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## The Effect of High Pressure Oxygen on the Experimental Tumors in Rats to Radiotherapy

### Differences on the Regression Pattern of the Size of two Kinds of Experimental Tumors

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実験腫瘍の放射線治療における高圧酸素の効果

ラットの2種の実験腫瘍の縮少の差について

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ラットの大腿皮下に移植された yoshida ascites hepatoma AH 130と N-Nitroquinoline N-Oxide induced Donryu rat pulmonary carcinoma(Sato and Shimozato) を用いて放射線の3気圧酸素下照射の効果を検討した。

1. AH-130腫瘍では、1500R, 3000R照射で、腫瘍の縮少の促進、消失率の増加がみとめられた。
2. 4-NQO induced tumorでは、1000R, 1500

R, 2000R照射で、大気下照射群と3気圧酸素下照射群の間に、腫瘍の縮少、消失共に差が認められなかった。

3. 3気圧酸素下照射後、数日間は体重増加は停止するが、以後は、コントロールと同様に増加する。

4. 脱毛や、湿性皮膚炎は、3気圧下照射群の方が強い反応を示した。

Tumor cells are located at various distances from the blood capillary, so the oxygen tension at each location of the surrounding media of tumor cells is varied, according to the gradient of the oxygen tension.

Many authors<sup>1,4,6,7,5,15,16,17,18</sup> agree that this different oxygen tension in tumor is closely related to the radiosensitivity of the tumor cells, and measurement of oxygen tension in tissue were made<sup>2,3,16,18</sup>.

W.E. Powers<sup>1</sup> demonstrated a survival curve with two components corresponding to a cell population which consists of 99 per cent sensitive and 1 per cent resistant cells in vivo in C<sub>3</sub>H mice. He has shown that the resistant population was increased to twenty times in the anoxic condition, and decreased to the 0.1 per cent level in the hyperbaric condition of oxygen. In 1957, L.H. Gray<sup>4</sup> demonstrated nine kinds of experimental tumors, the radiosensitivity of which was closely related to the oxygen tension. In 1966, H.B. Hewitt<sup>9</sup> reported the experimental results in which surviving fractions of sarcoma cells irradiated in air was greater than that in high pressure oxygen by a factor ranging from 0.73 to 4.8, and suggested that the enhancement ratio of radiosensitivity in this range would have a meagre therapeutic advantage. H. Suit has shown TCD<sub>50</sub> using C<sub>3</sub>H mice mammary carcinoma, which were irradiated under 44 psi of pure oxygen, in air and in anoxic condition. The ratio of TCD<sub>50</sub> in anoxia and in 44 psi of pure oxygen was 2.6.

Here we demonstrate two different kinds of solid tumors, Yoshida ascites hepatoma AH 130 and 4-Nitroquinoline-N-Oxide induced Donryu rat pulmonary carcinoma (Sato and Shimozato)<sup>12</sup> (abbreviat-

ed as AH 130 tumor and 4-NQO tumor). The former showed higher radiosensitivity under three atmospheric pressures of pure oxygen, while the latter does not show the increase of radiosensitivity under the same conditions.

### Materials and Methods

**Animals and tumors:** Male Donryu rats, 120 to 150 gram of body weight were used. AH 130 ascites tumor of 0.2 ml, which contains about  $10^7$  cells was injected subcutaneous into femur of rats. For the experiment using 4-NQO solid tumors, a piece of solid tumors was transplanted in the femur of rats subcutaneously with a transplantation needle. Both transplanted tumors were allowed to grow until a mass was noticed. Between the 5th and 7th day, the tumors grew to the size of 1.0 to 2.0 cm in diameter which were used for the experiment. The animals which bore the solid tumors were divided in three groups randomly, namely control group, a group which were irradiated in air, and a group which were irradiated in three atmospheric pressure of pure oxygen.

**Irradiation:** X-rays of 6 MeV produced from Linear accelerator (Varians) was delivered to tumors which were covered with 1.5 cm thickness of dental modeling as an absorber to yield a maximum build up at the tumor surface. The irradiated area was limited to one femur and leg which bore a tumor, so that other body part was out of the radiation field by carefully protecting with lead blocks. There was some scattered radiation to the body part but this amount was negligible. The total radiation dose was delivered by single irradiation. For the radiation therapy under high pressure oxygen, a portable type of the hyperbaric oxygen chamber<sup>13</sup> made by Vickers Co. was used. It took about 5 minutes to reach 3 atmospheric pressure. Animals were maintained for 10 minutes in this pressure before irradiation in order to obtain sufficient oxygenation of tumor tissue. Soon after a total dose was delivered, pressure in the oxygen chamber were decreased to the atmospheric pressure in 4 minutes. All animals were anesthetized with Nembutal during treatments.

