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Studies on the Biological Action of Hyperthermia on Tumor Cell Mortality (1)*

Man-Sik Kang and Chung Choo Lee
(Dept. of Zoology, College of Natural Sciences, Seoul National University)

腫瘍細胞의 致死에 미치는 溫熱處理의 生物學的 作用에 관한 硏究(1)

姜 萬 植 · 李 廷 珠
(서울大 自然大 動物學科)

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摘 要

溫熱處理가 SCK 腫瘍細胞에 미치는 生物學的 影響을 몇가지 측면에서 檢討하였다.

첫째로, 溫熱處理가 ***in vitro***와 ***in vivo***에서 어떤 차이가 있는 지를 알아보기 위해서 生殘曲線을 구하여 비교하여 본 바, ***in vivo***의 경우에 더욱 큰 腫瘍細胞 致死效果를 보았다.

둘째로, 이러한 細胞致死作用의 차이가 나타나는 原因을 알아보기 위해서 溫熱處理가 腫瘍組織과 筋肉組織의 溫度와 pH에 어떤 차이를 나타내는 지를 측정하여 본 바, 腫瘍의 경우 溫度는 筋肉보다 높았겼으며, pH는 초기의 上昇을 제외하고는 계속해서 낮은 狀態를 유지했다. 이와 같은 腫瘍내의 酸性狀態가 溫熱處理의 細胞致死效果를 增强시키는 것으로 보였다.

셋째로, 이와같은 腫瘍내 pH의 酸性化의 原因을 구명해 보기 위해서 腫瘍과 肝 속의 乳酸含量을 정량하여 본 바, 腫瘍의 경우 溫熱處理 후 1시간에는 增加하였으나 그후에는 계속 減少된 상태를 유지했다. 이에 반해서 肝의 경우 5시간에 最大値를 보였고 24시간 동안 계속해서 上昇된 값을 유지했다. 이러한 腫瘍내 乳酸含量의 減少는 예기치 못했던 결과였는데, 이는 溫熱處理로 말미암아 腫瘍내의 細胞가 계속해서 死滅되는 결과로 腫瘍 단위 重量당 乳酸量의 감소를 가져오는 것으로 풀이할 수 있었다.

넷째로, 溫熱處理에 의한 細胞致死效果의 標的을 찾아 보기 위한 한가지 試圖로서 膜表面蛋白質의 變化를 관찰하였던 바, 高分子蛋白質의 減少와 더불어 低分子蛋白質의 增加라는 一般的인 現象을 볼 수 있었고, 특히 70K 蛋白質이 特異的 增加를 보인 것과 이의 증가가 41°C에서 最大의 값을 보인 것은 興味

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溫熱處理가 腫瘍내 pH의 酸性化를 招來하는 原因과 膜表面蛋白質에 特異한 影響을 미치는 機作에 대해서는 앞으로 더욱 많은 硏究가 傾注되어야 할 興味있는 問題로 생각된다.

INTRODUCTION

It has been demonstrated that hyperthermia causes greater damage to malignant cells in solid tumor *in vivo* than to the same cell line cultured *in vitro* (Kang, *et al.*, 1980; Marmor *et al.*, 1977). It has also been reported that the malignant tumors *in vivo* may be more heat-sensitive than are the surrounding normal tissues (Storm *et al.*, 1979; Mendecki *et al.*, 1976; Overgaard and Suit, 1979; Kim and Hahn, 1979). These phenomena may be attributed, at least in part, to the well-known facts that intratumor milieu is intrinsically acidic and that acidic environment greatly enhances hyperthermic killing of mammalian cells (Gerweck, 1977; Overgaard and Bichel, 1977; Freeman *et al.*, 1977; von Ardenne and Reitnauer, 1971).

Indications are that acidic environment may not only potentiate initial hyperthermic cell killing but also suppress the repair of thermodamage and development of thermotolerance (Henle and Dethelefsen, 1978; Nielson and Overgaard, 1979; Gerweck *et al.*, 1980; Goldin and Leeper, 1981). Song *et al.* (1980) and Bicher *et al.* (1980) reported that intratumor acidity further increases upon heating. It is conceivable that understanding the mechanisms and kinetics of pH change in the heated tumors would help clinicians plan effective protocols of fractionated hyperthermia of human tumors.

The neoplastic tissue is characterized by its high aerobic and anaerobic glycolysis. Therefore, the most plausible cause for the heat-induced increase in acidity in the heated tumors appears to be an increase in glycolysis accompanied by an increase in lactic acid synthesis and/or accumulation of lactic acid. Gullino *et al.* (1964) reported that the lactic acid content in the interstitial fluid of Walker tumor of rat was 2-3 fold greater than that in the subcutaneous fluid or aortic serum of rats. In light of well-known high glycolytic activity in neoplastic cells, the rather large difference in the lactic acid content between the tumor and the normal tissue is not surprising. In fact, as much as 37% of glucose utilized dy Walker tumors is converted into lactic acid by aerobic and anaerobic glycolysis (Gullino *et al.*, 1967).

