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Temperature Distribution and pH Changes During Hyperthermic Regional Isolation Perfusion

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Abstract—*Hyperthermic perfusion was given as a palliative treatment in three patients with a bulky tumour in the leg. During the treatment, temperature and pH data were collected in both tumour and normal tissues. The hyperthermia dose administered was* 2 h *at* 41.9–42.7°C. *It was found that the temperature distribution was far from homogeneous. In one case tumour pH could be monitored throughout the whole treatment procedure. The tumour pH showed a steep decrease during the initial phase of the perfusion, from a mean value of* 7.11 *to* 5.94 *at the start of the hyperthermic phase. Subcutis pH decreased only* 0.29 *units during the whole procedure. In all three patients considerable tumour regression was observed, without severe toxicity.*

Hyperthermic perfusion appeared to be an effective debulking treatment. The pH decrease in tumour tissue, immediately before hyperthermia, may be responsible for the remarkable effectiveness of the hyperthermic treatment.

1. INTRODUCTION

CONTROLLED normothermic regional isolation perfusion (RIP) with cytostatic drugs is part of the therapeutic arsenal in the Dr. Daniel den Hoed Cancer Center and is performed in cooperation with the Netherlands Cancer Institute (Antoni van Leeuwenhoek Huis) in Amsterdam [1]. In the Rotterdam Center, experience has also been obtained with the clinical application of hyperthermia, including whole body and local hyperthermia [2, 3]. Besides, human tumour pH data are collected as part of the research on the influence of tumour physiology on hyperthermia effectiveness [4, 5]. This combination of experiences has led to the treatment of some patients with large tumours of the lower limb with hyperthermic perfusion, during which temperature and pH measurements were performed.

Two of the important factors determining hyperthermia effectiveness are hyperthermia dose and tissue physiology. Hyperthermia dose is determined by the extent and the duration of temperature increase [6]. The hyperthermia dose reported to be given during perfusion in combination with chemotherapy is mostly relatively low [7–10], probably on the basis of unacceptable toxicity experienced with the simultaneous combination of high dose hyperthermia and drugs [11]. We therefore decided not to give these two types of treatment simultaneously.

Low environmental pH has been found to increase cell sensitivity to hyperthermia [12, 13]. However, more recent data indicate that cells adapt to a low environmental pH [14], which suggests that only a pH decrease shortly before administration of hyperthermia might induce an enhanced sensitivity. This study presents evidence in favour of this hypothesis, since the pH decrease which we observed during the initiation of the hyperthermic perfusion may have played a role in the remarkable therapeutic effect of the treatment.

2. MATERIALS AND METHODS

2.1. *Patients*

In the period November 1984 to July 1986, three patients were treated with hyperthermic RIP. All three patients had a bulky tumour in the lower limb which caused much inconvenience, and had been treated previously with various modalities. Standard treatment in these patients would have been amputation of the limb. The perfusion treatment

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was aimed at palliation. All patients had given informed consent.

The first case was a 59-year-old male with recurrent fibrosarcoma in the leg, ulcerating through the skin, with maximum tumour diameters of 11.5 × 13.5 cm, extension in depth about 5 cm. After the primary treatment of this tumour, 26 months earlier, recurrences had developed after 12 and 6 months, respectively. The second case was a 71-year-old female with recurrent leiomyosarcoma in the calf muscle, with a maxmimum diameter of 7 cm. This recurrence had developed 8 years after the primary treatment; in this period there had been no local problems and no systemic therapy. The third case was a 61-year-old male with multiple metastatic nodules of non-Hodgkin's lymphoma located in the skin of the leg. The nodules were confluating and ulcerating, the largest nodule measuring 5 cm. The local problem existed for 40 months and had been treated with various modalities almost continuously; during this period no local control had been achieved. The history of this third patient is reported in detail elsewhere [15].

