Experimental and Clinical Aspects of Hyperthermia Applied to the Treatment of Cancer with Special Reference to the Role of Ultrasonic and Microwave Heating

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I. Introduction

Temperatures above 37°C have been used to treat cancer in man for many years. Indeed treatment by means of a hot iron was used at the time of Hippocrates. More recently, less crude techniques have been tried by means of

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which the temperature of a tumor has been raised by only a few degrees (41°–45°C). Even at these temperatures a lethal effect on cells has been observed.

Hyperthermia has been induced by pyrogenic toxins or a variety of external heating methods. The earliest clinical report in recent years was published by Busch in 1866. It was the clinical observation of a histologically confirmed case of sarcoma of the face that disappeared completely after two attacks of erysipelas associated with a high fever. Further similar clinical observations led to the next step in which cancer patients were deliberately and repeatedly infected with erysipelas (Coley, 1983) or treated by local heat applications (Westermark, 1898). Thus Coley prepared a toxin of bacterial products that was widely used, and the collective results have been analyzed retrospectively by Nauts et al. (1946). In a further review, Nauts et al. (1953) include 30 cases of histologically confirmed inoperable malignancies treated in the period 1893–1933. There was a ten-year survival of 80%, with patients alive up to 50 years after treatment.

The application of local heat was first reported by Westermark (1898), who treated carcinoma of the cervix by douching with hot water. Soon, other methods of external heating were applied, such as hot air, rf diathermy, and radiant heat; numerous papers on this subject were published during the first half of this century.

Evidence has been obtained that malignant cells may show an increased differential sensitivity when compared with the response of untransformed cells. Hyperthermia has also been shown to potentiate the effect of ionizing radiation (x-rays) and of some cancer chemotherapeutic agents.

Warren (1935) wrote a classic paper in which he reported on 32 cases of hopeless malignant disease, with widespread metastases, that had been treated by artificial fever of 41.5°C, induced by diathermy or radiant energy. One young woman with an embryoma of the ovary was subjected to several treatments, one of them for 21 consecutive hours. A few patients received x-irradiation immediately after the fever treatment. In most of the cases the treatment brought immediate improvement in the general condition and shrinkage of the tumor masses. Although there was an increase in the expected length of life, Warren stresses in his conclusions that no results approaching cure had yet been obtained by this procedure.

Later work on the possibility of combining hyperthermia and x-irradiation was reviewed by Selawry and colleagues (Selawry et al., 1958). Since that time there has been an interest in the role of hyperthermia for cancer therapy, which has recently become more intense.

This review will discuss limited aspects of the subject, since there have been several extensive recent reviews (Cavaliere et al., 1967; Overgaard and Overgaard, 1972a; Block and Zubrod, 1973; Suit and Shwayder, 1974; Dietzel, 1975). Little detail concerning in vitro work will be mentioned; these are discussed in

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another chapter of this volume (Thrall et al.). The techniques available for inducing hyperthermia will be discussed with particular reference to ultrasonic and microwave sources of energy. The factors that affect the response to heat such as the magnitude of temperature and duration, and other modifying factors such as pH will be mentioned with a brief discussion of their possible mechanisms of action. Combined modality treatment with x-irradiation and drugs will also be considered.

Although there has been much laboratory work carried out, few clinical reports of the recent use of hyperthermia are available. These will be discussed in subsequent sections of this paper. Some of these clinical results are difficult to assess because of the multiplicity of therapeutic modalities used (von Ardenne, 1967; Issels, 1970) and these will not be mentioned further.

II. Methods of Inducing Hyperthermia

A. Introduction

The induction of hyperthermia may be achieved by a variety of techniques. The temperature that may be used will depend, in practice, on the need to exceed 41°C to obtain a significant therapeutic effect and the upper limit of tolerance of normal tissues (around 45°C). The magnitude of the effect is also directly related to the duration of exposure to that temperature. Whole-body hyperthermia is limited to an upper temperature of 42°C in man, but under appropriate conditions this can be maintained for many hours (Warren, 1935; Pettigrew et al., 1974).

More exciting, and probably offering a wider range of therapeutic possibilities, is the heating of a selected region, limb, or organ, by localized hyperthermia. The temperature tolerance limit of most of the normal tissues is within the range 44°C–45°C for a few hours of exposure. There are some reports that normal and tumor tissues are similarly sensitive to heat (Love et al., 1970; Auersperg, 1966). However, most of the evidence would suggest that tumor cells are specifically more sensitive to heat (Schreck, 1966; Bender and Schramm, 1966; Cavaliere et al., 1967; Muckle and Dickson, 1971; Kim et al., 1974; Stehlin et al., 1975). This acquisition of heat sensitivity seems to be an early finding after in vitro induced malignant transformation (Chen and Heidelberger, 1969). These conclusions are derived from in vitro work carried out at temperatures higher than 42°C and may not be applicable to whole-body hyperthermia.

Dickson and Muckle (1972) compared, in the same animal system, whole-body heating with local tumor heating. In both cases the intratumor temperature was maintained at 42°C. Local heat cured 50% of the animals, while with general heat only 7% were cured.

The method for producing in vitro, animal and human whole-body or

Heating method	In vitro		Animal
			Whole-body hyperthermia
Radiant heat (visible and infrared)	Auersperg	(1966)	Dickson and Muckle (1972)
			Martin and Schloerb (1964)
			Sapozink et al. (1973)
			Birenbaum et al. (1975)[a]
Hot water heating	Robinson and Wizenberg (1974)		Woeber (1965)
	Auersperg	(1966)	Schmidt et al. (1968)
	Har-Kedar	(1976)	von Ardenne (1967)
	Ben-Hur et al.	(1972)	
	Giovanella et al.	(1973)	
Hot air heating	Johnson and Pavelec	(1973)	Klärner and Klärner (1958)
	Woodhall et al.	(1960)	Burger and Fuhrman (1964)[a]
	Chen and Heidelberger	(1969)	Suzuki (1967)
	Dickson and Shah	(1972)	
Warmed fluid perfusion of limb/organ			
Inoculation of pyrogenic toxins/bacteria			Donaldson et al. (1968)
			O'Malley et al. (1962)
			Lipton and Fossler (1974)[a]
Shortwave diathermy	Häkkinen et al.	(1975)	Sugiura (1941)
			Woeber (1965)
Ultrasound	Todd and Schroy	(1974)	Southam et al. (1953)
	Bleaney et al.	(1972)	Woeber (1965)
	Kremkau et al.	(1974)	Fujita and Sakuma (1974)[a]
	Loch et al.	(1971)	
Microwaves	Moressi	(1964)	Dietzel (1975)
	Yao and Jiles	(1969)	Lappenbusch and Gillespie (1973)[a]
			Birenbaum et al. (1975)[a]
			Schrot and Hawkins (1974)[a]
Miscellaneous			

[a]Physiological studies on healthy animals or human volunteers.
[b]Accidental exposure (nonfatal).

TABLE I: Methods of Inducing Hyperthermia

	Animal experiments		Clinical use	
	Whole-body hyperthermia	Localized hyperthermia	Whole-body hyperthermia	Localized hyperthermia
	Dickson and Muckle (1972) Martin and Schloerb (1964) Sapozink et al. (1973) Birenbaum et al. (1975)[a]	Hahn et al. (1974) Allen (1955)	Warren (1935) Wüst et al. (1975)	Lehmann et al. (1966)[a]
	Woeber (1965) Schmidt et al. (1968) von Ardenne (1967)	Dickson and Muckle (1972) Crile (1963)	Crile (1962) Suryanarayan (1966)	Crile (1962) Heyn and Kurz (1967)
	Klärner and Klärner (1958) Burger and Fuhrman (1964)[a] Suzuki (1967)	Allen (1955) Yerushalmi and Har-Kedar (1974)	Sterling (1975) Pettigrew et al. (1974)	
		Shingleton et al. (1962) Cockett et al. (1967) Cavaliere et al. (1967) Rochlin et al. (1961) Suzuki (1967)		Woodhall et al. (1960) Hall et al. (1974) Cockett et al. (1967) Stehlin et al. (1975) Cavaliere et al. (1967) Shingleton et al. (1961)
	Donaldson et al. (1968) O'Malley et al. (1962) Lipton and Fossler (1974)[a]		Nauts et al. (1946, 1953) Review Miller and Nicholson (1971) Review Chandler et al. (1965) Johnston and Novales (1962)	
	Sugiura (1941) Woeber (1965)	Overgaard and Overgaard (1972a) Dittmar (1949) Shingleton et al. (1962)	Wüst et al. (1975)	Fuchs (1952) Birkner and Wachsmann (1949) Korb (1939)
	Southam et al. (1953) Woeber (1965) Fujita and Sakuma (1974)[a]	Saigusa (1973) Southam et al. (1953) Woeber (1965) Linke et al. (1973)[a] Lehmann and Krusen (1955) Crile (1962) Cater et al. (1964) Copeland and Michaelson (1970) Zimmer et al. (1971) J.C. Lin et al. (1973)[a] Gessler et al. (1950)		Horvath (1944) Woeber (1965) Heimburger et al. (1974)
	Dietzel (1975) Lappenbusch and Gillespie (1973)[a] Birenbaum et al. (1975)[a] Schrot and Hawkins (1974)[a]		Rosenthal and Beering (1968)[b]	Hartman and Crile (1968) Brenner (1975) Lehman et al. (1966)[a]
		Popovic and Masironi (1966b) (electric heating coil) Gilchrist et al. (1965) (electromagnetic field-hysteresis) Sutton (1971) (surgical diathermy)		Sutton (1971) Crile (1962) (surgical diathermy)

volunteers.

localized hyperthermia are summarized in Table I. These include the use of radiant heat, hot water bath, hot air, ultrasound, microwaves, heated perfusate, and exogenous pyrogens. A few examples of clinical applications of whole-body and localized heating will be briefly described.

