Eur J Cancer Clin Oncol Vol. 17, No. 7, pp 781–795, 1981.
Printed in Great Britain

0014-2964/81/070781-14 $02.00/0

Enhancement of Thermal Damage to the Microcirculation of 'Sandwich' Tumours by Additional Treatment

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Abstract—*The effects of hyperthermia on tumour microcirculation were investigated. For this purpose, transparent tumours were grown in the 'sandwich' system in the dorsal skin flap of the rat (Rhabdomyosarcoma* BA1112 *in WAG/Rij rats). Heating was performed with air; the temperature of the cover slip overlying the tumour was kept at either* 42.5° *or* 42°C *by means of an electronic controller. Evaluation of the effect was based on microscopic observation and photographic recordings and was expressed as the proportion of tumours with intact microcirculation, i.e., the proportion of tumours not showing heat damage. The results indicate that, at* 42.5°C *after a latent period of about* 1 hr, *the microcirculation of the tumours begins to slow down. At the end of a treatment time of* 180 min, *about* 70% *of the tumours show microcirculatory damage. It appears that some time is required before the majority of the tumours show heat damage. The effect is still essentially the same at* 42°C, *but the damage is to a much lesser extent. When the animals received additional treatment, i.e. misonidazole, glucose or* 5-*thio*-D-*glucose, the damaging effect of the hyperthermic treatment at* 42°C *appeared to be increased. The effects of all combined treatments approximated the degree of inactivation obtained with* 42.5°C *treatment.*

INTRODUCTION

THE POTENTIAL of heat in the treatment of tumours has been well established. Recent reviews on the subject with regard to the effects of heat on cells and tumours have been published by Overgaard [1], Suit and Gerweck [2], Bhuyan [3], Field and Bleehan [4] and Dewey *et al.* [5]. Many possible mechanisms for the destructive effect of heat on tumours have been proposed and investigated. No conclusive evidence has yet been obtained that tumour cells as such are necessarily more heat sensitive than normal cells when the clonogenic viability of cells is used as the end point. However, cells which are nutritionally deprived or have a low extracellular pH are known to be highly sensitive to moderate temperatures. Such conditions are assumed to exist in central areas of tumours [6–8]. This would imply that the center of a tumour would be more sensitive to the same heat treatment than its peripheral rim of viable tumour cells. Moreover, the effect of heat under these conditions is likely to depend on factors that may influence the metabolism of the cells in such areas. Some of these aspects could be studied quite well in our transparent tumour system. Temperatures of 42.5 and 42°C for 3 hr were applied and the metabolically active substances investigated included glucose, 5-thio-glucose and misonidazole.

Accepted 30 January 1981.

MATERIALS AND METHODS

The tumour system consisted of the Rhabdomyosarcoma BA1112, which is isogeneous in the WAG/Rij strain of rats. The tumour originated in 1962 in the submandibulous masculature of a total body irradiated rat and can be presently considered as an undifferentiated sarcoma. Small pieces of tumour of about 0.2 mm^3 were implanted in 'sandwich' chambers [9, 10]. The latter consists of a skin flap of the rat in which a part of the subcutis is enclosed between a glass cover slip and a mica base plate. The tumour grows in a sheet-like manner in this system. The

thickness of the tumour is about 150–200 μm, the diameter 3–5 mm. For orientation purposes, a few carbon microspheres of 80 μm diameter are implanted along with the tumour tissue.

The heating and temperature measuring system consisted of a proportionally controlled air heating device (Fig. 1). The skin flap with its supporting plastic splint extended into a box which was made of perspex and lined with an additional 2 mm thick layer of polystyrene foam. The heat was delivered through five radiotype resistors (total power 50 W) which were mounted in front of a small blower. The sensor of the electronic temperature controller was attached by means of a small piece of adhesive gum to the cover slip overlying the tumour area. This small piece of adhesive gum also served for the attachment of the thermocouple needle of the Ellab (Ellab Instruments, Copenhagen, Denmark) medical thermometer. The piece of adhesive gum was always placed next to the tumour in order not to interfere with observations. Because of the good thermal contact provided by the adhesive gum between the sensors and the cover slip, the cover slip and the tumour and the close proximity of sensors, adhesive gum and tumour, the tumour temperature was assumed to be sufficiently close to the measured temperature. Microthermocouple measurements are not possible in this set-up, as the microthermocouples are too fragile to penetrate the skin, chamber, etc., from the side. Also, infrared measurements proved not to be feasible, due to the high absorption of infrared by the (200 μm thick) glass cover slip. Some temperature measurements in a similar 'sandwich' system by Cetas and Boone [11] support the view that a reasonable approximation of the tumour temperature is obtained in the manner described. Because of the experience that proportional controllers reach their preset temperature in a slightly undulating way, the final temperature was always approached by manually increasing the preset temperature of the controller by 0.1°C increments. In this way, we could be sure that the tumour was never exposed to an 'overshoot' temperature. The temperature of the circulating air in the box in the direct vicinity of the cover slip was usually about 0.5°C higher than that of the indicated thermocouple needle temperature.

The recording of changes occurring in the tumour was made by observation (every 15 min) and photography (every hour). To avoid any contribution of heat by the HBO-100 lamp, the shutter was normally closed and the green-coloured, heat filtered light was admitted only during the brief moments of observation or photography. The additional

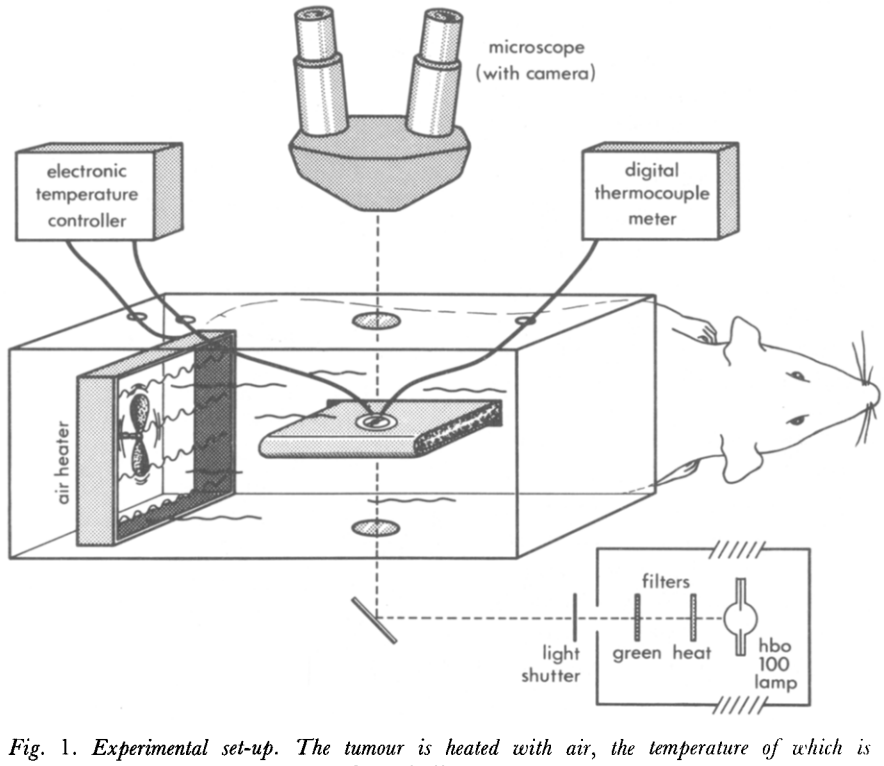

Fig. 1. Experimental set-up. The tumour is heated with air, the temperature of which is electronically controlled.

