* (29) state that tumor blood flow, as measured by an isotope dilution technique from surgically excised material, was 2%–15% of surgically excised normal material. They claim that the success of RF energy, in hyperthermia, is due to differential cooling of the normal tissue by the improved normal tissue perfusion. Using transplanted tumors in rat's ovaries or kidneys, Gullino and Grantham (25) showed that the tumor blood flow per milligram of tissue was 10–20 times less than the blood flow per milligram of tissue of the host ovary or kidney. However, it must be pointed out that in this experiment, the tumor was 10–100 times greater in weight than the host ovary or kidney and the actual total perfusion to the isolated ovary or kidney increased following the growth of tumor.

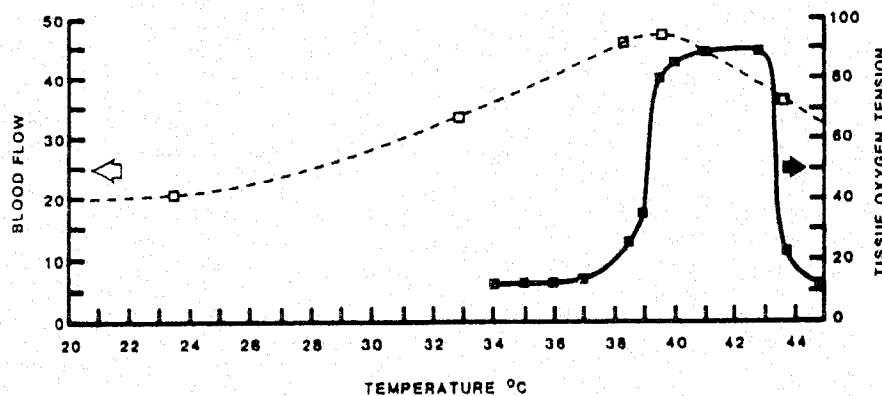


Fig. 6. Mouse tumor blood flow and tissue oxygen tension plotted as a function of temperature. All readings were taken in the same series of animals. Steady state values obtained for blood flow (\square) and TpO_2 (\blacksquare) in this series are plotted. It is clear that a strong correlation exists between change in blood flow with temperature and the change in tissue oxygen tension with temperature.

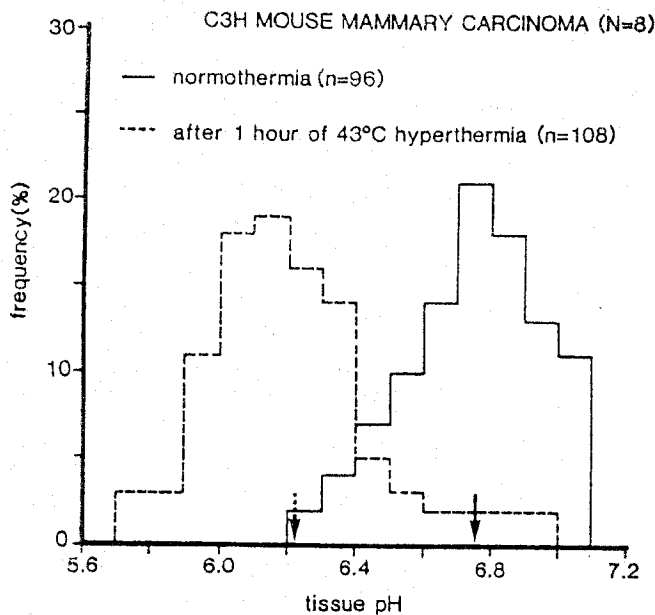


Fig. 7. Histogram of tissue pH levels obtained in mouse tumor. A total of 96 determinations were made at normal temperature, while 108 were made following one hour of 43°C microwave hyperthermia. The mean value of pH obtained in the control group was approximately 6.75, while in the group after one hour of hyperthermia the mean value was approximately 6.2.

By using radioactive microspheres in very large human tumors, Shibata and Maclean (37) showed microsphere normal tissue-to-tumor distribution ratios ranging from 3-1 to 20-1. Bierman *et al.* (7) via a differential between arterial and venous blood pO₂ as an index of blood flow in 12 patients with metastatic, neoplastic lesions showed that there was a greater increase in blood flow through the tumors than in comparable normal tissue, possibly due to the presence of arteriovenous shunts. Bierman *et al.* (8) also noted an increase in the thermal index of superficial tumors, as compared with the normal tissue.

