Interaction Between 2450-MHz Microwaves and Ionizing Radiation in Tribolium Confusum

PING-KWONG LAI, MEMBER, IEEE, CHARLES A. CAIN, MEMBER, IEEE, AND HOWARD S. DUCOFF

Abstract—**The combined effect of single or fractionated ^{137}Cs γ radiation and 2 h of 2450-MHz microwave radiation at two different power levels, resulting in specific absorption rates of 680 or 760 W/kg in distilled H_2O, was investigated in the flour beetle, Tribolium confusum. The potentiating effect of microwave treatment after single γ irradiations was observed only if applied within 60 min; in contrast, the potentiating effect of microwave treatment given before γ -irradiation lasted at least 8 h. Microwave treatment also altered the kinetics of split-dose recovery. At the higher power level, split-dose recovery was abolished for at least 36 h; at the lower power level, split-dose repair of ionizing radiation damage was delayed for 8–10 h, and then, with longer interfraction intervals, survival increased. All of the foregoing observations essentially mimic those previously reported for appropriate hyperthermic treatment by means of immersion in hot water. These findings are in keeping with the hypothesis that heating, whether by microwaves or by water immersion, affects the repair capabilities of the beetle, either by damaging some of the enzymes that repair radiation-damaged DNA or by altering the cytostructural integrity of the DNA-chromatin membrane complex, rendering the DNA lesions less amenable to repair.**

I. Introduction

A. Hyperthermia and Cancer Therapy

USE of hyperthermia in treatment of malignant tumors has a long history. Recent renewed interest stems from encouraging data obtained with *in vitro* cell cultures, as well as with transplantable tumors in small animals. Among the effects that have contributed to this renewed interest are the following.

1) Hyperthermia reduces oxygen enhancement ratios for killing of tumor cells [1] by ionizing radiation.

2) Alone, hyperthermia causes greater killing of hypoxic tumor cells than of euoxic cells [2].

3) Cell-age dependence of sensitivity to heat differs from that of sensitivity to ionizing radiation; radioresistant *S*-phase cells are more sensitive to killing by hyperthermia than are the radiosensitive G_1 cells [3].

4) Hyperthermia induces selective damage to malignant cells *in vivo* [4] with little or minor reversible damage to normal tumor-bed tissue.

Manuscript received July 10, 1977; revised January 20, 1978. This paper was supported by Grant CA 13779, awarded by the National Cancer Institute.

P. K. Lai was with the Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801. He is now with the Department of Radiology, University of Pittsburgh Medical School, Pittsburgh, PA.

C. A. Cain is with the Department of Electrical Engineering and the Faculty of Bioengineering, University of Illinois, Urbana, IL 61801.

H. S. Ducoff is with the Department of Physiology and Biophysics and the Faculty of Bioengineering, University of Illinois, Urbana, IL 61801.

5) Hyperthermia alters the capacity of cells to accumulate and repair sublethal radiation damage [5].

B. Microwave Heating and Cancer Therapy

Regression has been observed in tumors treated with microwave-induced hyperthermia. Overgaard and Overgaard [6] treated twelve isologous experimental mouse tumors of different origin, degree of differentiation, and histology with short wave diathermia (27.12 MHz) in the temperature range 41–43°C. Some permanent cures were obtained after treatment, but curability varied from 0 to 25 percent for different tumors. Yerushalmi [7] investigated the effects of microwave heating (2450 MHz) on SV-40 induced fibrosarcoma in hybrid mice. A significantly higher cure rate was obtained when microwaves and X-ray were applied simultaneously. Mendecki *et al.* [8] reported 100-percent complete disappearance of mammary adenocarcinoma implanted in the C3H mouse after two treatments of microwave-induced hyperthermia of 43°C for 45 min. Thus microwave hyperthermia may be used as an antitumor agent either alone or as an adjunct to radiotherapy.

