Total-Body Hyperthermia versus Primary Tumor Hyperthermia in the Treatment of the Rabbit VX-2 Carcinoma

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SUMMARY

The respiration of rabbit VX-2 carcinoma cells heated in vitro increased at temperatures between 37.5° and 40.0° and was inhibited after 2 hr at 42.0°. Anaerobic glycolysis of the cells, in the presence and in the absence of added glucose, was unaffected by elevated temperature. The $\text{O}_2$ uptake of metastatic VX-2 cells isolated from involved lymph nodes was significantly depressed but not completely inhibited by 42.0° in vitro.

Established VX-2 tumors were treated by local hyperthermia (water bath immersion of the tumor-bearing hind limb) or by total-body hyperthermia (radiant heating of the rabbit). In each case, the intratumor temperature was maintained above 42.0° for 1 hr on Days 35, 36, and 37 following tumor inoculation, the fractionated therapy being completed within the mean generation time of the tumor cells. There was a significant difference in the regression rates of tumors treated by the two methods. In the case of local heating, tumor volume was halved every 0.97 week from the 2nd week following treatment while, after total-body heating, tumor volume was halved every 2.9 weeks over the same period. Four of 8 rabbits treated by primary tumor hyperthermia are alive 2 years after heating, whereas only 1 of 14 animals treated by total-body hyperthermia is alive 1 year after therapy; all control rabbits died with lung and lymph node metastases at 70 ± 6 days after tumor-cell inoculation into the limb. Intrathoracic and intraabdominal thermocouple sensors indicated that central body (core) temperature of the rabbits fluctuated considerably over the period of total-body heating, with an average temperature in the region of 40°.

It is suggested that the inability to maintain a central body temperature of 42° may have contributed to the failure of total-body hyperthermia to increase animal survival. Stimulation of metastatic cells by the 40° central body temperature is a possibility, but a different response of the immune system of the tumor-bearing host to the two types of heating also requires consideration.

INTRODUCTION

Over the past 10 years there has been a renewal of interest in the treatment of cancer by hyperthermia (temperatures of 40° and above). This has stemmed from the publications of Von Ardenne (33) in East Germany, who proposes hyperthermia as the basis of his multiphase approach to cancer therapy (Krebs-Mehrschritt-Therapie), from the work of Cavaliere et al. (3) in Italy on hyperthermic perfusion for limb tumors, and from the work of Cavaliere et al. and Mondovi et al. (17, 18) on the biochemical mechanism of the selective heat sensitivity of cancer cells. A beneficial synergistic action of elevated temperature and cytocidal drugs on cancer cells has recently been demonstrated clinically (29) and experimentally (9).

We have been interested in total-body hyperthermia as a means of destroying metastatic as well as primary cancer cells. We have used the rapidly growing and metastasizing VX-2 carcinoma in the rabbit as an experimental model; and, in a previous communication, hyperthermia was shown to exert a selective inhibitory effect on the metabolism and growth of VX-2 cells (19). Immersion of the tumor-bearing limb in a water bath at 46° (tumor temperature, 42° for 1 hr) on 3 occasions within the mean tumor cell generation time produced necrosis and lysis of tumor cells within 24 hr of the 3rd heating. Fifty % of the treated series of rabbits are still alive 2 years after therapy, while the control animals all died within 10 weeks of tumor-cell inoculation; the remaining 50% of treated animals died at the same time as the controls from lung and lymph node metastases.

The present report compares the effects of total-body heating with heat applied to the tumor-bearing limb, and the 2 forms of hyperthermia have been equated in terms of intratumor temperature. Previous results on the effects of local heating have also been extended.

MATERIALS AND METHODS

The VX-2 carcinoma, an anaplastic derivative of the Shope virus papilloma, was propagated by serial transfer of 1,000,000 cells into the hind limbs of New Zealand white rabbits (19). The cells gave rise to a palpable, relatively nonnecrotic tumor, 3 to 4 cm in diameter (tumor volume, approximately 40 ml), by 5 weeks after inoculation, and treatment of the tumor was given at this time. Untreated rabbits died with metastases (primary tumor volume, approximately 230 ml) within a further 5 weeks (mean survival time, 70 ± 6 days). Tumor volume was calculated from caliper measurements made at weekly intervals, with allowance being made for normal tissues of the animal (19).
In Vivo Studies. The in vivo studies were performed on anesthetized animals given i.v. Nembutal, 0.6 ml/kg body weight. Local hyperthermia was applied to the tumors by immersion of the hind limb in a water bath at 46°; once the desired intratumor temperature (42°) was reached, it was maintained for 1 hr. Tumor, rectal, thoracic muscle (flank), and skin (ear) temperatures were measured throughout the experimental period by use of a Cambridge potentiometer (Type 44228) with copper-constantan thermocouple needle electrodes sensitive to temperature change only at the needle tip; the instrument records temperature with an accuracy of ±0.1°.

