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Differential Response of Normal and Tumor Microcirculation to Hyperthermia[1]

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ABSTRACT

RBC velocity and vessel lumen diameter were measured in individual microvessels in normal (mature granulation) and neoplastic (VX2 carcinoma) tissues grown in a transparent rabbit ear chamber. Blood flow rates were determined before, during, and after local hyperthermia treatments at 40–52° for 1 hr. Blood flow in normal tissue increased dramatically with temperature, but stasis occurred at higher temperatures and/or longer durations of heating. In tumors, blood flow rate did not increase as much, and stasis occurred at lower levels of hyperthermia.

Both the magnitude and the time of maximum flow appeared to be bimodal functions of temperature. That is, both of these parameters increased with temperature up to a certain critical temperature, and then decreased at higher temperatures. This critical temperature was approximately 45.7° in normal tissue and 43.0° in tumors. Normal tissue required temperatures greater than 47° to bring about vascular stasis in less than 1 hr, while stasis occurred in tumors in the same time frame at temperatures greater than 41°. Normal tissue could increase its maximum flow capacity up to 6 times its preheating value, while neoplastic tissue could only double its maximum flow capacity. This differential flow response in individual microvessels was used to develop a theoretical framework relating various mechanisms of blood flow modifications due to hyperthermia.

INTRODUCTION

The microcirculation plays a critical role in determining the effectiveness of hyperthermia, either alone or in combination with radiotherapy and chemotherapy (19, 37). A number of investigators have measured the effect of hyperthermia on blood flow in normal and neoplastic tissues using various macroscopic, tissue-averaged methods as summarized (19, 37). However, only a limited number of quantitative studies have focused on blood flow modifications in individual microvessels (5, 14).

The objective of this work was, therefore, to measure RBC velocity and lumen diameter in individual microvessels in normal (mature granulation) and neoplastic (VX2 carcinoma) tissues grown in a rabbit ear chamber preparation. Blood flow modifications were determined in both tissue types before, during, and after local hyperthermia treatments for 1 hr at 40–52°, using the instrumentation and techniques described (8, 10). The observed differential flow responses between normal and neoplastic tissues are compared with the published data and are explained in terms of various mechanisms involved in blood flow regulation during hyperthermia.

MATERIALS AND METHODS

Animals and Tumor. Male New Zealand White rabbits (Three Springs Kennel Co., Zelienople, PA), 3 to 4 kg, were fed Purina laboratory chow (Ralston Purina Co., St. Louis, MO). The normal tissue studied was mature granulation tissue, and the transplantable tumor studied was VX2 carcinoma, both grown in modified Sandison-Clark transparent ear chambers.

Normal and Neoplastic Tissue Preparation. Transparent chambers (One of a Kind, Ltd., Lincoln Park, NJ) were surgically implanted in the right ears of male rabbits following the procedure described (8, 10, 24, 44). The chamber design allowed for the formation of a thin granulation tissue bed (40 ± 10 μm (S.D.) thick and 5.4 mm in diameter). Granulation tissue began to grow into the chamber approximately 1 week postimplant and reached maturity approximately 6 weeks postimplant (10). At this time, the chamber was used either for granulation tissue studies or for tumor implantation.

For tumor implantation, the animal was anesthetized (30 to 40 mg/kg Nembutal i.p.), and the coverglass which formed the top plate of the chamber was carefully removed. VX2 carcinoma was excised from the flank of a tumor-bearing host, minced, and placed in 0.9% NaCl solution (saline), and then spread uniformly over a new coverglass. The chamber was then reassembled with the new tumor-bearing coverglass (25). The success rate for tumor takes was 50%. Damaged tissues were not used for experimentation. When the tumor invaded the granulation tissue, the vessels became dilated and tortuous, and their density increased significantly (25). This angiogenic response was observed 3 to 4 days postimplant. The chamber was used for tumor studies, on the average, 13 days post-tumor implant. In a limited number of animals, transverse sections of the tissues were examined histologically to confirm the existence of neoplastic tissue (25).

Blood Flow Measurements. RBC velocity and lumen diameter of blood vessels were measured both on-line and off-line using the instrumentation described in detail (8) and shown schematically in Chart 1. The rabbit was lightly anesthetized and placed in a restraining device. The chamber was locked into an x, y travel microscope stage, and the tissue was transilluminated by a 100-watt tungsten-halogen lamp (Carl Zeiss, Inc., Oberkochen, West Germany) driven by a stabilized DC power supply (Model 6263B; Hewlett Packard, Palo Alto, CA). For on-line measurements, the desired blood vessel was viewed on a television monitor (Model EVM-11; Electrohome Ltd., Kitchener, Ontario, Canada), at a final magnification of × 3500, via a low-light television camera (Model 4410/SIT; Cohu, Inc., San Diego, CA). For off-line measurements, a number of blood vessels were recorded on a video cassette recorder (Model HR-3600AU; JVC Vidstar, U. S. JVC Corp., Maspeth, NY) and simultaneously observed on a second television monitor (Model EVM-11) via a high frame rate television camera (Model 4410A/SIT; Cohu). This setup allowed us to monitor and analyze a single vessel during the experiment and a number of surrounding vessels at a later time.

The diameter of individual vessels was measured using an image-splitting and -shearing monitor (Model 907; IPM, Inc., San Diego, CA).

