RADIATION RESEARCH 87, 646–656 (1981)

Cell Surface Charge and Cell Division in *Escherichia coli* after X Irradiation

CHIKAKO SATO,* KIYOHIDE KOJIMA,‡ KIMIKO NISHIZAWA,* AND YUKINORI HIROTA‡

*Laboratory of Experimental Radiology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan
†Laboratory of Oncological Pathology, Nara Medical University, Kashihara 634, Japan
‡Laboratory of National Institute of Genetics, Mishima 411, Japan

SATO, C., KOJIMA, K., NISHIZAWA, K., AND HIROTA, Y. Cell Surface Charge and Cell Division in *Escherichia coli* after X Irradiation. *Radiat. Res.* **87,** 646–656 (1981).

Simultaneous detection of electrophoretic mobility (EPM) and morphology of individual irradiated *Escherichia coli* cells under the phase microscope revealed a concurrent decrease in EPM and arrest of cell division. EPM decreased with time and reached a minimum 15 min after irradiation with doses ranging from 100 R to 80 kR. Cells elongating due to the division block retained the minimum EPM. After a recovery phase, separated small-sized daughter cells and some long filamentous cells, which had a few cleavages at the termini, returned to the normal EPM. This finding indicates that recovery in EPM, which represents recovery in the surface architecture, precedes or coincides with the resumption of cell division. Nuclear staining of the recovering cells leads to the suggestion that the cleavage of the cell takes place whenever the EPM has recovered, irrespective of the segregation of DNA, which gives rise to anuclear cells having normal EPM. It is suggested that the mechanism of EPM decrease is Ca^{2+}-dependent conformational change of the membrane accompanying vertical translocation of charged groups.

We have investigated the role of membrane damage in impaired proliferation and death of cells after X irradiation using cell electrophoresis to detect membrane damage. The advantages of this method are its sensitivity as a probe to detect the alteration of dynamic physiological charge-related properties on the surface of living cells (*1–3*), and the simultaneous detection of the electrophoretic mobility (EPM) and the morphology of individual cells under the phase microscope. *Escherichia coli* was used as a model system to study the relationship between cell division and the cell surface. Bacteria inhibited in cell division are easily seen during measurement of EPM because of their filamentous form. Decrease in EPM after irradiation has been reported in several types of mammalian cells and yeasts (*4–6*). Our previous work on three lines of cultured mammalian cells revealed that the fraction of cells whose EPM did not recover within 24 hr after irradiation was statistically in agreement with the fraction of non-colony-forming cells (*7–9*). The present experiments utilizing *E. coli* clearly indicated that EPM decreased in cells when cell division was inhibited, and that recovery of EPM occurred at the resumption of the cell division.

0033-7587/81/090646-11$02.00/0

MATERIALS AND METHODS

Cells and culture. An *Escherichia coli* K-12 strain, PA3092 (*F⁻thr⁻leu⁻lacy⁻trp⁻his⁻thy⁻str⁻malA⁻xyl⁻mtl⁻arg⁻suII⁻*), was used. The cells were cultured with aeration in L-tubes at 37°C in L broth (1% bactotrypton, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose) supplemented by 50 mg/liter thymine. Only cells growing exponentially with the shortest doubling time were used. The cell maintains a constant electrophoretic mobility during the logarithmic phase of growth but shows a reduced mobility at the stationary phase.

Irradiation. X irradiation of the cells was carried out in culture medium in a 3-cm-diameter plastic Petri dish (2-mm depth of cell suspension) at 3°C on ice. The physical factors of exposure were: 200 kVp, 20 mA, 0.5 mm Al + 0.5 mm Cu filter added, half-value layer 1.13 mm Cu, 25-cm target–sample distance, and exposure rate in air 425 R/min.