treatment consisted of the administration of either glucose, 5-thio-D-glucose or misonidazole. Glucose in a concentration of 50 mg. ml^{-1} was given as a single intraperitoneal injection of 1 g.kg^{-1} 2 hr before the beginning of the heat treatment. The drug 5-thio-D-glucose (Pfanstiehl, Waukegan, IL) was administered in the drinking water for the whole week preceding the treatment. The concentration in the drinking water was 1 mg.ml^{-1}. As the animals were always kept in individual cages, the average amount of 5-thio-D-glucose intake could be estimated. The animals ingested approximately 200 mg.kg^{-1}. day^{-1}. Misonidazole (Roche Products; 30 mg.ml^{-1}) was given i.p. at the beginning of the heat treatment. The dose was 300 mg.kg^{-1}. The animals were anaesthetized i.p. with "Hypnorm" (Philips–Duphar) at 1 ml.kg^{-1}.

A total of 140 tumours were exposed to the various treatments. The heat exposure time was invariably kept at 180 min at continuous temperature. Variations in measured temperature remained within ± 0.1°C of the preset temperature.

RESULTS

The results of the heat treatment at 42.5 and 42°C for 3 hr are shown in Fig. 2. It appears that the longer the exposure time, the more tumours show a stoppage of the microcirculation. This stoppage is frequently limited to the inner part of the tumour, leaving a small outer ring of circulating vessels. The dotted lines drawn in Figs. 2, 4, 5 and 6 were calculated via a Weibull curve [12]. There is an obvious difference in effect between the 42.5 and 42°C exposures. The exposure time for 50% effect at 42.5°C is about 160 min, while extrapolation of the 42°C curve gives 226 min. The administration of glucose 2 hr before the heat treatment appears to enhance the effect. This is demonstrated in Figs. 3(a)–(e). In this example, the circulation seems to first be affected after about 2 hr with an increase in extent for the last hour of treatment. The results of 41 treatments are shown in Fig. 4. A treatment time of about 160 min is required to reduce the number of tumours with intact circulation to one half of the initial number. The effect of 5-thio-glucose is shown in Fig. 5. It appears that more than

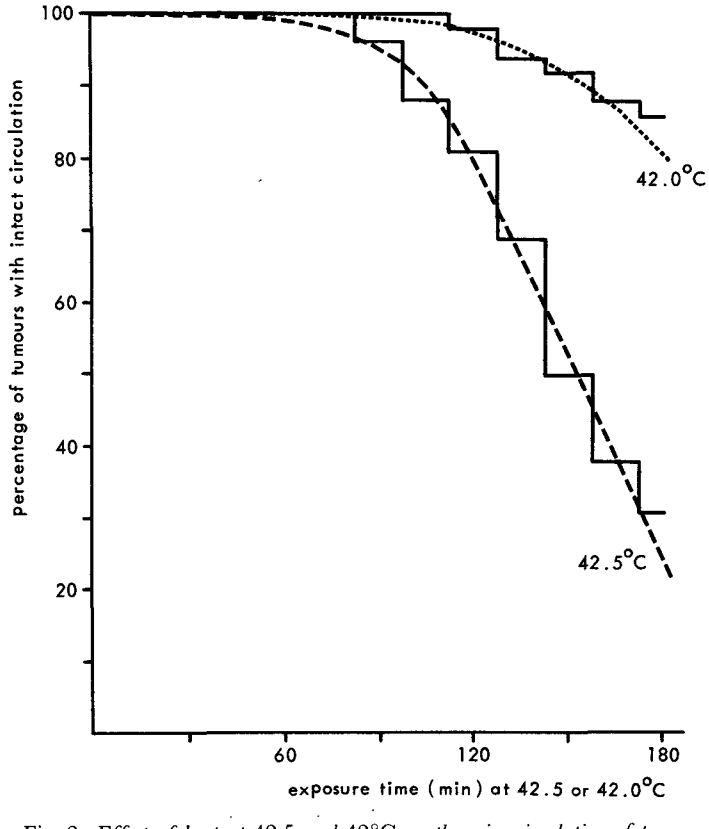

Fig. 2. Effect of heat at 42.5 *and* 42°C *on the microcirculation of tumours* (26 *tumours were used for the* 42.5°C *exposure and* 50 *tumours for the* 42°C *exposure). Ordinate: percentage of tumours with intact microcirculation. Abscissa: Exposure time. All tumours were exposed for the full* 180 min. *The curve through the observed data was calculated according to* [12].

3 hr is required for a 50% reduction. The (extrapolated) value for this is about 190 min. The effect of misonidazole is about the same as that of 5-thio-glucose. Misonidazole appears to enhance the effect of 42°C hyperthermia, as shown in Fig. 6. The (extrapolated) 50% effect time is about 190 min.

On the following day, the tumors showing microcirculatory inactivity almost inevitably show extensive necrosis in the parts affected. This is shown in Fig. 7. For the 42.5°C treatment, only 5 of 24 (21%) of the tumours treated for 180 min showed intact circulation on the day following the treatment, while the 42°C treatment resulted in 34 of 49 (69%) tumours without visible damage. The observations on the intensification of the microcirculatory damage during the 180 min treatment at 42°C by the various additional treatments showed that, with glucose, only 7 of 39 (18%) of the treated tumours had intact circulation on the next day. For 5-thio-glucose and misonidazole, these values were, respectively, 2 of 11 (18%) and 3 of 12 (25%), as evident from Fig. 7.

DISCUSSION

Hyperthermia induced damage of the microcirculatory system of tumours is now a well-recognized phenomenon. The curves shown in Fig. 2 indicate that some time at a hyperthermic temperature is required before this phenomenon takes place. However, many authors mention in their reports only the treatment temperature rather than the thermal exposure, which by definition consists of a given temperature applied over a defined time period. Moreover, the intensity of the thermal effects on the microcirculation of tumours may quite possibly depend on rather specific conditions, for instance, the type of animal, of tumour, the implantation site and the way in which heat is applied.

In human tumours, Sugaar and LeVeen report that, as a result of a rather ill-defined heat treatment, prominent vascular changes appeared in the surgical specimen [13]. Also, the observation of Herbst and Sauer [14] that during radiofrequency treatment, the temperature in human tumours increases slowly during the first 30 min but then rapidly increases to 46°C may probably have to be interpreted as being the result of a shutdown of the circulation in these tumours 30 min after the start of the heat treatment. Histological investigations in experimental tumour systems also tend to support this interpretation. Piro *et al.* [15] found marked vasodilation and stasis at 42°C and massive haemorrhage and necrosis at 44.5°C. Similar findings were made by Von Ardenne [16] at 41°C in a situation where the tumour was acidified to a pH of 6.1.