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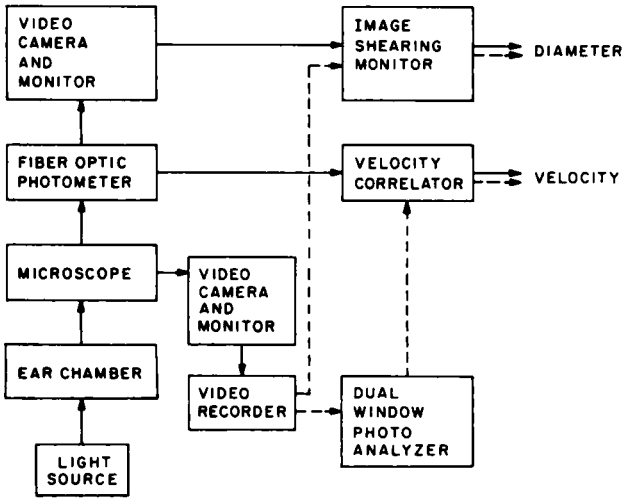

Chart 1. Schematic of the experiment setup. ——, on-line; - - - -, off-line.

The RBC velocity was measured on-line with a fiber optic photometer (IPM Inc., San Diego, CA) and off-line with a video photoanalyzer (Model 204H; IPM, Inc.); both instruments were connected to a tracking velocity correlator (Model 102; IPM, Inc.). The voltage signals from the image-shearing monitor and the velocity correlator were displayed on a strip chart recorder (Model 595; Omega Engineering, Inc., Stamford, CT) and stored on the main computing facility disk space via a data acquisition unit (Autodata Nine; Accurex, Inc., Mountain View, CA). These voltages were converted to diameter (D) and velocity (V) using calibration functions determined previously (8).

The mean volumetric flow rate (Q) for each vessel was calculated as:

$$Q = \left(\frac{V}{1.6}\right)\left(\frac{\pi D^2}{4}\right)$$

This equation is based on the empirical relationship $V_{RBC\,(centerline)} = 1.6\ V_{mean}$ (22).

Temperature Control. The tissue grown in the chamber was heated using a temperature-controlled circulating water jacket mounted beneath the chamber (Chart 2). Step changes in jacket temperature were achieved by simultaneously turning the 2 valves which controlled the flow from a cold bath (30°) and a hot bath (40–70°). The following equation, based on a heat transfer model and previously determined calibration functions, allowed the calculation of the average chamber tissue temperature (T_t) from the measured ambient temperature (T_a) and water jacket temperature (T_j) to within ± 0.3° accuracy:

$$T_t = T_j - 0.1957\ [T_j - T_a]$$

The temperature uniformity in the tissue was checked using a heat transfer model based on the bioheat transfer equation (8).

Rectal temperature was continuously monitored using a veterinary implant probe (Model VIP-T; Omega Engineering Inc.). Surface ear temperature adjacent to the chamber was measured by a surface thermocouple (Model CO1-E). Ambient temperature was recorded by a similar thermocouple (Model CO3-E). Water jacket temperature was recorded by a hypodermic thermocouple (Model HYP-T). All thermocouples were individually calibrated ranging 20–60°, using an NBS mercury thermometer, and allowed temperature measurements with an accuracy of ± 0.2°. Data acquisition and processing were performed in a manner similar to the velocity and diameter cases.

Experimental Protocol. Once the normal or neoplastic tissues reached the desired stage of growth, the rabbit was lightly anesthetized (27.2 ± 4.6 mg/kg Nembutal i.p.) and secured supine on a restraining device which restricted head movement but maintained normal circulation to the chamber. The water jacket was mounted to the chamber, and the assembly was secured to the stage. A section of chamber was carefully selected so that a well-perfused vessel could be monitored for on-line measurements, and 1 to 3 neighboring vessels could be recorded for off-line measurements. Once this position was determined, its coordinates were recorded, and the chamber was locked in place for the duration of the experiment.

Water from the cold bath (30°) was circulated through the jacket. The base-line temperature of 30° is close to the normal rabbit ear temperature. On-line measurements of RBC velocity, lumen diameter, and ear surface, rectal, ambient jacket temperatures were recorded continuously over a 15-min base-line period. Other vessels were videotaped for later off-line analysis. After 15 min, water from the hot bath was circulated through the jacket to obtain the desired tissue temperature (40–52°). At the end of 60 min of heating at constant temperature, the jacket was reconnected to the cold bath, and the tissue was cooled to and maintained at the base-line temperature for 45 min. Recording of all parameters was continued for the length of the experiment. The videotape of the experiment was analyzed off-line for diameter and velocity data in as many vessels as possible. The ear chamber was observed microscopically the following day, and qualitative changes in tissue appearance and microcirculation were noted.

In this study, hyperthermia experiments were conducted on mature granulation tissues at 11 different temperatures ranging 40.4–52.4°, and on neoplastic tissues at 8 different temperatures ranging 40.1–44.8°. These experiments permitted analysis of blood flow in 20 vessels in granulation tissue network (11 on-line and 9 off-line; diameter, 26.8 ± 8.5 μm), and 13 vessels in neoplastic tissue network (8 on-line and 5 off-line; diameter, 37.8 ± 11.3 μm). The average age of the granulation tissue was 68 days, and that of the tumor was 12 days (see Table 1 for the detailed age distribution). None of the tumors studied was visibly necrotic prior to the hyperthermia experiment.

RESULTS

Typical temperature profiles during a hyperthermia experiment are shown in Chart 3. Note that the rectal temperature was considerably higher than the skin temperature. The jacket temperature closely approximated a step change. The calculated tissue temperature reached 99% of its final steady-state value in about 7 min.

During hyperthermia, blood flow in normal tissue increased with increasing temperature, and remained elevated up to approximately 45.7°. Higher temperatures increased blood flow initially, but eventually caused the blood flow to shut down. The higher the temperature, the less time was required for the

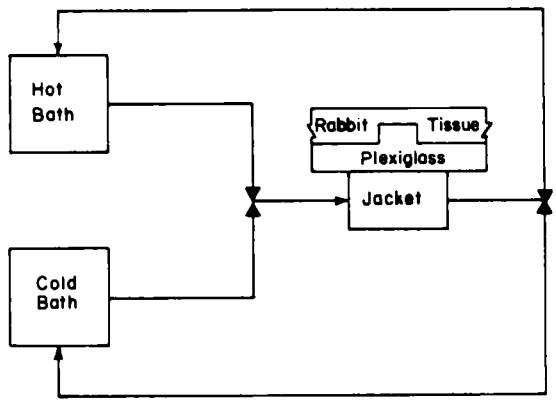

Chart 2. Block diagram of hyperthermia water bath system. A water jacket is placed beneath the ear chamber. Changes in the temperature of the jacket can be obtained by simultaneously turning the 2 valves which control the flow from either of 2 circulating water baths set at different temperatures.