During total-body heating, the flank, intrathoracic, intraabdominal, and intratumor temperatures were measured. The intraabdominal and intrathoracic electrodes were used for measurement of central body or "core" temperature; the intrathoracic needle was inserted into the midline mediastinum in such a way as to avoid perforation of the pleural or pericardial sacs, and the intraabdominal sensor was introduced under the liver in a right paramedian position.

Several methods of total-body heating were investigated, including total immersion of the rabbit, with the exception of its head, in a thermostatically controlled bath, and radiant heat. Bath immersion was technically difficult because of the temperature-monitoring electrodes; it was also hazardous for the animal, with involuntary movements resulting very easily and rapidly in drowning. Radiant heating was found to be the most satisfactory method of elevating body temperature. For this, the anesthetized rabbit, with the temperature electrodes in place, was installed on its side in a 24- x 18- x 18-inch stainless steel Forttech cage, which had been heavily insulated on all sides with 1.5-inch-thick walls of polystyrene, with a removable trapdoor for observation of the animal. Heat was supplied by 2 300-watt electric bulbs mounted at diagonally opposite corners of the cage, and a small fan inside the box minimized the formation of heat pockets. Experiments were performed in an insulated, draft-free room without windows, and the ambient temperature of the room was maintained at 25.0 ± 0.5°. With this arrangement, it was possible to raise the core temperature of the rabbit to 42° within 30 min. A temperature in this region (air temperature in the box, 46°-48°) could be maintained for 1 hr without apparent distress on the part of the animal. Immediately following heat therapy, each rabbit was given 10 to 50 ml 4% dextrose-0.18% NaCl solution i.p. to replace fluid loss. This protocol resulted in no animal distress or death, despite the fact that the box temperature rose to 49° on 1 occasion (maximum skin temperature, 47°) for 30 min.

In Vitro Studies. Cells were obtained from heated and unheated tumors and lymph nodes by the enzymatic disaggregation method detailed previously (19). Tumor and other tissue slices (less than 1 mm thick) were obtained by freehand cutting with a razor blade.

Warburg Manometry. For Warburg manometry, 5 to 10 × 10⁶ tumor cells, or 100- to 200-mg tumor slices, were incubated simultaneously at 37.5° and at 42° in independent water baths. A Tris-HCl-sucrose buffer, pH 7.4, was used for respiration studies, and a Tris-HCl-bicarbonate buffer, pH 7.4, was used for anaerobic glycolysis measurements (19); results were expressed as μl gas exchanged per mg tissue, dry weight, per hr (μl O₂ consumed = respiration, QO₂; μl CO₂ produced = glycolysis, QCO₂).

RESULTS

Chart 1 records the O₂ uptake values for 5,000,000 to 10,000,000 VX-2 cells incubated at temperatures above 37.5° in Tris-HCl-sucrose buffer over 6 hr in Warburg flasks. At temperatures up to and including 41.0°, there was no quantitative difference in O₂ uptake over the 1st hr; even as early as 60 min, however, there was a significant depression in O₂ consumption by cells heated at 42.0°, compared to that of control cells at 37.5° (p < 0.001). After 2 hr in the flasks, there was a marked increase in respiration in cells heated at 39.0° or 40.0°, but the O₂ uptake values for populations heated at 41.0° were similar to the values recorded for the control cells (p > 0.05). The cells heated at 42.0° respired at a very low rate after the 1st hr; on transfer of these flasks to 37.5° at the end of 4 hr, oxygen uptake ceased after a further 2 hr.