Microcirculatory studies with direct observation systems have also indicated that the microcirculatory flow is very responsive to heat [17]. Endrich *et al.* [18] demonstrated a substantial decrease in the arterial inflow after exposure to 41.3°C. The latter was quantified on the basis of velocity measurements on the erythrocytes of tumours *in vivo*. Endrich *et al.* also observed increased leukocyte adhesiveness, stasis in the blood vessels and the development of petechiae. Eddy [19] noted a rapid decrease in microvascular flow in tumours in the hamster check pouch. In his experiments, it was only a few minutes before the flow decreased, depending upon the temperature (range 41–45°C for the 30 min). Eddy reported constriction of arterioles, petechial haemorrhages and 'bulbous segments'. At 45°C, there was a stoppage after 20–25 min. It should be expected that there will be discrepancies in conclusions reached with different assay systems. For example, Gullino with his 'tissue isolated tumour' system observed no appreciable change in the Walker 256 carcinoma at various times after exposure to 43°C for 20 min [20] and Song *et al.* [21] also found the blood flow in this tumour to be unchanged by an exposure to 43°C for 1 hr. The difference between the above-mentioned investigations and the present results may be due to a multitude of factors. Not only are different tumours involved but also the site of implantation differs. Moreover, the heat delivery systems differ considerably and there may be essential differences between the temperatures used as well as the exposure times. As mentioned earlier, Fig. 2 gives the impression that a prolonged exposure time is required before the majority of the tumours show circulatory stoppage at 42.5 and 42°C. Whether or not a minor decrease occurs in the velocity of the erythrocytes moving in the tumour capillaries during heat treatment could not be evaluated in the present experiments, as this requires more extensive instrumentation. However, with the same 'sandwich' tumour system as described in these experiments but with a different heating method, Endrich *et al.* [18] were able to show that the arteriolar inflow decreased as early as 5–10 min after treatment. Apparently, tissue pH and oxygenation also decrease during

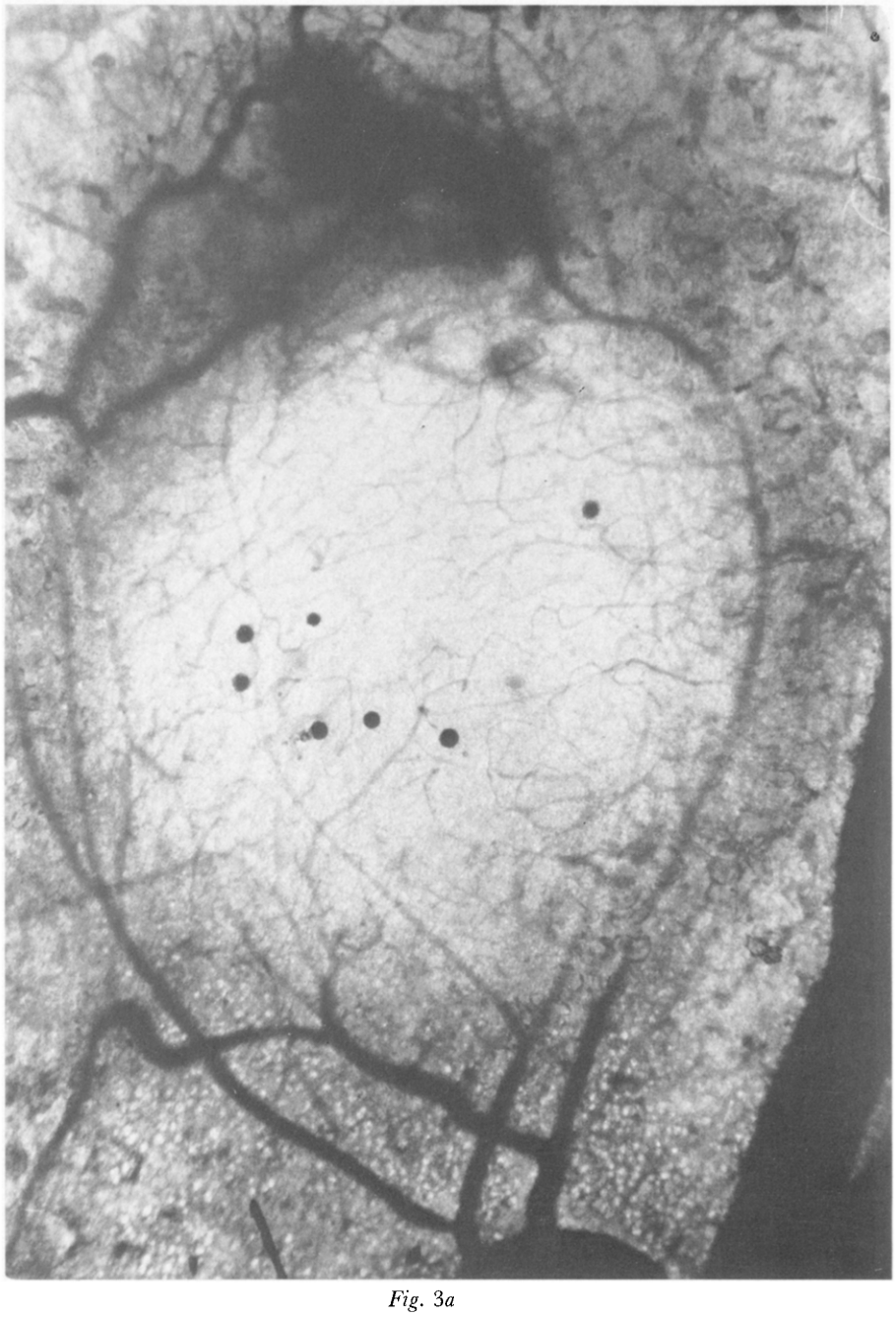

Fig. 3a

Fig. 3. Example of the effect of 42°C *heat treatment in combination with* 1000 mg.kg^{-1} *glucose i.p.* 2 hr *before treatment on the tumour microcirculatory system (tumour is Rhabdomyosarcoma BA* 1112). *The black dots in the tumour tissue are the* 80 μm *diameter microsphere markers. (a) Beginning of treatment; good circulation. (b) Same tumour after* 1 hr *at* 42°C; *good circulation. (c) Same tumour after* 2 hr *at* 42°C; *slow down in the circulation and areas of haemorrhages. (d) Same tumour after* 3 hr *at* 42°C; *no circulation, extensive haemorrhages. (e) Same tumour next day; no circulation, extensive necrosis.*