Table 1
Experimental results

Temperature	Tissue age[a]	Vessel no.	Q_m[b]	t_m (min)	t_s (min)
Normal tissue					
40.4°	93	1	1.0	2	
		2	1.6	2	
41.3°	47	1	2.2	11	
		2	1.9	10	
		3	1.0	11	
43.3°	69	1	1.7	11	
		2	1.6	10	
44.3°	113	1	2.5	20	
45.7°	37	1	6.8	50	
		2	2.5	41	
46.5°	122	1	2.0	14	
47.1°	100	1	3.3	10	50
		2	1.5	10	42
		3	2.0	8	45
		4	3.2	13	42
47.4°	35	1	2.9	10	33
		2	2.1	5	32
49.0°	40	1	2.3	8	12
49.7°	40	1	1.1	5	22
52.4°	57	1	1.5	5	14
Neoplastic tissue					
40.1°	10[c]	1	1.0	0	
		2	1.0	0	
40.2°	8[c]	1	1.0	8	
		2	1.5	31	
41.2°	17[c]	1	1.1	11	
41.2°	9[c]	1	1.1	9	51
		2	1.0	7	23
42.2°	7[c]	1	1.2	20	
42.5°	9[c]	1	2.0	3	47
43.8°	9[c]	1	2.1	25	38
		2	1.0	28	42
44.8°	24[c]	1	1.3	8	23
		2	1.0	22	23

[a] Number of days since the chamber implant.
[b] Q_m, maximum blood flow rate measured during hyperthermia relative to maximum prehyperthermia blood flow rate; t_m, time after the onset of hyperthermia when the flow rate was maximum; t_s, time required for vascular stasis.
[c] Number of days since the VX2 implant.

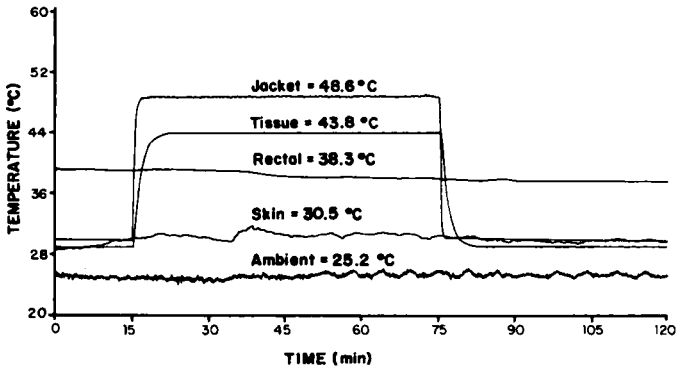

Chart 3. Hyperthermia temperature *versus* time profiles. Typical profiles of ambient, skin, rectal, and water jacket temperatures during hyperthermia experiments are shown. A 15-min control period is followed by a 60-min heating period, which is then followed by a 45-min period at the preheating control temperature. The average temperature of tissue in the chamber is calculated from the measured ambient and jacket temperatures.

vascular stasis (t_s). Both the normalized peak blood flow rate (Q_m) and the time required for the blood flow to reach its peak value (t_m) appeared to be bimodal functions of temperature; *i.e.*, 40–45.7°, these 2 parameters increased with temperature and, above 45.7° they decreased with temperature (Table 1; Chart 4).

Neoplastic tissue blood flow exhibited similar trends, but was

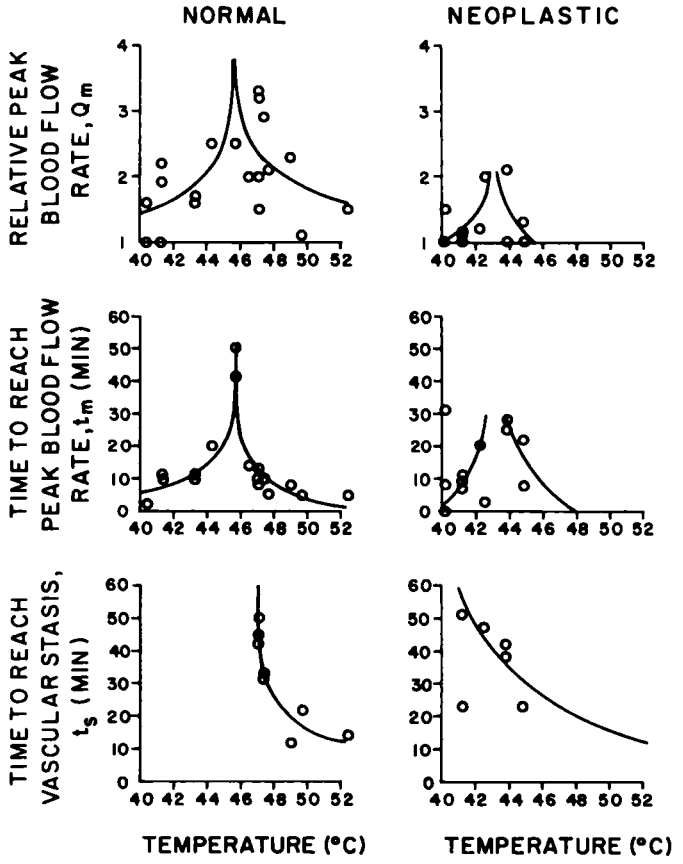

Chart 4. Normal *versus* neoplastic experimental results. The combined on- and off-line hyperthermia experimental results (see Table 1) show normal *versus* neoplastic differential response. Three factors are considered: (*a*) relative increase in blood flow capacity (Q_m) which represents the amount by which a blood vessel is able to increase its maximum flow rate during hyperthermia relative to its maximum flow rate during normothermia; (*b*) time required for the flow to reach its maximum value after the onset of heating (t_m); and (*c*) time required for the onset of vascular stasis (t_s). *Lines*, least-square fits to the data (note that the *point* indicating Q_m = 6.8 for normal tissue at 45.7° is not shown here because it is out of scale; see Table 1).