C. The Tribolium System

Flour beetles of the genus Tribolium have been used extensively in genetic, ecological, and nutritional studies, and they provide a radiobiological tool of growing importance [9]-[14]. The coupling effect of magnetic fields with temperature, ionizing radiation, and oxygen tension has been examined in detail by Amer [15]. Tribolium was also used in the Biosatellite-II experiments, in which the effects of weightlessness, gravity compensation, and ionizing radiation were examined [16]. This paper examines the modification by 2450-MHz microwaves of the repair of γ radiation in young adults of Tribolium confusum.

II. Materials and Methods

Methods of husbandry and handling of Tribolium confusum, of γ irradiation with ^{137}Cs, and of statistical analysis and graphic representation were described earlier [17]. Only young male beetles were used for the present study. The γ-radiation dose is expressed in gray (Gy), the new international unit for absorbed radiation (1 Gy is defined as the absorption of 1 J/kg and is thus equal to 100 rad). For 2450-MHz microwave treatment, 50 beetles were

0018-9480/78/0800-0530$00.75

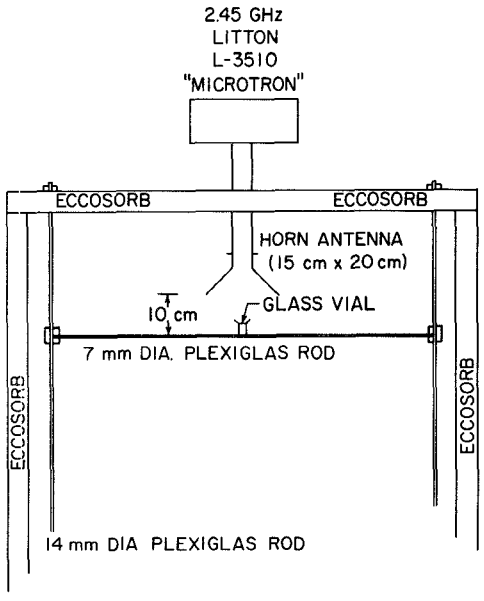

Fig. 1. Chamber for 2450-MHz irradiation.

placed in a 25-mm diameter glass vial 10 cm from the aperture of a horn antenna located inside an Eccosorb-lined exposure chamber (Fig. 1). The insects were free to move on the vial bottom during exposure; the electric field vector was parallel to the plane of the vial bottom. The microwave source was a 2.45-GHz continuous-wave Litton 3510 "Microtron."

The beetles were not strong microwave absorbers, having only 48-percent H_2O by body weight. Live insects averaged 2.48 mg of body mass, and were approximately 4 mm long. In view of the small size, temperature measurement of individual beetles was not attempted. Calorimetric measurements were made by replacing the beetles in the vial with 1 ml of distilled H_2O, and resulted in specific absorption rates (SAR) of 680 and 760 W/kg in distilled H_2O at the two different levels of input power used to irradiate the insects by microwaves. These SAR values in water were estimated from a series of temperature-rise measurements made after 10- and 15-s irradiation intervals. The initial water temperature was 25°C. Vials used for calorimetric measurements were insulated in styrofoam blocks. Final temperatures were determined within 3 s following the irradiation period, using a digital thermometer (Bailey BAT 8C) and a small retractable probe constructed from 2-mil diameter thermocouple wire.

The incident power levels resulting in 680- and 760-W/kg SAR in distilled H_2O were well above 200 mW/cm^2, the upper limit of measurement capability with the Narda 8100 power density meter. At an incident power density of 200 mW/cm^2, measured with the Narda 8122A probe, the SAR in 1 ml of distilled H_2O was 123 W/kg. Accordingly, we estimate the power levels which result in measured SAR values of 680 and 760 W/kg as approximately 1106 and 1236 mW/cm^2, respectively. The values of SAR in distilled H_2O are used to establish the level of radiation intensity, and are *not* intended to imply similar SAR values in intact beetles. The small size of the insects precluded accurate temperature measurements. For the same reason, heating curves were not undertaken with either intact insects or equivalent phantoms.

No detailed survival curve after microwave irradiation was obtained. The two power levels were well below those resulting in microwave killing.