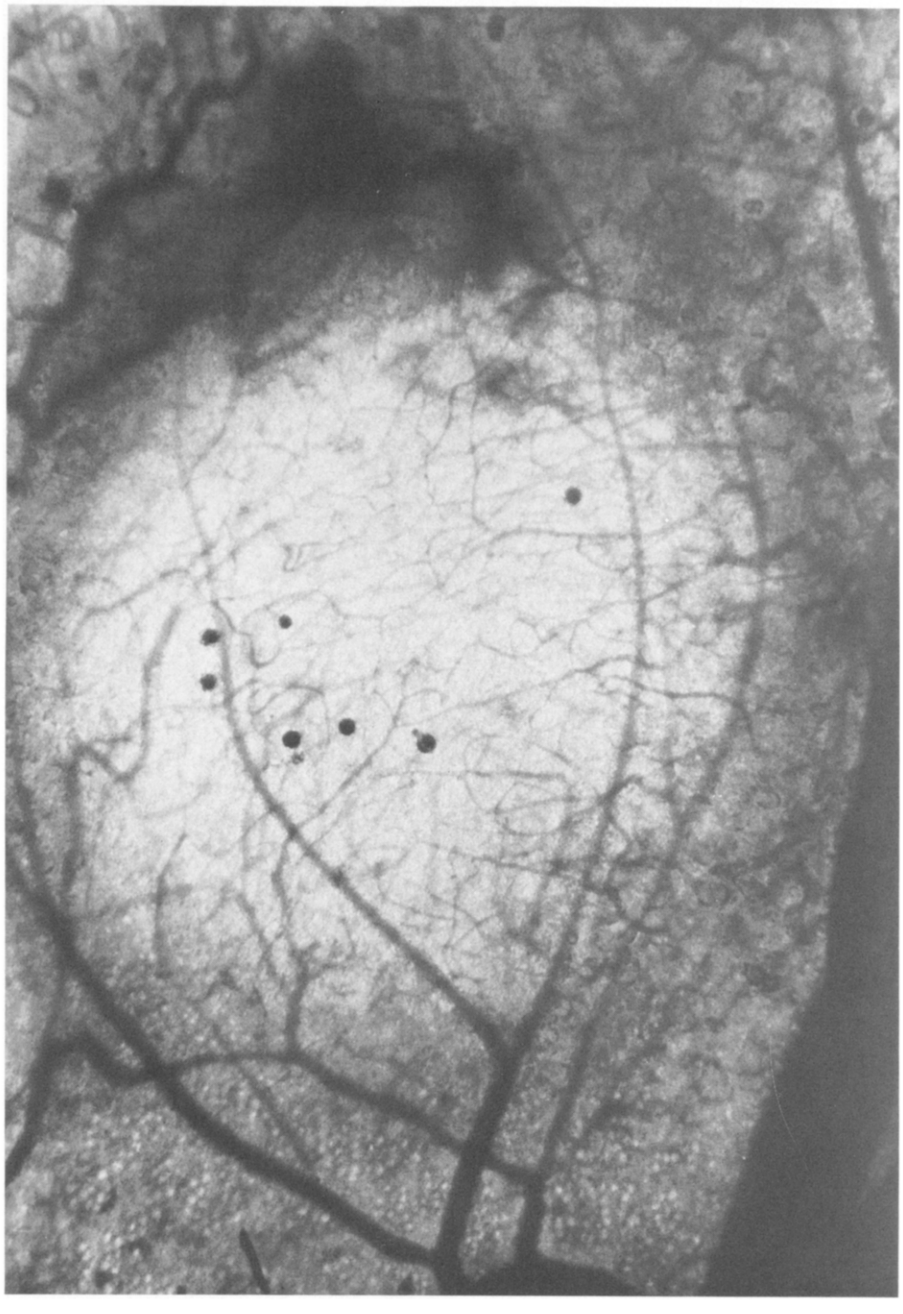

Fig. 3b.

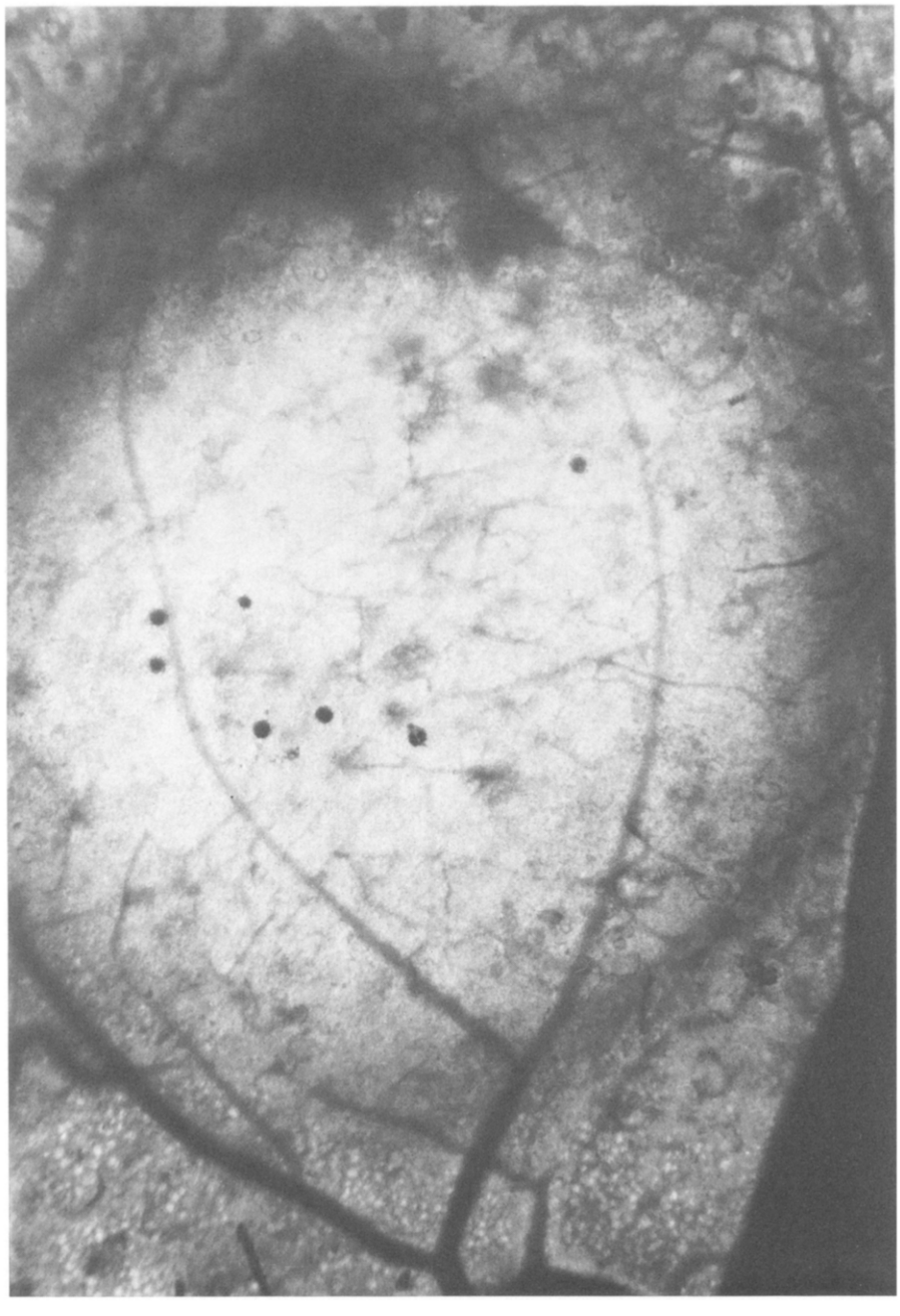

Fig. 3*c*.