more sensitive to the level and duration of heating. Blood flow increased only slightly during the onset of heating and did not remain elevated for very long. Stasis occurred at much lower temperatures than in normal tissues. At approximately 40–43°, the normalized peak flow rate (Q_m) and the time required for the blood flow to reach its peak value (t_m) increased with temperature. Above 43°, both of these parameters decreased with temperature. Also, the magnitude of peak flow was less than that in normal tissues (Table 1; Chart 4). The normal tissue was able to increase its blood flow rate up to 6.8-fold (at 45.7°), while the neoplastic tissue was only able to increase it by a factor of 2.1 (at 43.8°).

The response of multiple vessels in the same rabbit ear chamber is also summarized in Table 1 in terms of the 3 parameters discussed above: Q_m, t_m, and t_s. Although the general trends were similar, there was some degree of variability among vessels. For example, while vessels 1, 2, 3, and 4 at 47.1° in normal tissue reached peak flow in approximately the same time (10, 10, 8, and 13 min), and reached vascular stasis in about the same time (50, 42, 45, and 42 min), they differed in the magnitude of peak blood flow rates (3.3, 1.5, 2.0, and 3.2).

The RBC velocity profiles in both normal and neoplastic tissues exhibited the same trends as the blood flow rate profiles dis-

cussed above. RBC velocity and blood flow were intermittent in tumors before, during, and after the heating step. In normal tissues, the vessel diameter increased up to 50% during moderate heating but decreased slightly at higher temperatures (>47°). Vessel diameter in neoplastic tissue generally did not increase; in fact, it even decreased slightly at moderate temperatures. WBCs sticking to the vessel walls during hyperthermia decreased the "functional" diameter further.

In normal tissues heated up to 46° and tumor tissues heated up to 41°, blood flow returned to the prehyperthermia level in about 45 min after heating was suspended. In normal tissues heated at higher temperatures, blood flow did not always return to the base-line level in 45 min postheating. In most tumors, the vascular statis was permanent due to hyperthermia treatment at temperatures greater than 42°. Observations of tissues at 24 hr after heating up to 45–46° for 1 hr showed that normal tissue microcirculation returned to control level, while the neoplastic tissue microcirculation remained impaired.

DISCUSSION

The objective of this work was to quantify responses of normal (mature granulation) tissue and tumor (VX2 carcinoma) microcirculation to local hyperthermia treatment at temperatures 40–52° for 1 hr. Blood flow in granulation tissue increased significantly with temperature, but vascular stasis occurred at higher temperatures and/or longer durations of heating. Tumors could not increase their blood flow as much, and stasis occurred at lower levels of hyperthermia. The observed flow trends in normal and neoplastic tissues were characterized in terms of 3 parameters: (*a*) relative increase in blood flow capacity (Q_m), which is the ratio of the maximum blood flow rate during hyperthermia with respect to the maximum blood flow rate during normothermia; (*b*) time required for the flow to reach its maximum value after the onset of heating (t_m); and (*c*) time required for the onset of vascular stasis (t_s). Chart 5 compares the best-fit curves to these parameters for granulation and neoplastic tissues (the details of curve fitting are given in Ref. 9). Note that both Q_m and t_m appear to be bimodal functions of temperature; *i.e.*, both of these parameters increase up to a certain critical temperature, and then decrease at higher temperatures. This critical temperature was approximately 45.7° in granulation tissue, and 43° in VX2 carcinoma (note that the empirical equations used to fit the Q_m and t_m data are not adequate for describing the behavior around the critical temperatures). The granulation tissue required temperatures greater than 47° to cause vascular stasis in less than 1 hr, while stasis occurred in tumors in the same time frame at temperatures greater than 41°.

In what follows, these findings are compared with the published data for other normal and neoplastic tissues (19, 37, 42), and a theoretical framework is presented which relates various mechanisms of the blood flow modifications due to hyperthermia (9).

Effect of Hyperthermia on Normal Tissue Blood Flow Rate. Published data on the effects of hyperthermia on blood flow rates in various normal tissues are summarized in Table 2, in terms of the 2 parameters, Q_m and t_m. All these data show that the peak blood flow rate (Q_m) of skin and muscle in various animals increases with increasing thermal dose (up to 45° for 2 hr in rats, and 47° for 1 hr in dogs). Our results show a similar increase in Q_m up to a critical temperature of 45.7° in mature

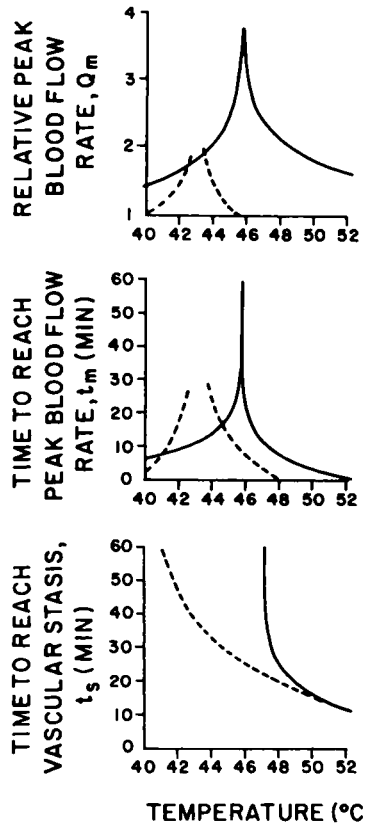

Chart 5. Best fits to the experimental results. Chart 4 is redrawn here to better show the differential response of normal and tumor microcirculation in terms of the least-squares fits to the experimental results (9). Normal tissue, ——; neoplastic tissue, - - - -.