In patient 1, two hyperthermic perfusions were performed with a time interval of 4 weeks. The second perfusion was performed after the observation of partial regression following the first perfusion, with evidence of progression after 3 weeks, and given in combination with a series of radiotherapy. In patient 2, one hyperthermic perfusion was given without additional treatment. In patient 3, the hyperthermic perfusion was followed 10 days later by a normothermic perfusion with L-phenylalanine mustard (melphalan, 11 mg/l perfused tissue [16]).

2.2. *Perfusion technique*

The extracorporeal circulation system was connected to the superficial femoral artery and vein. The flow rate varied from 350 to 400 ml/min. The tourniquet was applied with a pressure of 200–300 mmHg. The technique has been reported in detail by Wieberdink [1]. In all cases, the pH of the perfusate was measured at least once during the perfusion, on the arterial as well as the venous side. These data are given in Table 1.

In the hyperthermic perfusion procedure the following phases can be distinguished:

(a) the surgical phase, during which the blood vessels are dissected; when the vessels are cannulated to make the connection to the extracorporeal circulation system, the limb circulation is clamped;
(b) the heating phase, which starts when the perfusion runs;
(c) the hyperthermic phase during which the tissue temperatures are kept at the desired level for 2 h;
(d) the cooling phase.

During a RIP with chemotherapy in our institute, phases (c) and (d) are as follows: (c) the controlled normothermic phase (tissue temperatures 37–38°C) during which the perfusate contains the chemotherapeutic agent for 1 h and (d) rinsing of the limb's circulation with dextran solution (Macrodex®).

2.3. *Hyperthermia*

Hyperthermia was induced by increasing the temperature of the perfusate up to 44.2°C and, after reaching the desired temperature, maintained by controlling the temperature of the perfusate (41–44.8°C). The leg was wrapped by a mattress with water circulating at a temperature of 39–44°C.

2.4. *Temperature measurements*

Temperatures were measured by thermocouples. In the first two patients, single-point thermocouple needles were used, whereas in the third patient multi-point thermocouple probes placed within closed-tip catheters [17] were used. Temperatures were recorded every 2 min during the heating and cooling phases, and every 10 min during steady state hyperthermia.

2.5. *pH measurements*

pH was measured by a Philips C 902S tissue pH electrode, using the method described by Van den Berg *et al.* [4]. Following calibration in NBS buffers at pH = 6.841 and 7.385, the electrodes were sterilized in Cidex solution (Johnson and Johnson, Benelux BV). To allow the introduction of the fragile glass electrode into tissue, a small incision in the skin or tumour surface was made. The pH was continuously measured with a Knick pH meter (model 645; input impedence 2.10^{12} ohm) in conjunction with a chart recorder. In patient 1, no tissue pH measurements were performed during the first hyperthermic perfusion.

2.6. *Tumour response and normal tissue reaction*

The superficially located tumours were measured by callipers and documented by colour photographs. The intramuscular tumour in patient 2 was documented by CT scanning. Any erythema, oedema and dysfunction of the peripheal nerves was recorded.

3. RESULTS

3.1. *Temperature measurements*

The desired hyperthermic temperature levels were reached 35–50 min (mean 44 min) after the

Table 1. Perfusate pH values

		1-1*	1-2	2-1	3-1	3-2†
pH arterial	a‡	7.32	7.32	6.98	7.27	7.50
	b§	n.m.	7.30	7.11	7.24	7.43
	c‖	n.m.	7.31	7.10	7.22	—
pH venous	a	7.29	7.29	7.09	7.24	7.50
	b	n.m.	7.30	7.08	n.m.	7.39
	c	n.m.	7.32	7.09	7.22	—

*Patient and treatment number.
†One hour normothermic perfusion, time b = time c.
‡a = at start perfusion.
§b = after 1 h perfusion at the desired temperature.
‖c = at the end of perfusion at the desired temperature.
n.m.: not measured.

start of perfusion. During the second perfusion in patient 3 (with melphalan), controlled normothermia was reached after 20 min.

