Influence of Temperature, Anoxia and Cysteamine on Radiosensitivity of Thymocytes (in Vitro)※

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胸腺細胞の放射線感受性に及ぼす温度，無酸素状態及びシステアミンの影響

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細菌や酵母等の放射線感受性に対する phase state（液相，固相）の効果についての研究は種々あるが，哺乳動物細胞に対する照射時の温度の影響に関する研究は少く，又一致した見解がない．

我々は−20°Cから37°C迄の種々の温度で照射された白鼠の胸腺細胞の放射線感受性の一連の変化を調べ，之を無酸素，酸素飽和状態，システアミン（空気中）附加で比較し，次の如き結果が得られた．

1） 酸素で飽和した場合には，氷点下温度で感受性は減少し，氷点の所で不連続的な変化を示した．

2） 無酸素状態，システアミン（空気中）附加，及び無酸素状態でシステアミンを加えた場合には，氷点で感受性の著明な減少が見られなかつた．

3） 無酸素状態でシステアミンを加えた場合には，システアミン（空気中）のみの時，或いは，無酸素状態の時に比べ更に強い防禦効果を認めた．

4） 一般に氷点以上の温度（即ち液相）の場合には温度の変化により殆んど感受性の変化がなかつた．

5） −20°Cでは，又逆に放射線感受性の増加が見られた．

1. Introduction

The results concerning the influence of temperature at the time of irradiation on the radiation response of living cell especially mammalian cells is now conflicting. There are many reports about the effect of phase state on the radiosensitivity of certain bacteria[23)11)], yeast[28)], bacteriophage particles[1)] and pollen grain[6)]. In all cases studied, the sensitivity was remarkably reduced at temperature below the freezing point for X-irradiated cells. However, the study about the entire spectrum of response about the modification of X-ray sensitivity by such treatments as anoxia, temperature changes, and addition of various chemicals is lacking.

※ Dedicated to Professor M. Fukuda on the occasion of his 60th birthday.

In the recent literatures[9)24)5)2)], it has been emphasized that the problem whether the radioprotective action of a group of sulfhydryl compounds as cysteine and cysteamine is based on the removal of oxygen or is caused by another mode of action on the cellular level is still not solved.

In the experiments reported in this paper, we examined the entire spectrum of radiosensitivity of thymocytes irradiated at various temperature from −20°C to 37°C and compared it with anoxic, oxygenated condition and addition of cysteamines.

2. Experimental Methods

Female rats weighing about 150 to 200 g were anesthetized with ether. The thymus was removed aseptically, dissected free of fat and minced with scissors on a sterile watch glass by a standard method[20)16)]. The mince was suspended with 10 cc Krebs Ringer's solution and centrifuged for 5 minutes at 600 g, the supernate discarded and the packed cells resuspended in Krebs Ringer. The concentration of the suspension was approximately 10^8 cells per milliliter.

The thymocyte suspensions were treated with following ways;

1) They were saturated with oxygen by bubbling with pure gas for about 10 minutes at 0°C.

2) They were bubbled with nitrogen at 0°C in the same way and were freed of oxygen.

3) They were added with 7.0×10^{-4}M cysteamine at 15 minutes before irradiation (pH=7.2) and immediately after they were saturated with nitrogen.

After one of these treatments, the tubes of suspension were immersed in either a freezing mixture or water, depending on the temperature desired for a particular experiment. Cold temperature was obtained with dry ice plus ethyl alcohol. The test tube with thymocyte suspensions were immersed gradually in the freezing mixture or water for 15–20 minutes prior to irradiation. Irradiation was performed with gamma rays of ^{60}Co (Gamma cell 150), 37 cm target distance. The dose was measured in air at the flask center with a Victoreen chamber. The total radiation dose ranged from 50 to 900 R.