Table 1 shows that after in vivo heating of the VX-2 tumor at 42.0° by water immersion of the affected limb, there was also a depression of O₂ uptake, measured at 37.5°, in tumor cell populations. The decrease in respiration was evident 2 hr after heating and was highly significant at all time points (p < 0.001). By 10 days after heating, the O₂ uptake values had increased again; the cell suspensions obtained from the tumors at this time contained several cell types, which probably included fibroblasts, macrophages, and some tumor cells. Few

![Chart 1](chart1.png)

Chart 1. Oxygen uptake of VX-2 cells (5 to 10 × 10⁶) with time in Tris-HCl-sucrose buffer at temperatures above 37.5°. Each point is the mean ± S.D., and the number of tumors studied at each point on the appropriate curve is indicated by the figures in brackets. All manometric observations were carried out in duplicate flasks.
cancer cells could be seen in sections of tumors 4 weeks after heating; at this time, the tumor mass consisted chiefly of fibroblasts (19), and prolonged trypsinization was required to obtain suspensions of these cells. The respiration of slices of the regressing tumors was therefore examined at 4 weeks following heating. Table 1 illustrates that the depression of O₂ uptake observed with VX-2 cells after in vivo exposure of the tumor to 42.0° also occurred with slices of the tumor, indicating that the enzymatic disaggregation process was not damaging tumor-cell respiration. The decrease in respiration of VX-2 cells and slices that occurred at 42.0° in vitro and also after heating of the tumor in vivo was not altered by addition of glucose (2 g/liter) or succinate (0.01 M) to the incubation medium.

When anaerobic glycolysis of the VX-2 cells was examined at 37.5° (Chart 2), CO₂ production in the absence and in the presence of added glucose was linear for 4 hr; subsequently, endogenous CO₂ production decreased, while CO₂ production in the presence of added glucose continued unabated. The difference between the 2 lines of the graph was significant at the 6-hr point (p < 0.001). At 42.0° in vitro, the values for CO₂ production, in the absence and in the presence of added glucose, did not differ significantly from those at 37.5° (p > 0.05 at all time points). The effects of in vitro heating at 42° on the respiration and glycolysis of VX-2 cells confirm essentially the results of previous workers with other types of cancer cells (3, 18). When glycolysis was measured after in vivo heating of the tumor by water immersion for 1 hr on 3 occasions, endogenous CO₂ production was significantly reduced following the 4th hour (p < 0.001), compared with endogenous CO₂ production when the cells were maintained at 37.5° or 42.0° in Warburg flasks. With addition of glucose (2 g/liter) to the medium, however, glycolysis increased to values similar to those for glycolysis in cells heated at 42.0° in vitro in the presence of glucose, indicating the unimpaired integrity of the glycolytic enzymes of the cells.

The effect of 42.0° in vitro on cells of the primary limb tumor and on VX-2 cells that had metastasized to the regional lymph nodes is compared in Table 2. Metastatic tumor cells were obtained from lymph nodes in the groin draining the tumor; by 5 to 6 weeks following tumor inoculation, the nodes were grossly enlarged and almost totally replaced by

<table>
<thead>
<tr>
<th>Time after heating</th>
<th>Tumor sample</th>
<th>O₂ uptake (µl/mg, dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control at 37.5°</td>
<td>Suspensions</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>42° in vivo 2 hr</td>
<td>Slices</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>10 days</td>
<td>Suspensions</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Slices</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>4 wk</td>
<td>Slices</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Slices</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

Chart 2. CO₂ production of VX-2 cell populations with time at 37.5° in Tris-HCl-HCO₃ buffer. The experiments on cells heated at 42° in vivo were performed 2 hr after the final heating of tumors as described in the legend to Table 1. Otherwise, legend as for Chart 1.

Table 2 also records respiration values for slices of normal rabbit skin, from which the VX-2 tumor originated, and for slices of normal inguinal lymph nodes and lung, to which the tumor metastasized. Oxygen consumption was linear at 37.5° over the time period examined for these 3 tissues. Increase in

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**Table 1**

O₂ uptake of VX-2 tumor at 37.5° following local in vivo hyperthermia

Heat was applied by immersion of the tumor-bearing limb in a water bath at 46°, and the intratumor temperature was maintained at 42° for 1 hr on 3 occasions at 24-hr intervals on Days 35, 36, and 37 after VX-2 cell inoculation. All manometric readings were made in duplicate flasks, which contained 5,000,000 to 10,000,000 VX-2 cells, or 100- to 200-mg slices, from the same tumor in Tris-HCl-sucrose buffer. The values are the means ± S.D. for 6 tumors in each case, except the control figures, which are from 10 tumors.
Table 2