Electrophoresis. At different times of incubation in shaking L-tubes at 37°C after irradiation, an aliquot of cell suspension was placed on ice, centrifuged, and then washed in cold 67 m*M* phosphate buffer supplemented with 5.4% sorbitol for electrophoresis. The electrophoretic mobility of individual cells was measured at 25 ± 0.5°C with a Zeiss cytopherometer as reported in (*5, 10, 11*). Each cell was allowed to move 16 μm in a scaled thin chamber under the phase microscope alternatively in both directions following reversal of current (4 mA) in the 67 m*M* phosphate buffer supplemented with 5.4% sorbitol. The 67 m*M* phosphate buffer (pH 7.3) contained 50.1 m*M* Na_2HPO_4 and 16.5 m*M* KH_2PO_4. The ionic strength and osmolarity of the buffer were usually indicated as 0.167 and 183 mosm with the assumption of complete dissociation of phosphates. However, the conductivity of the buffer supplemented with 5.4% sorbitol was 7.471×10^3 $\mu\mho$/cm. This value was about one-half the conductivity of the 167 m*M* NaCl solution, thus indicating about 50% dissociation of phosphates. Since 5.4% sorbitol is isotonic (about 300 mosm), the electrophoresis medium is hypertonic by about 183/2 mosm due to phosphates. For the measurement of mobility at lower ionic strengths of solution, phosphate buffers diluted stepwise (6.7, 13.4, 26.8, 40.2, 53.6 m*M*) were supplemented with 5.4% sorbitol to maintain the same viscosity. The conductivities of the medium were 1.076×10^3, 1.811×10^3, 3.413×10^3, 4.784×10^3, and 6.141×10^3 $\mu\mho$/cm, respectively. Osmolarity of the medium varied from about 309 to 373 mosm. We chose the same concentration of sorbitol because EPM is dependent on the ionic strength and the viscosity of the medium, but not on its osmolarity. The mobility was determined from separate experiments on 10–100 cells for each set of conditions and calculated as $\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$.

Morphological observations. Cells were fixed in 10% formalin for 5 min, washed with phosphate-buffered saline, and then spread on a glass slide coated with poly-L-lysine using a cytocentrifuge (Shandon Elliott) for 5 min at 1500 rpm. The cells were dried on the slide, treated with 1 *N* HCl for 5 min at 60°C to digest ribonucleic acid, washed by running water, and then stained in freshly diluted Giemsa solution (Merck). Distribution of chromosomes and the size of the cells were detected using a microscopic photograph. The cells in the suspension were counted with a hemocytometer after different incubation periods.

RESULTS

Morphological change after irradiation. Figure 1 shows the nuclear staining of cells indicating changes in cell size and distribution of nuclear mass with time at 37°C after irradiation with 50 kR. Nonirradiated cells (Fig. 1A) were about 2.5 μm in mean length and contained one to four nuclei per cell. The cells with one nuclear mass were only 3.2% of the cells in nonirradiated culture, but were 47 and 91% (Fig. 1B) of the cells 15 and 30 min, respectively, after irradiation with 50 kR. As shown in Fig. 2, the mean number of nuclear masses in a cell decreased from 2.86 to 1.11 during the first 30 min of incubation after the exposure. During this 30 min, the number of cells increased about 1.6-fold, and the increase in cell length was slight. These results suggest that cell separation proceeded in those cells in which the chromosomes had segregated before irradiation. Enlargement of condensed nuclear masses at the central site of cells and the elongation of the cell progressed after 30 min without further separation of the cells (Figs. 1C, D). A cleavage of the cell was noticed at the central portion of some condensed nuclei. Morphological disintegration of cells appeared only after 2 hr of incubation when cell division resumed. At 3 hr of incubation, cell division was often observed in the filamentous cells in which chromosomes had been segregated to the daughter cells as shown by arrows in Fig. 1E. The filaments with a condensed nucleus at the center on occasion caused cell separation from the termini, thereby giving rise to anucleated cells. During the next 2 hr, normal-sized cells proliferated and became predominant over lysed cells and long filaments (Fig. 1F).

Change in electrophoretic mobility (EPM) with time after irradiation. Figure 3 shows the time course of change in EPM after irradiation with different doses. Decrease in EPM was detectable even after irradiation with 100 or 500 R, but it returned to normal rapidly during the subsequent incubation for 15 or 45 min, respectively. The EPM reduction was not different and was maximum 15 min after irradiation with doses ranging from 15 to 80 kR. EPM recovery, however, was dependent on dose; recovery began earlier and reached a higher value after smaller doses of irradiation. Comparison of the time course change in cell length and EPM indicated that EPM recovery began 2 hr after irradiation with 50 kR when the length of the cell was maximum.

Frequency distribution of EPM and cell size. Figure 4 illustrates the frequency distribution of EPM of unirradiated cells and of cells exposed 30 min earlier to 50 kR. Every irradiated cell showed reduced EPM, and the two distributions are clearly separated with the boundary at $-1.2\ \mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$. The cells irradiated with 50 kR progressively elongated to form filaments with incubation periods up to 2 hr and kept the minimum EPM. Thereafter separation of normal-sized cells took place from the termini of the long filaments as shown in Fig. 1. Three hours after irradiation with 50 kR, about 31% of the cells were of small size (below 4 μm). Figure 5 exhibits the relationship between EPM and the length of the cell. The distribution of the points is separated into three groups. All the short cells (below 4 μm) had EPM higher than $-1.2\ \mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$ as nonirradiated cells. EPM of the longer cells distributed mostly below -1.2, but 30% of them showed