granulation tissue. However, unlike these findings, our investigation shows that Q_m decreases with increasing temperature beyond 45.7° (Table 1; Chart 4). Similarly, all of the published data on muscle and skin suggest a decrease in the time required for the blood flow to peak (t_m) with increasing thermal dose. Our data show this behavior beyond 45.7°. However, at lower temperatures, our data indicate an increase in t_m with temperature. To our knowledge, this bimodal response of Q_m and t_m has not been demonstrated prior to this work. It is also possible that the granulation tissue or tumors grown in the chambers are so thin that the tissues are quickly heated and, as a result, maximum blood flow is achieved in a short time at low temperatures. Such a rapid rise in temperature may not be possible in organs or tissues in natural site.

Similar to our results, several investigators observed a decline (with increasing time of heating) in blood flow rate after it reached its peak value (6, 34–36). However, with the exception of the findings of Dewhirst *et al.* (5), none of the investigators reported complete vascular stasis in normal tissues. Note that the thermal doses used in the present investigation to cause vascular stasis were higher than those used by other investigators. Normal tissues adjacent to a tumor may, however, be subjected to such high thermal doses during local hyperthermia (39).

A limited number of investigators have measured vasodilation in normal tissues during hyperthermia. Asano *et al.* (2) reported an increase in diameter of vessels of granulation tissue grown in the rabbit ear chamber. Unlike the present investigation, their studies were conducted only at 40°. Song *et al.* (35) found a 3-

Table 2
Effect of hyperthermia on normal tissue blood flow rate

Host	Treatment (°C × time)	Tissue	Q_m[a] (ratio)	t_m (min)	Comments	Refs.
Mouse	42.5° × 1 hr	Skin	3	60		38
Rat (Sprague-Dawley)	42° × 2 hr	Skin	2	120	Continuous increase in blood flow rate during treatment	34–36
		Muscle	3.5	120		
	43° × 2 hr	Skin	6	120		
		Muscle	3.5	120		
	44° × 2 hr	Skin	12	30	Flow decreased after reaching maximum	
		Muscle	6	60	Flow did not decrease	
	45° × 2 hr	Skin	15	15	Flow decreased to 0.5 times control value at the end of treatment	
		Muscle	9	30	Flow returned to control value at the end of treatment	
Rat (Fischer)	43.5° × 1 hr	Skin	9	60		27
		Muscle	7–8	60		
Rat (Wistar)	42° × 3 hr	Skin	20	60	Flow rate declined after 1 hr of heating	6
		Muscle	10	60		
Rat	Continuous increase in temperature 38–46°	Mature granulation tissue	2.2–10		Vascular stasis at temperatures as low as 40° at 1°/min heating rate	5
Rabbit	40°	Mature granulation tissue			Increase in vessel diameter and RBC velocity	2
	40° × 1 hr to 52° × 1 hr	See Table 1 and Chart 4	See Table 1 and Chart 4	See Table 1 and Chart 4		This work
Dog	43° × 1 hr	Muscle	2–3	40	Thermal clearance method used to measure blood flow rate. Peak blood flow rate increased to 40, 59, and 183 ml/100 g/min at 43, 45, and 47°, respectively.	23
	45° × 1 hr	Muscle		24		
	47° × 1 hr	Muscle		16		

[a] Q_m, maximum blood flow rate measured during hyperthermia relative to maximum prehyperthermia blood flow rate; t_m, time after the onset of hyperthermia when the flow rate was maximum.

fold increase in vascular space of the skin and 1.5-fold in that of the muscle of Sprague-Dawley rats subjected to 43° hyperthermia for 1 hr. Our results on vasodilation up to 50% agree with these values.

Effect of Hyperthermia on Tumor Blood Flow Rate. Summarized in Table 3 are the published tumor data of various investigators in terms of 4 parameters: Q_m; t_m; $t_{1/2}$, time required for 50% blood flow inhibition; and t_s, time required for complete vascular stasis. Despite the variability in these parameters due to tumor type, size, location, host, method of blood flow measurement, and method of inducing hyperthermia, tumor microcirculation is, in general, more sensitive to heat than is normal microcirculation. Most tumors exhibit a decrease in blood flow and even complete vascular stasis at moderate hyperthermia. Walker 256 carcinoma is the most resistant tumor in this respect. Times required for 50% blood flow inhibition ($t_{1/2}$) and for complete inhibition (t_s) decrease with increasing temperature, similar to our results.

Unlike normal tissues, not all tumors are able to increase their blood flow rate during hyperthermia. Moreover, the increase in tumor blood flow rate is smaller ($Q_m < 2.5$). Unlike normal tissues, the dependence of Q_m and t_m on temperature has not been studied extensively. Bicher *et al.* (3, 4) reported that the tumor blood flow increased up to 40–41°, and then decreased; however, in their experiments, the tumor temperature was not held constant for 1 hr, but increased continuously 32–45° in 30 to 40 min. As a result, the bimodal response observed in the present investigation can not be compared with the available data.