In the present work we investigated the heat-induced change in the intratumor temperature, pH and lactic acid, and in membrane surface proteins, as well as in cell survival in experimental tumor system.

MATERIALS AND METHODS

TUMOR

The SCK tumor used in the present study was a mammary carcinoma of female A/J mice which arose spontaneously in 1974 and was adapted to grow both *in vivo* and *in vitro* by Dr. Clement in the Radiobiology Laboratory, University of Minnesota Medical School. We have been maintaining it by alternate passage *in vitro* and *in vivo*, routinely subculturing the cells *in vitro* several times before injecting them back into animals. Cells obtained from cultures or from tumors *in vivo* grow well in RPMI 1640 medium supplemented with 10% fetal calf serum. For *in vivo* studies, the exponentially growing cells in culture were treated with 0.25% trypsin for 15 minutes, washed three times with plain RPMI 1640, and the cells able to exclude trypan blue were counted. About 5×10^4 cells were injected subcutaneously into the right leg of A/J mice. When the tumors grew to 7-8 mm in diameter, which took about 9-10 days, we used them as an *in vivo* experimental tumor system.

HYPERTHERMIA OF TUMOR CELLS IN VITRO

Cell Survival Studies. Exponentially growing cells in culture were trypsinized and the viable cells were counted. The appropriate number of cells were plated in T-type glass culture flasks. Fifteen hours after incubation at 37°C in a humidified mixture of 5% CO_2 and 95% air, the medium was replaced with prewarmed fresh medium, and the flasks were plugged with rubber stoppers. Two 21-gauge needles were pierced through the stoppers and water-saturated mixture of 5% CO_2 and 95% air was flushed into the flasks through one of the needles at a flow rate of 0.5 liter/min/flask. After incubation for 45 minutes at 37°C with continuous gassing and gentle rocking, the needles were removed and the flasks were immersed horizontally in a thermostatically controled water bath.

After heat treatment for various length of time, the flasks were removed and the rubber stoppers were replaced with ordinary flask caps, and the cells were cultured for 9-12 days at 37°C in a CO_2-incubator. After fixing with 95% alcohol and staining with crystal violet, the macroscopic clones containing more than 50 cells were counted and the surviving fractions were calculated.

Analysis of Membrane Surface Proteins. Lactoperoxidase used was purchased from Sigma Chemical Co. and Na ^{131}I was obtained from the Korea Energy Research Institute.

Surface membrane protein labeling with ^{131}I was essentially based on the method of Hubbard *et al.* (1972). Briefly, culture media were removed and cells were washed three times with 0.9% saline. Cells were scraped with a rubber policeman and were collected by centrifugation. Cell pellet was resupended in 0.5 ml phosphate-buffered saline(PBS) and added by 100 μl of lactoperoxidase (500 μg/ml) and 150 μCi of Na^{131}I (1 mCi/ml). Iodination reaction was initiated by the addition of 20 μl of 90 mM H_2O_2 and another 20μl

of the H_2O_2 was added 10 minutes later. After 10 minutes of incubation, reaction was stopped by the addition of 10 ml PBS containing 0.5mM of $Na_2S_2O_3$ and 5mM phenylmethylsulfonylfluoride (PMSF). Cells were washed three times by centrifugation with phosphate buffered-sodium iodide to remove excess radioiodine. The cells collected were suspended in 0.1 M tris-HCl buffer, pH 6.8.

The discontinuous SDS-polyacrylamide gel electrophoresis was performed according essentially to Laemmli (1970). Aliquots of homogenate were counted for radioactivity and equal amounts of radioactivity were applied to separate slots of 7.5% slab gels. The samples were dissolved in 0.65 M tris-HCl, pH 6.8, containing 2% SDS, 5% mercaptoethanol, and 0.5% bromophenol blue. Samples were boiled for three minutes and electrophoresis was carried out with a constant current of 50-70 mA for 4-6 hr. Gels were stained with 0.2% Coomassie brilliant blue in 40% methanol and 10% acetic acid for 3 hr, and destained with 35% methanol and 7% acetic acid until the background became clear.

The destained gels were dried under gentle vacuum for autoradiography. Autoradiograms were prepared by exposing the gels on an X-ray film for a few days. Protein in homogenate was determined by the method of Lowry *et al.* (1951).