The mean temperatures achieved in tumour tissue ranged from 41.9 to 42.7°C, and the maximum temperatures from 42.6 to 43.0°C. A summary of the thermometry data is given in Table 2. Tumour temperature was higher than normal tissue temperature in patient 3, about equal in patient 2 and lower during both perfusions in patient 1. During two hyperthermic perfusions more than one tumour temperature was measured. The data are given in Table 3. It was found that the intratumoural temperature distribution during hyperthermic perfusion is not homogeneous. This was clearly demonstrated in patient 3, where tumour temperature was measured at nine sites. In this patient, tumour temperature increased with increasing depth (Fig. 1). At the sites with the higher temperatures the heating rate after the start of perfusion was also higher. The lowest temperature in this case was found at a depth of 5 mm in the part of the tumour

Table 2. Temperatures during hyperthemic perfusions

Patient and treatment number		Temperatures (°C) Subcutis	Muscle	*n**	Tumour Range	Average
1-1	T_{mean}	42.4	42.0	1		41.9
	T_{max}	43.0	42.7			42.6
	Eqt43	49				27
1-2	T_{mean}	42.4	42.2	2	41.4–42.3	41.9
	T_{max}	42.9	42.8		42.3–42.9	42.6
	Eqt43	51			16–49	33
2-1	T_{mean}	42.6	42.8	1		42.7
	T_{max}	43.0	43.0			43.0
	Eqt43	85				82
3-1	T_{mean}	42.1	42.3	9	40.2–43.1	42.2
	T_{max}	42.6	42.8		40.6–43.4	42.6
	Eqt43	25				53
3-2	T_{mean}	38.0	38.5	5	38.4–38.6	38.5
	T_{max}	38.3	38.8		38.7–38.9	38.8

T_{mean}: (the average of) the mean temperature(s) during the hyperthermia 'plateau phase'; T_{max}: (the average of) the maximum temperature(s) achieved; Eqt43: the equivalent time at 43°C in minutes calculated as described in a previous paper [3].
**n*: number of thermometry probes within the tumour.

Table 3. Intratumoural temperatures during hyperthermic perfusion

		T_{mean}	T_{max}
1-1	tumour	41.9	42.6
1-2	tumour centre	41.4	42.3
	tumour periphery	42.3	42.9
2-1	deep in tumour	42.7	43.0
3-1	tumour surface*	42.3	42.6
	tumour, depth 5 mm†	40.2	40.6
	tumour, depth 8 mm (average, $n = 5$)	42.3	42.7
	tumour, depth 12–15 mm (average, $n = 2$)	43.0	43.3

*At the tumour surface which was covered by the mattress.
†At the site where the mattress did not cover the skin, to allow safe positions for the pH electrodes.