Comparison between the modifying effect of microwave treatment (i.e., microwave hyperthermia) and that of hyperthermia by water-immersion of vials of beetles was made for three different protocols:

1) hyperthermia applied either immediately before or immediately after various graded γ-ray doses;
2) hyperthermia applied either before or after a particular γ-ray dose, with various intervals at the standard incubation temperature of 30°C between the two modalities;
3) hyperthermia applied immediately before or immediately after the first of two γ-irradiations with varying intervals of time at 30°C between; this procedure measures the kinetics of repair of radiation damage [19] as well as the magnitude of the sparing effect of dose-fractionation (SDF).

Microwave treatment was applied for 2 h in each case. Because of the distance between the microwave- and the γ-irradiation facilities, there was an unavoidable lag of 5–10 min between application of the two modalities even when the intent was "immediate." The sample for each point in the following figures consisted of 50 beetles; one standard error about the mean is shown on each data point.

III. Procedures and Results

The effect of hyperthermia (by water-immersion of vials of beetles) on radiation damage and on repair kinetics has been examined in great detail by Lai and Ducoff [17], [18]. Readers are referred to these publications for background information. Briefly, preirradiation hyperthermia exerts a long-lasting effect (at least 5 h); by contrast, postirradiation hyperthermia must be applied within 60 min to exert a sensitizing effect. In split-dose experiments, hyperthermia before or after the first γ-radiation dose, delayed split-dose repair for 8–10 h or suppressed split-dose repair for at least 36 h, depending on the level of hyperthermia.

A. Microwave and γ Irradiation in Immediate Sequence

Microwave treatment was applied for 2 h, either immediately before or immediately after various graded γ-ray doses, with the results depicted in Fig. 2. The broken lines represent previously reported data for hyperthermia by water-immersion of the vials of beetles. The sensitizing effect of microwave treatment (680-W/kg SAR in distilled H_2O) is indicated by the shift of the survival curve to the

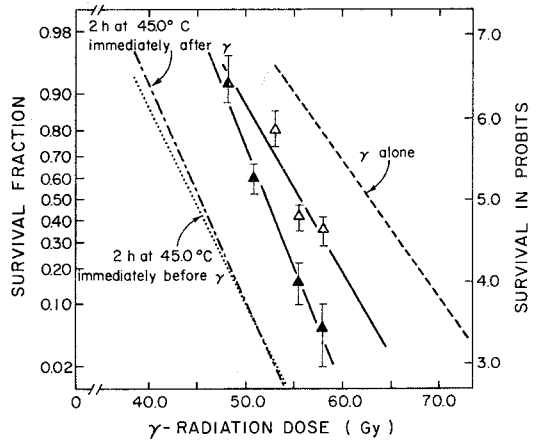

Fig. 2. Probit plot, survival of beetles after γ irradiation, and 2 h of hyperthermia by microwave treatment (680-W/kg SAR in distilled H_2O) or by water immersion in immediate sequence. Open triangles, microwave treatment following γ irradiation; filled triangles, microwave treatment before γ irradiation.

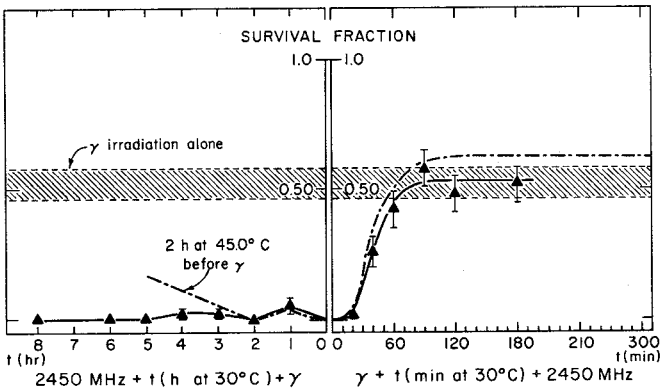

Fig. 3. Time course of interaction of microwave treatment (760-W/kg SAR in distilled H_2O for 2 h) and γ irradiation (62.8 Gy) in T. confusum ebony.