O$_2$ uptake of primary and metastatic VX-2 cells at 37.5° and 42°

All readings were made in duplicate flasks, which contained 5,000,000 to 10,000,000 tumor cells, or 100- to 200-mg slices, of the normal tissues. Each series of observations was recorded with material from the same tumor or tissue maintained simultaneously in waterbaths at 37.5° and 42.0°. The values are the means ± S.D. for 10 tumors in the case of the VX-2 cells, for 3 different experiments with skin, and for 4 different experiments with the other cells and tissues.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>VX-2 cells</th>
<th>Metastatic VX-2 cells</th>
<th>Skin</th>
<th>Lymph node</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.5°</td>
<td>6.3 ± 1.0</td>
<td>6.9 ± 0.6</td>
<td>10.2 ± 0.8</td>
<td>9.3 ± 0.7</td>
<td>10.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10.5 ± 1.9</td>
<td>13.6 ± 1.0</td>
<td>15.8 ± 1.0</td>
<td>15.5 ± 1.4</td>
<td>16.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>18.9 ± 2.5</td>
<td>16.8 ± 1.8</td>
<td>21.6 ± 1.4</td>
<td>21.2 ± 2.0</td>
<td>23.5 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>31.0 ± 3.2</td>
<td>22.3 ± 2.4</td>
<td>30.2 ± 2.8</td>
<td>29.5 ± 2.7</td>
<td>30.6 ± 2.5</td>
</tr>
<tr>
<td>42.0°</td>
<td>3.9 ± 0.7</td>
<td>3.7 ± 0.3</td>
<td>11.6 ± 1.0</td>
<td>9.1 ± 1.6</td>
<td>10.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>4.7 ± 0.7</td>
<td>8.8 ± 0.3</td>
<td>18.9 ± 1.4</td>
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<td></td>
<td>5.1 ± 0.9</td>
<td>11.4 ± 0.7</td>
<td>27.6 ± 2.0</td>
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<tr>
<td></td>
<td>5.7 ± 0.8</td>
<td>14.2 ± 0.8</td>
<td>38.2 ± 2.9</td>
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Temperature (42.0°) produced little alteration in the respiration of lymph node and lung; respiration in skin slices was stimulated at the elevated temperature. The resistance of normal tissues to damage by temperatures in excess of 40° has been reported by other workers (3, 5, 34).

In Chart 3, the results of local heating and general body heating are compared in terms of the effect on primary tumor volume. From an initial transplant of 1,000,000 cells into the hind limb of the rabbit, the VX-2 tumor increased in volume exponentially from the 5th to the 9th week after cell inoculation. Subsequently, tumor volume increased at a much slower rate until the rabbits died at 70 ± 6 days. Heating was applied at 35 days at the beginning of logarithmic growth. Following 3 heatings, during which an intratumor temperature of 42° was maintained for 1 hr by local or general body heat at 24-hr intervals, tumor volume continued to increase for a further 2 weeks; subsequently, all heated tumors decreased in volume. Four of the 8 rabbits treated by limb immersion are alive, with no signs of tumor, 2 years after treatment. Of the 14 animals treated by total-body heating, 4 died at 20 weeks after tumor inoculation, 1 survived to 30 weeks, and 1 is alive 1 year after therapy; the other 8 rabbits died within 10 weeks of tumor inoculation (mean survival time, 65 ± 8 days). All animals that died contained metastases in the lungs and inguinal and paraaortic lymph nodes at autopsy.

In Chart 4, regression lines have been fitted to the log tumor volume measurements following local heating and total-body heating. For local heating (Chart 4A), the equation to the line is:

$$\log y = 3.98 - 0.311x$$

and for total-body heating (Chart 4B), the equation to the line is:

$$\log y = 2.60 - 0.104x$$

As the variances about the 2 lines are significantly different ($F$ with 39 and 56 d.f. = 6.585; $p < 0.001$), the slopes were compared by use of Cochran's approximation to the $t$ test. This revealed that the difference in slope of the regression lines

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**Table 2**

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<td>23.5 ± 1.4</td>
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<td>31.0 ± 3.2</td>
<td>22.3 ± 2.4</td>
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**Chart 3.** Changes in mean VX-2 tumor volume following local heating applied to the tumor-bearing limb (water bath immersion) or following total-body hyperthermia induced by radiant heating of the rabbit. In both methods, heat was applied for 75 min on 3 consecutive days at 24-hr intervals, beginning on Day 35 after VX-2 cell inoculation. The figures in brackets denote the number of tumors (animals) studied at each point.
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1.5

Chart 5 shows that, once an intratumor temperature of 42.0°C was achieved, it was maintained above this minimum while the heat was applied, with an average temperature of 42.7 ± 0.3°C between 15 and 75 min. During this 1-hr heating period, the average thoracic muscle temperature was 40.4 ± 0.3°C, while rectal readings remained within the temperature range considered normal for the rabbits.