B. Whole-Body Heating

The inoculation of pyrogenic toxins such as the Coley's toxin (mixed toxins of *Streptococcus pyogenicus* and *Serratia marcescens*) or of a bacterial polysaccharide from *Serratia marcescens* will induce a rise of temperature in man. The temperature and duration of fever achieved vary, but they are generally less than 41°C maintained for some ho·

Induction of artificial fever by external heating sources enables a higher temperature to be reached and held for a longer time. Warren (1935) introduced his patients into a chamber, heated by six 250-watt bulbs, where they were maintained at 41.5°C without anesthesia for several hours (the longest treatment reported was for 21 hr).

Henderson and Pettigrew (1971) and Pettigrew *et al.* (1974) have used the alveolar surface of the lungs, which has 20 times the area of the skin surface, as a heat exchanger. This procedure is carried out under general anesthesia, with the loss of body heat through sweating prevented by covering the whole skin surface with a hot wax layer. Induction of hyperthermia is achieved quite quickly by heated, dry air, thermostatically controlled and circulated through the respiratory system. When 41.5°C is reached the basic metabolic processes will maintain this high temperature. Overheating is easily avoided by exposure of some skin regions to allow sweating.

The system proposed by Wüst *et al.* (1975) consists of a bed cabin with a double heating system. Shortwave diathermy heats the patient from underneath, while infrared lamps are on the upper side. The patient is sedated and his head is outside the cabin. Temperatures of 40°–40.5°C are reached and maintained for 1 hr.

C. Localized Heating

Hyperthermic limb perfusion has been achieved either by heating the perfused blood to 48°–49°C (Cavaliere *et al.*, 1967), or by adding external heat to a perfusate at 43.3°C (Stehlin *et al.*, 1975). Intratumor temperatures of more than 40°C have been maintained for several hours. In some of the cases reported by Cavaliere *et al.* (1967) the intratumor temperature rose to 44°–45°C.

A combination of whole-body cooling to 30°C and a heated perfusate at 42°C containing cytotoxic drugs was applied to pelvic and abdominal malignancies by Shingleton *et al.* (1961) and Leone *et al.* (1962). It is probable that the

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intratumor temperature was less than 40°. However, the temperature gradient created (±10°C) between the tumor surroundings and other regions of the body could have been of value to protect the healthy tissues, while the drug reached the higher concentration and activity in the tumor region.

Perfusion of the bladder by heated isotonic fluid at 45°C is technically very easy and was well tolerated by most of the patients (Hall et al., 1974). When radiation was added to the heated perfusion treatment (Cockett et al., 1967) a lower intravesical temperature (43°C) should probably be used.

Superficial tumors have been heated by several sources such as ultrasound (Woeber, 1965), microwaves (Brenner, 1975), hot water (Heyn and Kurz, 1967), and diathermy (Korb, 1939).

Localized and selective heating of deep-located tumors by external sources of heat has not yet been achieved. However, the works of J. C. Lin et al. (1973) with microwaves and Heimburger et al. (1974) with ultrasound would suggest that with appropriate focusing the above sources of energy could be useful. These two methods are considered in more detail in subsequent sections.

III. Mechanisms of Action

Many mechanisms have been proposed to explain the effects of hyperthermia on cells in general and also the higher sensitivity of cancer cells. These are based on experiments using different cell or tissue systems treated under a variety of conditions with varying end points. It is therefore dangerous to extrapolate conclusions from these experiments too far, but certain mechanisms of action do seem possible. Thus, effects have been reported on the cell membrane, RNA and DNA synthesis, lysosomes, and cell pH.

The involvement of the cell membrane as the primary target is suggested by some studies. Thus, changes were seen in the ultrastructure of the surface of heated lymphocytes (P. S. Lin et al., 1973). Allan and Norman (1974) have suggested that the presence of an increased biowater content in the tumor cells, as compared with untransformed cells, could be the main reason for the selective heat sensitivity. The possible final result of hyperthermia might be the rupture of the already less structured cell membrane, which then allows its subsequent destruction by the host's natural defenses. Also, the permeability of the cell membrane to adriamycin is increased at 43°C (Hahn et al., 1975).

Heat sensitivity of the nucleolar RNA has been described by Simard and Bernhard (1967). Exposure of normal fibroblasts to 42°C for 15 min produced striking nucleolar lesions as seen by electron microscopy. These were irreversible after exposure to higher temperatures and for longer exposure times. Similar findings were described by Heine et al. (1971) using HeLa cells. Autoradiographic studies by Simard and Bernhard (1967) indicated that nucleolar RNA synthesis was inhibited, while extranucleolar synthesis was less affected. Inhibi-

tion of RNA synthesis was also confirmed by tritium-labeled uridine studies on HeLa cells (Warocquier and Scherrer, 1969; McCormick and Penman, 1969; Palzer and Heidelberger, 1973a) and on Ehrlich or Yoshida ascites tumor cells (Strom et al., 1973).

The inhibition on DNA synthesis has been suggested as a mechanism associated with the lethal action of heat. Several studies have shown a very high heat sensitivity of the S phase in normal and tumor cell lines (Westra and Dewey, 1971; Sisken et al., 1965; Watanabe and Okada, 1967; Palzer and Heidelberger, 1973b). A selective heat sensitivity of tumor cells for DNA synthesis inhibition is suggested by the biochemical studies of Giovanella and Heidelberger (1968). At the same exposure conditions of temperature and time (42°C for 2 hr) DNA synthesis was lower by 20% in hepatoma 5123 or by 50% in the Novikoff hepatoma, while the regenerating liver doubled its DNA synthesis rate. Similar results with the same cell lines are described by Mondovi et al. (1969), who also included human osteosarcoma cells in their study. After 3 hr at 43°C these cells showed a 60% inhibition of the incorporation of tritiated thymidine. Radioautographic studies on human cells (Sisken et al., 1965) exposed for 1 hr to a range of temperatures (30°C–43°C) show that above 39°C there is a continuous drop in DNA synthesis. Only after 5 hr exposure to 43°C is the damage irreversible.

The relationship between lysosomes and the effect of hyperthermia has been suggested by Turano et al. (1970). In isolated lysosomes both from Novikoff hepatoma and Ehrlich ascites tumor cells, after incubation at 43°C for 30 min, the release of hydrolytic enzymes is enhanced by a factor of 2–3 when compared with normal or regenerating liver lysosomes. The authors, however, do not accept that the lysosomal damage is the primary mechanism responsible for the heat effect on tumor cells. The conclusions of Overgaard and Overgaard (1972a) are rather different, in their work with an animal system in which they study the histological and biochemical changes at varying times after the heat treatment. An autolytic disintegration of the heat-damaged tumor cells is described, whereas the intermingled stroma and vascular cells are not affected. The authors assume that the direct effect of heat is due to an elective activation of the acid hydrolases localized in the lysosomes of the tumor cells.

The fact that tumors have a lower pH than normal tissues has been shown in different animal systems (Eden et al., 1955; Tagashira et al., 1953; Kahler and Moore, 1962; Gullino et al., 1965; Burk et al., 1967) and in a wide range of human tumors (Meyer et al., 1948; Pampus, 1963; Ashby, 1966). The injection of glucose will induce a further drop in the intratumor pH (Eden et al., 1955; Kahler and Moore, 1962; Ashby, 1966). Overgaard (1975) reported that in the pH range of 7.2–6.5 the growth of unheated cells was not impaired, while at a temperature of 42.5°C for 1 hr the survival dropped from 100% at pH 7.2 to 10% at 6.5. The suggested mechanism has two pathways. A short one is by

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heat-induced lysosome activity and cell destruction (Overgaard and Overgaard, 1972a). The longer pathway is through steps of repair inhibition: a relative increase of anaerobic glycolysis with consequent higher lactic acid content and intensified lysosomal activity with consequent cell death. In our own experience (Har-Kedar, 1975), the surviving fraction of early plateau-phase cells exposed for 3 and 6 hr at 42°C was increased to a considerable extent if the heating was performed in fresh medium (pH 7.4) instead of medium from 24-hr-old cultures (pH 6.8).

The various mechanisms of action involved in the hyperthermic effect would probably suggest that many of these effects are related and that the search for one primary target is perhaps not justified. The availability of methods for heating a small part of a single cell, either by use of a tunable organic dye laser microbeam (Berns, 1972) or by an electrical microheater (Nicklas, 1973) might help to throw further light on this subject.

IV. Effects of Hyperthermia Alone

In vitro studies are reviewed in another paper in this issue (Thrall et al.).

In animal experiments, heat alone has cured tumors in a few systems (Overgaard and Overgaard, 1972a; Dickson and Muckle, 1972; Crile, 1963) but failed in others (Hahn, 1974) even when the intratumor temperature was more than 44°C (Cater et al., 1964; Thrall and Gillette, 1974; Thrall et al., 1973; Yerushalmi and Har-Kedar, 1974). There are reports of heat stimulation of tumor growth (Brett and Schloerb, 1962) and the induction of an earlier spread of metastases (Dickson and Ellis, 1974; Mikawa, 1937), possibly when the temperature or time of exposure is below a therapeutic threshold.