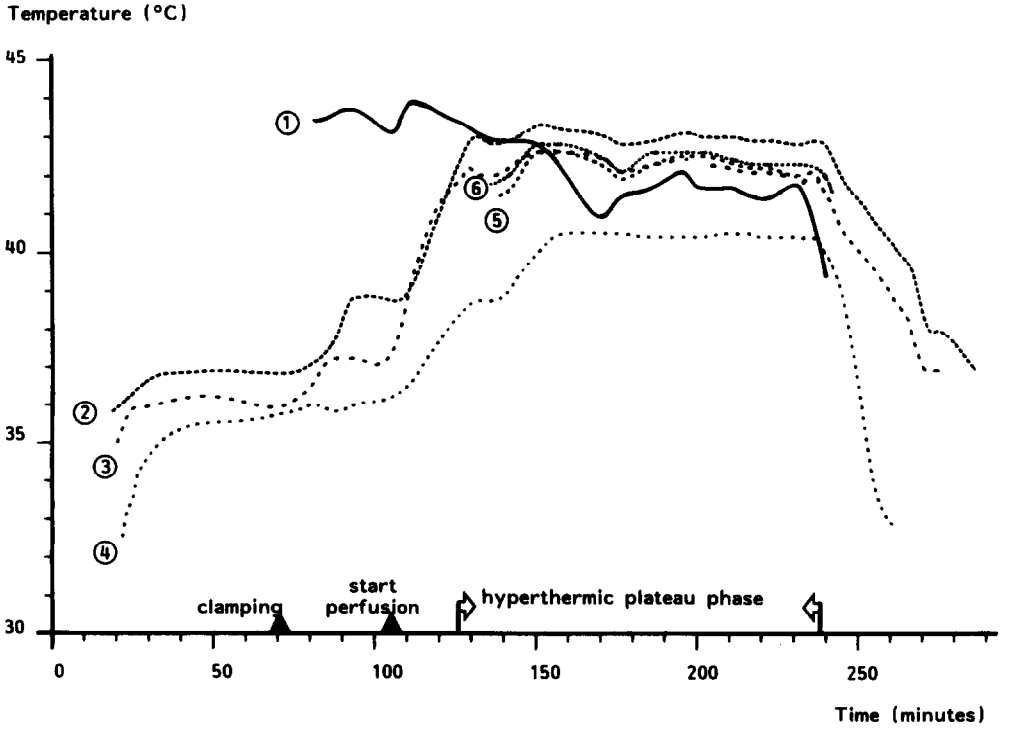

Fig. 1. Temperature distribution during hyperthermic perfusion in patient 3. 1: Temperature of the perfusate; 2: average tumour temperature at a depth of 12–15 mm (n = 2); 3: average tumour temperature at a depth of 8 mm (n = 5); 4: tumour temperature at a depth of 5 mm (n = 1); 5: intramuscular temperature; 6: subcutaneous temperature.

which was exposed to the environmental air. This site was not insulated by the water mattress to allow undisturbed pH measurements.

3.2. *pH measurements*

Evaluation of tumour pH during the whole hyperthermic perfusion procedure was possible only during the first treatment in patient 3. During the second perfusion in patient 1, the probes were inserted at the end of the surgical procedure. As the time required to reach equilibrium following insertion of the probe in tumour tissue was found to be in the order of 50–90 min [5], the values obtained were considered unreliable in this case. During the treatment in patient 2 and the second, normothermic, perfusion in patient 3, tumour pH measurements were not reliable due to movements of the probes, caused by handling of the water mattress.

Reliable readings of the pH in the subcutis could be recorded for the three treatments in patients 2 and 3.

The course of tumour pH during the first treatment of patient 3 is given in Fig. 2. Tumour pH at the end of the 'surgical phase' (values of 7.09, 7.11 and 7.13) is much lower than the subcutis pH