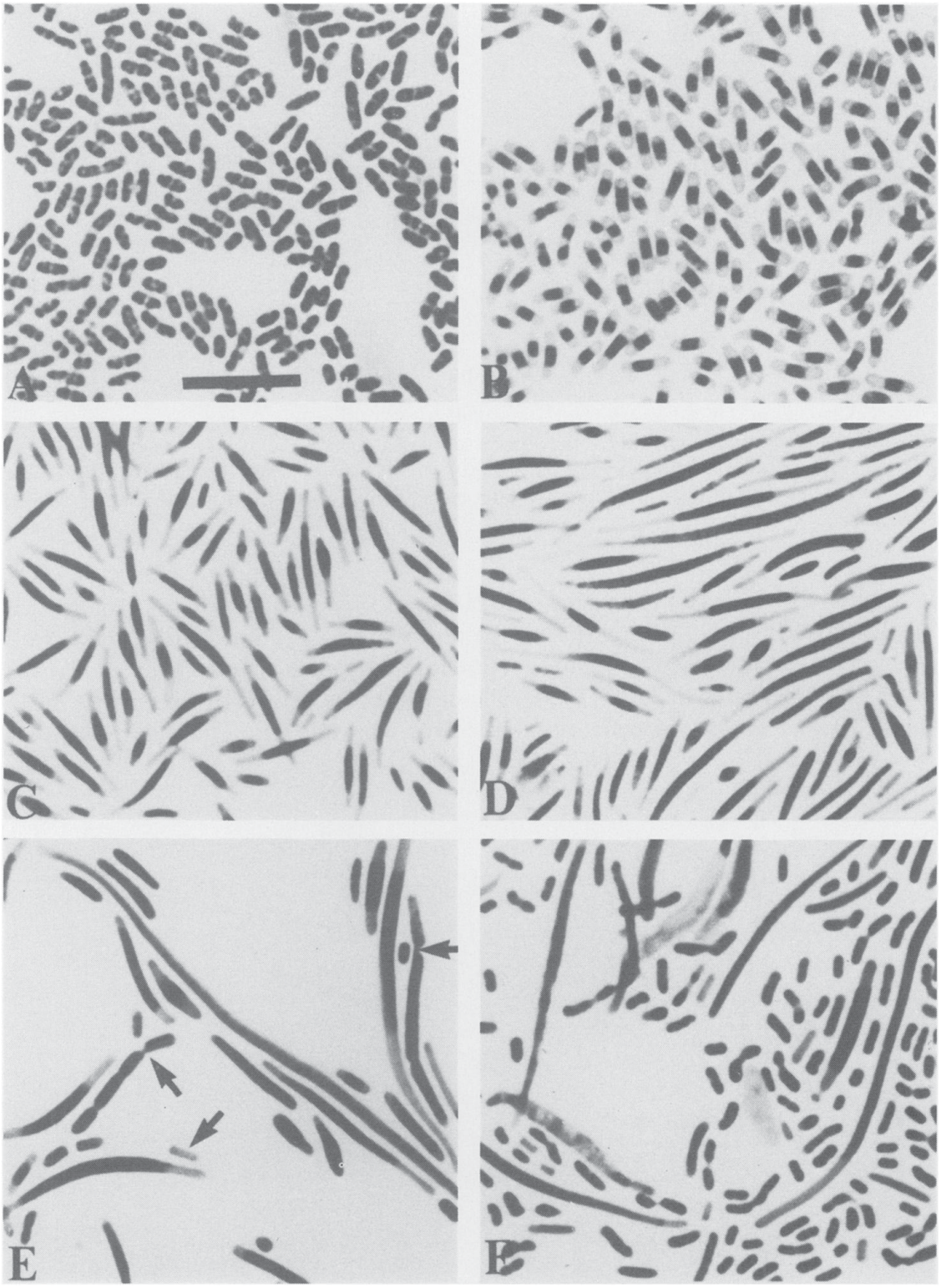

FIG. 1. Nuclear staining of *Escherichia coli* K-12 indicating cell size and distribution of chromosomes. Cells were fixed with 10% formalin at 30 min (B) or 1 (C), 2 (D), 3 (E), or 5 hr (F) after irradiation with 50 kR, treated with 1 *N* HCl for 5 min at 60°C, and then stained in Giemsa solution. Arrows in (E) indicate cleavages of elongated cells and an anuclear cell. (A) Nonirradiated cells. Bar in (A) is 10 μm.