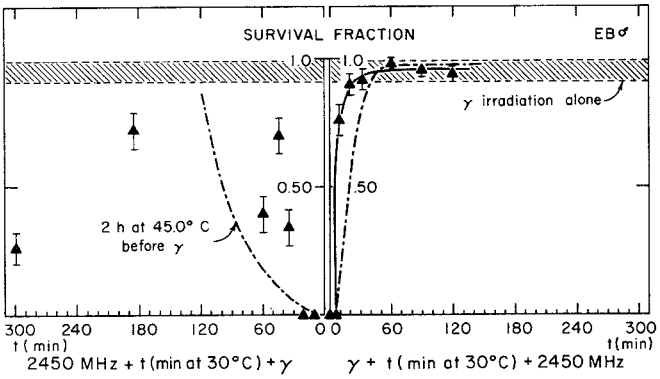

Fig. 4. Time course of interaction of microwave treatment (760-W/kg SAR in distilled H_2O for 2 h) and γ irradiation (53.1 Gy) in T. confusum ebony.

left of that for beetles exposed to γ rays alone (the dashed line in Fig. 2). The amount of the shift produced by this level of microwave irradiation is less than the amount of the shift produced by immersion at 45.0°C for 2 h but greater than that produced by immersion at 43°C.

B. Temporal Relationship Between Microwave and γ Irradiation

Microwave treatment was applied for 2 h, either before or after a fixed γ-ray dose, with various intervals at the normal incubation temperature of 30°C between the two modalities. These procedures are represented symbolically in Figs. 3 and 4 as "2450 MHz + t (h or min at 30°C) + γ" and "γ + t (min at 30° C) + 2450 MHz," respectively. Two different γ- radiation doses were used; the shaded regions in Figs. 3 and 4 represent one standard error about the mean of the survival of beetles exposed to γ-radiation alone.

1) γ-Ray Dose, 62.8 Gy; Microwaves, 760 W/kg SAR in Distilled Water: A single γ-ray dose of 62.8 Gy reduces the surviving fraction of the beetles to ~0.5. When γ-irradiation and microwave irradiation were applied in immediate sequence, survival dropped to zero (Fig. 3). For at least 8 h after microwave treatment there was still marked interaction with subsequent γ irradiation, so that survival of the beetles was virtually nil. In contrast, survival was indistinguishable from that of beetles exposed to γ irradiation alone, if microwave treatment was delayed for 60 min or longer after γ irradiation. These findings are similar to those obtained with immersion in a water bath at 45.0°C for 2 h [17] as summarized in Fig. 3.

2) γ-Ray Dose, 53.1 Gy; Microwaves, 760 W/kg SAR in Distilled Water: This combined mode of treatment is essentially the same as that for the experiment depicted in Fig. 3, except that the γ-ray dose was 53.1 Gy, which alone causes very little killing of the beetles. Nevertheless, the two modalities applied in immediate sequence produced complete lethality (Fig. 4). Note, however, the rapid increase in survival, which is unaffected if microwave treatment is given 60 min or longer after γ irradiation.

C. Microwave Treatment and the Sparing Effect of Dose-Fractionation (SDF)

A single γ-ray dose of 73.4 Gy reduces survival to 0.02–0.06 (2–6 percent); when given in two equal fractions (36.7 Gy), however, with various intervals in between, survival increases with increasing interfraction time to a peak at about 4 h, then falls slightly, and then rises again, as shown by the dashed lines in Figs. 5 and 6. A similar rise and fall is found for split-dose irradiation of mammalian cells [19], though the peak occurs earlier at the 37°C incubation temperature of mammalian cells. The sparing effect represents repair of sublethal damage.