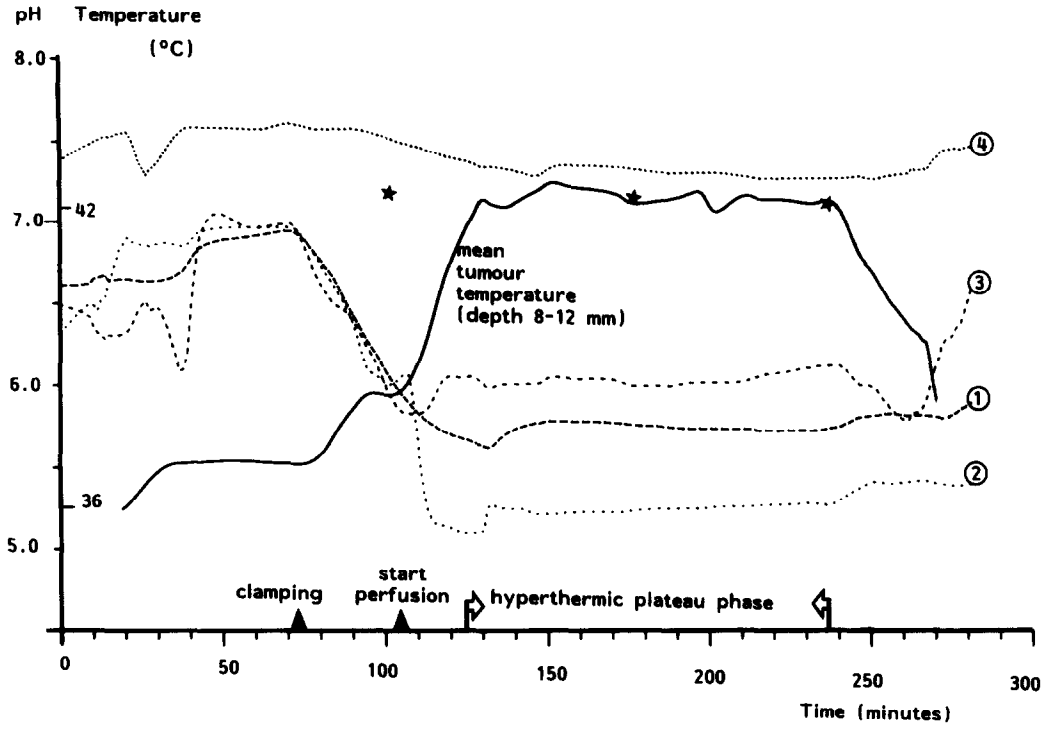

Fig. 2. Tissue pH values during hyperthermic perfusion in patient 3. 1, 2 *and* 3: *tumour pH at different sites*; 4: *subcutis pH*; *perfusate pH at the arterial site.*

(7.66). Immediately following clamping, tumour pH values started to decrease. After the start of perfusion (pH of the perfusate 7.27), the pH decreased further, reaching values of 5.99, 5.51 and 6.33 respectively, the mean pH at the start of the 'hyperthermic phase' thus becoming 5.94. During the hyperthermic phase, no further decreases were observed. The subcutis pH shows much less change during the perfusion: −0.11 after clamping, −0.10 during the 'heating phase' and no change during the 'hyperthermic phase'. During the second perfusion in patient 3, the subcutis pH also showed little change: −0.01 following clamping, −0.07 during the warming phase and −0.07 during the phase of normothermia. In patient 2, the subcutis pH dropped by 0.34 units following the start of perfusion, from a value of 7.30 to a value of 6.96 (pH of the perfusate in this case 6.98).

3.3. *Tumour response*

In patients 1 and 3, hyperthermic perfusion resulted in immediate tumour regression. In both patients a blueish discoloration of the formerly red cutaneous tumour nodules was observed at the end of the perfusion procedure. During the following days massive tumour necrosis could be observed, which was confirmed by histological examination. In patient 1, where the follow up was expectative, tumour regression continued during 3 weeks. At the end of this period, only the original tumour margins were left, but these appeared vital and started to progress again. The second hyperthermic perfusion, which was given in combination with a series of radiotherapy (55 Gy in 45 days) on part of the leg, resulted in a complete tumour response within the radiation field for a duration of 4 months. A subcutaneous metastasis on the back, which had been treated in about the same period with radiotherapy only (40 Gy), showed no regression.

In patient 3, the combination of hyperthermic perfusion with a normothermic melphalan perfusion applied 10 days later resulted in a complete tumour response for a duration of 7 months.

In patient 2, relief from the previously severe pain was achieved immediately after treatment. Tumour regression was observed from 1 month after the hyperthermic perfusion. This regression continued until 10 months after the treatment and the palliative effect was still present at that time, when the follow up was discontinued by the start of systemic chemotherapy for progression elsewhere.

3.4. *Toxicity*

Erythema and oedema, grade II [16], was observed only in patient 2. A decubitus ulcer of the heel was observed in case 1 (second perfusion) and case 2. In patient 2 also a small area of necrosis developed at the site where the intravenous catheter for pressure monitoring had been sutured to the skin. Partial and transient nervous function loss was

observed in patient 1 (motor function loss after the first perfusion) and patient 3 (sensibility loss after the first perfusion, motor nerve lesion after the second perfusion). In both patients this was at the level of the peroneal and posterior tibial nerve.

4. DISCUSSION

The three treated patients all showed a remarkable tumour regression following hyperthermic treatment at doses of 2 h at 41.9–42.7°C. In patients 1 and 3 the hyperthermia was combined with another treatment modality, which in both cases resulted in a complete response. In patient 2, hyperthermic treatment alone resulted in a long-lasting partial regression and palliation. This excellent palliative effect may be due to the relatively high hyperthermia dose (2 h at an average of 42.7°C), in combination with the low growth rate of the tumour (local recurrence after 8 years without local or systemic treatment).