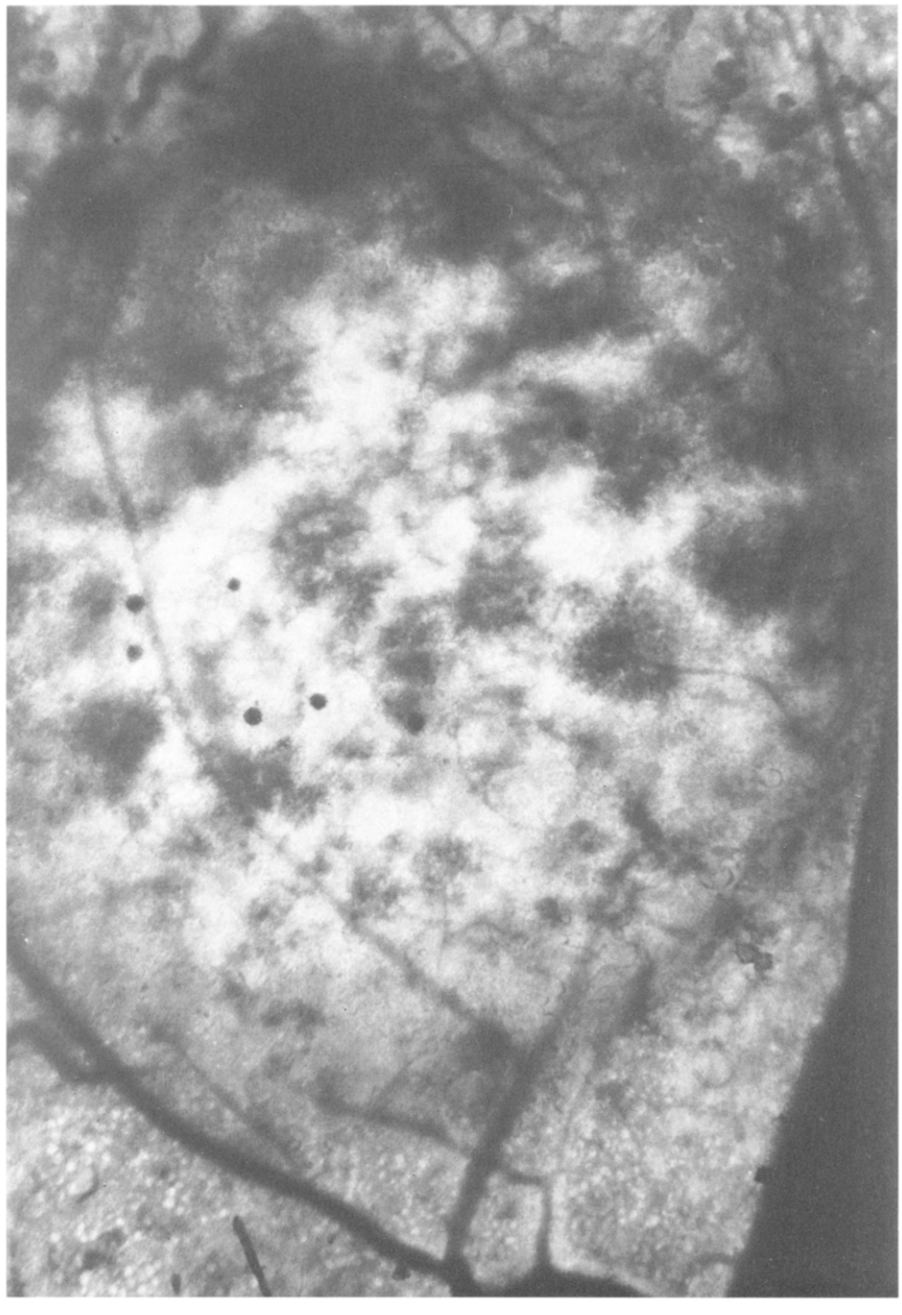

Fig. 3d.

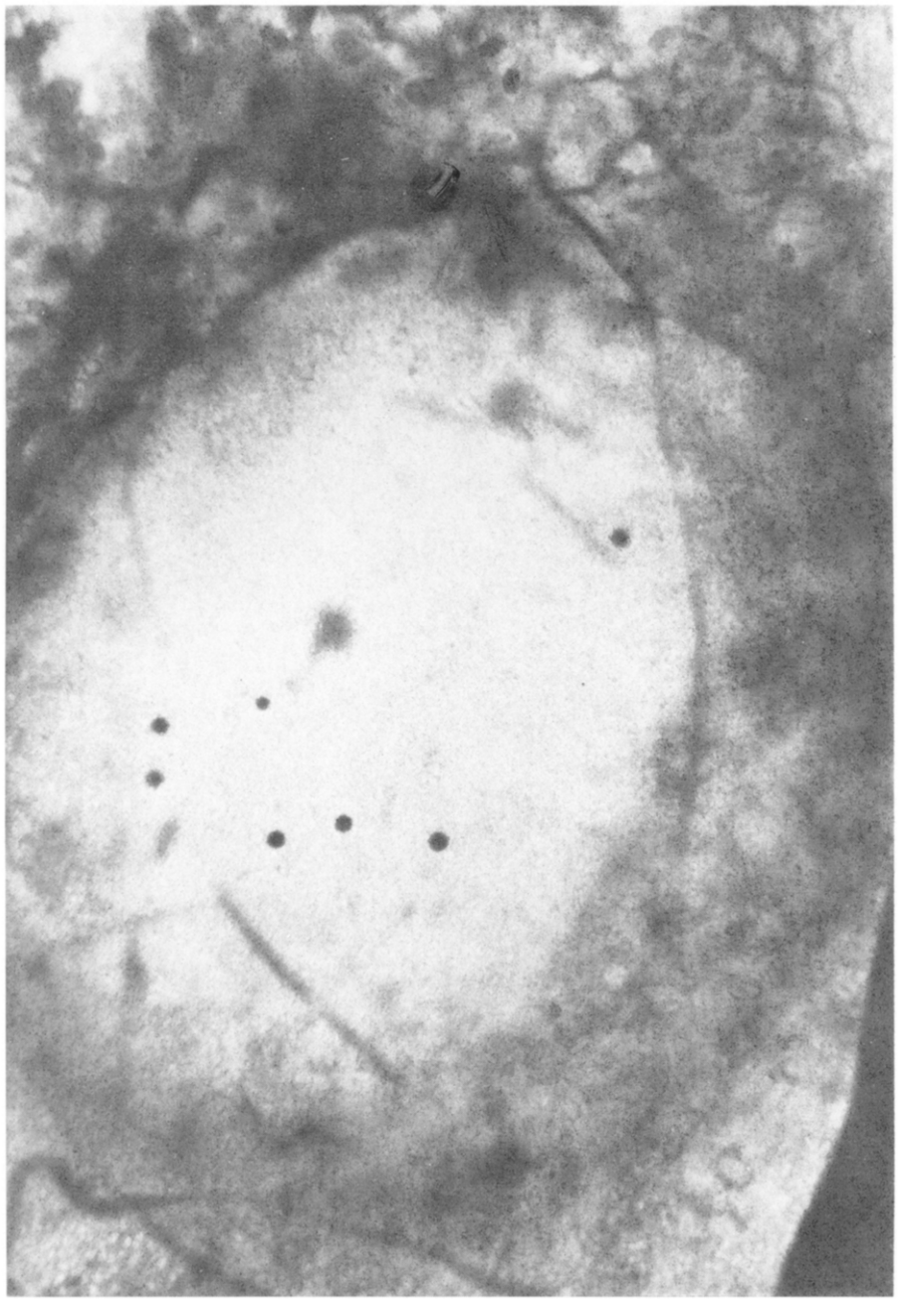

Fig. 3e.