Mechanisms of Blood Flow Regulation. Local perfusion rate in a tissue is a function of the number of perfused blood vessels per unit volume (n) and blood flow rate through each vessel (Q). The simplest equation that relates flow rate (Q) to the pressure drop (ΔP), vessel diameter (D), vessel length (L), and blood viscosity (μ) is the well known Hagen-Poiseuille law:

$$Q = \frac{\pi \Delta P D^4}{128 \mu L}$$

Therefore, blood perfusion can be increased by increasing the number of functional vessels, blood pressure, or vessel diameter, and/or by decreasing the blood viscosity or vessel length. The various neural, humoral, metabolic, and myogenic mechanisms that regulate each of these parameters are not known precisely. Based on the experimental observations of other investigators and our own findings, we propose the framework shown in Chart 6 to explain the differential response of normal and tumor microcirculation to hyperthermia. In the discussion to follow, note that some mechanisms increase blood flow while others decrease it. The overall response is the net result of these opposing factors. Also note that differences exist in these mechanisms between normal and tumor tissues.

A rise in tissue temperature may increase the cardiac output, raise the systemic blood pressure, or redistribute the available flow to the heated region. The net result is an increased pressure drop (ΔP) across the vasculature in the heated region, which serves to increase the blood flow. We refer to this as the remote response.

The local response in normal tissues is to actively dilate the heated vessels by relaxing the vascular smooth muscles in order to increase the flow. Neoplastic tissues lack this local control mechanism because their vessels are devoid of smooth muscle

Table 3

Effect of hyperthermia on tumor blood flow rate
Data are updated from the work of Jain (19).

Tumor (host)	Treatment (°C × time)	Q_m[a] (ratio)	t_m (min)	$t_{1/2}$ (min)	t_s (min)	Comments	Refs.
Mammary carcinoma (C3H mice)	Continuous increase in temperature from 32–45° in 30–40 min	2.4				Blood flow increased up to 40–41°, and then decreased	3, 4
Transplants of spontaneous tumors (C3H mice)	Continuous increase in temperature, 37.5–44°					No significant change	30
Ependymoblastoma (C57BL/6 mice)	40° × 1.5 hr		30	60–75		Not all tumors responded uniformly	40
	42° × 1 hr		30	60			
	45° × 1 hr			15			
Mammary carcinoma (mice)	41°					Blood flow increased 38–41°, and then decreased	20, 21
SCK carcinoma	41–44° × 30 min				30	Vascular space was considered a measure of blood flow	36
SAFA tumor (mice)	42.5° × 1 hr		30			Blood flow returned to control level at the end of heating and decreased significantly at 1 to 2 days after treatment	38
Walker 256 carcinoma (Sprague-Dawley rats)	40–43° × 1 hr					No significant change during hyperthermia	17
	43° × 0.5 hr					No significant change 18, 48, and 72 hr after heating	18
	43° × 1 hr					No significant change	33–36
	45° × 1 hr					Slight increase in blood flow in small tumors	
	45° × 1 hr					Significant decrease at 3 hr after hyperthermia	
Yoshida sarcoma (Wistar rats)	42° × 1 hr					Blood flow stopped 1 hr after heating and recovered to control value 12 hr after heating	6
	42° × 3 hr				3 hr		
DS-carcinoma sarcoma (Sprague-Dawley rats)	39.5° × 20 min	1.23	20				42
	43–44° × 20 min	1.05–2	20–15		20	Blood flow increased in 60% of tumors	41
	42.5–43.5° × 100 min				100	Blood flow became one-tenth of control level	1
Mammary carcinoma 13726A (Fischer rat)	43.5° × 1 hr	1.1	1 hr			Blood flow decreased significantly 1–24 hr after heating	27
BA-1112 rhabdomyosarcoma (WAG-Rij rats)	41° × 40			40			13
	42° × 40			30			
	43° × 40				40		
	44° × 40				10		
Sandwich tumor (rat)	42–43° × 3 hr				140 ± 60		29
	Increased ambient temperature 25–35°	2				Whole-body hyperthermia	
	Local 40–42° × 60				60	Local hyperthermia	14
Sandwich tumor (rat)						44.5–45° RBC velocity dropped to zero	5
Squamous cell carcinoma (hamster cheek pouch)	41° × 30			15		Immediate vasoconstriction followed by vasodilation	11
	43° × 30				30		
	45° × 30				20–25	Fractionated heat at 42° with 1-hr interval led to vascular stasis	12
VX2 (rabbit)	40–45° × 1 hr	See Table 1 and Chart 4	See Table 1 and Chart 4	See Table 1 and Chart 4			This work

[a] Q_m, maximum blood flow rate measured during hyperthermia relative to maximum prehyperthermia blood flow rate; t_m, time after the onset of hyperthermia when the flow rate was maximum; $t_{1/2}$, time required for 50% blood flow reduction; t_s, time required for vascular stasis.

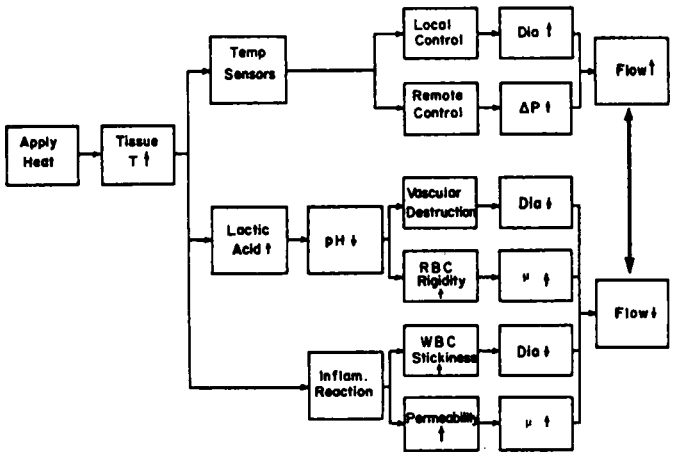

Chart 6. Theoretical framework for blood flow regulation during hyperthermia. Physiological mechanisms are thought to be responsible for regulating blood flow during hyperthermia. Differences exist in these mechanisms between normal and neoplastic tissues. Note that some factors increase flow, while others decrease flow. The overall response is the result of these opposing factors.

cells. Our experimental observations on vessel diameter (D) in normal and neoplastic tissues corroborate this hypothesis.