The temperature distribution within the tumour tissue was far from homogeneous. The difference between mean intratumoural temperatures at different sites during the hyperthermic phase was as much as 0.9°C in patient 2 and 2.9°C in patient 3, respectively. It was observed that at the sites at which the heating rate was the highest, the mean and maximum temperatures also were the highest. This probably reflects a thermal contact with the vascular system, through which the tissue is heated during perfusion. Thus, the relationship between blood flow and tissue temperature during perfusion is opposite to that during heating with electromagnetic radiation, where blood flow tends to cool the tissue [18].

The normal tissue temperature was, as expected, not much different from the tumour temperature. A higher tumour temperature was only observed during one perfusion. So, therapeutic gain by hyperthermic perfusion can only be expected if there is a difference in sensitivity between normal and tumour cells.

Temperature and duration of temperature increase determine the effectiveness of hyperthermia [6], but in addition the sensitivity of cells to hyperthermia is considerably influenced by environmental factors such as hypoxia, low pH and insufficient nutrient supply [12]. Recent reports indicate that cells can adapt to less than optimal environmental conditions, in particular pH, and that the sensitivity to hyperthermia then is equal to that of cells in a normal environment [14].

Unfortunately, the pH measurements performed failed to give both complete and reliable results in three of the four perfusion procedures. This was due to various technical problems such as insufficient time available to reach equilibrium and the impossibility of avoiding inadvertent movements of the probes. In the one case where a reliable and full reading was achieved, a sharp decrease of pH level was observed following clamping and during the first period of perfusion, immediately before the actual hyperthermic treatment. We assume that such a pH decrease may also have occurred during the other perfusions, by anaerobic glycolysis within the tumours (the glucose concentration in the perfusate fluid was relatively high: 23–39 $mmol.l^{-1}$ at the start of perfusion) and accumulation of lactate. The pH decrease immediately before the hyperthermic treatment then may have increased the sensitivity of the tumour cells. Such a steep decrease was not observed in the subcutaneous tissue, probably because the normal cells do not metabolize glucose anaerobically to the same extent as tumours, and any lactate can be removed efficiently by the blood. The only perfusion during which the subcutaneous pH dropped after the start of the perfusion was that in patient 2. In this case the perfusate pH was relatively low, and the subcutaneous pH reached levels close to that of the pefusate pH.

Patient 2 was the only case in whom toxicity of the normal tissues was observed in the whole treated volume (erythema and oedema). This may be related to the disturbance of the physiologic conditions within the normal tissues, and then can be prevented by using a perfusion fluid at normal pH. Further toxicity was limited to pressure sites, where hypoxia is expected to occur: ulcers at the heel in two cases and transient peripheral nervous function loss in two cases, probably caused by the pressure exerted through the tourniquet, or, in patient 2, by oedema. Pressure related toxicity can be prevented by using anti-decubitus cushions at the sites at risk, and by decreasing the pressure applied by the touriquet to a minimum acceptable level: leakage of the perfusate to the systemic circulation is not as serious as during a perfusion with chemotherapy.

We conclude that hyperthermic perfusion can be a valid additional treatment for bulky tumours in the limbs, for which another treatment modality is expected to be only partly effective. During hyperthermic perfusion the temperature distribution is not homogeneous. The pH decrease in tumour tissue, immediately before hyperthermia, may be responsible for the remarkable effectiveness of the hyperthermic treatment. Toxicity of hyperthermia can be limited to a minimum by restoring physiologic conditions within the normal tissues during the perfusion.