HYPERTHERMIA OF TUMORS IN VIVO

Cell Survival Studies. Tumors growing in the leg of mice were excised, minced, and treated with 0.25% trypsin and a smll amount of DNase in plain RPMI 1640 for 20 minutes at room temperature with continuous stirring. The dispersed cells were washed once with medium containing 20% calf serum and twice with plain medium. About 5×10^4 viable cells in 0.05 ml were injected subcutaneously into the legs of female mice.

When the tumors grew to 7-8 mm in diameter, the animals were anesthetized with pentobarbital (0.04 mg/g) and placed on a Plexiglas board with semicircular holes. The tumor bearing legs were passed through the holes and immobilized by anchoring a toe with thread to Plexiglas support adjacent to each hole. The tumors were heated by placing the board directly over a water bath, immersing the legs. The intratumor temperature, measured by a 29-gauge thermoprobe, rose from 34-35°C prior to heating to about 0.5°C lower than the water temperature within 2-3 minutes. The tumors were excised after heating and trimmed and weighed. For each experiment, three tumors were minced and dispersed to single cells, as described above. The number of cells per gram of tumor was calculated and the cells were cultured in Falcon 3013 plastic culture flasks with 8 ml RPMI 1640 supplemented with 10% fetal calf serum and antibiotics. The clones were stained and counted 9-12 days later.

Measurement of Intratumor Temperature. The temperature of tumors was measured with a 29-gauge copper-constantan thermoprobe (Bailey Instrument Co., Saddle Brook, N.J., type MT-3). The probe was connected to a chart recorder (Omniscribe, Model A5223-15,

Houston Instrument Division, Bausch & Lomb, Austin, Texas) which was calibrated with a digital readout monitor(Bailey Instrument Co., Model BAT-8).

Measurement of Intratumor pH. The pH of tumors of mice was measured during and after hyperthermia, using a needle electrode (Micro Electrode, Londonderry, N.H.) with a diameter of 0.8 mm. Reference electrode with a diameter of 0.1 mm was constructed and both electrodes were carefully inserted into the center of the tumor. The change in pH was monitored using a strip-chart recorder. The electrode and thermoprobe were immobilized by firmly attaching them to a stand placed above the tumor.

Measurement of Lactic Acid Content. At various times after heating, the tumors and liver were dissected under light ether anesthesia and immediately frozen in liquid nitrogen. Preliminary studies indicated that delaying of freezing the tissues for only a minute after dissection significantly increased the lactic acid content in the tissues.

The lactic acid content was measured using a procedure described by Gutmann and Wahlefeld (1977). The principle of this method is that the L-(+)-lactic acid is oxidized to pyruvic acid in the presence of nicotinamide adenine dinucleotide(NAD^+) by the enzymatic reaction of lactate dehydrogenase(LDH).

$$\text{L-(+)-Lactic acid} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvic acid} + NADH_2$$

The pyruvic acid formed is trapped by hydrazine in the reaction mixture so that the reaction can be completed to one direction in the presence of excess amounts of NAD^+. From the amount of $NADH_2$ formed, the lactic acid content is calculated.

The frozen tissues were quickly weighed, 10 volume of ice-cold 8% perchloric acid was immediately added, and then homogenized with a glass homogenizer. Following centrifugation for 10 minutes at $3,000 \times g$ in a 4°C centrifuge, 0.2 ml of supernatant was mixed with 2.8 ml of reaction mixture (pH 9.2) containing 0.5 mmole of hydrazine, 50 units of LDH (Sigma, Stock No. 826-6), 5mg of NAD^+ (Sigma Stock No. 260-110) and 0.6 mmole glycine in water. The mixtures were incubated in 37°C water bath for 30 minutes. The $NADH_2$ content was measured spectrophotometrically by the increase in absorbance at 340 nm, and the lactic acid content was computed.

RESULTS

The kinetics of the SCK tumor cell death following hyperthermia *in vitro* and *in vivo* are compared in Fig. 1. The survival of heat-treated cells was normalized to the control. As Fig. 1 indicates, there was a significant difference in cell death between the cells *in vitro* and those *in vivo*. When the heat sensitivity is compared in terms of D_0 value for each group at same temperature, D_0's for *in vivo* and *in vitro* at 42.5°C are 34 min and 140 min respectively, whereas those at 43.5°C are 12 min and 32 min respectively. The difference in D_0's is greater at 42.5°C than at 43.5°C.