The most important factor governing the differential response of normal *versus* neoplastic tissue is the selective accumulation of lactic acid in neoplastic tissues (4, 6, 19, 37). The resulting decrease in pH causes an increase in RBC membrane rigidity which increases the viscosity of blood (μ) (7). Song *et al.* (36) reported that pH does not change appreciably during hyperthermia in normal tissues. However, there is evidence that pH may drop up to one full unit during hyperthermia in tumors (4). Lower pH also renders the endothelial and parenchyma cells more sensitive to heat. The resulting swelling of endothelial cells and tissue parenchyma reduces the effective diameter of the blood vessels, causing a flow reduction (11, 12).

The inflammatory reaction to thermal injury is more severe in tumors than in normal tissues. As a result, WBC stick to the vessel walls and decrease their "functional" diameter. Ferguson *et al.* (16) reported that postcapillary resistance increased by as much as 300-fold due to leukocyte adherence in venules of rat skeletal muscle following thermal injury. Eriksson and Robson (15) found that 16 WBC stuck in a venule approximately 25 μm in diameter and 200 μm long at 1 hr after thermal injury. Other investigators using transparent chamber preparations (5, 11, 12, 14, 29), including ourselves, have observed significant increases in WBC flux in tumors during hyperthermia, giving credence to this hypothesis. Finally, an increase in the microvascular permeability in tissues due to thermal injury may increase the blood viscosity by increasing the hematocrit and protein concentration due to plasma leakage (7, 26, 28) or by hyperfibrinogenemia (31, 32, 43).

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REFERENCES

1. Ardenne, M. V., and Reitnauer, P. G. Selective inhibition of microcirculation in tumor tissue. Naturwissenschaften, *67:* 154–158, 1980.
2. Asano, M., Ohkubo, C., and Sawanobori, K. Studies on the local regulatory mechanism of microcirculation within the rabbit ear chamber (8). Comparison of microcirculatory effects of cutaneous warming and adrenergic alpha-receptor blockade with phentolamine. J. Physiol. Soc. Jpn. *41:* 8–9, 1979.
3. Bicher, H. I., Hetzel, F. W., Sandhu, T. S., Frinak, S., Vaupel, P., O'Hara, M. D., and O'Brien, T. Effects of hyperthermia on normal and tumor microenvironment. Radiology, *137:* 523–530, 1980.
4. Bicher, H. I., and Matagvaria, N. Circulatory responses of malignant tumors during hyperthermia. Microvasc. Res., *21:* 19–26, 1981.
5. Dewhirst, M. W., Gross, J. F., and Kundrat, M. Effect of rate of heating on tumor and normal tissue microcirculation (Abstract No. Ac-4). *In:* 31st Annual Meeting of the Radiation Research Society, February 27 to March 3, p. 7, 1983.
6. Dickson, J. A., and Calderwood, S. K. Temperature range and selective sensitivity of tumors to hyperthermia: a critical review. Ann. N. Y. Acad. Sci., *335:* 180–205, 1980.
7. Dintenfass, L. Viscosity of blood at high haematocrits measured in microcapillary (parallel-plate) viscometers of r = 3–30 microns. *In:* A. L. Copley (ed.), Hemorheology, pp. 197–209. Oxford: Pergamon Press, 1968.
8. Dudar, T. E. Flow Modifications in Normal and Neoplastic Tissue During Growth and Hyperthermia. Ph.D. Dissertation, Carnegie-Mellon University, 1982.
9. Dudar, T. E., and Jain, R. K. Mathematical models for microcirculatory flow modifications in normal and neoplastic tissues during hyperthermia. American Institute of Chemical Engineers Symposium Series, *79:* 153–162, 1983.
10. Dudar, T. E., and Jain, R. K. Microcirculatory flow changes during tissue growth. Microvasc. Res., *25:* 1–21, 1983.
11. Eddy, H. A. Alterations in tumor microvasculature during hyperthermia. Radiology, *137:* 515–521, 1980.
12. Eddy, H. A., Sutherland, R. M., and Chmielewski, G. Tumor microcirculation response: hyperthermia and radiation combination. Natl. Cancer Inst. Monogr., *61:* 225–229, 1982.
13. Emami, B., Nussbaum, G., TenHaken, R. K., Hahn, N., and Hughes, W. Effects of local hyperthermia on tumor microcirculation: blood flow rate studies (abstract). Int. J. Radiat. Oncol. Biol. Phys., *5*(Suppl 2): 175, 1979.
14. Endrich, B., Zweifach, B. W., Reinhold, H. S., and Intaglietta, M. Quantitative studies of microcirculatory function in malignant tissue. Influence of temperature on microvascular hemodynamics during the early growth of the BA 1112 rat sarcoma. Int. J. Radiat. Oncol. Biol. Phys., *5:* 2021–2030, 1980.
15. Eriksson, E., and Robson, M. C. New pathophysiologic mechanism explaining the generalized edema after a major burn. Surg. Forum, *28:* 540–543, 1977.
16. Ferguson, M. K., Seifert, F. C., and Replogle, R. L. Leukocyte adherence in venules of rat skeletal muscle following thermal injury. Microvasc. Res., *24:* 34–41, 1982.
17. Gullino, P. M. Influence of blood supply on thermal properties and metabolism of mammary carcinomas. Ann. N. Y. Acad. Sci., *335:* 1–21, 1980.
18. Gullino, P. M., Yi, P. N., and Grantham, F. H. Relationship between temperature and blood supply or consumption of oxygen and glucose by rat mammary carcinomas. J. Natl. Cancer Inst., *60:* 835–847, 1978.
19. Jain, R. K. Bioheat transfer: mathematical models of thermal systems. *In:* F. Kristian Storm (ed.), Hyperthermia in Cancer Therapy, pp. 9–46. Boston, MA: G. K. Hall Medical Publisher, 1983.
20. Johnson, R. J. R. Effect of hyperthermia on tumor blood flow. *In:* J. E. Robinson and M. J. Wizenberg (eds.), Proceedings of the International Symposium on Cancer Therapy by Hyperthermia and Radiation, pp. 154–155. Chevy Chase, MD: American College of Radiology, 1976.
21. Johnson, R. J. R. Radiation and hyperthermia. *In:* C. Streffer, D. van Beuningen, F. Dietzel, E. Rottinger, J. E. Robinson, E. Scherer, S. Seeber, and K. R. Trott (eds.), Proceedings of the Second International Symposium on Cancer Therapy by Hyperthermia and Radiation, pp. 89–95. Baltimore: Urban and Schwarzenberg, 1978.
22. Lipowsky, H. H., and Zweifach, B. W. Application of the "two-slit" photometric technique to the measurement of microvascular volumetric flow rates. Microvasc. Res., *15:* 93–101, 1978.
23. Milligan, A. J., and Panjehpour, M. Mathematical predictions of tumor and normal tissue blood flow during hyperthermia (Abstract No. Ab-2). Thirty-first Annual Meeting of the Radiation Research Society, p. 4. San Antonio, TX, February 27 to March 3, 1983.
24. Nugent, L. J., and Jain, R. K. Monitoring transport in the rabbit ear chamber. Microvasc. Res., *24:* 204–209, 1982.
25. Nugent, L. J., and Jain, R. K. Extravascular diffusion in normal and neoplastic tissues. Cancer Res., *44:* 238–244, 1984.
26. Papenfuss, H. D., and Gross, J. F. The interaction between fluid exchange and blood viscosity in single capillaries. American Institute of Chemical Engineers Symposium Series, *74:* 10–18, 1978.
27. Rappaport, D. S., and Song, C. W. Blood flow and intravascular volume of mammary adenocarcinoma 13726A and normal tissues of the rat during and following hyperthermia. Int. J. Radiat. Oncol. Biol. Phys., in press, 1983.
28. Reemtsma, K., and Creech, O., Jr. Viscosity studies of blood, plasma, and plasma substitutes. J. Thorac. Cardiovasc. Surg., *44:* 674–680, 1962.
29. Reinhold, H. S., Blachiewicz, B., and Berg-Blok, A. Decrease in tumor microcirculation during hyperthermia. *In:* C. Streffer, D. van Beuninger, F. Dietzel, E. Rottinger, J. E. Robinson, E. Scherer, S. Seeber, and K. R. Trott (eds.), Proceedings of the Second International Symposium on Cancer Therapy by