An animal system was described by Popovic and Masironi (1966a,b) which even if it does not fit completely within the definition of hyperthermia is a heat treatment with possible prospects for clinical adaptation. The methods consisted of cooling the animal and selectively heating the tumor region (hamster cheek pouch tumor) to 37°C. The animal was maintained at a temperature differential of 7°C (i.e., body temperature of 30°C) for 24 hr, or when the body temperature was lowered to 4°C treatment was given for 10 hr. In both cases the authors claim that 100% cures were achieved.

Clinical data about treatments with hyperthermia alone have been less encouraging. There are those cases already cited of cures after spontaneous and induced erysipelas and toxin therapy, as reviewed by Nauts et al. (1946, 1953); Everson and Cole (1956), and Miller and Nicholson (1971), among many others.

There have been relatively few clinical trials with whole-body hyperthermia. This has been achieved by different induction methods (see Section II on Methods) such as radiant heat (Warren, 1935), inhalation of heated air (Petti-

grew et al., 1974), hot water bath (Crile, 1962), shortwaves and infrared lamps (Wüst et al., 1975), and hot air chamber (E. W. Sterling, personal communication, 1975).

Those treatments were carried out in advanced and hopeless cancer patients. Their results suggest that there was a temporary subjective and objective improvement and possibly some prolongation of survival, but no cures.

Localized heat treatments, without the addition of radiation or drugs, have been performed using hot water baths for skin metastases (Crile, 1962) and carcinoma of the penis (Heyn and Kurz, 1967), perfusions for bladder cancer (Hall et al., 1974), and limb tumors (Cavaliere et al., 1967).

Cavaliere and his colleagues (1967) have treated many patients by regional limb perfusions with prewarmed blood. The intratumor temperature was raised to 41.5°–43.5°C for 2–8 hr in a group of patients of ages in the range of 13 to 74 years. Histological diagnoses included 12 sarcomas, seven melanomas, two squamous cell carcinomas of the skin, and one metastatic leiomyosarcoma of the uterus. Although there were many complications in more than half of the treated patients, the fact remains that gross disappearance of the tumor was achieved in ten of the 22 patients. Four of the patients with melanoma were reported alive and with functional limbs 28, 27, 11, and 7 months after treatment.

Another long-term cure was reported by Heyn and Kurz (1967). They have a 10-year follow-up on one patient treated for carcinoma of the penis. There was no evidence of recurrence or functional damage.

V. Effects of Hyperthermia with Radiation

In vitro and animal experiments are reviewed in another paper in this issue (Thrall et al.).

At the beginning of this century, the clinical use of x-ray therapy had two recognized limitations. Some tumors did not respond to the treatment and others were deeply located and therefore not accessible to a therapeutic dose of radiation without severe skin damage. The knowledge, at that time, that heat treatment has some effects on cancer resulted in trials of the combination of heat and radiation. The first paper published on this combined therapy (Müller, 1912), reported on 100 patients with advanced histologically confirmed tumors, treated by diathermy and x-rays. The follow-up time was 2 years at the most, and a rough summary of the results indicates that one-third of the cases showed complete regression; one-third had a faster regression than expected from radiation alone, and one-third failed to show a clear potentiating effect. These results are difficult to evaluate because no temperature measurements were made and the comparative results are the evaluation of the author based on his personal

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experience. The conventional diathermy used was probably of low heating efficiency for many of the deeply located tumors such as those of bronchus or esophagus.

Warren (1935), treating a patient with embryoma of the ovary and widespread metastases, added two doses of 250 rads of x-ray therapy to the upper lung fields containing secondary deposits after 21 hr of induced fever at 41.5°C. The diagnostic chest x-ray films seemed to indicate that the masses in the parts of the lungs that were irradiated disappeared, while in other parts they shrank but regrew rapidly. This was confirmed at autopsy when the radiation-treated regions had almost uninvolved parenchyma as compared with the huge masses in other parts. In this case and others where radiation was given after heat, a definite synergistic effect could be obtained. However, in several other patients who received the radiation therapy prior to the induced fever, the effect was no more intense than that expected from fever therapy alone.

Interesting results were reported by Korb (1939) in one patient with two skin basal cell carcinomas. Eq   radiation doses were given to the patient's tumors, while one of them was also treated by shortwave diathermy. The tumor treated by the combination regressed completely, while the other persisted.

Shortwave diathermy (6-m wavelength) was applied before radiation, to a wide range of tumors, by Birkner and Wachsmann (1949). In one patient with squamous cell carcinoma of the skin the intratumor temperature reached a maximum of 44°C and was maintained for 2–3 hr. Other tumors (larynx, lips, penis, cervix) had shorter heating times. The combined treatment did not decrease the amount of radiation needed but was said to improve the results that could be achieved by radiation alone. Similar results with lung and breast tumors have been reported by Fuchs (1952). The lack of comparative groups leaves the reviewer without data for evaluation of these treatments.

The results achieved by Woeber (1965) with ultrasonic heating are given in a later section of this review.

Crile (1962) treated superficial tumors or metastases with heat followed by radiation. Immersion in a water bath at 44°–46°C for 1 hr sensitized the tumors to radiation by a factor of 2.5. Hartman and Crile (1968) analyzed five cases of osteosarcoma treated by combined therapy. The first of these was given local treatment with the water bath at 43°C for 93 min, followed by 1000 rads of telecobalt twice weekly to a total of 5000 rads. After some months lung metastases were resected and the patient was reported free of disease 6½ years after treatment. The other 4 patients were treated by microwave heating and radiation, and are discussed in another section.

After studies on dogs, Cockett et al. (1967) applied hyperthermia to seven elderly patients with inoperable carcinoma of the bladder. During the bladder perfusion with sterile water at 43°C, the patients had their planned conventional megavoltage irradiation. The authors were impressed by the marked response,

and their conclusion is that in those cases in which irradiation can be beneficial, combined therapy is probably more effective.

A combined triple measurement (hyperthermic perfusion with chemotherapy and radiation) was applied by Stehlin (1969) to 11 patients with primary or recurrent sarcomas of the lower extremities. Most of the patients had a full radiation course (5000–6000 rads in 5–6 weeks) after the perfusion treatment. In six of the cases there was no evidence of tumor at a maximum follow-up of 18 months.

The timing of the heat treatment with relation to radiation is a subject still under investigation in experimental systems (for references, see discussion in Section VII on microwaves). In many such systems the sequence over a few hours seems relatively unimportant (Overgaard and Overgaard, 1972b; Hahn et al., 1974; Kim et al., 1974). However, in man, the small clinical experience has been consistent with the view that heat needs to be given before radiation to achieve maximum potentiation (Warren, 1935; Birkner and Wachsmann, 1949; Fuchs, 1952; Crile, 1962).

VI. Effects of Hyperthermia with Chemotherapy

The possibility of using hyperthermia as a potentiator of the effects of cytostatic drugs was suggested by the work of Woodhall et al. (1960). VX-2 rabbit tumors were excised and aliquots of cells were treated by four different alkylating agents at temperatures of $20°$, $37°$, and $42°C$ for time intervals of between 1 and 24 hr. The treated cells were reinoculated into animals and after 26 days the tumors were excised and weighed. There was a clear increase in the cytocidal effect of the drug after 3 hr drug incubation at $42°C$.

Rochlin et al. (1961) using ^{32}P-labeled thio-tepa, and Shingleton et al. (1962) using ^{14}C-labeled nitrogen mustard, showed that a higher content of the drug could be achieved in a dog limb heated to $40°–42°C$. This might be expected to produce an increased local cytotoxic effect.

An increasing number of drugs have since then been tested in vitro and in animal experiments, and contradictory results have been achieved in the different systems. Alkylating agents, for example, are reported to show an enhancement by heat (Giovanella et al., 1970; Johnson and Pavelec, 1973; Dickson and Suzangar, 1974) or no enhancement (Crile, 1963).

Woodhall et al., whose animal experiments were reported earlier, also treated 20 patients with cancer of the head by regional perfusion combined with alkylating agents. The perfusate was maintained at a temperature of $41°–42°C$ for 20–30 min. Although the drug was administered at twice the minimum lethal dose, only one patient developed a severe leukopenia and died from pneumonia. A combination of whole-body cooling to $30°C$ and hyperthermic perfusion with

irradiation can be beneficial,

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hyperthermic perfusion with

alkylating agents of abdominal and pelvic malignancies gave objective improvement and partial remission in a few patients (Shingleton et al., 1961; Leone et al., 1962). Whole-body hyperthermia by water bath combined with cyclophosphamide was tried by Suryanarayan (1966) on nine patients with various advanced diseases. The author was impressed by the relief of pain and arrest in the growth of the tumors. However, there was only a short follow-up of 2 months and there have been no further reports on the later response of these patients.

After performing experiments on mice and dogs, Sutton (1971) made use of a system for localized heating of brain tumors by means of a miniature probe warmed to 42°C. The actual heating periods (6 hr daily for two days, or continuous heating up to 40 hr) were accompanied by the intravenous administration of 5-fluorouracil. After this treatment in seven patients the tumors were resected and studied histologically. Morphologically viable cancer cells were found outside the proximal probe region. The limited heating properties of this device are a clear handicap and a widespread cerebral hyperthermia needs to be achieved by other means. Better results could be expected from ultrasound focusing (Heimburger et al., 1974) or microwaves (J. C. Lin et al., 1973).

Short papers on heat and drug combinations were recently presented by Omar (1974) on head and neck regional hyperthermia, and by Heimburger et al. (1974) on the ultrasonic heating of brain tumors.