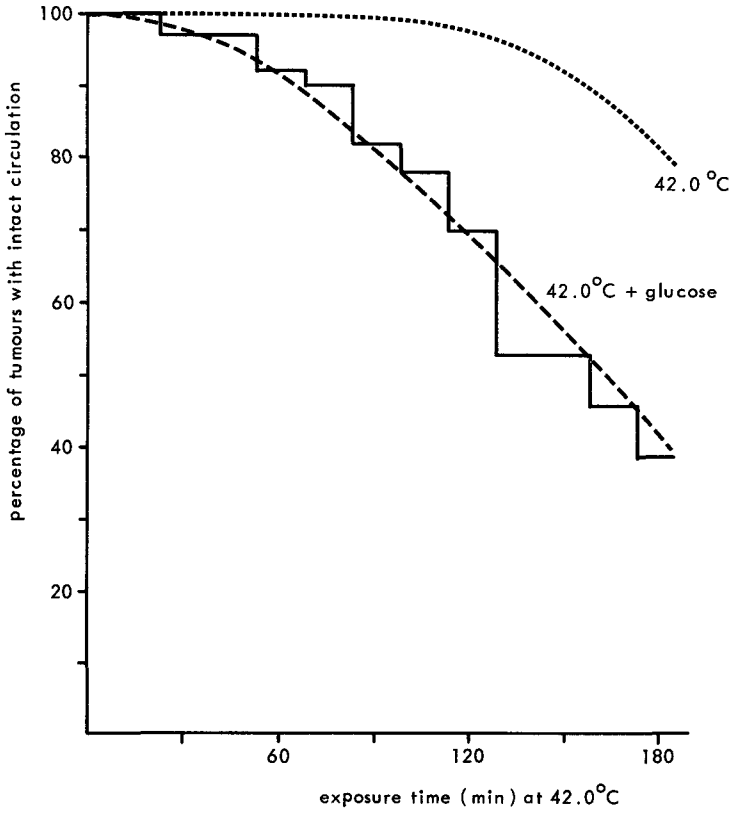

Fig. 4. Effect of 42°C *heat treatment in combination with* 1000 mg.kg^{-1} *glucose i.p. before treatment on the microcirculation of tumours* (n=41). *Parameters as in Fig.* 2. *Dotted line is taken from Fig.* 2 (42°C).

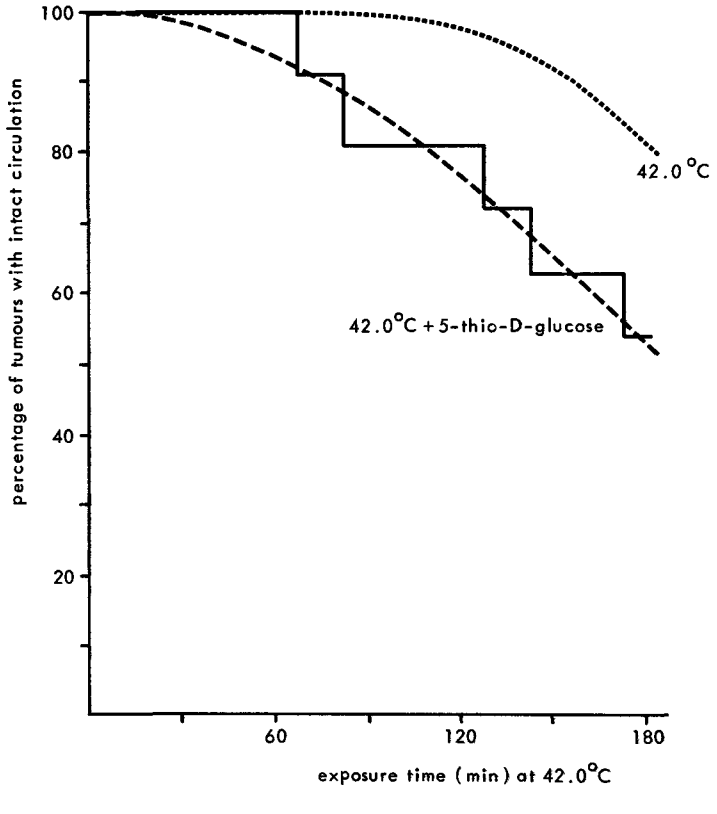

Fig. 5. The effect of a combination of 5-*thio*-D-*glucose and hyperthermia on the microcirculation of tumours* (n=11). *Parameters as in Fig.* 2. *Dotted line taken from Fig.* 2 (42°C).

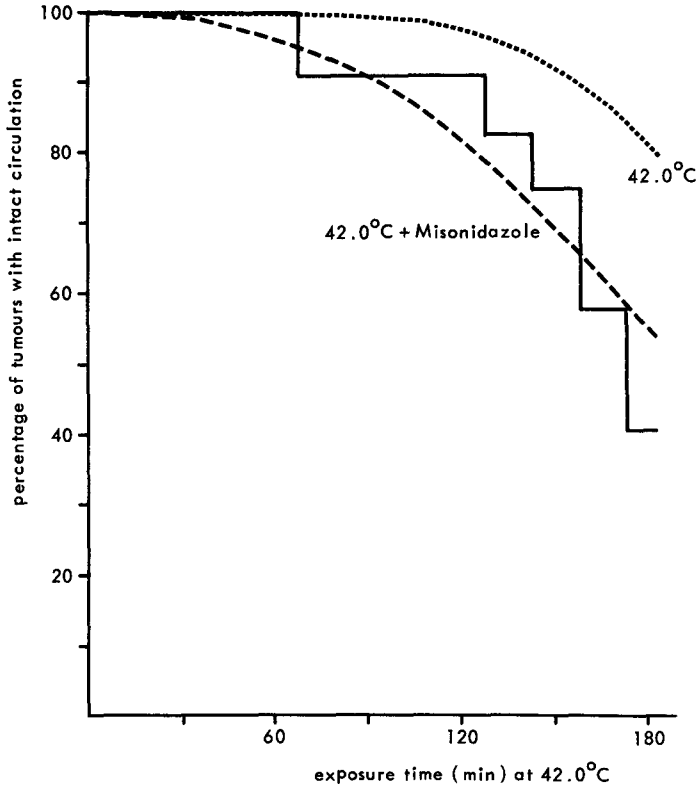

Fig. 6. The effect of a combined treatment of misonidazole and hyperthermia on the microcirculation of tumours (n=12). *Parameters as in Fig. 2. Dotted line taken from Fig. 2* (42°C).

