APPLICATION OF HEAT TO CANCER CHEMOTHERAPY
—EXPERIMENTAL STUDIES—

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SUMMARY

Many facts have been reported, that heat destroys some tumors and potentiates the effects of radiation on tumors. Recently, it was also showed *in vitro*, that heat enhances the effects of anticancer drugs. The first part of this work was designed to define this *in vivo*. Heat applied for 30 minutes inhibited the growth of Yoshida solid sarcoma implanted on the feet of rats and enhanced the Nitromin effectiveness on them at more than 42°C. Heat has been applied also to the regional perfusions to promote the action of chemotherapeutic agents. In the second part of this work, with the hyperthermic perfusions of the hind limbs of dogs, the tissue temperature could be elevated to up to 42°C without complications. These perfusions produced homogeneous distribution of temperature and did not affect the systemic temperature. The hyperthermic perfusion, therefore, was a most suitable method for local heating in combination with cancer chemotherapy. At the same time, elevated temperatures enhanced the action of Nitromin on normal tissue. The determination of the dose of drug should be carefully considered in hyperthermic perfusion.

PREFACE

About half a century ago heat was recognized as an agent which could destroy tumor tissues without causing severe damage to the surrounding tissues, and was used clinically. And, based on the fact that tumor sensitivity to radiation was elevated by heat, heat was applied in radiation therapy of cancer. But, as radiation therapy progressed rapidly, these facts were almost forgotten. Recently, these old findings have again received attention, and the usefulness of heat in the chemotherapy of cancer has been suggested as was the case in radiation therapy.

In this paper, the effect of local heat on the action of Nitromin* to Yoshida sarcoma of rat and hyperthermic perfusion as a method of local heating were studied.

I. EFFECT OF LOCAL HEATING UPON THE CHEMOTHERAPY
OF EXPERIMENTAL TUMOR

1) Introduction

Fifty years ago, Müller[1] observed that some human tumors could be selectively destroyed by prolonged heating and that the effects of irradiation were potentiated by heating. Since then, many works have been done on this problem, and all but some authors[2][3] supported Müller's observation both experimentally[4]−[13] and clinically[6][10]−[12][14][15].

The fact that the effect of irradiation is potentiated by hyperthermia suggests that an application of hyperthermia probably increases the effectiveness of the alkylating agents which have radiomimetic effects[16]. Further, a rise of temperature increases the rate of chemical reaction[17], and therefore the action of anticancer agents which is a chemical reaction is expected to be amplified by the hyperthermia.

Schmähl and Druckrey[18], as well as Mahaley and Woodhall[19], studied this subject *in vitro* and reported that anticancer drugs were more effective at higher temperatures than at lower ones. Woeber and his co-workers[20] showed this *in vivo*, but they used total body hyperthermia and could not elevate the temperature to above 40°C. Crile[10][11] applied local heating at temperatures of up to 49°C and showed the inhibiting effect of heat alone on the growth of some experimental tumors, but he observed that combined treatment with heat and conventional anticancer drugs had no additive or synergistic effect.

In this chapter, the effects of local hyperthermia at temperature levels of 40° to 46°C upon the Nitromin therapy of Yoshida solid sarcoma was investigated *in vivo*.

2) Materials and methods

Male Donryu rats weighing about 100 g were used. Ascitic fluid of Yoshida sarcoma containing about 10^7 tumor cells was injected into the dorsal aspect of the left feet subcutaneously. On the third day after the injection, solid tumors were established and the border of them became clear. These tumors were oval and the majority of them were of sizes of 1.0 to 1.4 cm by 0.6 to 0.9 cm. Few rats which had no tumors of this size were discarded.

The tumor-bearing feet were heated on the third day after inoculation by the following procedure. Rats were anaesthetized with 1 ml per 100 g body weight of 10% urethane injected subcutaneously, and were restrained with adhesive tape on plastic plates, 20 cm by 4 cm, with a slot 1 cm long and 1 cm wide, through which the tumor-bearing foot was passed down under the plate. Then the rats were placed on a water bath at the desired temperature, 40°, 42°, 44° or 46°C, with their tumor-bearing feet in the water for thirty minutes (Fig. 1). Five or ten mg/kg of Nitromin was injected subcutaneously just be-
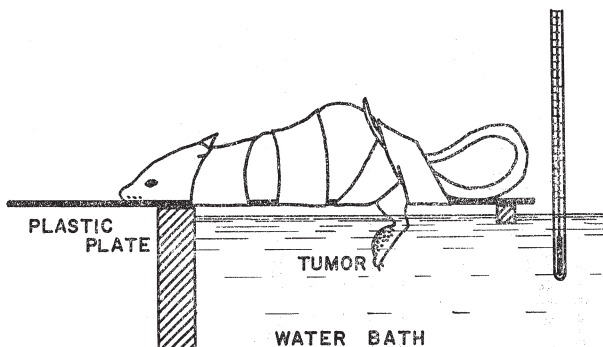
FIG. 1. Heating of the tumor-bearing foot in a water bath.

fore the beginning of heating. After these procedures, the tumor sizes were measured daily and autopsy was made when the rat died.

On the other hand, sixty rats were used for the purpose of microscopical study. Both feet were inoculated with an equal number of 10^7 Yoshida sarcoma cells and only the left feet were heated at 42°C for 30 minutes by the same procedure as described before. Rats were divided into two groups of equal number; 5 mg/kg of Nitromin was injected subcutaneously to one group, and none to the other. Both groups of rats, Nitromin-injected and non-injected, were sacrificed immediately after the heating, after 6 hours, 1, 2, 4 and 7 days respectively. Tumors of both sides of their feet were studied histologically, and the mitosis index was calculated by counting 2,000 viable tumor cells.