Whole-body hyperthermia has been, in a few cases, combined with bleomycin (Wüst et al., 1975). The aim was to achieve bleomycin-induced arrest of cells in late S and G_2. In these phases of the cycle cells have passed the position of maximum thermosensitivity, mid-S (Westra and Dewey, 1971). Eight to 12 sessions of 1-hr heating at 40°–45°C were performed on each of the seven patients. The choice of bleomycin is perhaps surprising, because the same authors (Wüst et al., 1973) could not demonstrate even an additive effect with heat in an in vitro system. From our experience (Har-Kedar, 1975) (Fig. 1) and that of others (Hahn et al., 1975) the effect of bleomycin has consistently been enhanced by hyperthermia of not less than 41°C in both animal and in vitro studies. For lower temperatures applied by Wüst, the exposure time would probably need to be much greater than the 1 hr for positive enhancement results to be seen.

Pettigrew et al. (1974) combined whole-body hyperthermia (41°–41.8°C) with chemotherapy in three patients with melanoma using phenylalanine mustard, and in patients with intestinal and breast cancers using a mixture of three drugs (cyclophosphamide, 5-fluorouracil, and vincristine). The first session lasted 90 min and two more treatments of 4 hr each followed at 3-day intervals. Drugs were injected during the last session. The authors' impression was that this combined treatment gave better results than did heat alone.

The recent report of Stehlin et al. (1975) is an extension of an earlier one
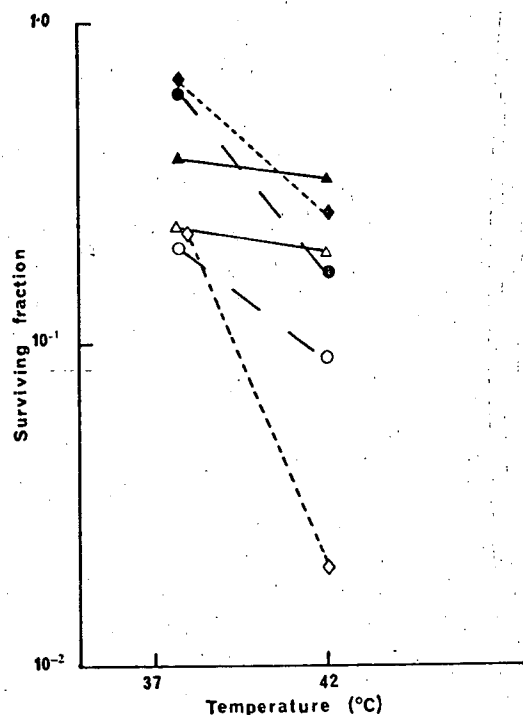
Fig. 1. Comparative *in vitro* effect of three drugs on EMT-6 cells, at 37°C and 42°C, for 1-hr exposure. Open symbols, exponentially growing cells; closed symbols, early plateau-phase cells. (○●) Bleomycin—20 μg/ml; (△▲) actinomycin D—1 μg/ml; (◇◆) adriamycin—0.5 μg/ml. (I. Har-Kedar, unpublished data.)

(Stehlin, 1969). This gives the results of hyperthermic perfusions at 39°–40°C with melphalan in 165 patients with melanoma of the limbs, carried out since 1967. In the 10 years from 1957 to 1966 the author applied normothermic infusions with the same drug in 339 cases. During the subsequent period from 1967 the improvement in surgical techniques and postoperative care could explain some higher 5-year survival, but the reported improvement by nearly 3-fold is likely to be a direct consequence of the introduction of the heat factor in the treatment schedule from that date, as seen in Fig. 2.

In some of the patients the limb perfusion had a therapeutic effect on distant metastases. The possibility of excessive drug leakage into the general circulation could, in some cases, explain the above findings. However, melanomas are known to be highly immunogenic tumors and their destruction by the treatment could liberate antigenic material inducing a stimulation of antitumor immunity. A similar response to heat therapy in an animal system was described by Goldenberg and Langner (1971).
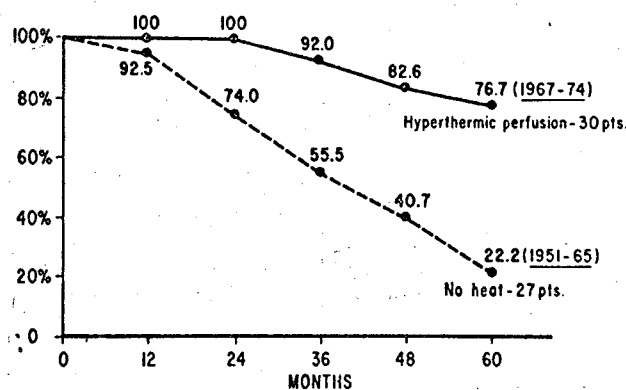
Fig. 2. The effect of adding hyperthermia to the perfusion treatment of patients with melanoma. The survival rates are given from the time of initial treatment of patients with similar staging of the disease. ——, Berkson-Gage; - - - Crude. Reproduced from Stehlin *et al.* (1975) by permission of *Surgery, Gynecology, and Obstetrics.*

A combined triple treatment of hyperthermic perfusion and radiation, together with melphalan and actinomycin D, was applied by Stehlin (1969) on patients with primary or recurrent sarcoma of the lower extremities. More recently he reported a 5-year survival of 61% (Stehlin, 1975).

Tables II, III, and IV summarize the different drugs tested in combination with heat *in vitro*, in animals, and in man.

VII. Microwaves in Cancer Therapy

A. Introduction and Physical Properties

Microwave radiation is commonly defined as that portion of the electromagnetic spectrum with wavelengths between 1 mm and 1 m (or the equivalent frequency range from 300 GHz to 300 MHz). Even at the highest frequency, the energy per photon is less than 10^{-3} eV which is too low to produce ionization.

Microwave energy can be propagated as a wave that is continuous or pulsed. The former is used in mostly communication and in fundamental research work. The pulsed wave is widely used in radar systems, industrial equipment, and medical diathermy devices.

The principal factors determining the absorption of microwave energy in a medium are the electrical permittivity and conductivity, which in turn depend upon the temperature of the medium and the frequency of the radiation. Part of the incident energy will be reflected from the surface of the material, and only a portion of the penetrating energy will be absorbed. The remainder passes through without any interaction with, or effect on, the exposed material. The

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MT-6 cells, at 37°C and 42°C, for
s; closed symbols, early plateau-
D—1 µg/ml; (◊♦) adriamycin—0.5

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TABLE II •
Combined Treatment by Hyperthermia and Drugs (*in Vitro*)

Drug	Cell line	Results	Method of assessment	References
Alkylating agents				
Thio-tepa	Yoshida sarcoma of rat	Potentiation	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
	Chinese hamster fibroblasts	Potentiation	Colony formation	Johnson and Pavelec (1973)
	VX-2 rabbit carcinoma cells	Potentiation	*In vivo* assay	Woodhall *et al.* (1960)
PAM (melphalan)	L1210 mouse leukemia	Potentiation	*In vivo* assay	Giovanella *et al.* (1970)
	Yoshida sarcoma of rat	Potentiation	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
MDMS	Yoshida sarcoma of rat	Potentiation	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
Nitrogen mustard	L1210 mouse leukemia	Potentiation	*In vivo* assay	Kremkau *et al.* (1974)
	Chinese hamster cells	Potentiation	Colony formation	Hahn (1974)
Cyclophosphamide	VX-2 rabbit carcinoma cells	Potentiation	*In vivo* assay	Mahaley and Woodhall (1961)
	Yoshida sarcoma of rat	Ineffective	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
	L1210 mouse leukemia	Potentiation	*In vivo* assay	Kremkau (1975)
A. 139, TEM, OPSPA	VX-2 rabbit carcinoma cells	Potentiation	*In vivo* assay	Woodhall *et al.* (1960)
Anti-metabolites				
5-Fluorouracil	Yoshida sarcoma of rat	Ineffective	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
Methotrexate	L1210 mouse leukemia	Ineffective	*In vivo* assay	Kremkau (1975)

Nitrogen mustard	L1210 mouse leukemia	Potentiation	*In vivo* assay	Kremkau *et al.* (1974)
	Chinese hamster cells	Potentiation	Colony formation	Hahn (1974)
Cyclophosphamide	VX-2 rabbit carcinoma cells	Potentiation	*In vivo* assay	Mahaley and Woodhall (1961)
	Yoshida sarcoma of rat	Ineffective	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
	L1210 mouse leukemia	Potentiation	*In vivo* assay	Kremkau (1975)
A. 139, TEM, OPSPA	VX-2 rabbit carcinoma cells	Potentiation	*In vivo* assay	Woodhall *et al.* (1960)
Anti-metabolites				
5-Fluorouracil	Yoshida sarcoma of rat	Ineffective	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
Methotrexate	L1210 mouse leukemia	Ineffective	*In vivo* assay	Kremkau (1975)
Mitotic inhibitors				
Vinblastine	L1210 mouse leukemia	Ineffective	*In vivo* assay	Giovanella *et al.* (1970)[a]
	Yoshida sarcoma of rat	Ineffective	Biochemical studies and *in vivo* assay	Dickson and Suzangar (1974)
Vincristine	L1210 mouse leukemia	Ineffective	*In vivo* assay	Kremkau (1975)
Antitumor antibiotics				
Bleomycin	EMT-6 mouse tumor cells	Potentiation	Colony formation	Har-Kedar (1975)
	Chinese hamster cells	Potentiation	Colony formation	Hahn *et al.* (1975)
	Rat Jensen sarcoma cells	Ineffective	Biochem studies	Wüst *et al.* (1973)
	6W-39 cells	Ineffective	Biochemical studies	
Adriamycin	EMT-6 mouse tumor cells	Potentiation	Colony formation	Hahn *et al.* (1975)
Actinomycin D	L1210 mouse leukemia	Potentiation	*In vivo* assay	Giovanella *et al.* (1970)
	Yoshida sarcoma of rat	Ineffective	Biochemial studies and *in vivo* assay	Dickson and Suzangar (1974)
	EMT-6 mouse tumor cells	Ineffective	Colony formation	I. Har-Kedar (unpublished)
Amphoteracin B	Chinese hamster cells	Potentiation	Colony formation	Hahn (1975)
Miscellaneous group				
BCNU	Chinese hamster cells	Ineffective	Colony formation	Hahn (1974)
	L1210 mouse leukemia	Potentiation	*In vivo* assay	Kremkau (1975)
Hydroxy urea	HeLa cells	Protection	Colony formation	Palzer and Heidelberger (1973b)
	Rat Jensen sarcoma cells 6W-39 cells	Addition	Biochemical studies	Wüst *et al.* (1973)
DHBA	L1210 mouse leukemia	Potentiation	*In vivo* assay	Giovanella *et al.* (1970)
	HeLa cells	Potentiation	Colony formation	Palzer and Heidelberger (1973b)

[a]Drug did not affect the cells at 37°C either.