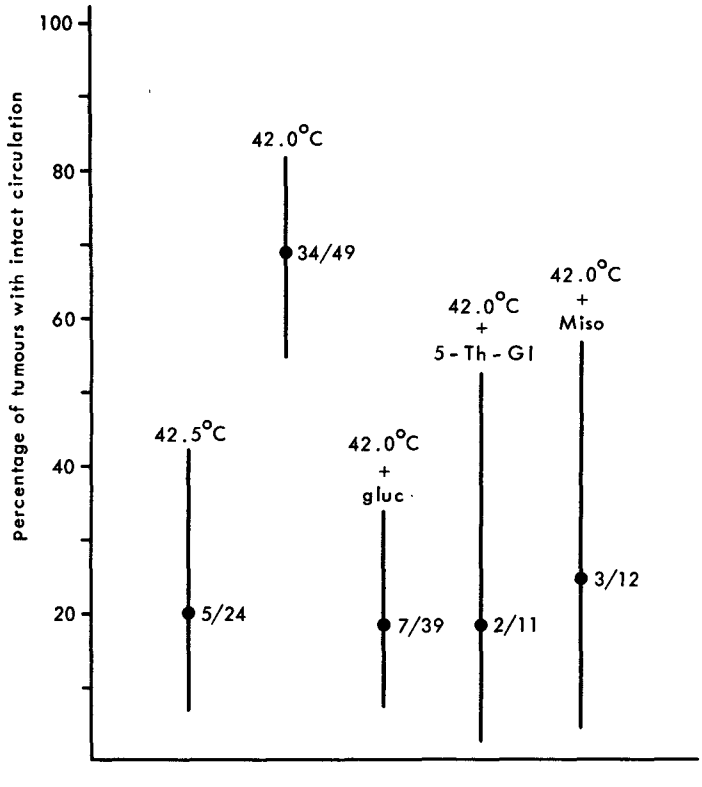

Fig. 7. Proportion of tumours with intact microcirculation on the day following treatment, indicating that the process of microcirculatory damage with subsequent necrosis is irreversible.

hyperthermic treatment, as reported by Bicher and Vaupel [22] and Vaupel *et al.* [23]. Von Ardenne and Reitnauer [24] postulate that tissue acidification by means of systemic glucose administration is an essential step in the development of a hyperthermia induced decrease in the 'intensity' of the microcirculation. The latter was approximated via the rate of light absorption by the tumour tissue after an i.v. injection of Evan's blue.

Our observations showed that, following a stoppage of the microcirculation, the stasis is almost inevitably irreversible. This effect can also be noted when heat treatment is followed on the next day by radiation for a cure [25, 26]. Logically, a prolonged stoppage of the microcirculation in any tissue is expected to lead to necrosis. That this occurs in our tumour system is adequately illustrated in Fig. 3. Moreover, while there is a stoppage of the circulation in the tumour, the tumour tissue is likely to be more sensitive to the hyperthermic treatment. This has been demonstrated *in vitro* by making various modifications in the 'physiological' conditions of the medium [1, 4, 5, 27] and clamping experiments in tumours *in vivo* also give confirmatory evidence [28]. Whether or not changes in the tissue pH as a result of hyperthermia are of importance is presently open for discussion. Von Ardenne and Krüger [29] refer to experiments of Schmid-Schönbein *et al.* [30] which have also shown that the flexibility of the erythrocyte membrane is dependent upon the pH of the medium. Eddy [19], however, refers to similar experiments of Crandall *et al.* [31] which indicated that quite low pH values (pH 4.6–6) are required and that a period of several hours must elapse before erythrocyte stiffening becomes apparent. It should be mentioned, however, that, in these experiments, an influence of time at pH 6.0 is hardly detectable. Therefore, the question of whether or not acidification of tumours is of importance for the development of heat induced microcirculatory stoppage remains an open question until appropriate determinations of the effects of both factors (i.e., the pH of the intracapillary blood and changes in the capillary flow) have been made. Other factors involved in a heat induced decreased microcirculatory flow may be those such as the development of increased tissue pressure through osmotic changes due to alterations in cellular or vascular permeability or changes in the surface properties of the capillary walls or those of some type of blood cell. Also, the possible influence of metabolic factors [27, 32] and whether a possible decreased tissue pH plays another role in heat induced cell killing is not presently clear [33, 34].

As shown in Figs. 4–6, the present experiments indicate that additional treatment may influence the microcirculatory response. Glucose, 5-thio-D-glucose and misonidazole all seem to be capable of increasing the damaging effect of hyperthermia on the microcirculation to a limited extent. All three additional treatments seem to substitute for about 0.5°C treatment temperature. This is apparent not only during treatment (Figs. 4–6 compared with Fig. 2) but also on the next day. Such data also illustrate that the microcirculatory damage induced by hyperthermia is an irreversible phenomenon which results in vast areas of necrosis on the following day.

Misonidazole has been found to be a potentiator for hyperthermia. This was reported in 1977 by several authors [35–39]. The dose of misonidazole in the present experiments where rats were used was somewhat lower (0.3 mg. g^{-1}) than that mostly used in mice, where the usual dose is 1 mg.g^{-1} [35–37]. This was purposely chosen in order to avoid lethality. Nevertheless, a limited, but significant effect was observed. The magnitude of the effect (i.e., an increase which is equivalent to 0.5°C) is of the same order as the value which may be inferred from the published cell survival curves [35–39]. The present observations seem to be in agreement with the conclusions drawn by Overgaard that the combined effects of misonidazole, hyperthermia and radiation on the C3H mouse mammary tumour may be explained as the result of independent actions of the combined modalities [40].

The glucose analogue 5-thio-D-glucose was recognized by Song *et al.* as a specific toxic agent for hypoxic tumour cells [41, 42]. In further experiments, Kim *et al.* in collaboration with Song demonstrated that 5-thio-D-glucose has an effect on hyperthermic cell killing [43], while Song *et al.* [44] established that hyperthermia potentiated the cytoxic effect of 5-thio-D-glucose on hypoxic cells. The present investigations confirm this finding by the observation that addition of 5-thio-D-glucose to the drinking water of the rats rendered the tumour more sensitive to hyperthermia.

Glucose has been proposed for many years by Von Ardenne as a substance for acidifying tumour tissue (for recent refs., see [16, 24, 29]). Indeed, in the present experiments, a high dose of glucose appeared to have a potentiating effect in the causation of hyper-

thermic damage to the microcirculation of the tumours.

The assay system used in these experiments was confined to observation and photography. No physiological determinations were made. This system was chosen on purpose in order to avoid any interference of external factors in the response of the delicate microcirculatory system of the tumours. It is difficult, therefore, to confirm any of the above-mentioned speculations regarding the mechanisms involved. Recent pilot experiments with high power optics have confirmed that the capillary flow comes to a full stop under hyperthermic conditions. However, even the high power observation of microcirculation stoppage does not reveal the mechanisms involved and a different experimental set-up will be required to answer this question.

Acknowledgements—The authors wish to thank Roche Products Ltd., Welwyn Garden City, Herts, for the supply of misonidazole and Drs. Wijnans and Van den Berg for help with the statistical evaluations.