The stopped tube were turned around the roller at 37°C for 17 hours. The number of viable cells was determined after incubation by staining with 0.02% erythrosin B[16)18)]. Unstained cells are assumed to be viable since they exhibit motility, protoplasmic streaming and pseudopod extension when cultured[16)]. On the other hand, resting cells which manifest varying degrees of molecular vacuolization stain with eosin.

The methods for obtaining cold temperature are as follows:

−2°C	dry ice+5% EtOH
−6°C	dry ice+15% EtOH
−20°C	dry ice+30% EtOH

In each experiment, the non irradiated controls were prepared; an aliquot of the cell suspension received both atmospheric and temperature treatment, but no irradiation. The number of eosin-resistant cells in the irradiated suspensions was expressed as a percentage of the non-irradiated population for each thymic preparation at 17 hours.

3. Experimental Results

A high percentage of non-irradiated thymocytes (60–70%) survived incubation for 17 hours at above freezing temperature. This results is in good agreement with those of Myers[15)]. However, an appreciable damaging effect of the freezing process were found as shown Table 1, and this effect were seen at

Table 1. Survival of non-irradiated thymocyte suspensions as a function of temperature (non-stained thymocyte count/total cell count)

	Oxygen	Nitrogen	Cysteamine	Nitrogen+ Cysteamine
−20°C	58.6 (0.77)	56.8 (0.75)	53.0 (0.70)	52.1 (0.69)
− 6°C	65.5 (0.86)	63.9 (0.84)	62.8 (0.83)	65.0 (0.86)
− 2°C	77.4 (1.02)	73.2 (0.96)	70.5 (0.93)	69.5 (0.91)
4°C	78.4 (1.03)	72.2 (0.95)	70.0 (0.92)	71.1 (0.94)
25°C	76.0 (1.00)	70.6 (0.93)	66.7 (0.88)	69.2 (0.91)
37°C	75.4 (0.99)			

( ) : Survival of non-irradiated, oxygenerated thymocyte at 25 C is set to 1.00.

every atomospheric conditions.

Irradiated thymocytes decrease their life span in vitrc, the percentage of surviving cells (eosin resistant) decreasing exponentially with increasing radiations as shown Fig. 1 and 2.

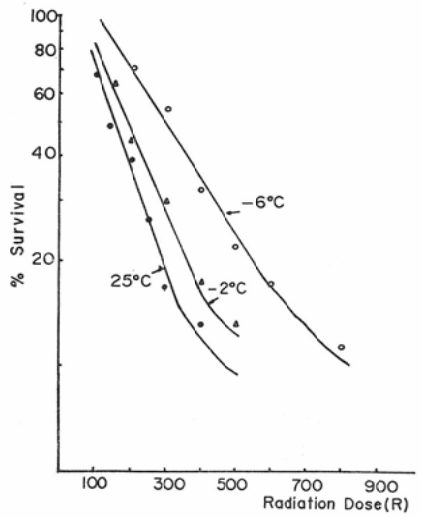

Fig. 1. Dose response curves for oxygen-saturated suspensions of thymocyte irradiated at various temperatures.

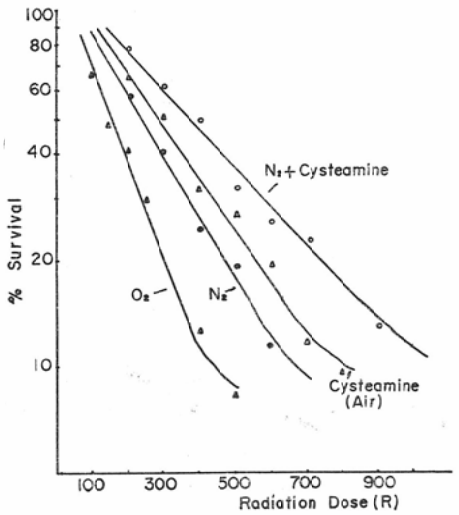

Fig. 2. Dose response curves of thymocytes irradiated at 25 C.