TABLE III: Combined Treatment of Hyperthermia and Drugs (Animal Systems)

Drug	Animal system	Results	Method of assessment	References
Alkylating agents				
Thio-tepa	Limb perfusion on dogs[a]	Potentiation	% retention of drug	Rochlin et al. (1961)
	Yoshida sarcoma of rat	Potentiation	Tumor growth	Dickson and Suzangar (1974)
PAM (melphalan)	Yoshida sarcoma of rat	Potentiation	Tumor growth	Dickson and Suzangar (1974)
MDMS	Yoshida-sarcoma of rat	Potentiation	Tumor growth	Dickson and Suzangar (1974)
Nitrogen mustard	Limb heating on dogs[a] — rabbits bearing VX-2 carcinoma	Potentiation	% retention of labeled drug in tissues or tumor	Shingleton et al. (1962)
	Dogs—chest cooled and lower body heated[a]	Potentiation	Comparison of bone marrow of pelvis and externum	Silberman and Hofmeyer (1966)
Cyclophosphamide	Yoshida sarcoma of rat	Ineffective	Tumor growth	Dickson and Suzangar (1974)
Anti-metabolites				
Methotrexate	VX-2 rabbit carcinoma	Addition	Biochemical studies and tumor growth	Muckle and Dickson (1973)
5-Fluorouracil	DMBA-induced rat tumors	Potentiation	Tumor growth	Zimmer et al. (1971)[b]
	Mice spontaneous mammary tumors	Potentiation	Tumor growth	Zimmer et al. (1971)[b]
	Toolan tumor in cheek pouches of hamsters	Potentiation	Tumor growth	Popovič and Masironi (1966c)[b]
	Yoshida sarcoma of rat	Ineffective	Tumor growth	Dickson and Suzangar (1974)
	Mouse ependymoblastoma	Potentiation	Histological studies	Sutton (1971)
Mitotic inhibitors				
Vincristine	Yoshida sarcoma of rat	Ineffective	Tumor growth	Dickson and Suzangar (1974)
Antitumor antibiotics				
Bleomycin	EMT-6 mouse tumor	Potentiation	Colony formation and tumor growth	I. Har-Kedar (unpublished)
Adriamycin	EMT-6 mouse tumor	Potentiation	Colony formation	Hahn et al. (1975)
Actinomycin D	Yoshida sarcoma of rat	Ineffective	Tumor growth	Dickson and Suzangar (1974)

[a]Healthy animals used for physiological studies.
[b]Tumors heated to 37°C in hypothermic animals.

Drug / Tumor	Effect	Result	Reference
tumors			
Toolan tumor in cheek pouches of hamsters	Tumor growth	Potentiation	Popović and Masironi (1966c)[b]
Yoshida sarcoma of rat	Tumor growth	Ineffective	Dickson and Suzangar (1974)
Mouse ependymoblastoma	Histological studies	Potentiation	Sutton (1971)
Mitotic inhibitors			
Vincristine	Tumor growth	Ineffective	Dickson and Suzangar (1974)
Yoshida sarcoma of rat			
Antitumor antibiotics			
Bleomycin	Colony formation and tumor growth	Potentiation	I. Har-Kedar (unpublished)
EMT-6 mouse tumor			
Adriamycin	Colony formation	Potentiation	Hahn et al. (1975)
EMT-6 mouse tumor			
Actinomycin D	Tumor growth	Ineffective	Dickson and Suzangar (1974)
Yoshida sarcoma of rat			

[a]Healthy animals used for physiological studies.
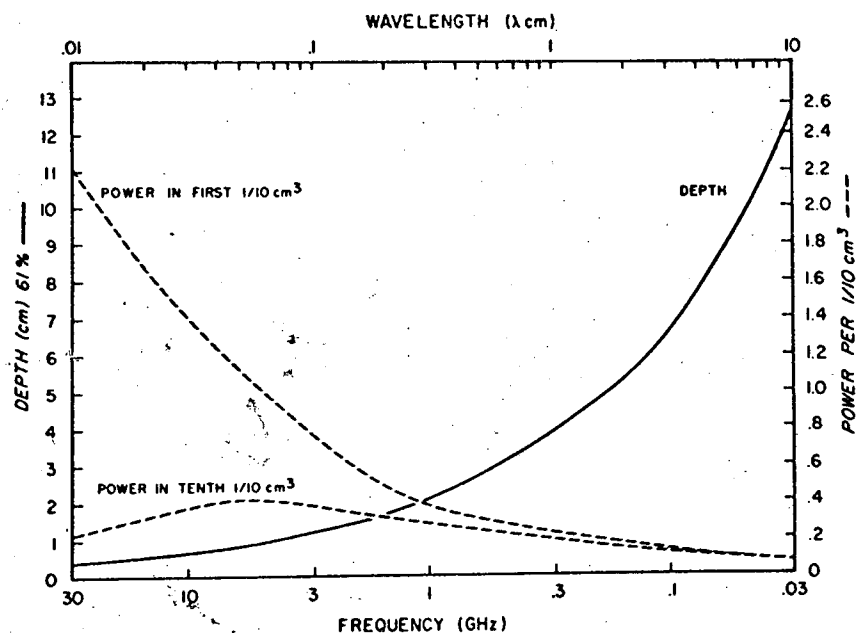[b]Tumors heated to 37°C in hypothermic animals.

Fig. 3. Microwave power in the first 0.1 cm^3 and in the tenth 0.1 cm^3, as a function of the frequency (or wavelength) and the depth of penetration to the point at which 61% of the 10 mW/cm^2 power is absorbed. Reproduced from Vogelman (1969), by permission.

absorbed radiation will generate heat in two ways. One of these is due to the dielectric relaxation of the dipolar molecules present in the material. The other mechanism of heat dissipation is due to the interaction of the electric field with the conductivity caused by free ions. The basic theory of these processes in relation to biological material has been described by Grant (1974). As an approximation, it turns out that for a typical human subject 40% of the incident microwave energy will be absorbed for frequencies below 1 GHz, between 20% and nearly 100% will be absorbed in the range of 1–2 GHz, and around 40% will be absorbed at higher frequencies (Seth and Michaelson, 1964). However, not only the integral amount of absorbed energy is important. Another critical factor for the therapeutic use of microwaves is the depth of penetration and uniformity of distribution of the energy, as a function of the wave frequency (Fig. 3) (Vogelman, 1969).

The primary biological effect of microwave radiation within the mammalian tissues is the conversion of the absorbed energy into heat. When a whole living organism or a large part of it is exposed and participates in the heat-transfer mechanisms then there is a local or general hyperthermia with, possibly, macroscopic damaging effects. Whether or not the damage is reversible will depend on several physical parameters related to the incident energy (e.g., frequency, power

TABLE IV

Combined Treatment of Hyperthermia and Drugs—Clinical Data

Reference	Drugs	Site of treated tumors	Heating method	Evaluation of treatment by:
Woodhall et al. (1960)	Cyclophosphamide, nitrogen mustard, Thio-tepa, A. 139	Face, mouth, nasopharynx (20 patients)	Localized heated perfusion (41–42°C)	Relief of pain, histological regression
Shingleton et al. (1961)	Cyclophosphamide PAM (melphalan) Nitrogen mustard A.139	Various abdominal or pelvic malignancies (25 cases)	General body hypothermia and regional perfusion (41–42°C)	Bone marrow protection
Leone et al. (1962)	Nitrogen mustard	Abdominal and pelvic tumors (11 cases)	General body hypothermia and regional perfusions (41°C)	Bone marrow protection, change in size of tumor
Suryanarayan (1966)	Cyclophosphamide	Various primary and metastatic tumors (9 cases)	Whole-body heating in a water bath	Relief of pain, changes in size of tumors and lymph nodes

	Thio-tepa, A. 139	(20 patients)		histological regression
Shingleton et al. (1961)	Cyclophosphamide PAM (melphalan) Nitrogen mustard A.139	Various abdominal or pelvic malignancies (25 cases)	General body hypothermia and regional perfusion (41–42°C)	Bone marrow protection
Leone et al. (1962)	Nitrogen mustard	Abdominal and pelvic tumors (11 cases)	General body hypothermia and regional perfusions (41°C)	Bone marrow protection, change in size of tumor
Suryanarayan (1966)	Cyclophosphamide	Various primary and metastatic tumors (9 cases)	Whole-body heating in a water bath	Relief of pain, changes in size of tumors and lymph nodes
Stehlin (1969)	PAM (melphalan)	Limb melanomas (39 cases)	Limb perfusion (46°C)	Change in size of tumor, increased survival
	PAM + actinomycin D (also radiotherapy)	Limb sarcomas (11 cases)		
Sutton (1971)	5-Fluorouracil	Malignant glioma of brain (7 cases)	Electrically heated microprobe	Posttreatment biopsy for histological studies
Pettigrew et al. (1974)	PAM (melphalan)	Metastatic melanoma (3 cases)	Whole-body hyperthermia by inhalation of hot air	Relief of pain, change in size of tumor and metastasis
	Cyclophosphamide, fluorouracil, vincristine	Gastrointestinal, breast, and osteoblastoma (10 cases)		
Wüst et al. (1975)	Bleomycin	Not specified (7 cases)	Whole-body hyperthermia by radiant heat	No details given
Stehlin et al. (1975)	PAM (melphalan)	Limb melanomas (165 cases)	Limb perfusion (skin temperature at 39–40°C)	Survival rates compared with similar group treated by perfusion and drug without hyperthermia

density, type of radiation source, time of exposure) and biophysiological parameters of the exposed organism (size and shape of the region or animal, thickness, composition, and electrical permittivity and conductivity of the exposed tissues). Continuous wave energy and pulsed microwaves (of the same average power level) will give similar biological effects (Schwan, 1972; Birenbaum et al., 1975).