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REFERENCES

1. Wieberdink J. *Physiological Considerations Regarding Isolation Perfusion of the Extremities.* Monograph available from The Netherlands Cancer Institute, Amsterdam. Krips Repro Meppel, The Netherlands, 1978.
2. Zee J van der, Rhoon GC van, Wike-Hooley JL, Faithfull NS, Reinhold HS. Whole-body hyperthermia in cancer therapy: a report of a phase I–II study. *Eur J Cancer Clin Oncol* 1983, **19**, 1189–1200.
3. Zee J van der, Putten WLJ van, Berg AP van den *et al.* Retrospective analysis of the response of tumours in patients treated with a combination of radiotherapy and hyperthermia. *Int J Hyperthermia* 1986, **2**, 337–349.
4. Berg AP van den, Wike-Hooley JL, Berg-Blok AE van den, Zee J van der, Reinhold HS. Tumour pH in human mammary carcinoma. *Eur J Cancer Clin Oncol* 1982, **18**, 457–462.
5. Wike-Hooley JL, Berg AP van den, Zee J van der, Reinhold HS. Human tumour pH and its variation. *Eur J Cancer Clin Oncol* 1985, **21**, 785–791.
6. Field SB, Morris CC. The relationship between heating time and temperature: its relevance to clinical hyperthermia. *Radiother Oncol* 1983, **1**, 179–186.
7. Schraffordt Koops H, Beekhuis H, Oldhoff J, Oosterhuis JW, Ploeg E van der, Vermey A. Local recurrence and survival in patients with (Clark level IV/V and over 1.5-mm thickness) stage I malignant melanoma of the extremities after regional perfusion. *Cancer* 1981, **48**, 1952–1957.
8. Stehlin JS, Giovanella BC, Ipolyi PD de, Anderson RF. Results of eleven years experience with heated perfusion for melanoma of the extremities. *Cancer Res* 1979, **39**, 2255–2257.
9. Vaglini M, Andreola S, Attili A *et al.* Hyperthermic antiblastic perfusion in the treatment of cancer of the extremities. *Tumori* 1985, **71**, 355–359.
10. Ghussen F, Krüger I, Groth W, Stützer H. The role of regional hyperthermic cytostatic perfusion in the treatment of extremity melanoma. *Cancer* 1988, **61**, 654–659.
11. Vaglini M, Ammatuna M, Nava M *et al.* Regional perfusion at high temperature in treatment of stage IIIA–IIIAB melanoma patients. *Tumori* 1983, **69**, 585–588.
12. Overgaard J, Nielsen OS. The role of tissue environmental factors on the kinetics and morphology of tumor cells exposed to hyperthermia. In: Jain RK, Gullino PM, eds. *Thermal Characteristics of Tumors: Applications in Detection and Treatment.* New York, The New York Academy of Sciences, 1980, 254–278.
13. Wike-Hooley JL, Haveman J, Reinhold HS. The relevance of tumour pH to the treatment of malignant diseases. *Radiother Oncol* 1984, **2**, 343–366.
14. Hahn GM, Shiu EC. Adaptation to low pH modifies thermal and thermochemical responses of mammalian cells. *Int J Hyperthermia* 1986, **2**, 379–387.
15. Jansen RFM, Geel BN van, Zee J van der, Hagenbeek A, Levendag PC. Intractible cutaneous non-Hodgkin's lymphoma of the lower limb: complete remission after sequential regional isolated hyperthermic perfusion and perfusion with l-phenylalanine-mustard (melphalan, L-Pam). *Cancer*, in press.
16. Wieberdink J, Benckhuysen C, Braat RP, Slooten EEA van, Olthuis GAA. Dosimetry in isolation perfusion of the limbs by assessment of perfused tissue volume and grading of toxic tissue reactions. *Eur J Cancer Clin Oncol* 1982, **18**, 905–910.
17. Zee J van der, Rhoon GC van, Broekmeijer-Reurink MP, Reinhold HS. The use of implanted closed-tip catheters for the introduction of thermometry probes during local hyperthermia treatment series. *Int J Hyperthermia* 1987, **3**, 337–345.
18. Patterson J, Strang R. The role of blood flow in hyperthermia. *Int J Radiat Oncol Biol Phys* 1979, **5**, 235–241.