In total-body heating experiments (Chart 6), the thoracic muscle temperature was above 42°C between 15 and 75 min (average, 43.2 ± 0.4°C), and the intratumor temperature over this time interval was similar to that in the primary during local heating (average, 42.6 ± 0.5°C). Intraabdominal and intrathoracic temperature patterns fluctuated over the heating period, however, between a maximum of 43.6°C and a minimum of 39.8°C, and the pattern of oscillation varied from animal to animal. Over the heating period, the average intrathoracic temperature was 40.5 ± 1.0°C, and the average intraabdominal temperature was 40.8 ± 0.7°C. During heating, the ears of the rabbits became bright pink, with temperatures in the region of 44—45°C, and there was marked hyperpnea. Animal recovery was rapid following general or local heating as detailed, but attempts to prolong either form of heating beyond 90 min led to animal distress.

In both local and general body heating, needle electrodes simultaneously placed in different regions of the same tumor revealed that the temperature did not vary significantly within a given tumor, provided that the electrode was inserted into nonnecrotic tumor tissue.

Chart 4. Regression lines fitted to log tumor volume measurements from the 7th week following tumor inoculation. Local hyperthermia to the tumor-bearing limb (A), or total-body hyperthermia (B), was applied on Days 35, 36, and 37 after cell inoculation. The individual tumor volumes for all experiments shown in Chart 3 have been plotted, and the equation to the regression line in each case is given.

for tumor volume following local and total-body heating is highly significant ($r = 5.357, p < 0.001$). In terms of volume, the regression equations mean that, in the case of local heating, tumor volume was halved every 0.97 week (95% confidence limits, 0.84 to 1.14 weeks) from the 7th week after inoculation; while, after total-body heating, tumor volume was halved every 2.9 weeks (95% confidence limits, 2.5 to 3.4 weeks) over the same period.

Chart 5 illustrates the temperature changes that took place during local heating of tumors. A rectal temperature sensor was difficult to keep in position, owing to the posture of the rabbit hindquarters during local heating. A needle electrode placed in the lower left deep thoracic muscles gave a recording of body temperature that followed a similar pattern to that of rectal temperature over the time of the experiment; the absolute values were approximately 1°C higher than temperatures recorded in the rectum (Chart 5). The average thoracic muscle temperature recorded in control unheated rabbits in the present work was 39.25 ± 1.0°C (25 animals), compared to an average rectal temperature of 38.20 ± 1.0°C in 10 members of the same series. Concomitantly, measured ear temperatures varied from animal to animal and followed no reproducible pattern over the experimental period. The thoracic muscle temperature was therefore adopted as a guide to the body temperature of the animals.
DISCUSSION

Although there are now over 300 papers in the literature describing the effect of elevated temperature on cancer cells [see the comprehensive article by Cavaliere et al. (3), and the recent review (32) for references], few of these reports detail tumor temperature during heating. In the present work, therefore, emphasis has been placed on intratumor temperature and the temperature of the metastases in relation to the applied temperature.

The older literature indicates clearly that, for the destruction of tumors in man and in animals, temperatures in excess of 40°C, often for prolonged periods, were required (see Refs. 21 and 34 for references). More recently, Selawry et al. (24) observed that, with 3 strains of human neoplastic cells in tissue culture (HeLa, H. Ep. 2, and J96), irreversible heat injury took place only at temperatures above 42°C, and Crile (5) found that the destructive effects of heat on Sarcoma 180 and S91 melanoma implanted into the hind feet of mice began at 42°C. Mondovi et al. (17), using Novikoff cells, concluded that 41°C was a critical temperature point at which maximum inhibition of thymidine uptake occurred, and Giovannella et al. (9) have also reported that the relationship between temperature increase and lethality is not linear; with L1210 leukemia cells, the killing effect became manifest between 41°C and 42°C. Dickson and Shah (6) recently found that the inhibitory effects of hyperthermia on a malignant cell line from rat breast became evident between 41°C and 42°C, as indicated by depression of respiration, increase in vital dye uptake, and decrease in proliferative potential of the cell populations both in vitro and in vivo. The work referred to above, the clinical results of Cavaliere et al. (3), and the present results (Chart 1; Table 1), indicate that 41—42°C may be the therapeutic temperature range to attain in heated tumors if the duration of heating is not to be prolonged and if subject tolerance is a prime consideration.