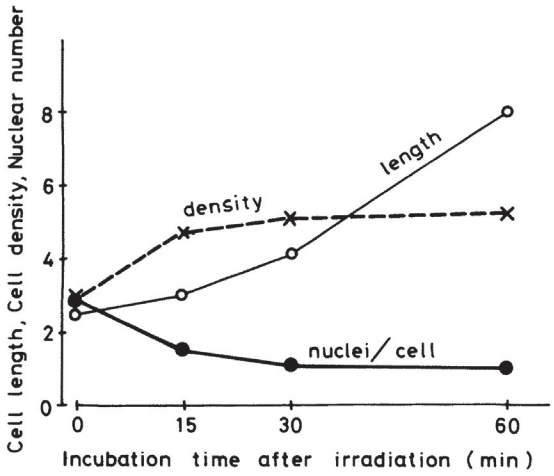

FIG. 2. Changes in (○) cell length (μm), (×) cell density (×10^7 cells/ml), and (●) number of nuclei/cell with incubation time after irradiation with 50 kR. Each point is the mean value of measurements on more than 400 cells. Results suggest early separation of cells in which the nuclei had previously segregated at the time of irradiation, and the later elongation of cells without nuclear segregation and cell division.

the higher EPM. A few cleavages per cell were often noticed in those filamentous cells having EPM higher than that of the ordinally filamentous cells. Closed circles in Fig. 5 represent those filamentous cells containing visible cleavage, which always showed EPM greater than −1.2. Morphology of these cells is exhibited in Fig. 1E. About 10% of elongated cells without visible cleavage also showed EPM above

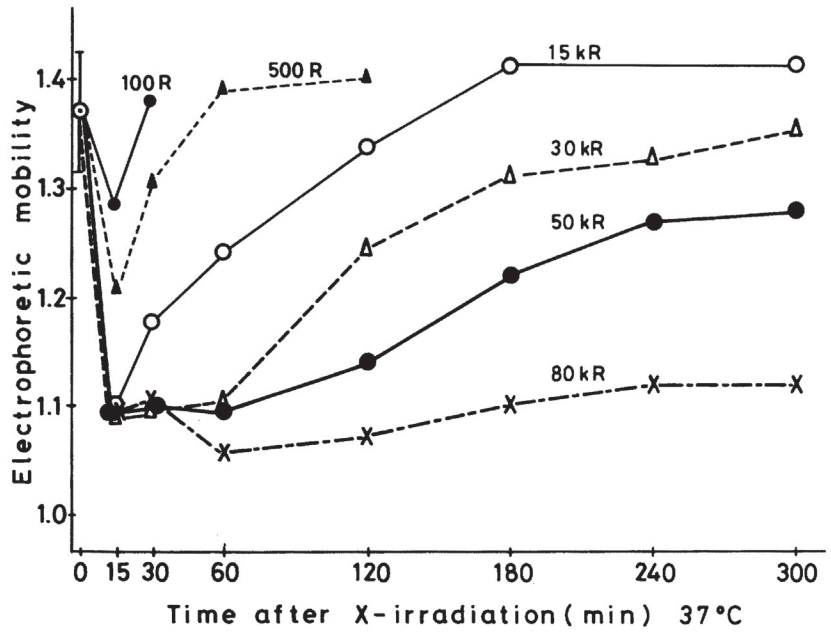

FIG. 3. Change in electrophoretic mobility ($-\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$) of *E. coli* with incubation time at 37°C after irradiation with 100 R (●), 500 R (▲), 15 kR (○), 30 kR (△), 50 kR (●), or 80 kR (×). Each point represents the mean value of measurements on more than 30 cells from three separate experiments.

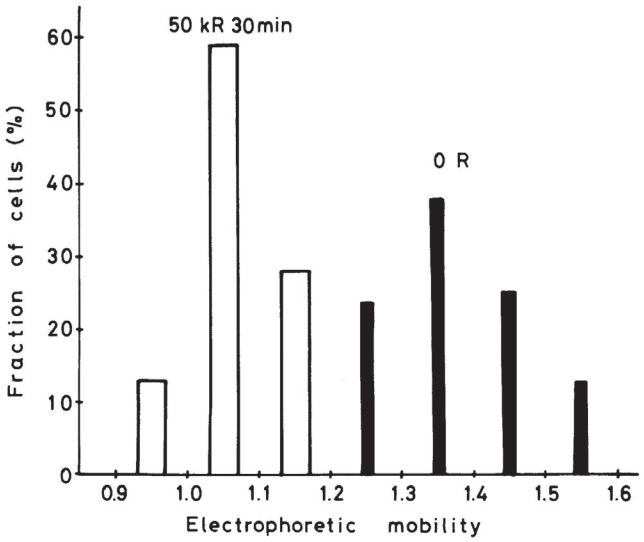

FIG. 4. Frequency distribution of electrophoretic mobility ($-\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$) of unirradiated cells (shaded columns) and cells exposed to 50 kR 30 min earlier (open columns).

