RECOVERY FROM RADIATION-INDUCED DECREASE IN CELL MEMBRANE CHARGE BY ADDED ADENOSINE TRIPHOSPHATE AND ITS MODIFICATION BY COLCHICINE OR CYTOCHALASIN B.

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Summary. Cell electrophoretic mobility of rat erythrocyte decreased with time after 3000 R X-irradiation without spontaneous recovery. On addition of 10^{-4}M ATP to the irradiated cells, recovery was observed within 10 minutes. Washing out of ATP and subsequent incubation for 1 hr resulted in the return of mobility to the low level. Preincubation with 0.1 μg/ml colchicine for 15 minutes or 1 μg/ml cytochalasin B for 30 min completely blocked the reversible effect of ATP on electrophoretic mobility. These results suggest the existence of tubulin-like polymerizing protein in the cytoplasmic membrane and changes in its conformation induced both by X-irradiation and by added ATP.

INTRODUCTION. In our previous papers (1-3), we reported the change in cell electrophoretic mobility after X-irradiation in cultured mammalian cells and lymphocytes. The electrophoretic mobility decreased with time and reached a minimum depending on dose 4 hours after exposure. After irradiation with doses higher than 1000 R, no recovery in the mobility was observed during the subsequent incubation. As the mechanism of the X-ray induced mobility reduction, we tentatively proposed a change in the conformation of the cell surface (4), based upon the findings that 1) the amount of acidic sugar contributing to the negative charge on the cell surface was not affected by X-irradiation, 2) the addition of low concentrations of sulfhydryl-

blocking agents, Concanavalin A or phytohemagglutinin completely blocked the change in mobility, as did fixation of the cell surface by a brief treatment with glutaraldehyde. In the present experiment, the effect of ATP in the presence and absence of colchicine or cytochalasin B were investigated under the hypothesis that ATP-dependent polymerizing proteins might be involved in conformation change of cell membrane.

MATELIAIS AND METHODS. Erythrocytes from Wistar King A rats were obtained by heart puncture. X-irradiation of the erythrocytes in a culture dish was carried out Dulbecco's phosphate buffered saline pH 7.2 at room temperature (15-20°C). The physical factors of exposure were: 200 kVp, 25 mA, 0.5 mmAl + 0.5 mmCu filter added, Half value layer 1.13 mmCu, 50 cm target-sample distance, and dose rate 105 R/min. After irradiation, erythrocytes were centrifuged and resuspended in culture medium RPMI#1640 (developed by Moore, Rosewell Park Memorial Institute) supplemented by 15% fetal calf serum, and incubated in a 5% CO_2 incubator at the cell density of 1×10^7 cells/ml.

The electrophoretic mobility of individual cells was measured at 25 ± 0.5°C as described by Fuhrmann and Ruhenstroth-Bauer (5,6). Each cell was allowed to move 16 μm alternately in both directions following reversal of current in 67mM phosphate buffer supplemented with 5.4% glucose (pH 7.3, ionic strength 0.167). The mobility was determined from separate experiments on 30-100 cells for each point and calculated in μm $sec^{-1}V^{-1}cm$.

RESULTS. The time-course change of the electrophoretic mobility of erythrocytes after irradiation with 250 R or 3000 R is indicated in Fig. 1. The mobility decreased progressively with time and reached a minimum 4 hours after exposure. Substantial recovery during the subsequent incubation for 20 hours in culture medium was observed with cells irradiated with 250 R, but not with 3000 R. The addition of 10^{-3}M ATP to the cell culture after irradiation with 3000 R for 24 hours resulted in rapid recovery in electrophoretic mobility at 37°C, but not at 3°C. The mobility of unirradiated cells remained constant during the incubation for 24 hours.

Fig. 2 shows the reversibility of the mobility changes on

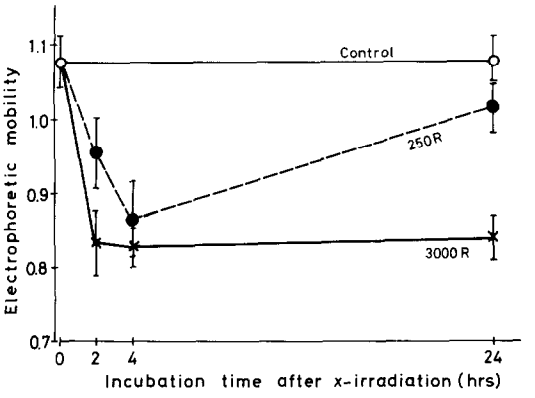

Fig. 1. Change in the electrophoretic mobility ($-\mu m\ sec^{-1}V^{-1}cm$) with time after x-irradiation with 250 R or 3000 R. The vertical lines represent one standard deviation for 30-100 measurements.