3) Results

The tumor sizes before and after the procedure are graphically shown in Fig. 2.

If no treatment was done, the tumors grew steadily and metastasis in the groin lymphnodes became palpable on the 4th to 6th days after the inoculation. Rats died on the 8th to 10th days, and autopsy revealed metastasis in the lumbar lymphnodes, lung and liver.

When the tumor-bearing feet were heated at 40°C, tumors progressed as fast as untreated. At 42°C, temporary regression of tumors was observed. Redness and swelling were seen on the feet after heating and disappeared on the next day. Tumors regressed for the subsequent three days but grew again thereafter. Rats died with metastasis on the 9th to 12th days after the inoculation, when tumors were smaller than that of controls. When the tumor-bearing feet were heated to a temperature of 44°C, temporary regression of tumors was not clear because swelling lasted for several days and made the measurement of tumor size impossible. Survival period and tumor size were almost the same as at 42°C. When heated to 46°C, swelling of the feet became

FIG. 2. Effect of combined Nitromin and local heating on Yoshida solid sarcoma implanted on the feet of rats. Local heating was applied for 30 minutes on the 3rd day after the inoculation.

remarkable and lasted longer. The feet became necrotic and finally fell off. Prolongation of survival was not observed at any temperature.

Tumors treated with 5 mg/kg of Nitromin alone grew without regression though somewhat slower than controls, and, even if heating at 40°C was applied with it, tumor growths were not affected. Rats died on the 12th day after inoculation on the average. When heating at 42°C was combined, tumors regressed gradually and some of them disappeared completely. When 10 mg/kg of Nitromin was administered, tumor regression occurred in both cases with and without heating, and the difference between them was not significant. The animals survived more than 17 days and some of them till the end of the fourth week when they were killed and proved to have no evidence of tumor. When heating at 44°C was combined with Nitromin treatment, the final results were similar to those of heating at 42°C though the course of tumor growth was not clear for several days after heating because of edema caused by heat. Heating at 46°C, with or without combined treatment with Nitromin, resulted in necrosis of the feet.

All of the dead rats had metastasis in the lung and liver, and all of the survived had not. The survival periods of rats were related not to the size of primary tumors but to the dose of Nitromin given systemically; about 9 days without Nitromin, 12 days with 5 mg/kg and more than 17 days with 10 mg/kg of Nitromin.

Histological studies were made of the tumors which were treated with none, with heat alone at 42°C for 30 minutes, with 5 mg/kg of Nitromin alone or with both (Fig. 3), and were summarized in Table 1. Edema in the skin and hemorrhage in the tumor tissue were seen just after the heating in the

TABLE 1. Histological findings in the tumor tissues treated. Of five rats each in experiment, numbers with findings are given

Treatment	Changes	Immediately	6 hours	1 day	2 days	4 days	7 days
		after treatment					
Control	Hemorrhage	0	1	0	1	0	0
	Nucleic Degeneration*	0	0	0	0	0	0
	Granulation	0	0	0	0	1	0
Nitromin Alone (5 mg/kg)	Hemorrhage	0	1	0	0	0	0
	Nucleic Degeneration*	0	2	0	0	0	0
	Granulation	0	0	0	0	0	1
Heat Alone (at 42°C for 30 min)	Hemorrhage	5	3	4	3	1	0
	Nucleic Degeneration*	0	0	5	5	4	0
	Granulation	0	0	0	0	0	5
Nitromin with Heat	Hemorrhage	5	4	5	3	0	0
	Nucleic Degeneration*	0	3	5	5	1	0
	Granulation	0	0	0	0	1	5

* Area of mass necrosis of the tumor were not subjected to study for nucleic change.
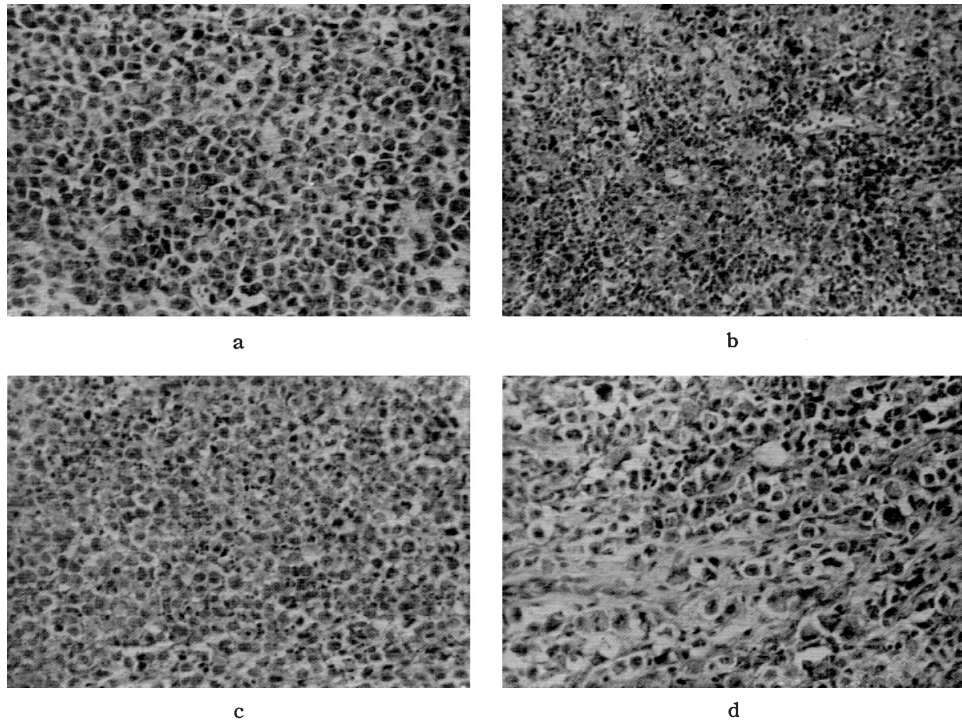
a

b

c

d

FIG. 3. Tumor tissue without heating (a) and tumor tissue treated with local heating; hemorrhage immediately after treatment (b), degenerative changes in nuclei on the next day (c) and granulation on the seventh day (d).