Nonthermal effects due to microwave radiation, and especially their biological importance, are still controversial. Some of these alleged effects may possibly be microscopic scale thermal damage, due to standing waves or to the fact that bound water has a much larger absorption coefficient than expected (Vogelman, 1969; Grant, 1974). At frequencies higher than about 30 GHz molecular resonance effects may also occur (Illinger, 1974).

Extensive analysis of the biological effects of microwaves, including excellent critical reviews of the nonthermal effects, were published by Michaelson (1969, 1972, 1974) and Milroy and Michaelson (1971). The physical background of the mechanisms that could explain nonthermal effects is discussed by Cleary (1973).

The human body, in normal good health, is able to dissipate heat from external sources by a complex sequence of homeostatic mechanisms aimed at restoring the normal temperature. This thermal regulatory process, even in regional heating, is mainly a function of the cooling action provided by the circulation of the blood. In areas or organs in which there is a relatively poor blood supply, the temperature may rise more rapidly and to a higher degree than in the rest of the body. Classical examples of such organs are the eye and the testes, and these are actually the most susceptible organs to microwave damage. From experiments carried out on rabbits (Carpenter, 1969) it would appear that the formation of cataracts of the eye is likely to be the principal health hazard, as in accidental overexposure of radar workers (Michaelson, 1969; Milroy and Michaelson, 1972; Zaret, 1974).

A tumor mass at the clinical stage generally has a poor blood supply, with extensive regions of necrosis or quasinecrosis which do not possess the bloodflow channels for a rapid dispersal of the heat produced by exposure to microwaves. These regions contain poorly oxygenated (hypoxic) cells, which may be resistant to conventional radiotherapy and to cytotoxic drug therapy. The introduction of hyperthermia using appropriate microwave equipment could, therefore, be more effective (either alone or combined with drugs or ionizing radiation) against those cells which are less sensitive to more conventionally available treatments.

B. Experimental Use

The worldwide use of radar installations for military and civil purposes and the introduction of microwave ovens into the home created an urgent need to know

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ly and to a higher degree than
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less sensitive to more conven-

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reated an urgent need to know

more about the potential hazards of such devices to human beings. A fair amount of literature is available on the biological effects and hazards (reviewed by Michaelson, 1969) and the safety procedures and standards for maximum exposure (reviewed by Michaelson, 1972).

Since their introduction in the late 1940's, there has been an increasing use of medical diathermy devices producing electromagnetic radiation in the microwave range, namely 2.45 GHz (Osborne and Fredrick, 1948; Wakim *et al.,* 1949). Their use has been only in physical therapy with similar indications for treatment to those for the shortwave devices already in use over the previous decades.

There were few reports of experimental work aimed at evaluating the use of microwave radiation in tumor therapy in the early postwar years (Gessler *et al.,* 1950; Allen, 1955). Only in the early 1960's did a renewed interest in hyperthermia stimulate a search for heating systems that could be applicable to clinical use. Discussing a paper by Woodhall *et al.* (1960), R. K. Gilchrist urged that the electronic industry be involved in the development of devices "that are just over the horizon . . ." and that would make it possible "to heat any organ inside the body to any degree."

A variety of animals, and microwave frequencies, have been used in various experimental trials. Spontaneous tumors in dogs were treated by a combination of ionizing radiation and heat from microwave diathermy and good control was achieved in several tumors (Crile, 1962). Unfortunately, this report does not give any further information about the type and size of tumors, degree of heating reached in the tumor, duration of heat, and timing in relation to the x-irradiation, thus making the results very difficult to interpret.

Cater *et al.* (1964) used rats bearing a transplantable hepatoma, heated by microwave radiation (10-cm wavelength) after a single x-ray treatment. All the unirradiated rats (either controls or those receiving heat alone) succumbed rapidly to the primary tumor. Of the remaining two groups, those treated by radiation alone or radiation plus microwave heating, better results were seen in the latter group when comparing the rate of tumor growth, length of survival, and number of long-lasting remissions. These results represent the pooled data from 14 experiments varying one from the other in dose of radiation (1310 to 2620 rads); exposure time to microwaves (8 or 10 min); intratumor temperature (45°C to 47°C) and time interval between radiation and heating (from a few hours to 4 days). This last factor needs further analysis. In most animal experiments in which the combination of heat, resulting from methods other than microwaves, and ionizing radiation was applied, a synergistic effect was achieved only if the interval between the modalities of treatment was less than 24 hr (Crile, 1963; Hahn *et al.,* 1974; Kim *et al.,* 1974; Robinson and Wizenberg, 1974; Overgaard and Overgaard, 1972b). Cater and his co-workers (1964), for reasons not explained in their paper, allowed an interval of 2 days or more in 10 of the 14 experiments. As the results from the individual experiments are not

given, it is difficult to assess whether or not microwave heating has its optimum conditions at a different time interval than for other heating modalities.

An interesting system has been described by Zimmer *et al.* (1971). After lowering to 4°C the body temperature of rats bearing DMBA-induced mammary tumors or mice with spontaneous mammary tumors, the tumor region alone was heated by microwave radiation (9.05 GHz for mice and 2.45 GHz for rats) to around 37°C. Such a temperature differential for a period of 1 hr was ineffective in curing the tumors, but was found to enhance the cytotoxic effect of 5-fluorouracil and to achieve a high rate of tumor regression.

A conflicting result which fails to show a synergistic killing effect is the work of Lappenbusch and Gillespie (1973). Chinese hamsters were exposed to whole-body irradiation by a microwave field of 60 mW/cm^2 for half an hour before and after x-irradiation of 0–1000 rads. The surprising results were that at the higher x-ray doses (750–90   ads) and when the microwave radiation was given afterward, the number of animals surviving was significantly higher than when compared with the group receiving x-irradiation alone. The authors suggest the possibility that microwaves immediately after x-ray treatment might stimulate the surviving cells of the bone marrow at a crucial time. However, this is only hypothesis, and this apparently radioprotective effect needs to be tested in other model systems.

The varied work described above employed microwave heating as a sensitizer for ionizing radiation or drugs, in circumstances in which heat alone was found to be ineffective. Dietzel *et al.* (1975) used 65-cm waves (461 MHz) alone to treat mice with Ehrlich ascites tumor. The whole peritoneal cavity was heated to different temperatures at various times after inoculation with the tumor cells. No significant therapeutic effect could be achieved. In earlier work (Dietzel *et al.*, 1971) the same method of heating cured 23% of mice with localized solid tumors.

An unusual application of the heating properties of microwaves has been suggested by Copeland and Michaelson (1970). Selective tumor heating increased 4-fold the concentration in the tumor of injected ^{131}I-labeled fibrinogen and therefore increased the tumor β-radiation dose.

In the past, waves longer than 1 meter (radio-frequency waves) have been used in cancer therapy trials, either in animal experiments (Dittmar, 1949; Sugiura, 1941) or with patients (Birkner and Wachsmann, 1949; Fuchs, 1952). Also, the more recent studies of Shingleton *et al.* (1962) and Overgaard and Overgaard (1972a) used shortwave diathermy at 27.12 MHz.

This frequency was also used by Häkkinen *et al.* (1975), who exposed human melanoma cells to combined shortwave and telecobalt γ-rays. This combined treatment left 15% more surviving cells than from radiation treatment alone. These results at first seem surprising, but can probably be explained on the basis that the temperature rise was less than 0.5°C. One would not therefore expect a

wave heating has its optimum
heating modalities.
Zimmer et al. (1971). After
ng DMBA-induced mammary
s, the tumor region alone was
ce and 2.45 GHz for rats) to
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nsters were exposed to whole-
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synergistic effect, as hyperthermia was not induced. At best we can exclude the existence of a nonthermic effect, and the higher number of survivors can possibly be explained by the stimulation of cell growth by temperatures under 40°C (Dickson and Ellis, 1974; Mikawa, 1937).

C. Clinical Use

The lack of convincing experiments with larger animals using microwave heating and the absence of a reliable method for continuous and nondestructive measurements of intratumor temperature have delayed fundamental clinical trials.