For total-body heating experiments, a reliable indicator of internal or "body core" temperature is essential, and the temperature of various deep viscera, including the heart, lower esophagus, liver, and rectum, have been proposed for this measurement (see Ref. 11 for references). There is evidence (16, 25) that, during rapid changes of body temperature, the small thermal gradients normally operative between tissues and organs in the animal body can increase considerably and that temperature recorded in the lower esophagus and heart is a sensitive indicator of such changes; whereas, in a thermally neutral environment, rectal temperature can be taken as a measure of core temperature, changes in rectal temperature are too sluggish to be of value when body temperature is changing rapidly (11, 16, 25). In the present work, the temperature of the deep thoracic muscles, as assessed against simultaneous rectal recordings, was an indication of (but approximately 1°C above) core temperature for local heating when the heat load imposed on the rabbit was small. Temperature measured in this way during total-body heating did not reveal the fluctuating intrathoracic and, to a lesser extent, intraabdominal temperature, as detected by sensors in the region of the heart and liver (Chart 6). The oscillation may have been due to the marked hyperventilation during hyperthermia, since the main mechanism of heat regulation in the rabbit in a hot environment is via the lungs (11). Oscillations in esophageal temperature during periods of hyperventilation have been described in man (4).

It is possible, therefore, that the average intrathoracic temperature of 40.5°C and average intraabdominal temperature of 40.8°C were inadequate to destroy the metastatic cancer cells. Although results were not obtained for VX-2 cells from the lung, the respiration of secondary cells from lymph nodes was less sensitive to 42°C in vitro than was respiration of the primary cells (Table 2), and temperatures in the region of 39—40°C produced a stimulatory effect on oxygen uptake of primary cells (Chart 1). Inability to maintain a higher core temperature may have contributed to death of the animals by stimulating the activity of secondaries. Although many reports indicate that, in general, the deleterious effect of high temperature on cancer cells depends on both the degree of heat applied and its duration (see Ref. 32 for references), there are also indications in the literature that the growth rate of malignant cells increases with temperature before the inhibitory temperature is reached. Selawry et al. (24) reported an increase in growth rate of cancerous cell lines in culture up to 39°C, and Rao and Engelberg (20) described a decrease in the mean generation time of HeLa cells at temperatures above 37°C.

The biological import of the statistically highly significant difference in the primary tumor regression rates after the 2 types of heating remains an open question at present. Crile (5)
has postulated that immunological mechanisms are unlikely to be involved in the disappearance of tumors following hyperthermia, since the effect is so rapid; and, in his experiments, mice cured of Sarcoma 180 succumbed to subsequent challenge of tumor cells. The Sarcoma 180 does not metastasize in mice, however (5), and in the rabbit VX-2 system, in which rapid and predictable metastasis occurs (7), an immunological element in the response to heating cannot be dismissed. It was postulated previously that survival of the rabbit after local heating to the tumor may depend on the ability of the immune system of the animal to destroy metastatic cells (19); and, in the present work, animals challenged by $1 \times 10^6$ VX-2 cells into the contralateral leg 1 to 3 months following local hyperthermia showed no signs of tumor growth. Total-body heating, on the other hand, may depress the immune response. Stimulation of the immune system by tumor breakdown products following local heating would be consistent with clinical observations. In the series of advanced melanomas and sarcomas (tumors with a high tendency to metastasize) subjected to regional hyperthermic perfusion by Cavaliere et al. (3), only 1 patient died from metastases. Stehlin (28) has greatly reduced the necessity for amputation in soft tissue sarcomas of the limbs by including hyperthermic perfusion in his treatment regimen of perfusion and irradiation and has raised the question of whether such treatment may initiate an immune response in the patients. Stehlin has also been impressed by the radiological disappearance of lung metastases following treatment of primary limb tumors by hyperthermia (J. S. Stehlin, personal communication).