-1.2. These results suggest that the recovery of EPM precedes or coincides with the separation of the daughter cells.

Effect of ionic strength on mobility. EPM was measured in a buffer solution of

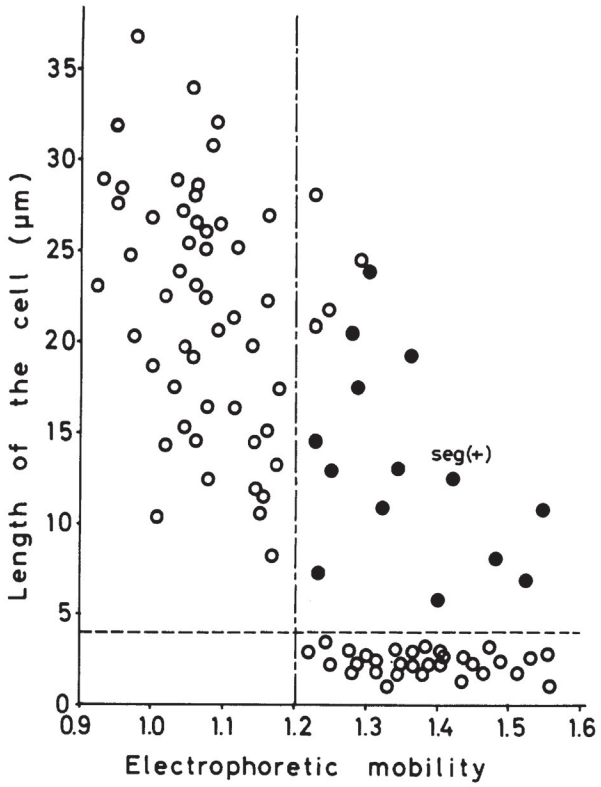

FIG. 5. Relationship between the electrophoretic mobility ($-\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$) and the length of the cell (μm) on 100 individual cells. Measurements were done 3 hr after irradiation with 50 kR. Closed circles are elongated cells with visible cleavage, which showed EPM above -1.2 $\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$ as separated small cells and unirradiated cells.

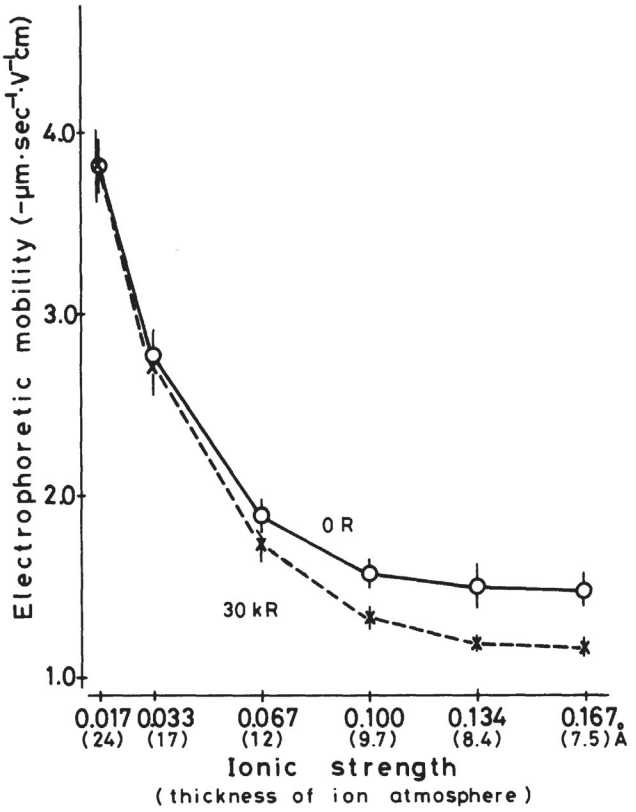

FIG. 6. Relationship between the ionic strength of phosphate buffer used for the measurement of electrophoretic mobility of *E. coli* irradiated with 30 kR 30 min earlier (×) and of unirradiated *E. coli* (○). The vertical lines represent one standard deviation for 30 to 100 cells.

various ionic strengths to determine whether a redistribution of charged groups in the membrane is responsible for EPM reduction. As indicated in Fig. 6, EPM decreased with increasing ionic strength. The difference in EPM between irradiated and nonirradiated cells was evident at ionic strengths of 0.100 and greater. At ionic strengths lower than 0.033, however, EPM was the same in irradiated and non-irradiated cells. Because the decay of potential with distance is less rapid in low-ionic-strength solution, the ionized groups embedded more deeply in the outer surface material exert their greater influence at lower ionic strength (*1, 2, 12*). Therefore with decreasing ionic strength, the thickness of the effective ionic layer increases. The thickness of the ionic layer was calculated according to the Debye-Hückel equation by $3.06 \times (\text{ionic strength})^{-1/2}$ Å as an approximate estimation assuming the complete dissociation of phosphates. These values are shown in parentheses under each value of ionic strength in Fig. 6. The data can be explained by a vertical translocation of negatively charged groups from the outermost layer of 0–7.5 Å into a deeper layer of 9.7–17 Å, or the inverse translocation of positive charges from the deep layer to the outermost layer occurred in irradiated cells. Similar translocation of acidic sugars was suggested in our previous experiments using cultured mammalian cells and erythrocytes (*11, 13*).