Dose response curves for oxygen-saturated suspensions of thymocytes irradiated at 25°C, −2° (pre-freezing point), −6°C (freezing point) were shown Fig. 1.

MLD (D37, mean lethal doses) were obtained from this curves. The reciprocal of MLD is one measure of radiosensitivity. When the radiosensitivity of oxygen saturated suspensions at 25°C was set to 1, the radiosensitivity of another various conditions were obtained (Table 2).

In the case of oxygen saturated suspensions, 12–15% decrease of radiosensitivity were found at non-freezing point (−2°C) compared with 25°C but decreased incontinuously at freezing point (about a half)

Table 2. MLD of thymocyte irradiated at various temperatures (R).

	Oxygen	Nitrogen		Cysteamine (Air)		Nitrogen + Cysteamine
−20°C	237±13.2 (6)	264±14.6 (6)	P <0.05 *	292±18.8 (4)	P <0.05**	335±22.5 (5)
− 6°C	380±10.8 (6)	428±15.0 (8)	P < 0.005	448±15.6 (4)	P <0.05	536±21.0 (4)
− 2°C	246±13.2 (6)	393±21.5 (6)	P <0.05	430±25.5 (5)	P < 0.2	479±25.6 (4)
4°C	243 (6)					
25°C	201±11.4 (10)	314±15.0 (8)	P < 0.001	366±19.0 (5)	P < 0.005	491±21.0 (5)
37°C	183±13.5 (5)	300±12.5 (5)	P < 0.001	325±16.5 (5)	P <0.01	395± 8.0 (5)

( ) : the number of experiment.
* : statistical differences between nitrogen and nitrogen + cysteamine
** : statistical differences between cysteamine and nitrogen + cysteamine.

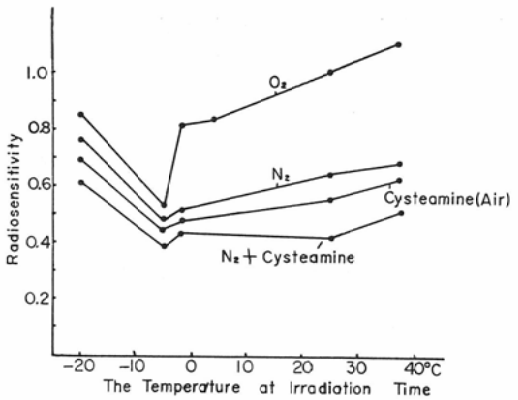

Fig. 3. The radiosensitivity of thymocytes in vitro.

and again increased at supercooled conditions (−20°C) as shown Fig. 3.

In the case of oxygen-depleted suspension, the radiosensitivity was 65% of oxygen saturated ones at 25°C, the degree of decrease at freezing point was not so remarkable.

In the cases of cysteamine and cysteamine under anoxic conditions, the similar results with oxygen depleted suspensions were obtained. But cysteamine under anoxic condition gave significantly. But cysteamine under anoxic condition gave significantly better protection than cysteamine only or anoxia only as shown Table 2. The radiosensitivity at supercooled suspensions (−20°C) were increased in all atmospheric conditions.

4. Discussion

It is well known fact that thymocyte has a great radiosensitivity and die a non-mitotic death (interphase death) within a few hours following low doses of ionizing radiation like other radiosensitive mammalian cells[21)22)25)26)].

The development of cell death dependent on the enviromental factors at the time of irradiation and conditions of incubation following irradiation as well as the dose administered[7)14)15)16)17)].

If diffusible free radicals are important in some types of radiation damage, their reaction ought to be inhibited in the solid state, i.e. the diffusion of free radicals is hindered, and reaction by indirect process should be sharply reduced when the water freezes.