heated feet whether Nitromin was given or not. On the next day, degenerative changes in the nuclei of the tumor cells were manifest. After one week, the debris was absorbed and granulation tissue replaced it. In the tumors treated with heat or Nitromin alone, mitosis decreased after the treatment but increased again after four days. When both heat and the drug were given at the same time, mitosis was largely depressed and showed no tendency to recover even after seven days (Table 2).

TABLE 2. Mitosis index (‰) after treatment with local heating and systemic administration of Nitromin

Treatment	Immediately	6 hours	1 day	2 days	4 days	7 days
	after treatment					
Control	14.4	15.1	13.2	14.6	12.6	14.3
Nitromin Alone (5 mg/kg)	7.4	4.3	4.7	6.0	14.1	11.4
Heat Alone (at 42°C for 30 min)	3.5	5.2	3.6	3.2	12.5	11.2
Nitromin with Heat	0.4	1.1	0.6	0.3	2.8	1.6

4) Discussion

Survival periods of rats inoculated Yoshida solid sarcoma on their feet were not affected by local heating at temperatures ranging from 40°C to 46°C for 30 minutes. In the case of experimental animals used in the present study, it is supposed that lymphogenous metastasis occurs easily and at an early stage because the inoculation was made by the injection of ascitic fluid containing free tumor cells. In fact, metastasis in the groin lymphnodes became palpable on the 4th to 6th days after the inoculation. It is conceivable, therefore, that metastasis into the groin or elsewhere out of the heated area had been established already on the 3rd day when heat was applied. It was apparent that the cause of death was metastasis in the lung and liver, and therefore it was presumed that local heating had no effect on the survival period.

On the growth of tumors, the inhibiting effect of local application of heat was demonstrated. As many authors have indicated, the effects of heat depend upon the degree of temperature used and the period heat is applied. The time of exposure required to obtain the same biologic effect can be decreased as the temperature rises. Rohdenburg and Prime[4] immersed fragments of Crocker mouse sarcoma 180 weighing 2 to 3 mg in Ringer's solution of various temperatures before inoculating them subcutaneously into the host. With a temperature of 42°C, the maximum lethal effect was attained after 180 minutes, with 44°C after 90 minutes, and with 46°C after 60 minutes. Westermark[5] observed that Flexner-Jobling's rat-carcinoma and Jensen's rat-sarcoma were caused to disappear if exposed to a temperature of 44°C for 180 minutes, to 46°C for 50 minutes and to 48°C for 20 minutes *in vivo*. Warren[6] exposed slices of the mouse sarcoma No. 180 and rat carcinosarcoma No. 256 with the proper precautions to temperatures of 41.5° and 42°C in water baths for definite periods of time and found that lethal time-temperature relationship was about twelve hours at 41.5°C and about five hours less at 42°C. Crile[10][11] immersed the feet of mouse implanted with sarcoma 180 in a water bath at desired temperatures. At 42°C, 120 minutes were required to destroy S180 in more than 50 per cent of the mice treated; at 44°C, 30 minutes; and at 46°C, only seven and one-half minutes. Selawry[7] observed thht tissue-cultured HeLa cells were killed after 14 hours exposure at 42°C, after 3 hours at 44°C and after 30 minutes at 46°C. In the present study, considering the clinical applicability especially in combination with chemotherapy, the period of thirty minutes was taken for the exposure time to heat. This dose of heat is less than those used by previous authors to destroy tumors. But the inhibiting effect of heat upon the growth of Yoshida solid sarcoma was observed at temperatures of more than 42°C.

Some authors hold opposite opinions. Piersol[21] considered heat treatment as contra-indication for malignant tumors. Urbach[2] and Baumeyer[3] observed that diathermy aggravated neoplastic disease. Selawry[7] studied on hyper-

thermia in tissue-cultured cells of malignant origin and found that there was a growth-stimulating temperature range, characterized by faster increase of cell number and higher mitotic index, between 36° and 38°C. He suggested that this might explain Urbach's and Baumeyer's observations. The existence of growth-stimulating temperature was not investigated in the present study, but at least there was no evidence that heat promotes tumor growth in the temperature range of between 40° and 46°C.