The same γ-ray treatment, 2 equal doses of 36.7 Gy, was used for the experiments shown in Figs. 5 and 6. In the experiments presented in Fig. 5, however, the beetles were exposed to microwaves at 760-W/kg SAR in distilled H_2O for 2 h just prior to the first γ irradiation; in the experiments of Fig. 6, the microwave irradiation immediately followed the first γ irradiation, and the SAR was 680 W/kg (in distilled H_2O loads). In both cases, the beetles were then kept at 30°C for various periods of time, designated as "t (h at 30°C)," until the second γ irradiation. Fig. 5 shows that SDF was completely abolished,

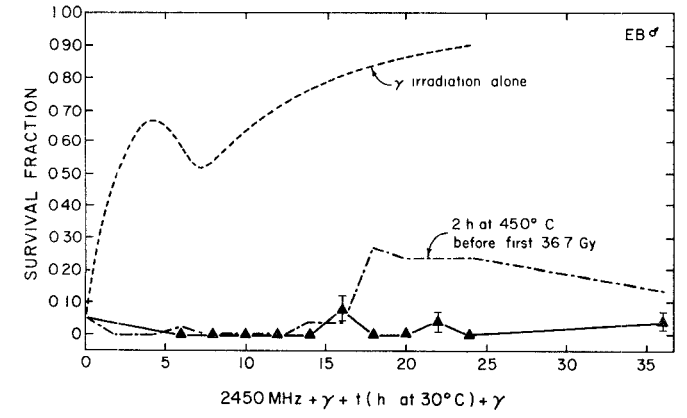

Fig. 5. Microwave hyperthermia and the sparing effect of dose fractionation in T. confusum. Triangles and solid line indicate survival of groups receiving microwave treatment (760-W/kg SAR in distilled H_2O) for 2 h immediately prior to the first of two γ irradiations (36.7 Gy each), with various interfraction intervals at 30°C.

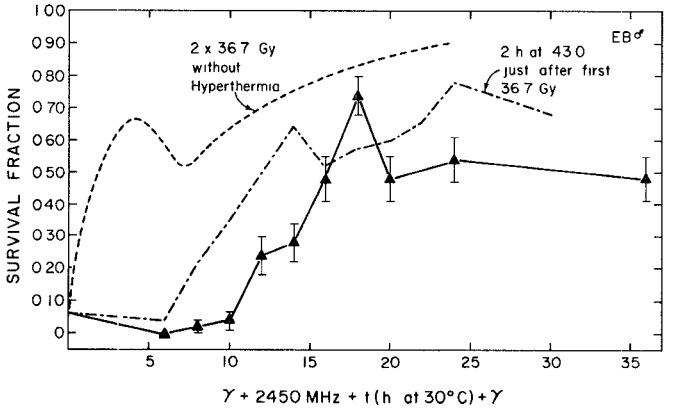

Fig. 6. Microwave hyperthermia and the sparing effect of dose fractionation in T. confusum. Triangles and solid line indicate survival of groups receiving microwave treatment (680-W/kg SAR in distilled H_2O) for 2 h immediately after the first of two γ irradiations (36.7 Gy each), with various interfraction intervals at 30°C.

even for interfraction intervals as long as 36 h at 30°C, by 2 h of microwave irradiation at the higher power level just before the first dose of 36.7 Gy. Similar suppression of SDF was observed [18] with water-immersion hyperthermia of 45.0°C for 2 h just before the first fraction of γ irradiation, as indicated by the dot-dash line of Fig. 5. The effect of microwave treatment was diminished when the lower power level was applied for 2 h immediately after the first γ-ray dose. As shown in Fig. 6, split-dose repair was delayed for 8–10 h, but eventually survival increased. This trend is similar to that with immersion hyperthermia of 43.0°C for 2 h given immediately after the first γ irradiation, as indicated by the dot-dash curve of Fig. 6. It should be emphasized that immersion hyperthermia, whether administered before or after the first γ irradiation, gives essentially the same suppression of SDF [18].

IV. Discussion

The findings of this study may be briefly summarized as follows.

1) Microwave irradiation in immediate sequence enhances the effect of ionizing radiation, regardless of the order of application.