Effect of temperature and reagents on EPM change. Cells were irradiated with 15 kR at 3°C on ice and then incubated in a water bath for 15 min with aeration

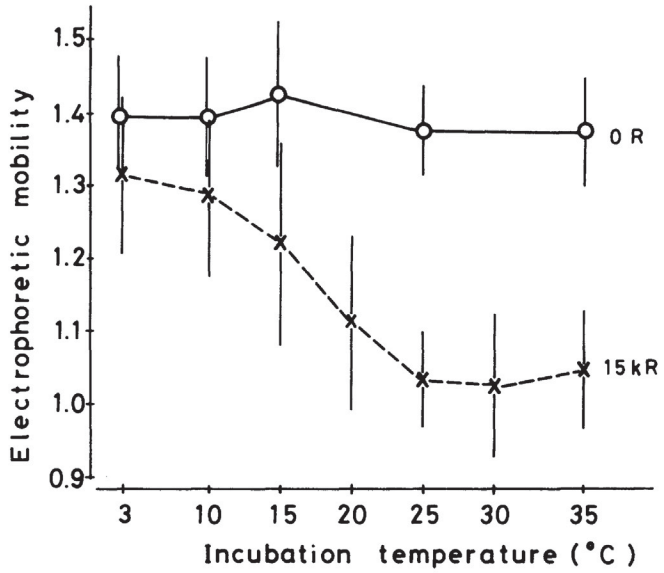

FIG. 7. Relationship between the incubation temperature and electrophoretic mobility ($-\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$) 15 min after irradiation with 15 kR. The vertical lines represent one standard deviation for 30 to 100 cells.

at different temperatures. Figure 7 indicates that the decrease in EPM was maximum and constant at temperatures ranging from 25 to 37°C, and smaller at temperatures of 10°C and lower. The slight difference of EPM between control and irradiated cells after incubation at the low temperature probably resulted from the EPM reduction during the measurement of EPM at 25°C for 5 min. The manifestation of the radiation effect on EPM depended markedly on temperature between 10 and 25°C. Incubation of unirradiated cells at different temperatures did not per se produce any effect on EPM.

To estimate the factors involved in EPM reduction after irradiation, effects of agents which could modify the membrane were examined. Table I indicates that *p*-(chloromercuri)benzoic acid (PCMB, a sulfhydryl-blocking agent), fluorescein mercuric acetate (FMA, a sulfhydryl-linking agent), glycerin, and ethylene glycol bis(β-aminoethyl ether)-*N,N,N'N'*-tetraacetic acid (EGTA, specific chelator of calcium ions) completely blocked EPM reduction after irradiation at the concentrations at which the agent itself had no effect on EPM.

DISCUSSION

The present experiments demonstrate the simultaneous occurrence of reduction in EPM and inhibition of cell division shortly after irradiation and their concurrent recovery in individual cells of *Escherichia coli.* The advantage of cell electrophoresis was the simultaneous detection of EPM and cell morphology on individual cells seen directly under the phase microscope. Cells that were elongating due to the arrest of cell division after irradiation retained their reduced EPM. It should be noted that EPM of the cell is determined by the charge density per unit surface area independently of cell size or shape (*3, 12*). EPM reached the minimum 15 min after irradiation, when the cells were only slightly longer than nonirradiated

TABLE 1

Blocking of Mobility Change after X Irradiation by Modifying Agents

Treatment	*Electrophoretic mobility* ($\mu m \cdot sec^{-1} \cdot V^{-1} \cdot cm$)	*Significant difference* ($P < 0.05$)
No treatment	-1.392 ± 0.076	Yes
30 kR, 30 min	-1.011 ± 0.059	
PCMB 5×10^{-6} *M* 30 min	-1.299 ± 0.062	No
PCMB 5×10^{-6} *M* + 30 kR	-1.252 ± 0.121	
FMA 10^{-6} *M*	-1.340 ± 0.074	No
FMA 10^{-6} *M* + 30 kR	-1.331 ± 0.069	
Glycerin 1 *M*	-1.336 ± 0.084	No
Glycerin 1 *M* + 30 kR	-1.292 ± 0.054	
EGTA 1 m*M*	-1.322 ± 0.079	No
EGTA 1 m*M* + 30 kR	-1.301 ± 0.086	
EGTA 1 m*M* + Ca^{2+} 1 m*M*	-1.329 ± 0.081	Yes
EGTA 1 m*M* + Ca^{2+} 1 m*M* + 30 kR	-1.042 ± 0.067	