When heat is combined with chemotherapeutic agents for cancer therapy, it is postulated that the former enhances the action of the latter, as was the case when combined with irradiation. A comparison of effects of temperature on the action of alkylating agents was studied by Schmähl and Druckrey[18], who exposed suspensions of Yoshida ascites sarcoma, Walker rat carcinoma and Jensen sarcoma for one hour to various concentrations of A 139, HN 2, tris-HN 2 or HN 2-oxide at 5° or 37°C. For A 139, HN 2 and tris-HN 2, concentrations required to produce the inhibiting effect of subsequent growth of tumors at 5°C were five to six times that at 37°C, and for HN 2-oxide (Nitromin), it was 3,000-fold. Bergel[22] also stated that the reactivity of nitrogen mustard increased twofold for each 10 degree rise in temperature. Mahaley and Woodhall[19] incubated clumps of VX 2 carcinoma cells *in vitro* at 20°, 37° or 42°C for 1, 2, 3 or 4 hours in the presence of given concentrations of TEM, OPSPA, TSPA, A 139, Cytoxan and AB 100, and then transplanted them to host rabbits. Tumors produced after 21 days from cells incubated at 20°C were always the largest and those produced from cells incubated at 42°C the smallest. A study *in vivo* was made by Woeber and his associates[20]. They used total body-immersion hyperthermia (39° to 39.5°C) of rats implanted with Walker carcinoma for 30 to 45 minutes prior to systemic administration of Nitromin and increased the rate of cure by 30 per cent. They observed that the rise of systemic temperature to above 40.5°C resulted in the death of animals. An *in vivo* study upon the combined treatment with chemotherapy and local hyperthermia of up to 46°C was made in this work. The enhancement of Nitromin action by heat was observed at the temperatures of more than 42°C for 30 minutes. It was most remarkable when 5 mg/kg of Nitromin was combined with heat treatment. While this dose of Nitromin alone was ineffective on the growth of Yoshida solid sarcoma implanted on the feet of rats, the tumor regressed gradually and some of them disappeared when combined with heat. This was supported by the histological study.

The feet of rats were destroyed and fell off when they were exposed to heat of 46°C for 30 minutes. This dose of heat is somewhat less than that for mice, which was 46°C for 45 minutes according to Crile[10]. As the intolerable dose of heat of the human extremity may differ also, care must be taken in the clinical use of heat.

The mechanisms of heat inhibiting tumor growth and potentiating the tumoricidal effect of chemotherapeutic agents are still unknown. Elevated temperatures result in active hyperemia which is mainly due to thermoregulatory mechanisms and serves the equalization of temperature. Higher blood supply leads to increased oxygen tension in the tissues. It has been reported that increased oxygen tension itself has tumoricidal effect[23] and potentiates the effect of irradiation[24][25] or nitrogen mustard[26][27] on tumors. The effect of heat on tumors may be due to the increased oxygen tension itself. Increased metabolic rate at higher temperatures also may have a relation to the effect of heat. Rochlin[28] performed isolation perfusion of the hind limb of the dog with P^{32}-tagged thioTEPA and found that the percentage of unbound drug returned in the wash was less at hyperthermic tissue temperatures than at normothermic ones. Shingleton and his co-workers[29] injected C^{14} labeled nitrogen mustard into the jugular vein or iliac artery of the dog with the hind limb cooled to 32°C or warmed to 42°C previously. There was seen a marked increase in radioactivity in the muscle sample from the heated extremity as compared with the sample from the cooled extremity, whether the drug was administered intravenously or intra-arterially. Okada[9] injected P^{32}-labeled TSPA into the abdominal cavity or iliac artery of rats having Yoshida solid sarcoma on their feet and heated the tumor-bearing feet at the same time. He found that the radioactivity in the tumor tissue increased as the tissue temperature rose from 35°C to 42°C. Since the action of chemotherapeutic agents is a chemical reaction and the rate of any chemical reaction is influenced by the temperature, it is reasonable that the uptake or binding of drugs would be increased in the tissue at higher temperatures. Mechanism of the effect of heat must be a subject of further research. Its elucidation might contribute to cancer therapy.

II. HYPERTHERMIC PERFUSION AS A METHOD OF LOCAL HEATING IN CANCER CHEMOTHERAPY

1) Introduction

Regional perfusion for cancer chemotherapy was first suggested by Klopp and his co-workers[31] in their report on intra-arterial injection of nitrogen mustard in 1950. Since 1957, when its experimental and clinical use was pioneered by Creech and his associates[16][32][33], many authors have applied this procedure to tumors of various organs[34]–[46].

The purpose of perfusion is to minimize the effects of drugs on the organs outside of the tumor-bearing area and to permit the delivery of a higher dose to the tumor zone by the localization of drugs. But, certain organs, such as the brain or liver, cannot be isolated completely, and a considerable amount of drug leaks into other parts of the body. And, when a large amount of drug is administered, the local toxic effect in the perfused region cannot be ignored

also. Therefore, an adjunct that enhances the action of chemotherapeutic agents or increases the sensitivity of tumor is desired.

As such an adjunct, Creech and his associates[16] used oxygen, since ionizing radiation is potentiated by high oxygen tension in the tissue.

Heat also was applied as such an adjunct to carotid perfusion by Woodhall and his co-workers[37] and to liver perfusion by Shingleton and his associates[33]-[40]. Their theoretical grounds were that heat destroys some kinds of tumors by itself and increases the effect of radiation or chemotherapeutic agents on tumor. These facts have been reported by many authors as described before. Woodhall and his co-workers heated perfusing blood to 41° or 42°C with the Harrison-Brown heat exchanger and maintained this temperature during the period of perfusion for 20 to 30 minutes in their carotid perfusion. By almost the same method, Shingleton and his associates brought the tissue temperature to 38° or 39°C with general body hypothermia at 31° to 32°C in their abdominal and liver perfusion.

A temperature of 38° to 39°C, however, seems to be insufficient to destroy tumors by itself, according to the results of the study presented in the first part of this report or other previous papers. Rohdenburg and Prime[4] reported that the first lethal effect of heat on Crocker mouse sarcoma 180 *in vitro* was evident after 60 minutes' exposure to a temperature of 42°C. Selawry[7] observed that the three strains of tissue-cultured human neoplastic cells, HeLa, J 96 and H. Ep, #2, reacted with increased rate of growth at elevated temperatures of up to 38°C, with temporary interruption of the mitotic cycle in the metaphase at between 39° and 40°C and with irreversible heat injury at temperatures higher than 42°C. Crile[10][11] described that the destructive effects of heat on the three types of transplantable tumors in mice, S 180, S 91 melanoma and carcinosarcoma in C 57, began at 42°C.