Crile (1962) reported the treatment of four children with osteogenic sarcoma by surgical exposure and microwave diathermy heating of the tumor and surrounding tissues to temperatures of 50°–60°C for 15 to 25 min. Shortly afterward, x-ray therapy was applied. The aim was to destroy the tumor, by heat, while leaving the dead bone as an autograft. Such a drastic approach falls far outside the range of the normally accepted limits of hyperthermia. In a later review of these patients, Hartman and Crile (1968), although impressed by the survival for more than 5 years of one of the children, recognized that other forms of treatment (e.g., massive irradiation of the bone) could be a better answer for their purposes.

Brenner (1975) treated 15 patients with superficial metastases by combined microwave heating and x-irradiation. Regression of some metastases was reported as being achieved with lower doses of radiation than commonly seen. Sarcomas were thought to be more sensitive than carcinoma.

D. Discussion and Conclusions

The fact that microwaves can generate heat within exposed tissues is well established. However, the theoretical advantages of microwaves over other methods of achieving hyperthermia remain to be demonstrated, although the fact that the heat can be localized in the region of interest is clearly a point in favor of their use. The precision of this technique can be seen in the work of J. C. Lin et al. (1973) using selective microwave brain heating of cats.

Since the first experimental work with microwaves, there have been some suggestions of biological effects that are unrelated to the rise of temperature in the tissues of animals under study (Imig et al., 1948; Teixeria-Pinto et al., 1960; Webb and Dodds, 1968; Carpenter and Livstone, 1971; Mayers and Habeshaw, 1973; Lebovitz, 1973). The possible existence of a specific frequency that will damage only the cancer cells is a dream that has been circulating since radiofrequency waves were used in diathermy half a century ago (Reiter, 1933; Hill, 1934). Since, however, the effect of microwaves on tissue is predominantly, if

not entirely, thermal, any such specific frequency is unlikely to exist. On the other hand, there may be a range of frequencies at which cancer cells are more vulnerable to microwaves than normal cells. It has been suggested that cancer cells contain more bound water than normal cells (Allan and Norman, 1974), and it has been shown that the absorption of microwave energy at certain frequencies is substantially higher for bound water than for free water (Grant *et al.*, 1975). Thus conditions exist for which malignant cells are likely to be more sensitive to microwave thermal damage than normal cells.

Recently published work reported different patterns for normal and cancer cells using microwave spectroscopy (Stamm *et al.*, 1974; Webb and Booth, 1972). Studies on the replication rate of bacteria showed that the microwave effects could be explained only as a result of temperature variations (Hamrick and Butler, 1973). More sr ifically, sarcoma 180 cells were studied by Moressi (1964) for nonthermal the: eutic effects at 2.45 GHz frequency. It was clearly demonstrated that at this frequency the damage produced was similar to that seen in cells that were heated to the same temperature in a water bath. In a more theoretical work, Sher *et al.* (1970) reached a similar conclusion that the pearl chain formation, a nonthermal biological effect supposedly related to genetic damage, cannot be induced in the absence of gross heating.

The possibility of localized heating of a deep tumor region is an exciting one. The selective microwave heating of the brain (J. C. Lin *et al.*, 1973) could be induced simultaneously with whole-body cooling, to create a higher differential temperature between the tumor (heated to 42°–43°) and other tissues (cooled to 30°–32°C). This might be a useful system for prolonged chemotherapeutic treatment. The appropriate drugs might be concentrated and active in the tumor region with fewer undesirable side effects on normal tissues. Possibly even the total dose of the cytotoxic agent could be lower than that usually administered because of a lower rate of excretion by cooled kidneys and reduced inactivation by the liver and other tissues. Technically, the cooling of the human body to 30°–32°C for many hours is feasible and has been widely used in cardiac surgery, neurosurgery, and even in infants (for treatment of acute pulmonary disease) as reported in a review on clinical hypothermia by Swan (1973). An even lower temperature of 28°C for 6–9 hr has been reported by Brehm *et al.* (1968) for the treatment of cancer patients in hibernation by x-irradiation. Microwaves could penetrate through cooled tissues without significant loss of heating, and by using an appropriate choice of frequencies and a multifocal device an acceptable uniform distribution of heating could be planned.

Another problem is the continuous monitoring of the temperature in tissues in order to avoid hot spots in sensitive points and to confirm the distribution of heat in the tumor and its surroundings. The technology to solve this problem exists. A system of remote thermography linked to a computer-controlled, variable frequency multifocal heating device would be one such solution. The

treated by ultrasound to only one of the sarcomas in each animal. In both these animals osteogenic sarcoma developed only in untreated bone tissue. The same group exposed healthy dogs to similar treatments without causing bone damage, as assessed after 3 years of follow-up (Janes et al., 1960).

In vivo experiments by Woeber (1954, 1965) suggested that ultrasound alone is ineffective for therapy and can even lead to growth stimulation if low intensities are used. His main effort was, therefore, to study the combination of ultrasound and x-ray therapy. Rats inoculated with Walker carcinoma were treated by 350 R x-irradiation and 5 min ultrasound (1.0 W/cm^2) either alone or in combination. The combined therapy cured all the rats and was equivalent to the effect of 600 R x-irradiation (enhancement factor 1.7), while x-rays or ultrasound alone did not significantly alter the progress of tumor growth.

Similar results were described by Lehmann and Krusen (1955), who treated mice bearing Ehrlich carcinoma in their tails by combining x-rays and ultrasound (1 MHz, 8.4 W/cm^2 for 4 min). When the tails were immersed in a water bath at $30°C$ the enhancement of the radiation effect by ultrasound was by a factor of 2. This could be prevented if the water in the bath was cooled to $10°C$, thereby suggesting that the essential mechanism was the hyperthermia generated by the ultrasound beams.

Clarke et al. (1970) attempted to assess whether the synergistic effect of ultrasound on x-irradiation is due to some specific ultrasonic effect separate from that of heating. Rats with a transplantable tumor (BICR/M1) were treated with x-rays (2000 rads in 35 min) and ultrasound (1 MHz frequency, 1 W/cm^2 average intensity for 35 min) either alone or in combination. During the treatment the animals were immersed in a water bath at $34°-35°C$. The temperature rise in the tumors receiving ultrasound was $3°$ or $4°C$ (to $38°-39°C$), at which level one would not expect thermal effects. Under the above conditions there was no significant effect on the growth rate of the tumors, either by ultrasound alone or combined with ionizing radiation.

The possibility that the timing of ultrasonic treatment in relation to cobalt therapy can be critical is suggested by the work of Fujita and Sakuma (1974), working with a conchal system. The simultaneous application of the two treatments, or ultrasound given prior to γ-irradiation, was the most effective, while the application of ultrasound after radiation was virtually without effect.

Ultrasonic treatment may potentiate the cytotoxic effects of some chemotherapeutic agents in a manner similar to the effect of direct heating.

The cytotoxic effect of nitrogen mustard in mouse L1210 leukemia can be enhanced by ultrasound (Kremkau et al., 1974). Cells were exposed in vitro to a continuous beam of ultrasound (1.9 MHz, 10 W/cm^2 for 10 min), treated by nitrogen mustard (1 μg/ml) alone, or given the two treatments in combination. The surviving cells were then assayed by inoculation into recipient animals

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whose survival time was evaluated. Control animals and the recipients of soni-
cated cells had a median survival of 10 days; for the drug-treated animals it was
13 days and for those with the combined treatment it was more than 30 days,
with 60% cures. The L1210 mouse leukemia line is highly thermosensitive
(Giovanella *et al.*, 1970), although the relatively short exposure time was
probably not enough to cause lethal heat damage. Synergistic effects of hyper-
thermia and alkylating agents are discussed in another section of this review, but
the above results probably represent another good example of heat–drug com-
bination therapy.

C. Clinical Use

The use of ultrasound for a variety of medical purposes was intermittently
advocated for many years, but was limited by fears of the biological hazards
involved. The successful use of ultrasound in several diagnostic procedures and
exposure of the most sensitive tissues (such as the central nervous system, eye,
fetus) brought the need for continuous basic studies and evaluation of the
biological hazards (reviews by Hill, 1968; Taylor and Dyson, 1972).

The first report of the successful clinical use of ultrasound in cancer therapy
(multiple skin metastases of sarcoma) was published by Horvath (1944), who
cited good results achieved in treatment of experimental tumors by Japanese
workers since 1934. The same author claimed cures of skin cancer in more
patients (references in Woeber, 1965). Woeber, following experiments in ani-
mals, treated 50 patients with primary skin cancer (including two melanomas)
by combining x-ray therapy reduced by one-third from the accepted dose, and
sonication of 0.2–0.3 W/cm^2 intensity and 1 MHz frequency (Woeber and Stein,
1963; Woeber, 1965). The results, although impressive, had little impact, be-
cause they could equally well and indeed more easily be achieved by the
traditional x-ray therapy with a slightly higher dose.

The fact that bones and air-containing tissues such as lungs are severe obstacles
to the transmission of ultrasonic energy is a serious limiting factor for its clinical
applicability. The fact that ultrasonic attenuation in brain tumor tissue (and
therefore its heating potential) is higher than in normal brain tissue (Lele, 1975)
also seems to have no immediate practical application. However, Heimburger *et
al.* (1974) treated some patients in an interesting way. Patients with intracranial
tumors had a segment of their skull removed and replaced by a stainless steel
wire mesh prosthesis. Diagnostic ultrasonic scanning through the intact scalp was
carried out to follow the changing size of the tumor under drug therapy. On the
assumption that ultrasound could increase the membrane permeability of tumor
cells to drugs, a few selected patients were treated with drugs as well. The
combined treatment appeared to be more effective than chemotherapy alone.