**Measurement of tumor size:** Tumor size was measured with measuring rule in three dimensions, the size was represented in cubic centimeter which is a product of three dimensions. This evaluation was a good measure as a representation of the tumor size. Tumor sizes were measured more than three times a week, until the tumor was completely disappeared or the animals were died with tumors. When tumors were cured, animals were observed up to the 60th day after irradiation.

### Results

#### 1. AH 130 solid tumor

The regression curves of the tumor size are shown in Figure 1-1 to 2-3. The two fifth of tumors to which 3000 R were delivered in air were regressed. This fraction could not be cured and grew again. The animals were died on 15th and 17th day after irradiation in this case. The respective group which were irradiated under three atmospheric pressures of pure oxygen, all tumors were disappeared from 7 to 15th day after irradiation. In the group to which 1500 R was delivered, 80 per cent of the tumors irradiated in air and 83 per cent of the tumors irradiated in high pressure oxygen disappeared. There is no difference in the cure ratio in both groups but the rate of regression of the tumors was faster in the group which was irradiated in high pressure oxygen than that in air, i.e. tumors irradiated in air were disappeared between 16th and 32nd day (mean: 24 days) after irradiation, while the tumors which were irradiated in three atmospheric pressures disappeared between 8th to 11th day (mean: 10 days) after irradiation.

Fig. 1-1 AH-130 Solid Tumors of Rats Femur

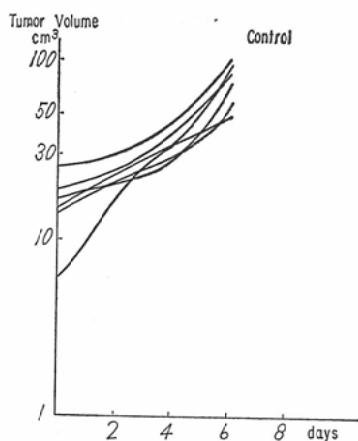


Fig. 1-2 AH-130 Solid Tumors of Rats Femur

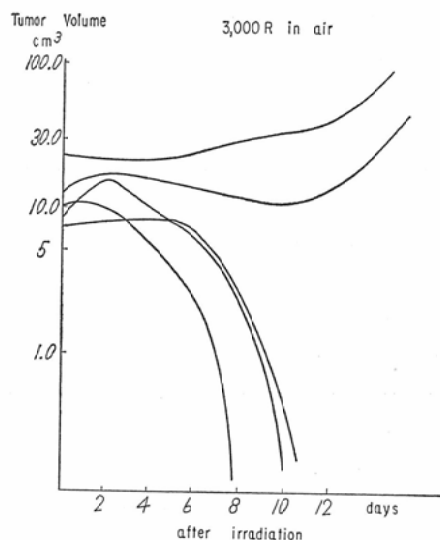
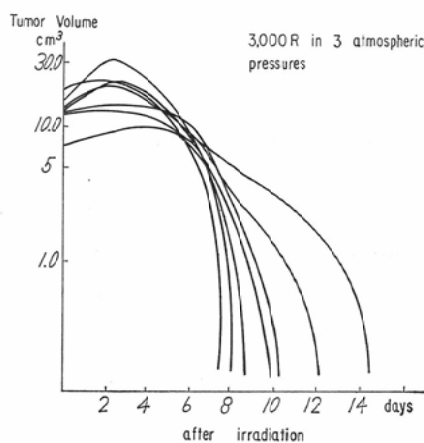


Fig. 1-3 AH-130 Solid Tumors of Rats Femur



tion.

## 2. 4-NQO tumors

In figure 3, regression curves of the tumor size are shown respectively to 1000R, 15000R and 2000R experimental groups. The solid curves were obtained for the experiment in which the animals were irradiated in air, while broken lines were those observed in three atmospheric pressures of pure oxygen. There was found no difference in the rate of regression and cure ratio between groups for which 1000 R and 1500R were delivered. For the 2000R group which was irradiated under high pressure oxygen, regression of tumors was accelerated but it was not so prominent as the regression of AH 130 tumors.