The rise and fall of intratumor temperature following application and cessation of heat

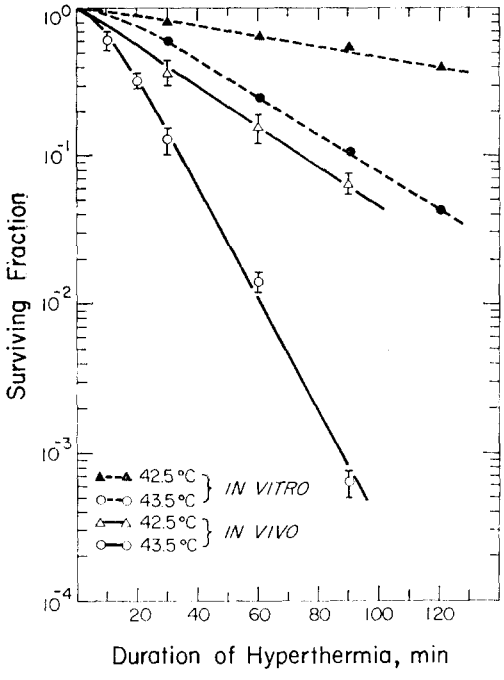

Fig. 1. The heat sensitivity of SCK tumor cells *in vivo* and *in vitro* were compared. The survival curves of the tumor cells heated *in vivo* at 42.5°C and 43.5°C were much steeper than those of cells heated at same temperatures. The *in vivo* tumor cells were significantly more heat sensitive than *in vitro* cells.

is shown in Fig. 2. The temperature varied considerably depending upon the size of tumor and the location of the tip of the thermoprobe. In general, it was higher in the core than at the periphery. Unlike the muscle (not shown), the tumor temperature did not drop following the initial rise, possibly due to the lack of increased blood flow in the tumors heated to 43.5°C. After 1 hr, the average core temperature of the small tumor was 42.9°C. It was apparent that the tumors reached higher temperature than the muscle upon being heated at the same temperature.

Fig. 2 also shows a representative profile of pH changes following hyperthermia at 43.5°C for 30 min. The pH change in the SCK tumor (1 cm in diameter) upon being heated at 43.5°C started out at 7.05 and increased to 7.18 during the first 7 min of heating, and then rapidly declined to 6.67 by 30 min. while it recovered to 6.8, it fell to 6.58 after being heated again at 43.5°C for 30 min. The pH of the leg muscle was 7.1-7.4. Contrary to the decrease in pH in the heated tumors, the pH in the muscle increased significantly when heated to 43.5-45.0°C (not shown).

The time course changes of lactic acid in the tumor and liver following hyperthermia are shown in Fig. 3. An average lactic acid content in the control tumor and liver was 4.1 μmole/g and 1.9 μmole/g, respectively. Following hyperthermia at 43.5°C for 30 min, maximum increase was observed in the tumor and liver at 1 hr and 3 hr, respectively. The increase in the tumor was followed by a gradual decrease below the control level, whereas the increase in the liver was maintained at quite a steady value for 24 hr after hyperthermia.

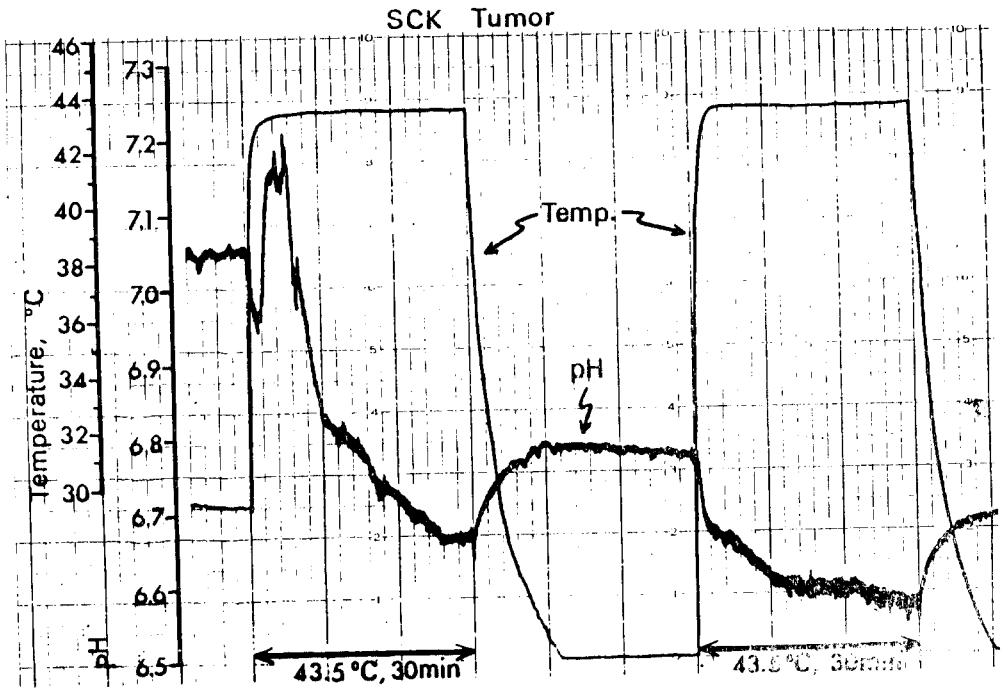

Fig. 2. The change of pH and temperature of SCK tumor mass upon heating at 43.5°C. The pH was measured by needle electrode of 800 μm in diameter and the temperature by thermoprobe.

