A STUDY ON RADIATION INJURY OF THE ERYTHROCYTE MEMBRANE

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It is of importance to study radiation injury in biological membrane as a step to clarify the membrane mechanism of cellular death by ionizing radiation. Since the cell membrane is understood to play a prominent part in cell activity especially in higher animals, there have been many observations^{2,3,8,10} made on the physicochemical changes of the membrane by ionizing radiation, such as those in the electrical properties of the cell membrane and the membrane permeability. No observations, however, seem to have been made on a systematic basis. In the present study it was attempted to obtain information systematically on the initial damage of the cell membrane caused by a relatively low dose of irradiation.

MATERIALS AND METHODS

Rat erythrocytes with a simple cell metabolism seem to be suitable materials for the study of the cell membrane. In this experiment, $^3\text{H}_2\text{O}$ β -rays were employed so that measurements of the dielectric constant and conductivity of erythrocyte suspension for a continuous irradiation might be made with more convenience. To 5 ml of erythrocyte suspension was added 0.1 ml $^3\text{H}_2\text{O}$ (1 Ci/0.1 ml), thus the concentration resulting in 200 mCi/ml. The dose rate calculated was about 40 rads/min.

To find out changes in permeability of the cell in a post-irradiation status, irradiation was carried out from a ^{60}Co γ -ray source at a dose rate of 40 rads/min, as determined by a Victoreen chamber. Throughout the experiment 5 ml of each cell suspension was kept usually at 25°C.

1. Biologically, the changes in electrical properties are of great importance, because they are generally detectable at a dose which is too small to produce any measurable chemical change but enough to produce a pronounced biological effect.

In observing changes in the cell membrane exposed to a continuous irradiation (40~1200 rads), measurements were made of the dielectric constant and conductivity of the erythrocyte suspension with Cole's bridge⁴) by using alternating current at a frequency of 4 Mc/sec and an oscilloscope. Besides, ^{60}Co γ -rays were used in a post-irradiation status.

2. To make the above results more clear, the erythrocyte suspension was subjected to the permeability test. As a result, changes in membrane permeability seemed to be related to the physical process of radiation injury. Erythrocytes which had been labeled with $^3\text{H}_2\text{O}$ as a tracer were suspended in 0.9% NaCl solution and irradiated with γ -rays (600~4000 rads). After irradiation, $^3\text{H}_2\text{O}$ effluxes were measured chronologically by a liquid scintillation counter. To modify the radiation effect on the cell membrane, experiments were carried out in solutions of different osmotic pressure; that is, erythrocytes

were suspended in 0.5 and 1.5% NaCl solutions.

3. To obtain any information on the biochemical process which would affect changes in cell membrane permeability, observation was made on a difference in $^3\text{H}_2\text{O}$ efflux rate between two temperatures, 10 and 25°C . Morphological changes (discoid $\rightarrow$ crenated) following the irradiation (1000 and 4000 rads) were examined photomicroscopically. Inosine and adenine (3 mg) were added to the erythrocyte suspension to observe a possible restoration to a normal shape⁹).

RESULTS

1. An increase in the dielectric constant and a decrease in the conductivity of the erythrocyte suspension were observed from the beginning up to 600 rads, as shown in

Fig. 1. Dielectric Increment of Erythrocyte Suspension.

Dose rate: 40 rad/min

Frequency: 4 Mc/sec

●—● continuous irradiation
 ×—× 20 minutes after irradiation
 ○—○ continuous irradiation of destroyed cells

Fig. 2. Changes in Conductivity of Erythrocyte Suspension.

Dose rate: 40 rad/min

Frequency: 4 Mc/sec

●—● continuous irradiation
 ×—× 20 minutes after irradiation
 ○—○ continuous irradiation of destroyed cells

Fig. 3. $^3\text{H}_2\text{O}$ Efflux Rate from Erythrocytes versus Dose Given.

●—● 0.9% NaCl solution ○—○ 0.5% NaCl solution
 ×—× 1.5% NaCl solution

Table 1. Effect of ^{60}Co γ -rays on $^3\text{H}_2\text{O}$ efflux rate (%/min) from erythrocytes in isotonic, hypotonic, and hypertonic solutions at 25°C

Solution	Dose in rads				
	0	600	1000	2000	4000
0.9% NaCl	36	38	45	54	94
0.5% NaCl	38	50	60	70	88
1.5% NaCl	39	38	48	55	90

Table 2. Effect of ^{60}Co γ -rays on $^3\text{H}_2\text{O}$ efflux rate (%/min) from erythrocytes at 25°C and 10°C

Temp.	Dose in rads				
	0	900	1000	2000	4000
25°C	36	38	45	54	64
10°C	34	39	43	52	60

Figs. 1 and 2. Irradiation with more than 800~1000 rads resulted in a decrease in the dielectric constant and an increase in the conductivity of the cell suspension. The dielectric constant and conductivity measured in a post-irradiation status underwent less changes than those measured for a continuous irradiation. This suggests that some of the changes observed for a continuous irradiation may apparently be reversible. The above findings may be interpreted as changes in ion permeability of the cell membrane^{8,9)}.