Wood[28] with haploid yeast, Feberge[6] with Tradescantia pollen, Rajewsky[19] with seeds, Stapleton[23] with E. coli B/r, have shown the importance of diffusible toxic substances intervending between the ionizing process and the biological effects of X-rays. In all cases, the sensitivity was discontinuously decreased at the freezing point as well as in this work. A remarkable similarity was found between the dependence temperature on the bacterial inactivation and thymocytes. Houtermans[11], Stapleton and Edigton[23] have demonstrated that frozen cells being approximately three times as radioresistant as unfrozen ones. The thymocytes in our case were about twice radioresistant in frozen state, but the radiosensitivity increased again and returned to that of liquid suspension in supercooled state (−20°C) as shown Fig. 3. It is suggested the mammalian cells are more vulnerable in supercooled state.

Wood[30] also found this and suggested that freezing produces dehydration by extracellular freezing of the water, whereas rapid freezing zt temperatures below about −35°C results in intracellular freezing of water as indicated by Meryman and Platt[13] for freezing of mammalian cells. Stapleton and Edington have also found that frozen, oxygen-depleted suspensions[23] are approximately twice as resistant as frozen, oxygen saturated suspensions. However, remarkable differences between them were not found in our results.

In oxygen saturated suspension, about 20% decrease of radiosensitivity at low temperature above freezing (4°C, −2°C) was found. Until recently, very little change in radiosensitivity of mammalian systems has been reported at temperature above freezing[4]. Weiss[30] could not to demonstrate a protective effect of low temperature on the reproduction of mammalian cells (Hela cells). However, Belli, J.A et. al.[3] has showed that low temperature at the time irradiation resulted a decrease in radiosensitivity of Hela cells. The nature of this protection is not known but he suggested that it is associated closely with slowed rate processes and recombination of molecules in the metionic state before deleterious reactions can take place. It may be said that cell processes such as enzyme reaction rate and mitotic division at low temperatures are slowed. The formation and reactivity of active radicals is diminished. This would tend to decrease the radiosensitivity of cells at 5°C than at 37°C.

It was reported that the oxygen effects observed with thymic cell and ascites tumor cell suspsnsions resemble closely microorganisms, insects and plants[10], the sensitivity in air being about 2.5 to 3 times that in nitrogen. In our case, the radiosensitivity in oxygen saturated condition were about 1.4 to 1.7 times that in nitrogen.

As well known, SH compounds easily undergo autoxiation and Gray[8] has explained that such autoxidation can lead to anoxia, thus protection is brought about indirectly through lack of oxygen. In our case, cysteamine under anoxic conditions showed significantly better protection than anoxia only in all temperature range at irradiation although the difference of sensitivity between them were minimum at freezing point as shown in Table 2. It may be said that the radioprotective effect of cysteamine is not caused by an anoxia only induced by its autoxidation. Our findings are essentially similar to those of Kohn and Gunter[9] who found that 1-cysteamine afforded a significant protection of irradiated E. coli B/r in suspensions in equilibrium with 0, 5, 20 and 100% oxygen at the time of irradiation. Cysteamine was a little more powerfull protector than anoxia only in our case. This is in good agreement with those of Vergrossen et. al[27].

5. Summary

The radiosensitivity of thymocyte has been studied as a function of the temperature during irradiation for oxygen-saturated, oxygen-depleted, cysteamine, and cysteamine under anoxic suspensions.

1) Anoxic condition, cysteamine (in Air) and cysteamine under anoxic condition gave much better protective effects compare with oxygen saturated condition.

2) In oxygen saturated suspension, a significant decrease in sensitivity at subfreezing temperatures and discontinuous change at freezing point were found.

3) Oxygen depleted suspension, cysteamine (in air) and cysteamine under anoxic condition showed no remarkable changes at the freezing point.

4) Cysteamine under anoxic condition gave significantly better protective effect than cysteamine only or anoxia only.

5) Oxygen saturated suspension showed a little changes in sensitivity at cold temperature above freezing (−2°, 4°C).

6) A further lowering of the irradiation temperature (−20°C) increased the radiosensitivity to that of liquid suspensions.

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