## 3. Body weight change

Fig. 2-1 AH-130 Solid Tumors of Rats Femur

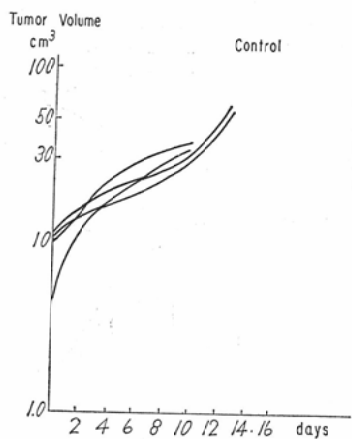


Fig. 2-2 AH-130 Solid Tumors of Rats Femur

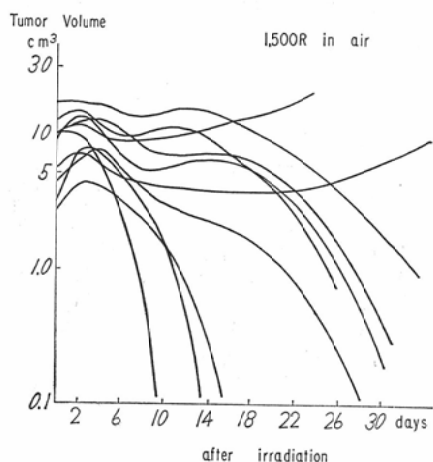
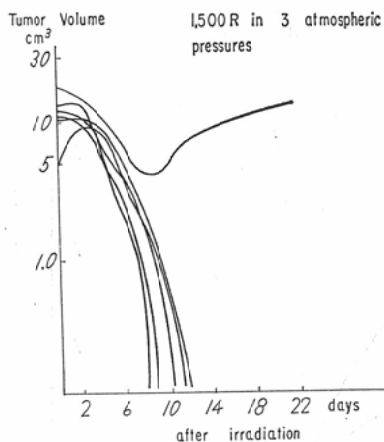


Fig. 2-3 AH-130 Solid Tumors of Rats Femur



Since the experimental animals were young and still growing, the body weight after the irradiation were measured. The effect of high pressure oxygen might be a factor which affect the change of body weight. As shown in Figure 4, on the next day after irradiation, the body weight of control animals which were irradiated in air were increased from 5 to 7 per cent. After third days, the rate of increase of body weight were the same in both groups.

#### 4. Skin reaction

In most cases incidence of epilation and moist desquamation of the femur of rats were higher in the group which was irradiated under high pressure oxygen. On the 13th day after 3000R are delivered under high pressure oxygen, all animals were epilated at the irradiated site. About a half of animals were epilated in the respective group which was irradiated in air.