controls. Detailed analyses on alterations in cell number, cell length, and distribution of nuclear mass during the first 30 min of exposure were carried out. It was observed that the division of the cell proceeded if the daughter nuclei had already been segregated in the irradiated parental cell at the time of irradiation. Presumably septa had already been formed in these parental cells. Partition of new nuclei, however, was completely inhibited. After 30 min of incubation, enlargement of the condensed nuclear mass at the center of the cell and the elongation of the cell progressed without accompanying cell division but retained the reduced EPM. An important finding at the recovery phase was that not only the separated small cells but also long filamentous cells regained high EPM. The latter cells had a few cleavages at the cell termini, indicating the formation of dividing cross walls. This finding suggests that recovery of EPM in a whole elongated cell precedes or coincides with the resumption of cell division at its termini. Since all small-sized cells, some of which lacked DNA, showed recovered EPM, cell division seemed to occur at the site where the surface structure had recovered irrespective of the distribution of DNA. This idea was supported by our recent experiment using a temperature-sensitive mutant which forms anucleated minicells at a high temperature (to be published).

Negatively charged molecular species which are responsible for EPM in the bacteria have not been identified because of the absence of purified specific enzymes. It was shown that the anionic groups of the cell surface of *E. coli* begin to dissociate between pH 2 and 4 and are carboxylic in nature (*14*). A hypothetical mechanism which might lead to the reduction of EPM of *E. coli* after irradiation is considered to be a conformational change of membrane as suggested in the case of mammalian

cells (*11, 13*). Detection of reduced EPM only at low ionic strengths suggested a vertical translocation of charged molecules from the peripheral layer to a deeper layer after irradiation. The character of molecular conformational change was supported by the blocking of EPM loss by adding sulfhydryl-blocking agents or protein-linking agents or lowering the temperature of the cell culture. All these treatments seem to restrict the movement and rearrangement of membrane molecules involving SH-proteins. The requirement of calcium ions for EPM reduction is another important feature of the mechanism. We reported the existence of a calcium-dependent process as an early step of EPM change after irradiation in erythrocytes (*15*). The target of X irradiation resulting in EPM reduction in mammalian cells is thought to be the membrane itself, because EPM reduction occurred in the erythrocyte ghosts containing neither nuclei nor cytoplasm as well as in whole erythrocytes and cultured mammalian cells (*11*).

Involvement of membrane damage in the loss of colony-forming ability of bacteria after irradiation has been proposed. A type of cell killing associated with the oxygen effect was suggested as the result of membrane damage (*16, 17*). Several membrane-specific drugs such as local anesthetics and tranquilizers preferentially sensitized the hypoxic cells to the same sensitivity as that of oxic cells (*18*). Further evidence in support of the hypothesis is as follows: (a) bacterial cells are sensitized by iodoacetamide under conditions that prevent the drug from entering the cells (*18*), (b) A mutants of *E. coli* carrying pol A^- mutation and a mutant strain Bs-1, both defective in a process of DNA repair, are not affected by the membrane-acting sensitizers (*19–21*). Because these membrane-acting reagents inhibit the repair of DNA (*22*), some interaction between the membrane and DNA was suggested in the process of sensitization. The DNA-membrane complex was indicated as a site which is particularly susceptible to enhancement of radiation damage by oxygen (*23*). Our results suggest that the inhibition and recovery of cell division are coordinated with the reduction and recovery of EPM, which reflects alterations in the structure of the cell surface after irradiation.

RECEIVED: August 6, 1980; REVISED: November 12, 1980; RE-REVISED: February 13, 1981