The studies reported here clearly demonstrate that localized microwave hyperthermia causes a rise in both blood flow and TpO₂ up to some temperature with a fall at higher temperatures. It also appears that the temperature at which the breaking point occurs is characteristic of the specific tissue. The mechanism of this effect seems to be predominantly mediated through the blood flow changes, any metabolic effects being secondary to a microcirculation that is activated at moderated hyperthermic temperatures and damaged at higher ranges. These results also indicate that the normal tissue microcirculation is better able to cope with hyperthermic stress.

The rise in tumor temperature up to 41°C leads to a significant increase in tumor blood flow (TBF). This effect has also been demonstrated by Englund *et al.* (20) and Sutton (46) for both the tumor region and host organ. As to the cause of this increased flow, presumably different factors including systemic and local autoregulation have to be taken into account. The oxygen partial pressures in several subcutaneous tumors in animals and in humans

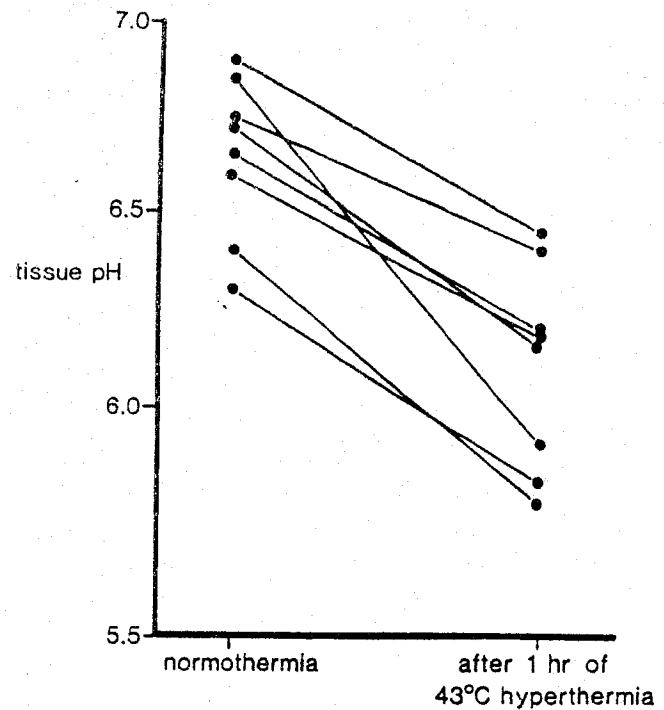


Fig. 8. This figure shows changes in pH induced by one hour of 43°C hyperthermia, examining single points within a single tumor. In each case a reading was taken with the pH microelectrode which was subsequently left in place throughout the period of hyperthermia, following which a second pH reading was obtained. It is clear that in each case the pH fell, the most dramatic being a drop of almost one complete unit of pH.

as measured with 100 μ m tip floating O₂ electrodes followed the change in blood flow (2).

A further rise in tissue temperature up to 42°C results in a marked breakdown of tumor blood flow to somewhat below the initial value. Similar results are obtained for *in situ* tumors, both in humans and mice. In metastatic lesions involving the skin, increases in flow occur due to elevations of temperature up to 40°C. With tumor temperature elevated to 46°C, the tissue oxygen tension in microareas of the tumor decreases following a drop in tumor blood flow. This correlates with the studies of Reinhold and Berg-Blok (35), who found that at 42°C, the center of a "sandwich" tumor becomes necrotic due to a decrease of tumor microcirculation at this temperature. These results, however, do not correspond with Song's finding that hyperthermia at 43°C does not change circulation in tumors, but increases it in normal tissues (40).

The restriction in blood flow at 42°C and the increase in total vascular resistance, respectively, presumably result from a series of factors. As main determinants of the decline of blood flow, a reduction of red cell deformability, multiple microthrombi as well as occlusions of microvessels have to be taken into account.

Reduced pH in tumors as compared to normal tissues has been reported by several authors (18, 26, 30, 31). The reduced pH of tumor fluid may be due in part to elevated lactic acid production resulting from poor tumor vascularity

and reduced oxygen tension. Reports also indicate that tumor cells produce lactic acid at an elevated rate even under oxygenated conditions (49). The significance of lowered pH on hyperthermia cell killing has been clearly demonstrated by Gerweck *et al.* and several other authors (22, 24, 32, 48). Cell killing is increased by factors of 5 and above when chronically hypoxic cells are heated to 42°C at a pH only 0.5 unit below normal.

The result of a pH drop in cancer tissue during or following hyperthermia is not surprising if one considers the familiar principle that temperature strongly influences the buffering processes and hence the pH. There is usually a shift to lower pH values if the temperature is elevated. Qualitatively, these changes are quite similar to those observed in blood. In addition, the increase in the CO₂ partial pressure during hyperthermia induced by changes in cellular metabolic pathways enhances tumor tissue acidosis. These factors, coupled with the observed breaking point in blood flow which would carry away metabolites, may provide a distinct therapeutic advantage for this modality.