2) When microwave treatment follows γ irradiation, interaction of the two modalities is detectable only if they are separated by less than 60 min.

3) With microwave treatment before γ irradiation, the enhancing effect persists for at least 8 h.

4) At the higher SAR (760 W/kg in distilled H_2O), SDF is completely abolished for interfraction intervals as long as 36 h.

5) Split-dose repair is delayed for only 8–10 h when the lower SAR (680 W/kg in distilled H_2O) is used for microwave irradiation.

In all of the foregoing respects, the effect of microwave treatment is very similar to that of water-immersion hyperthermia on the response to single or fractionated γ irradiation. It appears, then, that the sensitizing effect of microwaves on damage by ionizing radiation can be attributed entirely to heating by the microwave irradiation.

The death of the lethally-irradiated organism generally stems from the death, or loss of reproductive integrity, of proliferative cells; this is true both for mammals [20] and for beetles [9], [17]. The central role of damage to DNA in the cytocidal effect of ionizing radiation is also well established [21]. The cellular basis of thermal death of intact organisms is, however, still uncertain. Furthermore, thermodynamic calculations [22] suggest that the molecular basis of cell killing by heat is probably not DNA damage but may be damage to proteins. Possible specific sites of action of hyperthermia which have been suggested by various investigators include chromosomal protein [23], mitotic spindle protein [23], and the lipoprotein complex of the plasma membrane [24].

The results of the present study, as well as previous work with Tribolium [17], [18], emphasize the likelihood that the synergistic interaction between hyperthermia and ionizing radiation results from impairment by heat of the capcity to repair DNA damaged by the ionizing radiation. There is also some evidence that hyperthermia inhibits repair of sublethal radiation damage in cultured mammalian cells [5], [25]. The significance here is underscored by the finding that there is relatively little interaction between hyperthermia and densely-ionizing particles [26]; these produce cellular damage which is refractory to repair processes.

Hyperthermia could impair repair capacity by denaturation of enzymes necessary for repair or by alteration of the integrity of the DNA-nuclear protein-membrane lipoprotein complex which appears to play a role in repair. Note, however, that the molecular basis of thermal enhancement of the killing of cells by ionizing radiation need not be the same as that for cell killing by heat alone.

Results of the present study, together with previous work [17], [18] with the Tribolium system, suggest that the enhancement by microwave irradiation of damage by ionizing radiation is entirely the result of microwave-in-

duced hyperthermia. This work demonstrates the great importance of the time interval between γ irradiation and hyperthermia; microwave treatment may be the preferable method for rapid production of hyperthermia in tumors *in situ*.

References

[1] S. H. Kim, J. H. Kim, and E. W. Hahn, "The radiosensitization of hypoxic tumor cells by hyperthermia," *Radiology*, vol. 114, pp. 727–728, 1975.

[2] L. E. Gerweck, E. J. Gillette, and W. C. Dewey, "Killing of Chinese hamster cells *in vitro* by heating under hypoxic or aerobic conditions," *Europ. J. Cancer*, vol. 10, pp. 691–693, 1974.

[3] S. H. Kim, J. H. Kim, and E. W. Hahn, "The enhanced killing of irradiated HeLa cells in synchronous culture by hyperthermia," *Radiat. Res.*, vol. 66, pp. 337–345, 1976.

[4] J. Overgaard, "Ultrastructure of a murine mammary carcinoma exposed to hyperthermia *in vivo*," *Cancer Res.*, vol. 36, pp. 983–995, 1976.

[5] E. Ben-Hur, M. M. Elkind, and B. V. Bronk, "Thermally enhanced radioresponse of cultured Chinese hamster cells: Inhibition of repair of sublethal damage and enhancement of lethal damage," *Radiat. Res.*, vol. 58, 38–51, 1974.

[6] J. Overgaard and K. Overgaard, Personal communication.

[7] A. Yerushalmi, "Cure of a solid tumor by simultaneous administration of microwaves and X-ray irradiation," *Radiat. Res.*, vol. 64, pp. 602–610, 1975.