Hyperthermia and Radiation, pp. 231–232. Baltimore: Urban and Schwartzenberg, 1978.
30. Robinson, J. E., McCullough, D., and McCready, W. A. Blood perfusion of murine tumor at normal and hyperthermal temperatures. J. Natl. Cancer Inst. Monogr., *61:* 211–215, 1982.
31. Schoen, R. E., Wells, C. H., and Kolmen, S. N. Viscometric and microcirculatory observations following flame injury. J. Trauma, *11:* 619–624, 1971.
32. Shtykhno, Y. M., and Udovichenko, V. I. Comparative study of intravital microcirculation and blood rheology in rats after burns of different degrees of severity. Byull. Eksp. Biol. Med., *88:* 284–286, 1979.
33. Song, C. W. Effect of hyperthermia on vascular functions for normal tissues and experimental tumors: brief communication. J. Natl. Cancer Inst., *60:* 711–713, 1978.
34. Song, C. W., Kang, M. S., Rhee, J. G., and Levitt, S. H. Vascular damage and delayed cell death in tumors after hyperthermia. Br. J. Cancer, *41:* 309–312, 1980.
35. Song, C. W., Kang, M. S., Rhee, J. G., and Levitt, S. H. Effect of hyperthermia on vascular function in normal and neoplastic tissue. Ann. N. Y. Acad. Sci., *335:* 35–47, 1980.
36. Song, C. W., Kang, M. S., Rhee, J. G., and Levitt, S. H. The effect of hyperthermia on vascular function, pH, and cell survival. Radiology, *137:* 795–803, 1980.
37. Song, C. W., Lokshina, Aa., Rhee, J. G., Patten, M., and Levitt, S. H. Implication of blood flow in hyperthermic treatment of tumors. IEEE (Inst. Electr. Electron. Eng.) Trans. Biomed. Eng., in press, 1983.
38. Stewart, T., and Begg, A. Blood flow changes in transplanted mouse tumors and skin after mild hyperthermia. Br. J. Radiol., in press, 1983.
39. Storm, F. K., Harrison, W. H., Elliott, R. S., and Morton, D. L. Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials. Cancer Res., *39:* 2245–2251, 1979.
40. Sutton, C. H. Minipaper presented at the NYAS Conference on Thermal Characteristics of Tumors: Applications in Detection and Treatment. Ann. N. Y. Acad. Sci., *335:* 45–47, 1980.
41. Vaupel, P. Heat susceptibility of tumor blood flow (abstract no. Dg-1). Thirty-First Annual Meeting of the Radiation Research Society, p. 68. San Antonio, TX, February 27 to March 3, 1983.
42. Vaupel, P., Ostheimer, K., and Muller-Klieser, W. Circulatory and metabolic responses of malignant tumors during localized hyperthermia. J. Cancer Res. Clin. Oncol., *98:* 15–29, 1980.
43. Weaver, J. P. A., Evans, A., and Walder, D. N. The effect of increased fibrinogen content on the viscosity of blood. Clin. Sci., *36:* 1–10, 1969.
44. Zawicki, D. F., Jain, R. K., Schmid-Schoenbein, G. W., and Chien, S. Dynamics of neovascularization in normal tissue. Microvasc. Res., *21:* 37–47, 1981.