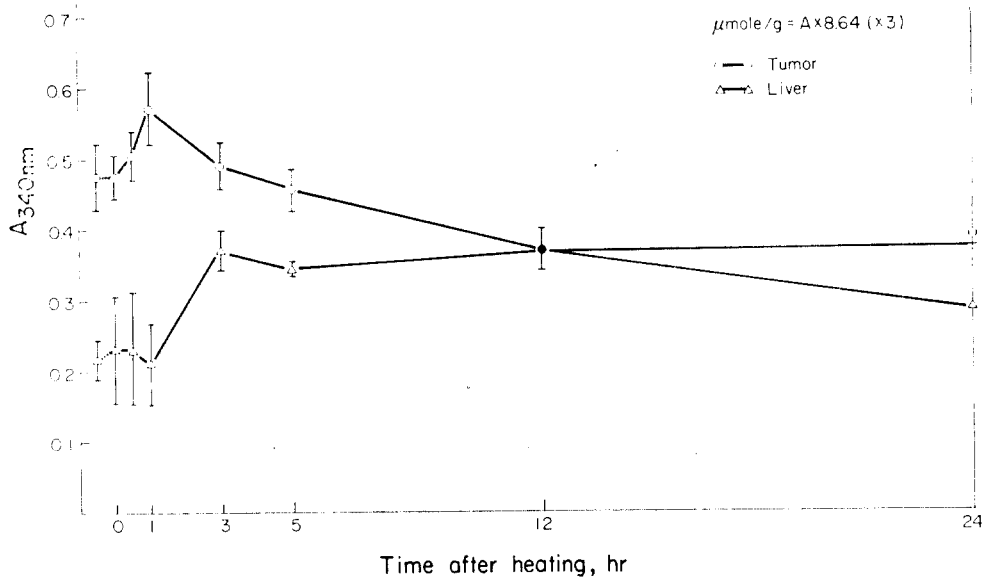

Fig. 3. Time course changes of lactic acid content in SCK tumor and in the liver following hyperthermia at 43.5°C for 30 min.

In Fig. 4 is shown the heat-induced changes in the surface membrane proteins in SCK tumor cells. Sample H_{24} was prepared by iodinating SCK cells 24 hr after shock at 43°C for 1 hr. A general finding was that band 1 and other high molecular proteins decreased markedly, whereas most of low molecular proteins increased. Major bands 5 and 7 were found to disappear almost completely by heat shock. The most prominent change was that the band 9, apparent molecular weight of 69~70K, increased significantly along with other low molecular proteins in heat shocked cells.

In an attempt to focus on the heat shock protein (hsp) 70K, various heat shocks were given to SCK cells as shown in Fig. 5. The most striking finding was that 69~70K protein appeared in large quantity at 41°C rather than at higher temperatures.

DISCUSSION

A comparison of the survival curve of SCK tumor cells *in vitro* and *in vivo* following hyperthermia demonstrated that the cytocidal effect of heating is far greater on the cells *in vivo* than on the cells *in vitro*. This finding is in good agreement with Bhuyan *et al.* (1977) that 7-day old L1210 ascites cells from mice were more heat sensitive compared to the same cells cultured *in vitro*. These investigators ascribed the difference in the growth phase of the cell.

Bichel and Overgaard (1977) reported that at pH 6.4 and 7.2 the plateau-phase cells were more heat sensitive than exponential phase cells. In addition, since acidity sensitizes cells to heat, the plateau-phase cells at pH 6.4 were about 1000-fold more heat sensitive than exponential phase cells at pH 7.2. In the present study, the pH of media for the *in vitro* study was at 7.2 and these cells were in exponential growth phase. On the other hand, the intratumor environment of the SCK tumor used for *in vivo* studies was probably acidic as is evident from Fig. 2, and at least part of the tumor cells might have been in plateau phase. Therefore, it might be that the greater heat sensitivity of tumor cells *in vivo* than that of tumor cells *in vitro* in this study was due, in part, to a high acidic intratumor environment and/or to the presence of plateau-phase cells. It should be pointed out, however, that several groups of investigators reported that plateau-phase cells are heat resistant rather than heat sensitive, as compared to exponential-phase cells (Kase and Hahn, 1976; Kim *et al.*, 1974; Power and Harris, 1977). Therefore, the role of the growth phase in the differential heat sensitivity of tumor cells *in vitro* and *in vivo* in our study is not certain.