Fig. 3-1 4NQO induced Tumors transplanted on Rats Femur

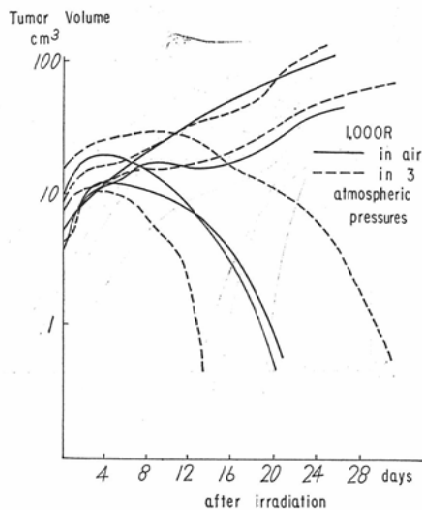


Fig. 3-2 4NQO induced Tumors transplanted on Rats Femur

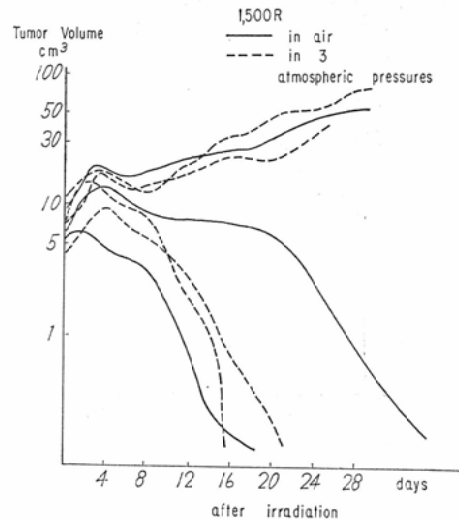


Fig. 3-3 4NQO induced Tumors transplanted on Rats Femur

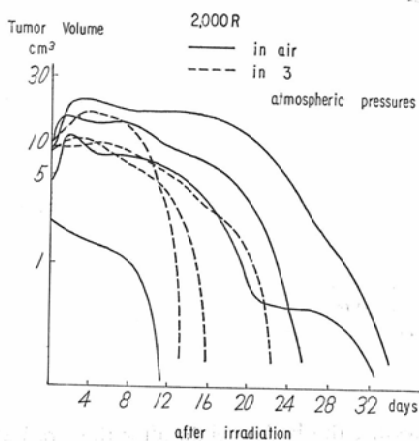
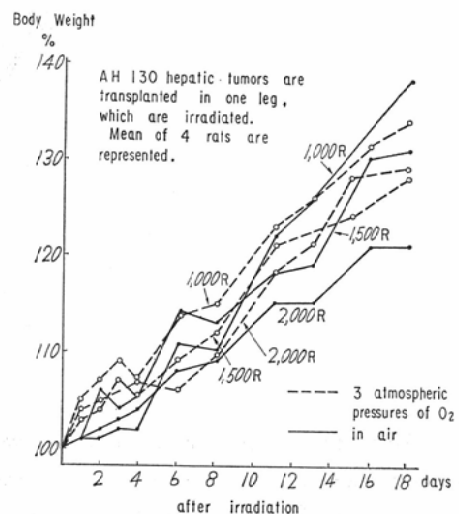


Fig. 4 Body Weight Changes of rats



**Discussion**

L.H. Gray<sup>4</sup> obtained a radiosensitivity curve regarding to the chromosome damage induced by X-rays in Ehrlich ascites tumor cells at different oxygen pressures. Those curves were supposed to fit to the other kind of tumors. Among them oxygen enhancement ratio of the radiation effect of the experimental tumors are 1.3 to 2.6<sup>7,15</sup>. We could not demonstrate the oxygen enhancement ratio of the tumors. W.E. Powers<sup>1</sup> demonstrated an anoxid component in the solid tumor cells. This population was varied

according to the degree of oxygenation. On the other hand, Thomlinson and Gray<sup>14</sup> studied the solid tumors histologically and demonstrated that the necrotic area of the tumors are located beyond  $160\mu$  apart from blood capillary. So it is easily supposed on the radiotherapy, the tumor cells within  $160\mu$  from the blood capillary are damaged, but beyond this range, the tumor cells might be still alive. This is the fundamental concept to support the radiation therapy under high pressure oxygen. Our results show that the cure ratio of the group which were irradiated with 3000R under high pressure oxygen is higher than the group which are irradiated in air, and the rate of regression of tumors was also higher in the former group when 1500R of radiation dose was delivered. Those are indirect evidence to show an anoxic component in the tumors. But 4-NQO induced tumors did not show any oxygen effect regarding to the radiosensitivity. The interpretation for this is difficult. Both experiments were performed under the same conditions, but the oxygen effect was manifested in one kind of tumor, but not demonstrated in another tumor. Further investigations to clarify the reason why the oxygen effect was found in one kind of tumor while absent in another kind may give some clue to the study of the oxygen effect.

The size of tumors is an important factor on the regression of tumors. AH 130 tumors have a variety of sizes, from 1 to 2 cm diameter at the 7th day even when a definite amount of ascites fluid was injected. The tumors grew as a round solid tumor or it has infiltration beyond the solid mass. In tumors larger than 1 cm diameter, necrosis at the center were often observed. All those tumors were used for the present experiment. Above situation might have affected the measurement of radiosensitivity in this experiment.

### Summary

Effect of three atmospheric pressures of pure oxygen on the irradiated solid tumors of femur of rats transplanted from Yoshida ascites hepatoma AH 130 and 4-Nitroquinoline-N-Oxide induced Donryu rat pulmonary carcinoma (Sato and Shimozato) were studied.

1. AH 130 tumors to which 1500R and 3000R were delivered in three atmospheric pressures of oxygen showed a faster regression of the size of tumors and a higher cure rate, compared with controls.
2. 4-NQO induced tumors, to which 1000R, 1500R and 2000R were delivered, showed no difference between the groups which were irradiated in air and three atmospheric pressures of oxygen.
3. Increase of body weight of rats was less in the animals which were irradiated in three atmospheric pressures of oxygen than the animals which were irradiated in air.
4. Skin reactions of irradiated femur were heavier in the animal which irradiated in three atmospheric pressures of oxygen than those which irradiated in air.

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