[8] J. Mendecki, E. Friedenthal, and C. Botstein, "Effects of microwave-induced local hyperthermia on mammary adenocarcinoma in C3H mice," *Cancer Res.*, vol. 36, pp. 2113–2114, 1976.

[9] H. S. Ducoff, "Causes of death in irradiated adult insects," *Biol. Rev.*, vol. 47, pp. 211–240, 1972.

[10] ——, "Form of the increased longevity of Tribolium after X-irradiation," *Exp. Geront.*, vol. 10, pp. 189–193, 1975.

[11] N. D. Glenn and H. S. Ducoff, "Acute lethality after fast-neutron and X-irradiation of Tribolium confusum," *Radiat. Res.*, vol. 65, pp. 120–129, 1976.

[12] H. E. Erdman, "Comparative X-ray sensitivity of Tribolium confusum and T. Castaneum (coleoptera: tenebrionidae) at different developmental stages during their life-cycle," *Nature*, vol. 195, p. 1218, 1962.

[13] ——, "Dose ratio of X-rays to fast neutrons in producing dominant lethals in flour beetles, Tribolium castaneum," *Nature*, vol. 205, pp. 99–100, 1965.

[14] ——, "Fast-neutron effects on productivity of young and old flour beetles, Tribolium castaneum Herbst, and alterations at different temperatures and after exposure of either or both sexes," *Int. J. Radiat. Biol.*, vol. 9, pp. 305–311, 1965.

[15] N. M. Amer, "The effects of homogeneous magnetic fields, ambient gas composition and temperature on development of Tribolium confusum," Ph.D. dissertation, Univ. of California, Berkeley, 1965.

[16] C. H. Yang and C. A. Tobias, "Interaction between radiation effects, gravity and other environmental factors," in *Life Science and Space Research XII*, P. H. A. Sneath, Ed. Berlin: Akademie-Verlag, 1974, pp. 21–30.

[17] P. K. Lai and H. S. Ducoff, "Kinetics of interaction of hyperthermia and ionizing radiation in Tribolium confusum," *Radiat. Res.*, vol. 72, pp. 296–307, 1977.

[18] ——, "Hyperthermia and the sparing effect of dose-fractionation in Tribolium confusum," accepted for publication in *Radiat. Res.*

[19] M. M. Elkind and G. F. Whitmore. *The Radiobiology of Cultured Mammaliam Cells*. New York: Gordon and Breach, 1967.

[20] V. P. Bond, T. M. Fliedner, and J. O. Archambeau, *Mammalian Radiation Lethality: A disturbance in cellular kinetics*. New York: Academic Press, 1965.

[21] K. I. Altman, G. B. Gerber, and S. Okada, *Radiation Biochemistry*, New York: Academic Press, vol. 1, 1970.

[22] A. Westra and W. C. Dewey, "Variation in sensitivity to heat shock during the cell-cycle of Chinese hamster cells in vitro," *Int. J. Radiat. Biol.*, vol. 19, pp. 467–477, 1971.

[23] W. C. Dewey, A. Westra, H. H. Miller, and H. Nagasawa, "Heat-induced lethality and chromosomal damage in synchronized Chinese hamster cells treated with 5-bromodeoxyuridine," *Int. J. Radiat. Biol.*, vol. 20, pp. 505–520, 1971.

[24] K. Bowler, C. J. Duncan, R. T. Gladwell, and T. F. Davison, "Cellular heat injury," *Comp. Biochem. Physiol.*, vol. 45A, pp. 441–450, 1973.

[25] L. E. Gerweck, E. L. Gillette, and W. C. Dewey, "Effect of heat and radiation on synchronous Chinese hamster cells: killing and repair," *Radiat. Res.*, vol. 64, pp. 611–623, 1975.

[26] E. W. Gerner and J. T. Leith, "Interaction of hyperthermia with radiations of different linear energy transfer," *Int. J. Radiat. Biol.*, vol. 31, pp. 283–288, 1977.