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applied for 10 min to Chinese hamster cells after various doses of radiation. The temperature rose to 42°C. Ultrasound was ineffective when given alone, but enhanced the radiation effect by a factor of 1.3. This effect could not be obtained when the same temperature at 42°C was achieved by heating in an incubator. The authors explain their findings by suggesting that probably more sonic energy will be absorbed in the nuclei, which have nearly twice the acoustic attenuation coefficient of whole cells and tissues. This could lead to localized microscopic heating damage in excess of any macroscopic heating damage.

An interesting system, exploiting some melanin-binding drugs and ultrasound, was suggested for the treatment of melanoma (McGinness and Corry, 1974; Corry and McGinness, 1974). Chlorpromazine and Kanamycin (drugs known to bind to melanosomes) were added for 1 or 2-hr to Chinese hamster ovary cells and three human melanoma lines of different pigment content. After removal of the drug the cells were exposed to an ultrasonic beam (no details given in the abstracts) and cell survival measured by DNA-synthesis and colony-formation assays. In the pigmen   cells the treatment resulted in a 10-fold greater killing. The possibility that the mechanical effects of ultrasound could release tumor cells was suggested by Chamberlain (1967), who exposed tumor pieces of sonication *in vitro*; an increased number of tumor cells were released into the medium. Temperature changes in the dishes were not reported.

In vivo experiments by Southam *et al.* (1953) compared the effect of ultrasound on normal mice and on animals inoculated with five different solid tumors and AK-4 leukemia. Normal mice were studied at a wide range of frequencies (0.5 to 3.8 MHz), intensities (1 to 10 W/cm²), and exposure times (1 to 25 min) of the central trunk area. Histological studies of 15 different tissues and organs were performed at scheduled intervals after the almost whole-body exposure. Although temperature measurements are not reported, the lethal effects and the pathological findings following the higher exposure intensities and frequencies are indicative of damage due to very high temperatures. The tumor-bearing animals were mainly treated with the maximum tolerated doses of ultrasound (10 mW/cm² at 0.5 MHz). The solid tumors were bilateral and one of each pair was shielded during the treatment. The leukemic animals were similarly treated. No differences were found when the growth rates and histological appearances of treated and untreated tumors were compared. Similarly, serial leucocyte counts of treated and untreated leukemic mice were not significantly different. The authors express their disappointment in the results in their closing sentence: "These studies give no reason to believe that ultrasonic energy might be useful in the treatment of inoperable neoplastic disease of man."

More optimistic conclusions are suggested by the work of Janes *et al.* (1957). Osteogenic sarcomas induced in five rabbits were exposed to ultrasound (1 MHz, 2–3 W/cm²), which produced varying degrees of necrosis in the tumors. Two additional rabbits, with radiographic evidence of bilateral limb tumors, were

quencies, may cause bone necrosis (Buchtala, 1948). The intensities of ultra-sound in use in diagnostic medicine (10–100 $\mu W/cm^2$) and in physiotherapy (1–3 W/cm^2) are below this potential hazard.

B. Experimental Use

In vitro experiments on the application of ultrasound to cancer therapy are relatively few. The reason is, perhaps, the variety and complexity of effects achieved by interaction of ultrasound with cells (Nyborg, 1972). Cells in suspension can be severely damaged by rupture of the cell membrane due to cavitation, a phenomenon that supposedly does not occur in organized tissues. Therefore, experiments *in vitro* need to be planned to avoid this artifact, either by using cells in gel suspension, as suggested by Clarke *et al.* (1970), by less frequent energy pulses (Clarke and Hill, 1969), or by increasing the environmental pressure (Ioshi *et al.*, 1973).

The cavitation effect is illustrated by the following: using L5178 Y mouse leukemia cells, Clarke and Hill (1969) showed that a continuous sonication of 10 sec (1 MHz, output of 15 W/cm^2) disrupted 50% of the cells. However, a 5-hr pulsed dose (avoiding the cavitational effect) gave a survival of nearly 100% despite a cumulative radiation dose 150 times that of the continuous exposure. In the same work it was also shown that the mitotic period is the most sensitive to ultrasound. Although the effect described was mainly due to cavitation, some thermal effect cannot be excluded.

Loch *et al.* (1971) treated human cells of three different lines (diploid embryonic brain cells, aneuploid cells derived from normal amniotic membrane, and HeLa cells) with ultrasonic clinical diagnostic and therapeutic devices (pulsed, 2.5 MHz frequency, output less than 10 mW/cm^2, exposure time 5–45 min; or continuous wave, 870 kHz, output of 0.05 to 3 W, exposure time of 10 min). A significant rise of temperature in the medium was avoided and was less than 0.5°C. In all three cell lines the growth rate was decreased by more than 25% when treated by the "therapeutic type" sonication.

Bleaney *et al.* (1972) exposed hamster lung cells in suspension to continuous and pulsed 1.5 MHz ultrasonic beams of 8.8 W/cm^2 and 15 W/cm^2 respectively, under conditions where no evidence of cavitation could be found. When the temperature of cells did not rise above 40°C, cell reproductive integrity was not affected.

The combination of ultrasound with ionizing radiation in an *in vitro* system with L5178Y mouse leukemia cells was studied by Clarke *et al.* (1970). Both heating and cavitation effects were deliberately avoided and no effect of ultrasound on the radiosensitivity of the cells could be demonstrated.

Recently Todd and Schroy (1974) suggested the possibility of useful thermal effects when ultrasound (frequency 920 kHz, intensity of 1.5 W/cm^2) was

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VIII. Ultrasound in Cancer Therapy

A. Introduction and Physical Properties

Ultrasonic energy can be defined as sound waves of a frequency higher than 20,000 Hz and therefore above the detection level of the human ear. The frequencies between 20 kilohertz (kHz) and 1 megahertz (MHz) are used for industrial purposes (e.g., ultrasonic cleaners). Medical devices employ frequencies in the MHz range. Ultrasound and sound require a transmission medium, in which they travel in the form of a wave. Unlike electromagnetic radiation, they are unable to propagate across a vacuum. Their velocity of propagation is variable and is directly related to the density and inversely to the elasticity of the medium. Therefore, in solid matter (and dense tissues such as bone) the sound velocity is more than twice that measured in water (and soft tissues) and 13 times the propagation velocity in air (Wells, 1972). In fact, it is quite difficult to propagate high-frequency ultrasound through air, and for practical purposes its study and applications are confined to solids and liquids.

When an ultrasonic beam passes through a medium most of the acoustic energy will be absorbed and turned into heat. The absorption, and therefore the induced rise in temperature, increases linearly with the frequency (Dunn, 1965; Taylor and Pond, 1970). Other physical and biophysical phenomena accepted as nonthermal effects of ultrasound may be present, and are described in the reviews of Taylor (1974) and Hill (1972). These include, for example, the phenomenon of cavitation, in which dissolved gases in fluid grow to bubbles that may behave as resonant cavities and vibrate at much higher amplitudes than the incident ultrasonic wave. This effect is actually the basis for the action of the ultrasonic cell disintegrator. Another example is the production of standing waves by the scattering of the ultrasonic beam at structural interfaces that are acoustically inhomogeneous (such as soft tissue and bone). However, Lele and Pierce (1972) point out that some of the nonthermal effects may in fact be producing hyperthermic damage at a microscopic level without overall measurable heating.

The macroscopic heating effect due to ultrasound has been suggested and tried as a means of inducing hyperthermia for cancer therapy. The general principles, described in the Section VII on microwaves, of the thermal regulatory processes in a living organism and the tumor mass and blood-supply relations are equally valid for ultrasound. But the high absorption of ultrasound in bone (Nelson *et al.*, 1950; Lehmann and Guy, 1972), and especially at high output and fre-

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D. Conclusions

The spectacular development of clinical diagnostic ultrasound, as shown by more than 3000 references collected by Clark and White (1975) over the period from 1971 to 1975, contrasts with the apparent lack of progress in its therapeutic use. The applicability of the thermal effect of ultrasound as a source of hyperthermia needs further study. Comparisons with other sources as to the related side effects, problems of focusing, uniformity of heat distribution, cost, hazards to personnel, etc. are required. Further studies are necessary on the specific nonthermal effects of ultrasound that may have applications in the therapy field, such as the intracellular mechanical effects suggested by Woeber (1965), and interaction with melanin-binding drugs (McGinness and Corry, 1974).

The work that has been carried out so far and partially reviewed in this chapter is only the first step in a field that promises to have interesting therapeutic possibilities for the future.

IX. General Summary and Conclusions

The data presented in this review show that there is unequivocal evidence that moderate hyperthermia (41°–45°C) may kill cells *in vitro* and *in vivo*. There is some evidence that suggests that malignant cells may be more sensitive to heat than normal cells, and that subpopulations of tumor cells normally protected *in vivo* from radiation and drug treatment by hypoxia, poor blood supply, and other less well-defined factors may be particularly sensitive to heat. Hyperthermic treatment may act in a synergistic way with ionizing radiation and with some cancer chemotherapeutic agents. The temporal relationship of the various treatments is of importance from the point of view of both sequence and duration.

There are numerous methods of increasing hyperthermia, only some of which are likely to have practical value in clinical practice. The most promising techniques at present under consideration for future work involve the use of microwave or ultrasound beams that can be collimated and focused through multiple ports at deep-seated tumors. Other methods currently being used in a limited number of patients, such as techniques for whole-body heating and isolated limb perfusion, will probably have only restricted application. Appropriate methods for the continuous monitoring of normal and tumor tissue temperature are being developed and should soon be generally available.

Ultimately the success or failure of this form of treatment will depend on the ability to produce a greater cytocidal effect in tumor than in normal tissues. Much experimental work is still required to determine the optimum conditions for achieving this differential effect. This will involve technologists, physicists,

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Conclusions

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