In order to expect the destructive effect of heat on tumors as well as the enhancement of the action of chemotherapeutic agents, therefore, hyperthermic perfusion should be undertaken by procedures which bring the tissue temperature to a level of 42°C for 30 minutes at least.

According to the study on hyperthermic blood perfusion of the dog hind limb by Reed and Hopkins[47], tissue temperatures of up to 45°C were tolerated for at least one hour. But their perfusion is not "regional", since the perfusing blood was taken out through the carotid artery instead of the femoral vein. So, the tolerance may be lower, if regional perfusion is performed. Moreover, it must be taken into consideration that the local effect of chemotherapeutic agents may alter the tolerance.

In this chapter, hyperthermic regional perfusions of up to 42°C with a chemotherapeutic agent were attempted, and the changes developed in the perfused area by this procedure were studied.

2) Materials and methods

The extracorporeal circuit consisted of a DeBakey roller pump; a disc oxygenator developed by Sawa[48]; a bubble trap; a filter for removal of fibrin clots; and venous and arterial blood reservoirs, afferent and efferent to the oxygenator, which were graduated and used as flowmeters also (Fig. 4). A perfusion pressure gauge was connected to the circuit, and a warm bath was used as a heat exchanger in which a part of the circuit was immersed. Total circuit was primed by about 450 ml of heparinized blood and one 1/min of 100% oxygen was administered into the oxygenator.
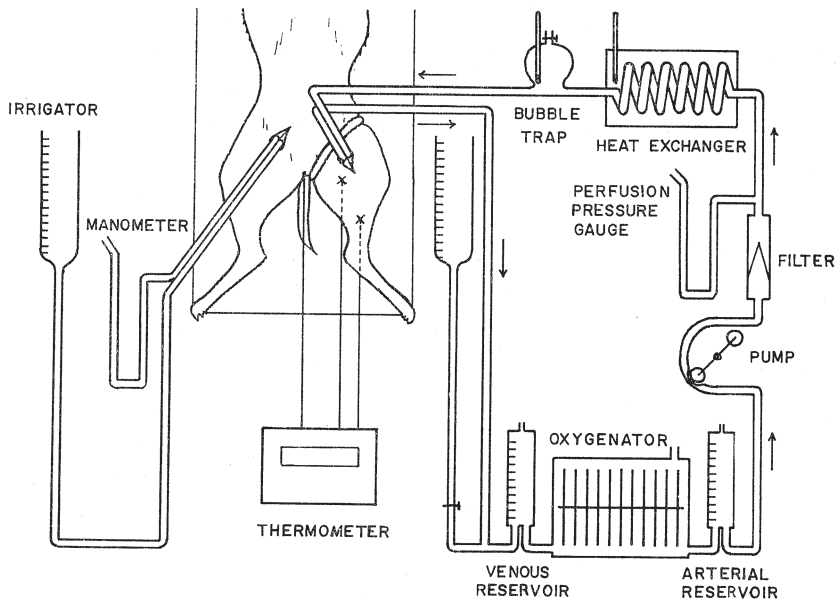

FIG. 4. Apparatus used in hyperthermic regional perfusion.

Adult dogs weighing about 10 kg were anesthetized with 0.03 g/kg of Isozol*, and the bilateral femoral arteries and veins were exposed by small incisions in the inguinal areas. After 2 mg/kg of heparin was given by intravenous injection, catheters for manometer and for drip transfusion of physiological saline were placed in the right femoral artery and vein respectively, directed proximally. In the left femoral artery and vein, the inflow arterial and outflow venous cannulas of the extracorporeal circuit were inserted respectively in a distal direction. A tourniquet was applied to the groin to isolate the perfused area from others. The extremity was wrapped with a blanket to prevent loss of heat from the surface.

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* Sodium 5-allyl-5-(1-methylbutyl)-2-thiobarbiturate.

Temperatures of the warm bath and the inflow blood were monitered. Rectal temperature was measured also.   To determine the tissue temperature, needle probes were placed in the thigh and leg muscle.

Perfusion was conducted as follows.   The torque of the pump was so regulated as to approximate the perfusion pressure to the systemic blood pressure, and when this was achieved, heating of the perfusate was begun. The temperature of the warm bath was controlled to maintain the desired temperature in the perfused tissue for 30 minutes.   Fifteen minutes after the tissue temperature reached the desired level, Nitromin was injected into the circuit near the inflow cannula.

When the heating ended, 200 ml of heparinized blood was poured into the perfused area to wash out Nitromin.   Polybrene was added to the drip of physiological saline to neutralize the action of heparin.   The tourniquet was released, the cannulas removed and the arteries and veins ligated.

Dogs were observed for four weeks after the perfusion and then killed for microscopical examination.