While the mechanism for the decrease in pH in heated tumors is not clear, it could be that lactic acid formation is increased due to enhanced metabolism without a concomitant increase in oxygen supply. It is also probable that draining of lactic acid in the heated tumor proceeds more slowly due to the vascular damage than heated control tissues, resulting in sustained depression of the pH. It is now well known that the cytocidal effect of heat is markedly enhanced under acidic conditions (Gerweck, 1977; Overgaard, 1976).

It is demonstrated in the present study that the lactic acid content in control SCK tumor was 4.1 μmole/g, while that in the liver was 1.9 μmole/g. This difference in the lactic acid content in the tumors and liver is consistent with the report by Gullino *et al.* (1964) that the lactic acid content in the interstitial fluid of Walker tumor of rat was 2-3-fold greater than that in the subcutaneous fluid or aortic serum of rats, although the method of sampling for quantitation was different from ours. In light of the well-known high glycolytic activity in neoplastic cells, the rather large difference in the lactic acid content between the tumor and the normal tissue is not surprising. In fact, as much as 37% of glucose utilized by Walker tumors is converted into lactic acid by aerobic and anaerobic glycolysis (Gullino *et. al.*, 1967). The gradual and sustained decrease of lactic acid content through 24 hr after initial increase at 1 hr in the heated tumor seems quite obsolete in view of the fact that acidic pH is thought to be attributable to the increased lactic acid, produced by increased anaerobic glycolysis and/or retained by impared blood flow. Therefore, the present result of lactic acid content following hyperthermia has to be discussed in connection with the delayed cell death, when the cells are left *in situ* after hyperthermia (Kang, *et al.*, 1980). The delayed cell death seems to be the principal cause for the decreased lactic acid content in the tumor for 24 hr after hyperthermia. Thus, it is probable that a large portion of damaged cells eventually disintegrate and thus, the formation of lactic acid by these cells declines gradually post-heating.

It is highly probable that an increase in the formation of glycolytic metabolites other than lactic acid also contributes significantly to the increase in acidity in the heated tumors. Furthermore, our present study does not exclude the possible presence of acid producing reactions other than glycolysis in the heated tumors. Indeed, it has been reported that despite the low intratumor pH, the glycolytic activity is slow and the lactic acid is small in hepatoma (Gullino *et al.*, 1964; Aisenberg and Morris, 1961), suggesting the presence of acid-producing reactions other than glycolysis in the hepatoma. In this context, Streffer *et al.* (1980) reported that whole-body hyperthermia at 40°C or 41°C decreased the hepatic content of lactate and pyruvate and increased that of β-hydroxybutyrate and acetoacetate, indicating an increase in lipolysis. Needless to mention, the energy metabolism, i.e., respiration, glycolysis and lipolysis, in normal liver and tumors may not be the same. Furthermore, whole-body and local heating might affect the energy metabolism differently, even in the same tumor.

Heat shock effect on membrane surface proteins was generally grouped to the marked decrease of high molecular proteins and the increase of low molecular proteins. This is in sharp contrast to the finding that several low molecular proteins of cytoplasmic origin were completely repressed at the same temperature as ours (Rubin *et al.*, 1982). The 70 K protein increased much higher in cell membrane iodinated 24 hr after heat shock than that iodinated immediately. The decrease to low level of this protein was shown 48 hr after heat shock. Landry *et al.* (1982) reported that heat shock-induced proteins were synthesized

during the first 4 hr following the conditioning treatment and maintained in the cells at high level for several hours, but became undetectable by 82 hr. At the same time, the synthesis of these heat shock proteins were involved in the development and decay of thermotolerance.

When temperature was raised from the control level of 37°C to values ranging 39-45°C, 70K protein was markedly induced at temperature above 41°C. Rubin *et al.*(1982) reported that 95K and 70K heat shock proteins appeared at 41°C and higher in mouse fibroblast cell line clone ID. Thus, 41°C is apparently a significent transition temperature for the heat shock proteins.

In view of the eminent influence of acidity on the thermal response, further investigation is warrented to elucidate the exact cause and kinetics of change in acidity in the tumors and normal tissues following hyperthermia. At the same time, the behavior of membrane surface protein, as one of the crucial targets of hyperthermic cell killing as well as development of thermotolerance, should await for further investigation.

SUMMARY

The biological effect of hyperthermia on the SCK tumor cells *in vitro* and *in vivo* were analyzed in several respects.

A comparison of the survival curve of SCK tumor cells in *vitro* and *in vivo* following hyperthermia demonstrated that the cytocidal effect of heating is far greater on the cells *in vivo* than on the cells *in vitro*.