REFERENCES

1. Overgaard J. Effect of hyperthermia on malignant cells *in vivo*. A review and a hypothesis. *Cancer* 1977; **39:** 2637.
2. Suit HD, Gerweck LE. Potential for hyperthermia and radiation therapy. *Cancer Res* 1979; **39:** 2290.
3. Bhuyan BK. Kinetic of cell kill by hyperthermia. *Cancer Res* 1979; **39:** 2277.
4. Field SB, Bleehan NM. Hyperthermia in the treatment of cancer. *Cancer Treat Rev* 1979; **6:** 63.
5. Dewey WC *et al.* Cell biology of hyperthermia and radiation. In: Meyn RE, Withers HR, eds. *Radiation Biology in Cancer Research* New York: Raven Press, 1980: 589.
6. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer* 1955; **9:** 539.
7. Tannock IF. Oxygen diffusion and the distribution of cellular radiosensitivity in tumours. *Br J Radiol* 1972; **45:** 515.
8. Gullino PM, Grantham FH, Courtney AH. Glucose consumption by transplanted tumors *in vivo*. *Cancer Res* 1967; **27:** 1031.
9. Reinhold HS, Blachiewicz B, Berg-Blok Avd. Reoxygenation of tumours in 'sandwich' chambers. *Eur J Cancer* 1979; **15:** 481.
10. Endrich B, Reinhold HS, Gross JF, Intaglietta M. Tissue perfusion inhomogeneity during early tumour growth in rats. *J Natl Cancer Inst* 1979; **62:** 387.
11. Cetas TC, Boone MR. Brief communication: Temperature measurements of a transplant access chamber for the rat dorsal fold. *Microvasc Res* 1979; **18:** 442.
12. Campos JL. Application of the Weibull distribution to some instances of cell survival; of neoplastic survival, and of ageing. *Br J Radiol* 1975; **48:** 913.
13. Sugaar S, LeVeen HH. A histopathologic study on the effects of radiofrequency thermotherapy on malignant tumors of the lung. *Cancer* 1979; **43:** 767.
14. Herbst M, Sauer R. Zur Strahlentherapie in lokaler Hyperthermie ein vorläufiger klinischer Erfahrungsbericht. *Strahlentherapie* 1980; **156:** 331.
15. Piro AJ *et al.* Effects of local hyperthermia on tumor microcirculation: II. Pathological studies. Abstract. *Am Soc Ther Radiologists, ROBP* 1979; **5:** 176.
16. Von Ardenne M *et al.* Histological proof for selective stop of microcirculation in tumor tissue at pH 6.1 and 41°C. *Naturwissenschaften* 1979; **66:** 1979.
17. Reinhold HS, Blachiewics B, Berg-Blok Avd. Decrease in tumor microcirculation during hyperthermia. In: Streffer C, ed. *Cancer Therapy by Hyperthermia and Radiation* Munich: Urban & Schwarzenberg, 1978: 231–232.
18. Endrich B *et al.* Quantitative studies of microcirculatory function in malignant tissue: influence of temperature on microvascular hemodynamics during the early growth of the BA1112 rat sarcoma. *Int J Radiat Oncol Biol Phys* 1979; **5:** 2021.
19. Eddy HA. Tumor microcirculation during hyperthermia. Abstract. *Am Soc Ther Radiologists, ROBP* 1979; **5,** suppl. 2: 177.
20. Gullino PM. Influence of blood supply on thermal properties and metabolism of mammary carcinoma. *Ann NY Acad Sci* 1980; **335:** 1.

21. Song CW, Rhee JG, Levitt SH. Blood flow in normal tissues and tumors during hyperthermia. *J Natl Cancer Inst* 1980; **64:** 119.
22. Bicher HI, Vaupel P. Physiological responses of intratumour pO_2 and pH to hyperthermia and radiation treatment. *Br J Cancer* 1980; **41:** 99.
23. Vaupel P, Frinak S, Bicher HI. Changes in the pH and pO_2 distribution within malignant tumors localized hyperthermia. *Pfluegers Arch* 1980; **384:** (suppl.): R12.
24. Von Ardenne M, Reitnauer PG. Gewebe-Ubersäurung und Mikrozirkulation. *Arch Geschwulstforsch* 1978; **48:** 729.
25. Van den Boogaard L. Enhanced tumor cure rates by combination of heat and irradiation with a one day interval. An experimental study. *Eur J Cancer* 1980; **16:** 93.
26. Marmor JB, Hahn N, Hahn GM. Tumor cure and cell survival after localized radiofrequency heating. *Cancer Res* 1977; **37:** 879.
27. Durand RE. Potentiation of radiation lethality by hyperthermia in a tumor model: effects of sequence, degree and duration of heating. *Int J Radiat Oncol Biol Phys* 1978; **4:** 401.
28. Hill SA, Denekamp J. The effect of vascular occlusion on the thermal sensitization of a mouse tumor. *Br J Cancer* 1978; **51:** 997.
29. Von Ardenne M, Krüger W. The use of hyperthermia within the frame of cancer multistep therapy. *Ann NY Acad Sci* 1980; **335:** 356.
30. Schmid-Schönbein H, Volger E, Weiss J, Brandhuber M. Effect of O −(hydroxyethyl)-rutosides on the microrheology of human blood under defined flow conditions. *Vasa* 1975; **4:** 263.
31. Crandall ED *et al.* Influence of pH on elastic deformability of the human erythrocyte membrane. *Am J Physiol* 1978; **235:** 269.
32. Streffer C, van Beuningen D, Zamboglou N. Cell killing by hyperthermia and radiation in cancer treatment. In: Abe M, Sakamoto K, Phillips TL, eds. *Treatment of Radioresistant Cancers.* Amsterdam: Elsevier/North-Holland, 1979.
33. Dickson JA, Calderwood SK. Effect of hyperglycemia and hyperthermia on the pH, glycolysis, and respiration of the Yoshia sarcoma *in vivo*. *J Natl Cancer Inst* 1979; **63:** 1371.
34. Haveman J. The pH of the cytoplasm as an important factor in the survival of *in vitro* cultured malignant cells after hyperthermia. Effects of carbonyl-cyanide 3-chlorophenylhydrazone. *Eur J Cancer* 1979; **15:** 1281.
35. George KC, Hirst DG, McNally NJ. Effect of hyperthermia on cytotoxicity of the radiosensitizer Ro-07-0582 in a solid mouse tumour. *Br J Cancer* 1977; **35:** 372.
36. Stone HB. Enhancement of local tumour control by misonidazole and hyperthermia. *Br J Cancer* 1978; **37:** 178.
37. Song CW, Rhee JG, Levitt SH. Blood flow in normal tissues and tumors during hyperthermia. *J Natl Cancer Inst* 1980; **64:** 119.
38. Stratford IJ, Adams GE. Effect of hyperthermia on differential cytotoxicity of a hypoxic cell radiosensitizer, Ro-07-0582, on mammalian cells *in vitro*. *Br J Cancer* 1977; **35:** 307.
39. Hall EJ, Astor M, Gerard C, Biaglow J. Cytotoxicity of Ro-07-0582 enhancement by hyperthermia and protection by cysteamine. *Br J Cancer* 1977; **35:** 809.
40. Overgaard J. Effect of misonidazole and hyperthermia on the radiosensitivity of a C3H mouse mammary carcinoma and its surrounding normal tissue. *Br J Cancer* 1980; **41:** 10.
41. Song CW, Clement JJ, Levitt SH. Brief communication: Preferential cytotoxicity of 5-thio-D-glucose against hypoxic tumor cells. *J Natl Cancer Inst* 1976; **57:** 603.
42. Song CW, Sung JH, Clement JJ, Levitt SH. Cytotoxic effect of 5-thio-D-glucose on chronically hypoxic cells in multi-cell spheroids. *Br J Cancer* 1978; **37:** 136.
43. Kim JH, Kim SH, Hahn EW, Song CW. 5-Thio-D-glucose selectively potentiates hyperthermic killing of hypoxic tumor cells. *Science* 1978; **200:** 206.
44. Song CW, Guertin DP, Levitt SH. Potentiation of cytotoxicity of 5-thio-D-glucose on hypoxic cells by hyperthermia. *Int J Radiat Oncol Biol Phys* 1979; **5:** 965.