3) Results

When the perfusate was not heated the temperature of the perfused limb dropped slowly to that of the perfusate, which was exposed to room temper-
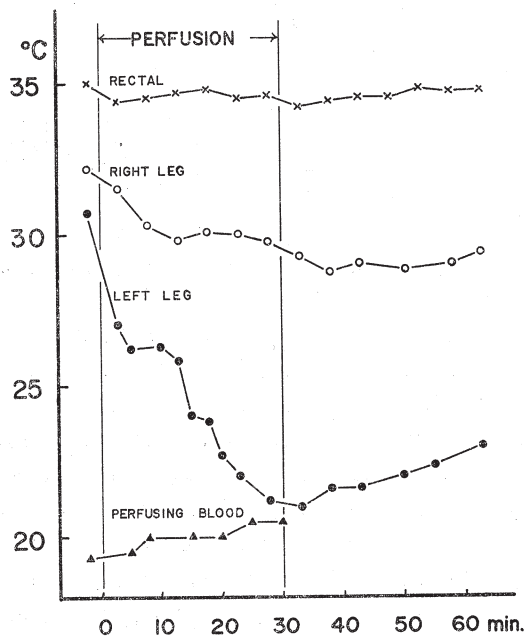

FIG. 5.   Graph shows data obtained during regional perfusion of hind limb of dog without heating of perfusate.   Note the fall in temperature in the perfused area.   Room temperature was 27°C.

ature in the extra-corporeal circuit (Fig. 5).

Table 3 shows the data obtained from the hyperthermic perfusions, and a case is graphically shown in Fig. 6. In the majority of cases, the rate of flow of perfusate was 4 to 6 ml/min/kg body weight when the torque of the pump was so regulated to approximate the perfusion pressure to the systemic blood pressure. And to elevate the temperature of the perfused area to up to 42°C, the temperature of the perfusate has to be about 46° to 47°C and the time required about 10 to 20 minutes. When the desired tissue temperature is lower, the temperature of the perfusate is made to be at a lower level and the time shorter.

TABLE 3. Summary of data in hyperthermic regional perfusion of dog's hind limb for 30 minutes with Nitromin

Dog No.	Blood Pressure (mmHg)	Perfusion Presure (mmHg)	Flow Rate (ml/min/kg)	Time required to elevate Temp. (min)	Temperatures in the				Change in Rectal Temp.	Dose of Nitromin (mg/kg)	Grade of Damage*
					Water Bath (°C)	Perf. Blood (°C)	Left Leg (°C)	Left Thigh (°C)			
1	130	140	4.0	10	48	42	36	36	+0.5	10	I
2	160	170	5.8	5	47	46	38	38	+0.4	0	I
3	140	140	6.0	21	44	43	39	39	−0.6	3	I
4	120	110	5.0	14	46	43	38	38	+0.2	5	II
5	110	120	6.0	11	45	43	38	38	+0.1	10	IV
6	180	180	4.8	10	47	45	38	38	+0.4	10	IV
7	130	120	5.9	5	48	43	38	38	+0.4	10	IV
8	160	150	5.9	22	47	45	40	40	+1.1	0	I
9	190	190	4 0	13	48	45	40	40	−0.8	3	II
10	150	160	4.7	5	44	41	40	40	+0.7	5	III
11	140	130	6.1	19	50	47	41	41	−1.0	0	II
12	140	140	6.2	20	54	46	42	42	−0.4	0	II
13	160	160	3.9	29	50	47	42	42	−0.9	3	III
14	210	200	5.4	25	54	46	42	42	−0.3	5	III

* I: No damage, II: Reversible damage, III: Irreversible damage. IV: Damage resulted in death of dog.

The temperatures of the leg muscle were always equal to that of the thigh muscle; *i.e.*, temperature distribution in the perfused area was homogeneous. Rectal temperature was not affected by the procedure.

The damages developing in the hind limbs of dogs after the hyperthermic perfusion fell into the following four groups according to grade; no damage, reversible damage, irreversible damage and severe damage which led to death. In the first group, no change was seen in the limb. The function of the limb was quite normal and the dog walked as usual on the next day. Histological study revealed no pathological finding. In the second group, signs of damage were seen only temporarily. Swelling appeared in the perfused hind limb, became most marked on the third day after the perfusion and thereafter decreased
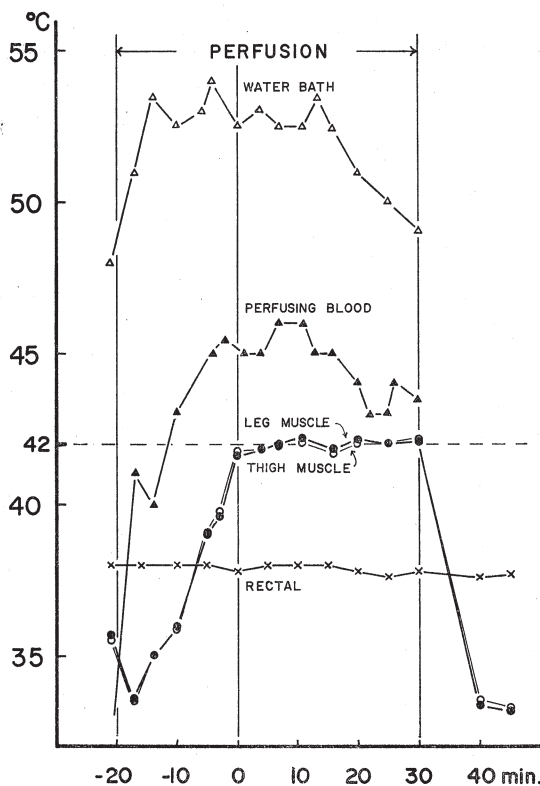
FIG. 6. A case of hyperthermic perfusion of dog's hind limb. Temperature in the thigh muscle was almost equal to that in the leg muscle always. There was no appreciable change in the rectal temperature.