The pH change in the SCK tumor upon being heated at 43.5°C started out at 7.05 and increased to 7.18 during the first 7 min of heating and then rapidly declined to 6.67 by 30 min. Contrary to the decrease in pH in the heated tumors, the pH in the muscle increased significantly when heated to 43.5-45.0°C.

Following hyperthermia at 43.5°C for 30 min, a maximum increase in the lactic acid content in the tumor and liver was observed at 1 hr and 3 hr, respectively. The increase in the tumor was followed by a gradual decrease below the control level, whereas the increase in the liver was maintained at quite a steady level for 24 hr.

The hyperthermia at 43°C for 1 hr exhibited a general tendency that high molecular proteins decrease markedly, whereas most of low molecular proteins increase. The most prominent change was that the heat shock protein 70K increased significantly along with other low molecular proteins in heat shocked cells.

REFERENCES

Aisenberg A.G. and H.P. Morris, 1961. Energy pathways of hepatoma No. 5123. *Nature* (*London*) **191**:1314-1315.

Bhuyan, B.K., K.G. Day, C.E. Edgerton, and O. Ogunbase, 1977. Sensitivity of different cell lines and of different phases in the cell cycle to hyperthermia. *Cancer Res.* **37**:3780-3784.

Bicher, H.I., F.W. Hetzel, and T.S. Sandhu, 1980. Effect of hyperthermia on normal tumor environment. *Radiology* **137**:523-530.

Bichel, P. and J. Overgaard, 1977. Hyperthermic effect on exponential and plateau ascites tumor cells in vitro dependent on environmental pH. *Radiat. Res.* **70**:449-454.

Freeman, M.L., W.C. Dewey, and L.E. Hopwood, 1977. Effect of pH on hyperthermic cell survival: Brief communication. *J. Natl. Cancer Inst.* **58**:1837-1839.

Gerweck, L.E., 1977. Modification of cell lethality at elevated temperatures. The pH effect. *Radiat Res.* **70**:224-235.

Gerweck, L.E., J. Jennings, and B. Richards, 1980. Influence of pH on the response of cells to single and split doses of hyperthermia. *Cancer Res.* **40**:4019-4024.

Goldin, E.M. and D.B. Leeper, 1981. The effect of reduced pH on the induction of thermotolerance. *Radiology* **141**:505-508.

Gullino, P.M., S.H. Clark, and F.H. Grantham, 1964. The interstitial fluid of solid tumors. *Cancer Res.* **24**:780-797.

Gullino, P.M., F.H, Grantham, and A.H. Courtney, 1967. Glucose consumption by transplanted tumor in vivo. *Cancer Res.* **27**:1031-1040.

Gutmann, L. and A.W. Wahlefeld. L-(+)-Lactate determination with lactate dehydrogenation and NAD. *In*: Bergmeyer H.U. ed. Method of Enzymatic Analysis, 2nd edition. New York and London, Academic Press, 1977, Vol. 3, 1464-1468.

Henle, K.J. and L.W. Dethelefsen, 1978. Heat fractionation and thermotolerance: A review. *Cancer Res.* **38**:143-1851.

Hubbard, A.L. and Z.A. Cohn, 1972. The enzymatic iodination of the red cell membrane. *J. Cell Biol.* **55**:390-393.

Kang M-S., Song C.W. and S.H. Levitt, 1980. Role of vascular function in response of tumors in vivo to hyperthermia. *Cancer Res.* **40**:1130-1135.

Kase, K.R., and G.M. Hahn, 1976. Comparison of some responses to hyperthermia by normal human diploid cells and neoplastic cells from the same origin. *Eur. J. Cancer* **12**:481-491.

Kim J.H. and E.W. Hahn, 1979. Clinical and biological studies of localized hyperthermia. *Cancer Res.* **39**: 2258-2261.

Kim, J.H., S.H. Kim, and E. Hahn, 1974. Thermal enhancement of the radiosensitivity using cultured normal and neoplastic cells. *Am. J. Roentgenol. Radium Ther. Nucl. Med.* **121**:860-864.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680-685.

Landry, J., D., Bernier, P. Chretien, L.M. Nicole, R.M. Tangnay, and N. Marceau, 1982. Synthesis and degradation of heat shock proteins during development and decay of thermotolerance. *Cancer Res.* **42**:2457-2461.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr, and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**:263-275.

Marmor, J.B., N. Hahn, and G.M. Hahn, 1977. Tumor cure and cell survival after localized radiofrequency heating. *Cancer Res.* **37**:879-883.