2. The effect of ^{60}Co γ -rays on $^3\text{H}_2\text{O}$ efflux rate are presented in Fig. 3 and Table 1. There was no significant difference membrane permeability for $^3\text{H}_2\text{O}$ between a sample irradiated with 600 rads and a non-irradiated sample in 0.9 or 1.5% NaCl solution. In 0.5% NaCl solution, however, an increase in permeability was observed in samples irradiated with 600 rads. In samples irradiated with 1000 rads even in 0.9 and 1.5% NaCl solutions, permeability increased and changes were enhanced in proportion to the dose given. In those in 0.5% NaCl solution, the enhancement was more obvious.

3. There was a slightly greater increase in $^3\text{H}_2\text{O}$ efflux rate at 10°C than at 25°C evidently due to the difference in thermal diffusion rate, as shown in Table 2. Morphologically, erythrocytes retained their normal shape even 180 minutes after irradiation with 1000 rads. At irradiation with 4000 rads, erythrocytes failed to maintain their normal discoid shape, turning to take a crenated disk shape⁹⁾. They were regenerated by the addition of inosine and adenine.

DISCUSSION AND CONCLUSIONS

Radiation injury to the erythrocyte membrane was detected initially as change in electrical properties. Changes in dielectric constant and conductivity of the erythrocyte were observed at a continuous irradiation even with a dose of 40 rads, although some of them were reversible in the post-irradiation status (Figs. 1 and 2). It is clear that the increase in conductivity was proportional to the ion concentration of the suspension. Moreover, the decrease in dielectric constant was replaced by the decrease in conductivity, as expected from the formula given by FRICKE⁵⁾. Thus, these changes can be explained in terms of the ion movement from the outside to the inside of the cell,

triggered by reactions between membrane molecule and ion or some free radical produced inside or outside the cell following irradiation.

After administration with a certain dose, the gradient of electrical changes proved to be inverse. The inversion occurred obviously at a dose between a low dose (600 rads) and a medium dose (1000 rads). So there must have been successive events in radiation injury of the membrane. After a certain dose, the development of ionic processes of the opposite direction was initiated. In this period, the overall electric charge in the system increased due to the liberation of a number of ions through the membrane, evidently because of the beginning of the destruction of the membrane¹⁾.

There was no significant difference in membrane permeability to $^3\text{H}_2\text{O}$ in an isotonic solution between non-irradiated and low irradiated samples. In a hypotonic solution, however, a low dose irradiation caused an increase in membrane permeability, as shown in Fig. 3. The membrane tension produced by osmotic pressure should be considered as a direct mechanical tension. So, the author is inclined to speculate that the factor of tension may be important for the intracellular membrane under irradiation. Namely, a stress of radiation effects in a hypotonic solution is assumed to indicate that membrane permeability for $^3\text{H}_2\text{O}$ intensively enhances changes when radiation cooperatively affects the mechanical tension of the membrane.

At irradiation with a medium dose, permeability increased even in an isotonic solution (Fig. 3). KAVANOU⁷⁾ proposed that pores in the biological membrane might be in two states; that is, an open configuration and a closed configuration, and that the transformation between the two configurations might be related intrinsically to the membrane function. Therefore, if the radiation dose amounts to a critical one, the closed configuration will be transformed into an open one, which is assumed to be rather unstable. If this assumption is valid, the change in membrane permeability in an isotonic solution at irradiation with a medium dose will reflect a cooperativeness of radiation with the changes of the membrane state⁶⁾ in living cells.

At irradiation with a low or medium dose, no biochemical damage seemed predominant. At irradiation with a high dose, erythrocytes underwent morphological changes, with their normal shape regenerated by the addition of inosine and adenine; namely, biochemical injury became predominant.

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赤血球膜の放射線損傷の研究

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放射線による間期の細胞死は、膜に起因することが多い。低線量で初期損傷は、膜の電気的特性で観察される。600 rads 以下では、その変化は可逆的であるが、1000 rads では、電気的特性は一方的に変化し、膜からのイオン流出として帰結される。 $^3\text{H}_2\text{O}$ を用いた流出実験でも、これを裏付ける。

膜を低張にして機械的張力を与えると、600 rads でも流出が見られる。これは正常の張力で、1000 rads の流出も生きてる膜のあり方が放射線に協力的に働くことを示唆する。

4000 rads では、上記の物理的損傷は生化学的損傷に進んでることが、アデニン、イノシンを用いた実験で解明された。