The importance of the proliferating fraction (growth fraction) of the cell population in tumor growth and response to therapy is now recognized; the position of the cell in its generation cycle at the time of exposure governs the response to chemotherapy (27) and to radiotherapy (8). Various workers have reported that both normal and malignant cells are most sensitive to damage by increased temperature during mitosis and, more specifically, in the stage of metaphase (see Ref. 3 for references). With human amnion cells in culture, Siskin et al. (26) found that all phases of the cell cycle were sensitive to temperatures above $39^\circ$, with metaphase especially so; Giovanella et al. (10) showed that, in HeLa cells, there was a large differential sensitivity to the action of heat according to the different phases of the cell cycle, the most sensitive part being early G2 phase. There is evidence that 1 factor influencing the response of tumors to fractionated radiotherapy is an increase in the rate of proliferation of surviving cells after each dose (8), and the effect of fractionated hyperthermia on the VX-2 may be attributable to a similar effect. Such a decrease in cell-cycle time following heating might explain the widespread tumor cell necrosis resulting from 3 applications of heat within a 48-hr period, when the mean cell generation time of the VX-2 is believed to be 87 hr, although this time is probably an overestimate (2). Fractionated heating may therefore have advantages for tumor therapy and would enable the applied heat to be kept within a range compatible with survival of the host; the classical experiments of Westermark (34), who showed that the difference in susceptibility to heat between normal and malignant tissues was greatest at the lower temperatures in the thermal death range for cancer cells, add strength to the proposal.

The bulk of the literature on the treatment of cancer by elevated temperature concerns the application of heat locally to tumors; many methods have been used for this, including shortwave diathermy (13), microwave diathermy (22, 34), water bath immersion (5, 31), ultrasound (23), and hyperthermic regional perfusion (3, 29, 31). Less information is available on total-body heating. Lovelock and Smith (15) have devised diathermy techniques for the rapid heating of whole frozen animals (hamsters and rabbits) without injury to the animal, and Bigelow et al. (1) found that diathermy was applicable to rewarming of hypothermic patients. At temperatures of $37^\circ$ and above, however, a danger of this technique is the differential sensitivity of normal tissues to overheating (15). Although other workers claim to be developing techniques for total-body heating (3), recent impetus for this approach to cancer therapy has come chiefly from Von Ardenne and his group, who use total-body immersion in an elaborately designed water tank to elevate the temperature. In over 50 papers and a book (33), this worker has discussed exhaustively the theoretical aspects of killing cancer cells by elevation of body temperature, but details of the effects of hyperthermia applied to animal or human tumors are difficult to obtain from his publications. Kirsch and Schmidt (14) and Suryanarayan (30) have treated patients by total-body immersion, but the results, as reported, are not impressive. The main disadvantage of the hot bath method is that the temperature difference between the heating fluid and the skin cannot be large because of the danger of burning; and recently, Henderson and Pettigrew (12) have devised an ingenious method of elevating body temperature in humans, using the large alveolar surface area of the lungs as heat exchanger. The patient's temperature is elevated by inhalation of a prewarmed mixture of 50% helium in oxygen, and heat loss is then minimized by insulating the body in a covering of paraffin wax. By this means, a controlled elevation of body temperature to $42^\circ$ can be achieved rapidly and reproducibly and has been maintained with little fluctuation for up to 6 hours; the therapy has been used repeatedly on several patients. The results of treating cancer patients have not yet been reported but appear encouraging (R. T. Pettigrew, personal communication).

In the present work, only transient heat storage conditions (16) were achieved in the rabbit, and the fluctuations in deep temperature may have been a reflection of insufficient time to stabilize thermal gradients. Oscillations of temperature have occurred after administration of pyrogens to rabbits, cats, and dogs, while such oscillations are less frequently seen in man during pyrogenic fevers (11). From this point of view, therefore, the rabbit may not be a suitable experimental animal for studying total-body hyperthermia. In this context, Vormel and Kuznetsova (32) have recently emphasized that the results of hyperthermia experiments on animals may give little indication of the response when man is the host, since thermoregulatory mechanisms in animals are different and less well developed than in humans. On the other hand, the different regression rates of the primary VX-2 tumor after local and total-body heating cautions against facile
interpretation of the effects of hyperthermia on the tumor-bearing host.

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Total-Body Hyperthermia versus Primary Tumor Hyperthermia in the Treatment of the Rabbit VX-2 Carcinoma

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