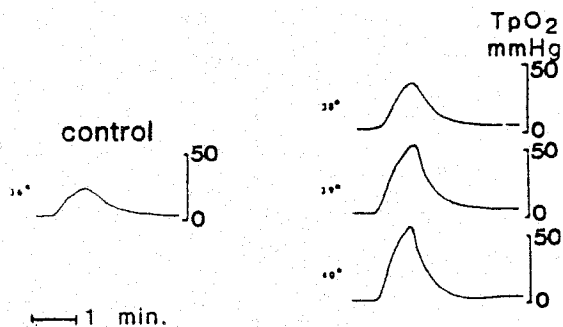
From the given data, a series of consequences results for this temperature range of maximum tumor blood flow. As the oxygen partial pressures in malignant tumors generally follow changes in blood flow, it can be expected that the radiosensitivity of cancer tissue may be improved during increased blood flow, thus producing a significant prolongation of survival time of tumor-bearing animals if they are treated with local hyperthermia in combination with irradiation. The clinical utility of the simultaneous combination of short-term, moderate tumor hyperthermia (41°C) and irradiation is currently being examined *in vivo* in our laboratory.

A second and perhaps more obvious conclusion can be derived from the data. It appears that the breaking point in blood flow (the temperature at which blood flow begins to decrease) in tumors is lower than in some normal tissues. Heating a region of the body which includes both tumor and normal tissues to a temperature which is above the breaking point for the tumor but only approaching that of the normal tissue would continue perfusion at levels even above normal while blood flow to the tumor would be reduced. With the increased metabolic activity and decreased flow, dramatic shifts in pH such as reported here would occur. As previously described by Gerweck and others (22-24), the combination of reduced pH and increased temperature is extremely cytotoxic. This effect alone should eradicate large areas of the tumor, including the radioresistant hypoxic areas, prior to the start of any combination with irradiation.

We may conclude from the results presented here that the therapeutic effectiveness of hyperthermia may result, at least partially, from several induced physiological modifications. First, moderate (41°C) hyperthermia in combination with ionizing radiation may result in improved tumor response by increasing oxygenation and hence, radiosensitivity coupled with a decrease in tumor pH. Second, higher levels of hyperthermia, 42°C and above,

TpO₂ RISE INDUCED BY MICROWAVE HYPERTHERMIA EFFECT ON O₂ BREATHING - HUMAN TUMORS

9a



TpO₂ RISE INDUCED BY MICROWAVE HYPERTHERMIA EFFECT ON O₂ BREATHING - MOUSE TUMORS

9b

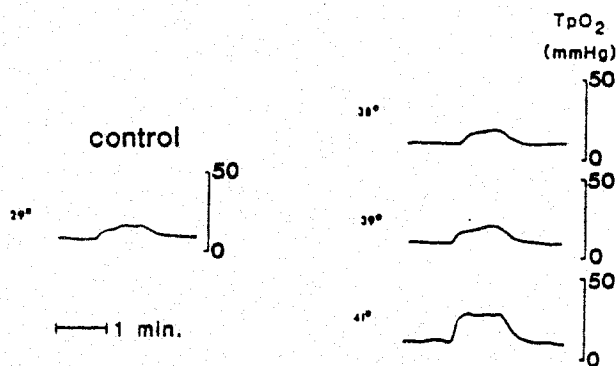


Fig. 9. Effect of microwave hyperthermia on tissue pO₂ response to oxygen breathing. In all cases the oxygen microelectrode was in place within the tumor. Under both normal and elevated temperatures the subject was given pure oxygen to breathe for a period of one minute and the resultant pO₂ profile in tissue determined.

a. In a representative human tumor, the normal (control) pO₂ response is seen on the left, while the response differences in temperatures up to 40°C are seen in the panel to the right.

b. In a mouse tumor, the resting temperature is significantly lower due to position of the tumor in the limb. The trend in pO₂ response profiles is similar to that seen in human tumors, the maximum response being obtained at 41°C.

may be directly tumoricidal because of elimination of tumor micro-blood flow and a concomitant sharp reduction in tumor pH while normal tissue can still be properly perfused.

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Haim I. Bicher, M.D., Ph.D.
 Therapeutic Radiology
 E & R Building, Room 3056
 Henry Ford Hospital
 2799 West Grand Blvd.
 Detroit, MI 48202