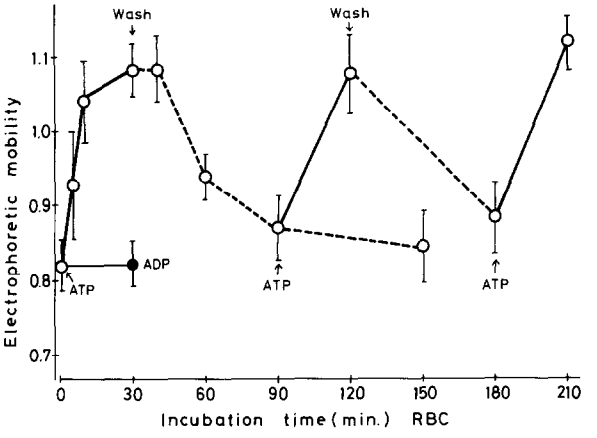

Fig. 2. Effect of ATP or ADP on the electrophoretic mobility of erythrocytes previously irradiated with 3000 R for 24 hours. ATP (10^{-3}M) (or ADP) was added to the cell culture in phosphate-buffered saline. The vertical lines represent one standard deviation for 30-100 measurements.

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addition and removal of ATP. Addition of Adenosin diphosphate(ADP) did not show the recovery effect. Washing out of ATP and subsequent incubation in phosphate buffered saline pH 7.2 for 1 hour at 37°C resulted in re-decrease in electrophoretic mobility to the level before the recovery.

The cells regained the normal electrophoretic mobility 10 minutes after the repeated addition of ATP into the phosphate buffered saline.

Fig. 3 shows the effect of increasing concentrations of ATP on the recovery of electrophoretic mobility of irradiated cells with 3000 R. The recovery effect was observed at concentrations above 10^{-5}, and remained constant above 10^{-4}M.

Table 1 shows the effect of pretreatment with colchicine or cytochalasin B on the recovery of electrophoretic mobility by added ATP. Treatment of cells with colchicine (0.1 µg/ml) or cytochalasin B (1 µg/ml) for 30 min did not alter the electrophoretic mobility. Longer incubation with higher concentration of the drugs caused a mobility reduction by itself. Pretreatment of irradiated erythrocytes with 0.1 µg/ml cytochalasin B for 30 min at 37°C completely blocked the ATP-induced recovery in electrophoretic mobility.

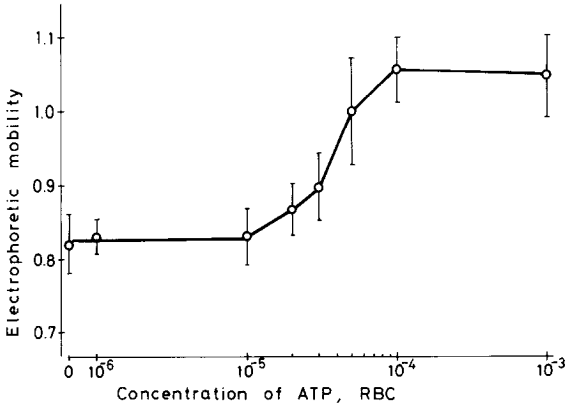

Fig. 3. Relationship between the concentration (M) of ATP and electrophoretic mobility of cells. Erythrocytes were incubated in culture medium (RPMI1640 + 15% calf serum) for 24 hours after irradiation with 3000 R, and then incubated with different concentrations of ATP for 30 min at 37°C. The vertical lines represent one standard deviation for 30-100 measurements.

TABLE 1

Effect of pretreatment with colchicine or cytochalasin B on the recovery effect of ATP

Condition of pretreatment				Electrophoretic mobility
Colchicine	0.1μg/ml	15min	37°C	-0.850 ± 0.035μm $sec^{-1}V^{-1}cm$
	0.1	30	37°C	-0.829 ± 0.047
	0.01	60	37°C	-1.002 ± 0.067
	0.1	30	4°C	-1.042 ± 0.047
Cytochalasin B	1.0μg/ml	15min	37°C	-0.909 ± 0.039
	1.0	30	37°C	-0.826 ± 0.037
	0.1	60	37°C	-0.868 ± 0.034
	1.0	60	4°C	-1.058 ± 0.045
Pretreatment(-), ATP treatment(+)				-1.140 ± 0.059
Pretreatment(-), ATP treatment(-)				-0.820 ± 0.041

Erythrocytes previously irradiated with 3000 R for 24 hours were pretreated with colchicine or cytochalasin B in phosphate buffered saline at 37°C or 4°C, and the drugs were removed by centrifugation. The cells were then incubated in culture medium with or without 10^{-3}M ATP for 30 min at 37°C.

Pretreatment with the drugs at 4°C showed no blocking effect.

DISCUSSION. In recent years, the existence of actin-like or myosin-like proteins in the cytoplasmic membrane has been reported (7-10). A protein (spectrin) extracted from erythrocyte membranes was induced to form coiled filaments which were similar in appearance to the filaments formed by F-actin (11), or by tubulin in the presence of ATP. Although differing in amino acid composition and other parameters, the proteins in membranes and muscle and tubulin of microtubules seem to share some common features. Non-muscle contractile proteins have generated great interest in recent years because

of their possible involvement in controlling cell shape and motility(12) and in cell agglutination by concanavalin A(13) and in other important cell functions. Colchicine and cytochalasin B are known to depolymerize microtubules and microfilaments, respectively. Reversible change of cell surface negativity by added ATP and its prevention by colchicine or cytochalasin B in the present experiments provide additional evidence for the possible involvement of polymerizing proteins in the conformation of the cell membrane.

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