REFERENCES

1. D. H. HEARD and G. V. F. SEAMAN, The influence of pH and ionic strength on the electrokinetic stability of the human erythrocyte membrane. *J. Gen. Physiol.* **43,** 635–654 (1960).
2. A. H. MADDY, The chemical organization of the plasma membrane of animal cells. *Int. Rev. Cytol.* **20,** 1–65 (1966).
3. J. N. MEHRISHI, Molecular aspects of the mammalian cell surface. In *Progress in Biophysics and Molecular Biology* (J. A. V. Butler and D. Noble, Eds.), Vol. 25, p. 1. Pergamon, Oxford/New York, 1972.
4. M. H. REPACHOLI, Electrophoretic mobility of tumor cells exposed to ultrasound and ionizing radiation. *Nature (London)* **227,** 166–167 (1970).
5. C. SATO and K. KOJIMA, Change in electrophoretic mobility of cultured cells after X-irradiation and their modification by Sh-blocking agents and hemagglutinin. *Radiat. Res.* **60,** 506–515 (1974).
6. H. RINK and H.-J. MEYER-TESCHENDORF, Influence of X irradiation on the electrophoretic mobility of yeast cells. *Radiat. Res.* **72,** 317–324 (1977).
7. C. SATO and K. KOJIMA, Irreversible loss of negative surface charge and loss of colony-forming ability in Burkitt lymphoma cells after X-irradiation. *Exp. Cell Res.* **69,** 435–439 (1971).

8. C. SATO, K. KOJIMA, M. ONOZAWA, and T. MATSUZAWA, Relationship between recovery of cell surface charge and colony-forming ability following radiation damage in three cell-lines. *Int. J. Radiat. Biol.* **22,** 479–488 (1972).
9. C. SATO, K. KOJIMA, T. MATSUZAWA, and Y. HINUMA, Relationship between loss of negative charge on nuclear membrane and loss of colony-forming ability in X-irradiated cells. *Radiat. Res.* **62,** 250–257 (1975).
10. G. F. FUHRMANN and G. RUHENSTROTH-BAUER, Cell electrophoresis employing a rectangular measuring cuvette. In *Cell Electrophoresis* (E. J. Ambrose, Ed.), pp 22–25. Churchill, London, 1965.
11. C. SATO, K. KOJIMA, and K. NISHIZAWA, Target of X irradiation and dislocation of sialic acid in decrease of cell surface charge of erythrocytes. *Radiat. Res.* **69,** 367–374 (1977).
12. C. C. BRINTON, JR., and M. A. LAUFFER, The electrophoresis of viruses, bacteria, and cells, and the microscope method of electrophoresis. In *Electrophoresis. Theory, Methods, and Applications* (M. Bier, Ed.), pp. 427–492. Academic Press, New York, 1959.
13. C. SATO, K. KOJIMA, and K. NISHIZAWA, Translocation of hyaluronic acid in cell surface of cultured mammalian cells after X-irradiation and its recovery by added adenosine triphosphate. *Biochim. Biophys. Acta* **470,** 446–452 (1977).
14. D. A. HAYDON and G. V. F. SEAMAN, An estimation of the surface ionogenic groups of the human erythrocyte and of *Escherichia coli*. *Proc. R. Soc. London Ser. B* **156,** 533–549 (1962).
15. C. SATO, K. NISHIZAWA, and K. KOJIMA, Calcium-dependent process in reduction of cell surface charge after X-irradiation. *Int. J. Radiat. Biol.* **35,** 221–228 (1979).
16. T. ALPER, Low oxygen enhancement ratios for radiosensitive bacterial strains, and the probable interaction of two types of primary lesion. *Nature (London)* **217,** 862–863 (1968).
17. M. A. SHENOY, J. C. ASQUITH, G. E. ADAMS, B. D. MICHAEL, and M. E. WATTS, Time-resolved oxygen effects in irradiated bacteria and mammalian cells: A rapid-mix study. *Radiat. Res.* **62,** 498–512 (1975).
18. M. A. SHENOY, K. C. GEORGE, B. B. SINGH, and A. R. GOPAL-AYENGAR, Modification of radiation effects in single-cell systems by membrane-binding agents. *Int. J. Radiat. Biol.* **28,** 519–526 (1975).
19. D. K. MYERS and K. G. CHETTY, Effect of radiosensitizing agents on DNA strand breaks and their rapid repair during irradiation. *Radiat. Res.* **53,** 307–314 (1973).
20. W. A. CRAMP and P. E. BRYANT, The effects of rifampicin on electron- and neutron-irradiated *E. coli* B/r and B_{s-1}: Survival, DNA degradation and DNA synthesis by membrane fragments. *Int. J. Radiat. Biol.* **27,** 143–156 (1975).
21. S. YONEI, Modification of radiation effects on *E. coli* B/r and a radiosensitive mutant B_{s-1} by membrane-binding drugs. *Int. J. Radiat. Biol.* **36,** 547–551 (1979).
22. C. K. K. NAIR and D. S. PRADHAN, Effect of procaine hydrochloride on DNA repair in *Escherichia coli*. *Chem. Biol. Interact.* **11,** 173–178 (1975).
23. W. A. CRAMP, D. K. WATKINS, and J. COLLINS, Effects of ionizing radiation on bacterial DNA-membrane complexes. *Nature (New Biol.)* **235,** 76, 77 (1972).