day by day and disappeared within one or two weeks. Dogs stood or walked with their swollen limb drawn up or limped at first, but this disturbance also disappeared within two or three weeks. They looked entirely healthy at the end of the fourth week when they were killed for histological examination. There was no evidence of damage microscopically too. In the third group, necrosis appeared in the limb, and the function of the limb was lost. Marked swelling lasted longer than in the second group, and necrotic ulcer with loss of hair appeared in the leg about ten days later. Three or four weeks after, the ulcer tended to heal with formation of scar tissue. The perfused limbs seemed to be paralyzed, and the dogs walked with the other three limbs. Histologically, there were acanthosis and capillary dilatation in the skin; degeneration, atrophy, dissolution and necrosis of the muscle cells; and granulation and fibrosis in places (Fig. 7). Dogs which died within two days after the perfusion belonged to the fourth group. Swelling of their limbs was marked. Severe edema, degeneration and necrosis of the roots of hairs and muscle cells
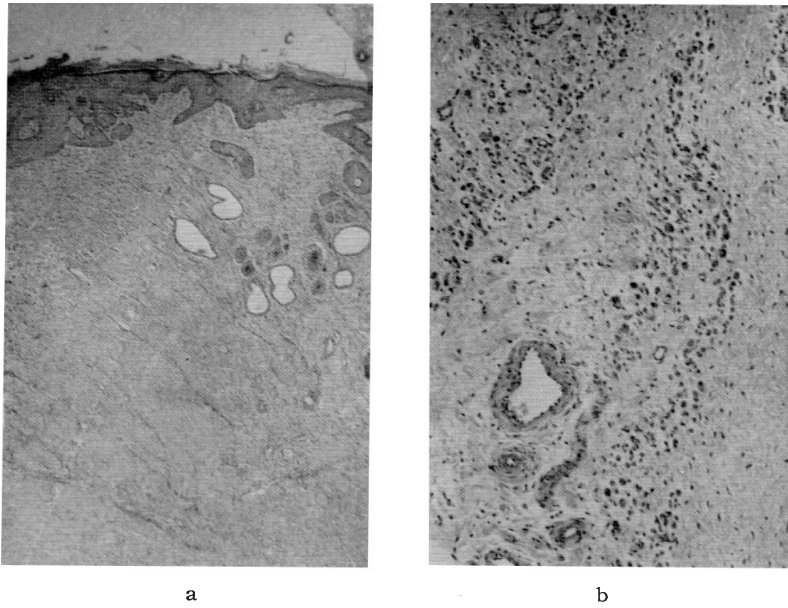
a   b

FIG. 7. The skin (a) and muscle (b) tissues in the third
group of damage.

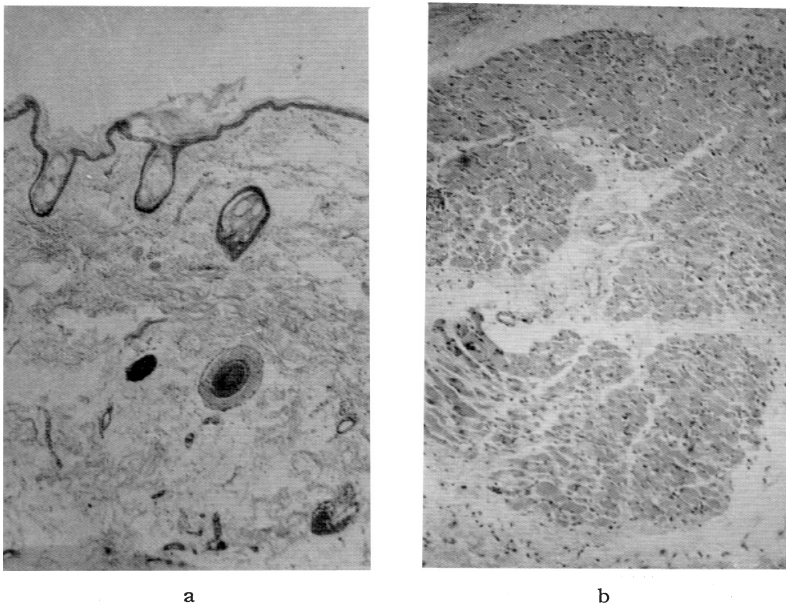a   b

FIG. 8. The skin (a) and muscle (b) tissues in the fourth
group of damage.

and central congestion of the liver were seen microscopically (Fig. 8). The cause of death was believed to be shock from dehydration and electrolytes imbalance, because there was expansive and marked edema in the perfused hind limb and abundant exsudate leaking foom the operations wound. They might have been saved, if treated properly. The grade of damage of the perfused limbs would have been more significant than the third group or at least the same.

Table 4 shows the grade of damage that occurred in the perfused hind limbs of dogs at various tissue temperatures and doses of Nitromin. At 36°C, no damage occurred with 10 mg/kg of Nitromin. But at 38°C, the limbs were injured irreversively with the same dose; at 40°C, with 5 mg/kg and at 42°C, with 3 mg/kg. When Nitromin was not administered, there was no significant damage even at 42°C tissue temperature.

TABLE 4. Grade of damage occurring in the perfused limb of dog by hyperthermic perfusion with administration of Nitromin

Tissue Temperature (°C)	Dose of Nitromin (mg/kg)			
	0	3	5	10
42	II	III	III	
41	II			
40	I	II	III	
39		I		
38	I		II	IV, IV, IV
37				
36				I

I: No damage, II: Reversible damage, III: Irreversible damage, IV: Damage resulted in death of dog

4) *Discussion*

During the course of isolation perfusion without heating of the perfusate, Rochlin and his co-operators[25] noted that the temperature of the tissue in the isolated area would drop to a hypothermic level, because the blood in the extracorporeal circuit was exposed to room temperatures. The present study also showed the same results. This is undesirable because the effect of chemotherapeutic agents will be diminished in the perfused area at hypothermic temperature and be relatively large in the systemic area at normothermic temperature. So, the temperature of the tumor-bearing area should be maintained at a hyperthermic level or at least normothermic level by heating of the perfusing blood, that is, by hyperthermic perfusion.