Mendecki, J., E. Friedenthal, and C. Botstein, 1976. Effect of microwave-induced local hyperthermia on mammary adenocarcinoma, in C3H mice. *Cancer Res.* **36**:2113-2114.

Nielsen, O.S. and J. Overgaard, 1979. Effect of extracellular pH on thermotolerance and recovery of extracellular pH on thermotolerance and recovery of hyperthermic damage in vitro. *Cancer Res.* 39:2772-2778.

Overgaard, J., 1976. Influence of extracellular pH on the viability and morphology of tumor cells exposed to hyperthermia. *J. Natl. Cancer Inst.* **56**:1243-1250.

Overgaard, J. and P. Bichel, 1977. The influence of hypoxia and acidity on the hyperthermic response of malignant cells in vitro. *Radiology* **123**:511-514.

Overgaard, J. and H.D. Suit, 1979. Time-temperature relationship in hyperthermic treatment of malignant and normal tissue in vivo. *Cancer Res.* **39**:2348-3253.

Power, J.A. and J.W. Harris, 1977. Response of extremely hypoxic cells to hyperthermia: survival and oxygen enhancement ratios. *Radiology* **123**:767-77.

Rubin, I., G. Getz, and H. Swift, 1982. Alteration of protein synthesis and induction of specific protein phosphorylation by hyperthermia. *Cancer Res.* **42**:1359-1368.

Song, C.W., M.S. Kang, J.G. Rhee, and S.H. Levitt, 1980. The effect of hyperthermia on vascular function, pH and cell survival. *Radiology* **137**:795-803.

Storm, F.K., W.H. Harrison, and R.S. Elliott, 1979. Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials. *Cancer Res.* **39**:2245-2251.

Streffer, C., B. Schubert, H. Lohmer, and P. Tamulevicius. Metabolic investigations in mouse liver after whole-body hyperthermia. *In*: Arcangeli G., and F. Maure, eds. Proceedings of the Ist Meeting of European Group of Hyperthermia in Radiation Oncology. Masson Italia Editor 1980, 209-213.

Von Ardenne, M. and P.G. Reitnauer, 1971. Basic in vitro experiment to shorten the duration of hyperthermia in multiple-step cancer therapy. *Arch. Geschwulstforsch.* **38**:264-269.

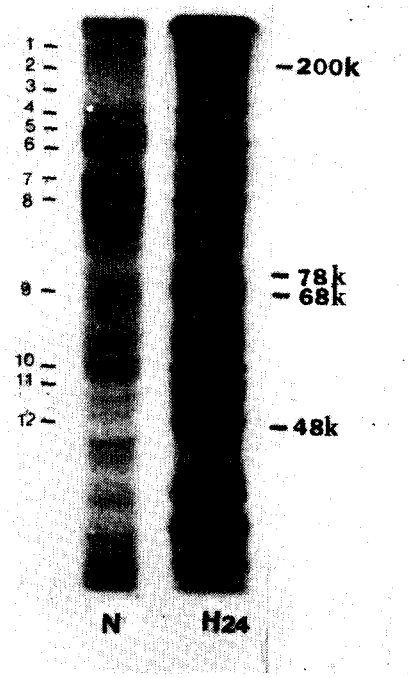

Fig. 4. Autoradiograms of ^{131}I-labeled surface membrane proteins separated by SDS-polyacrylamide gel electrophoresis. N, untreated control SCK tumor cells; H_{24}, iodinated 24 hr after the heat shock at 43°C for 1 hr.

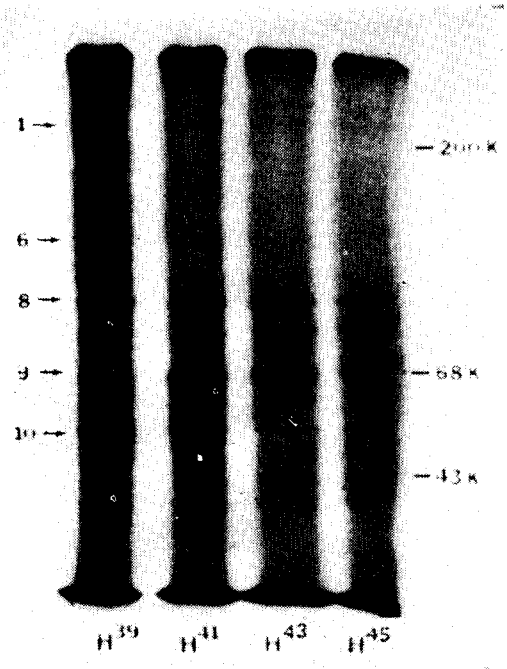

Fig. 5. Autoradiograms of ^{131}I-labeled surface membrane protein changes following various heat shocks. Superscript numbers refer to the temperature of heat shock given.