In hyperthermic perfusion, the temperature of the perfused area depends upon two cardinal factors; flow rate and temperature of the perfusing blood.

When the flow rate is too low, it fails to elevate the tissue temperature to a hyperthermic level[47]. When too high, on the contrary, circulatory disturbance in the perfused area and increase of leakage into the systemic area result[35][45]. Adequate and safety flow rate occurs when the perfusion pressure is equal to the arterial pressure[37][45]. As to the temperature of the perfusing blood, Ham and his associates[49] reported that human blood could be heated to up to 47°C for one hour without causing any morphological change in the erythrocyte, and hemolysis. So, to perform hyperthermic perfusion without complication, the flow rate and temperature of the perfusing blood should not exceed these limits. In this study, tissue temperatures of up to 42°C for 30 minutes showed these two factors to lie within safe range.

Creech and his associates[16] reported that the maximum safe dose of nitrogen mustard administered into the femoral artery of the dog was 0.4 mg/kg. In Shingleton's experiment[29], when the hind limb of dogs was heated with their inductive electromagnetic heating unit to 42°C, the safe dose decreased to 0.3 mg/kg. Ohashi[46] performed regional perfusion of the dog hind limb by the same procedure, except for heating, similar to that adopted in the present study. He described that maximum tolerable dose of Nitromin administered by the perfusion into the hind limb was 10 mg/kg. In the present study, the dose of 10 mg/kg of Nitromin was not tolerated at the tissue temperature of 38°C, 5 mg/kg at 40°C and even 3 mg/kg at 42°C. These results indicate that the toxic action of Nitromin on normal tissue was largely enhanced as the tissue temperature rose to hyperthermic levels. This is rather natural because the action of Nitromin is a kind of chemical reaction and is supposed to be governed by the van't Hoff-Arrhenius law, i.e., a rise of temperature increases the velocity of chemical reaction logarithmically[17].

This law is valid also for the lethal effect of chemotherapeutic agents on tumors as described in the former chapter. So, lesser doses of drugs would be sufficiently effective when local hyperthermia is combined and the systemic toxic effects could be decreased consequently. Moreover, when the tissue temperature is higher than 42°C, there is the possibility that the effect of heat itself inhibiting tumor growth might be expected at the same time as proved in the first chapter.

For heating tumors, many methods have been devised and used both experimentally and clinically[4][5][21][47]; hot air, steam, water bath, infra-red lamp, ultrasound and diathermy. Heat conducted or radiated from outside the body fails to elevate the temperature of deep situated tumors, because of the danger of burn injury in the skin. Diathermy by ultra-short wave or microwave is suitable for heating of deep situated regions. However, the distribution of temperature in the heated area is not homogeneous[3], and the use of thermoelectric thermometer is not feasible[6]. Temperatures of the tissue which are heated by dia-

thermy, therefore, cannot be kept under observation and control constantly. This is undesirable when heat is applied as an adjunct to cancer chemotherapy because the action of anticancer drugs is largely enhanced by a slight increase of temperature in the hyperthermic range as described before, and unexpected side effects may occur. Besides, heat is transmitted to other parts of the body by the blood circulation and sometimes elevates the systemic temperature. In comparison with these methods, hyperthermic perfusion produces homogeneous temperature distribution throughout the perfused area and does not affect the rectal temperature as observed in the present study. Even in liver perfusion accompanied by considerable leakage, Shingleton and his co-workers[33] observed no appreciable change in the rectal and esophageal temperatures and could combine this with general body hypothermia to protect the bone marrow outside of the perfused area.

Thus, hyperthermic chemotherapy perfusion is one of the most recommendable procedures for the combined treatment of cancer with heat and drugs, though care must be taken regarding the dose of drugs and the degree of temperature.

The tolerance to heat may be quite variable depending on the organs[33,40,47,50] or types of tumor[7,10,11,13] treated. A possibility also exists that some chemotherapeutic agents may be inactivated at the elevated temperature. Further studies on these problems are necessary.

CONCLUSIONS

Using Yoshida solid sarcoma implanted on the feet of rats and Nitromin, the effects of local hyperthermia in a range of between 40°C and 46°C upon tumors with or without chemotherapy were studied *in vivo*. Heat applied locally for 30 minutes inhibited the local tumor growth at temperatures higher than 42°C.

When combined with systemic administration of Nitromin, heat enhanced its effectiveness on the tumor also at more than 42°C. These findings *in vivo* supported previous studies *in vitro*.

At present, hyperthermic perfusion is most advisable as a method for local heating because it provides homogeneous temperature distribution throughout the heated area and does not affect the systemic temperature.

Tissue temperatures produced by hyperthermic regional perfusions were below 39°C in the past and were believed to be insufficient for the treatment of tumors.

Hyperthermic perfusion which reises tissue temperatures to up to 42°C was performed in the hind limb of the dog for 30 minutes without causing any complication.

The action of Nitromin on normal tissue is largely enhanced as the tissue temperature rises and therefore care must be taken to prevent unexpected tissue damage when chemotherapy is combined with heat.

Although there are many problems which need further investigations, heat is useful in the chemotherapy of cancer. Elucidation of the mechanisms responsible for heat to destroy tumor or enhance the effectiveness of anticancer drugs might contribute to cancer therapy.

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