Damages Caused by Irradiation in the Presence of NaCl in Macromolecular Components of *E. coli*

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Damages in macromolecular components and in their functions of *E. coli* produced by gamma irradiation in the presence of higher concentration of NaCl were determined with reference to the basic mechanism for NaCl radiosensitization. As the results, inhibitions of DNA repair, of DNA degradation, of macromolecular syntheses and of RNA species formation were found in DNA repair proficient strains. The number of radiation-induced DNA-strand breaks was not increased by the presence of 1 M NaCl during irradiation. It was suggested that inhibition of DNA repair may be of primary importance among these lesions for NaCl radiosensitization.

In a preceding paper,¹⁾ it was reported that the radiosensitivity of DNA-repair proficient strains of *E. coli* is increased by the presence of NaCl during irradiation. In order to account for the basic mechanism for NaCl-induced radiosensitization, a biochemical or biophysical approach has been attempted together with a radiation chemical study²⁾ in this laboratory. In respect to cellular damages caused by irradiation in the presence of 1 M NaCl, two types of lesions concerned with macromolecular components of *E. coli* B/r were observed,³⁾ i.e., damages in DNA strands and the inhibition of ribosomal subunit formation. However, the primary importance of those lesions in NaCl radiosensitization has not yet been confirmed. More detailed informations on cellular damages are required for elucidating the biochemical process leading to the enhancement of radiolethality by NaCl.

In the present paper, the results obtained in experiments to examine damages in macromolecular components of *E. coli* B/r cells irradiated in the presence of NaCl are described and the possible mechanism for NaCl radiosensitization is discussed.

MATERIALS AND METHODS

Bacterial strains and growth. Most studies were carried out with *E. coli* B/r provided by Dr. T. Alper

of the Hammersmith Hospital. In some experiments, *E. coli* K-12 P3478 (*polAI*) and its progenitor W3110 *thy*⁻ supplied by Dr. H. S. Kaplan of Stanford University were also used. Media and procedures for bacterial growth employed in this study have been described in the preceding paper.¹⁾

Irradiation. Irradiation was carried out by ⁶⁰Co-gamma rays. The range of dose rates was from 2.1 to 4.8 krad/min, unless otherwise specified.

Sedimentation experiments of DNA. The procedure of sedimentation experiments on DNA was essentially the same as the method described by McGrath and Williams⁴⁾ and Kaplan⁵⁾ with a slight modification. For labelling the cellular DNA of *E. coli* B/r, an overnight culture was diluted 20- to 40-fold into the MM-1 medium supplemented with 20 μ Ci/ml [methyl-³H]thymidine (Daiichi Pure Chemicals, 13.1 Ci/m mole), 200 μ g/ml deoxyadenosine and 100 μ g/ml Casamino Acid (Difco). Cultures were grown at 37°C for 2 to 3 generations. After chilling, the resulting labelled log-phase cells were harvested by centrifugation, washed twice and resuspended in 0.067 M phosphate buffer (pH 7.0, unless otherwise specified). From this master cell suspension, a set of four cell suspensions was prepared in chilled NaCl-free 0.067 M phosphate buffer, i.e., two suspensions of unirradiated cells presuspended for a period corresponding to the time of irradiation in NaCl-free and 1.0 M-NaCl-containing buffer and the other two suspensions of cells irradiated in NaCl-free and 1.0 M-NaCl-containing buffer. The irradiation dose was 28 krad. Immediately after irradiation or after the reincubation at 37°C in fresh MM-1, cells were collected by centrifugation, washed and resuspended in a

chilled solution containing 0.01 M Tris, 0.01 M NaCl and 0.01 M EDTA, pH 7.6 (TS-EDTA buffer) and subjected to the *n*-butanol treatment according to the method of Driedger and Grayston.⁶⁵ After washing with TS-EDTA buffer, cells were treated with 0.13 mg/ml lysozyme in the same buffer at 37°C for 10 min. Each 0.05 ml portion of the resulting protoplast suspension (containing 2×10^7 to 3×10^7 protoplasts) was gently mixed into 0.05 ml cap of 0.5 N NaOH layered on the top of a linear 4.2 ml 5~20% (w/v) sucrose gradient (pH 12.5) containing 0.01 M Tris and 0.01 M NaCl. After standing at room temperature for 30 to 50 min, gradients were then centrifuged at 30,000 rpm for 100 min at 20°C in a Hitachi 65P ultracentrifuge using an RPS 40T-2 rotor. Following centrifugation, the bottom of each tube was pierced and 10-drop fractions were collected onto Toyo filter paper disks. After drying, the disks were washed twice in chilled 10% trichloroacetic acid (TCA), once each in ethanol and ether, dried again, and assayed for TCA-insoluble radioactivity in a Beckman LS-150 liquid scintillation system. In later experiments with P3478 and W3110, the labelling medium was B2 broth containing 20 μ Ci/ml [methyl-³H]thymidine and sedimentation of DNA through alkaline gradients was performed by a procedure based on the method of Ginsberg and Webster⁷¹ and Town *et al.*⁸³ omitting the lysozyme treatment. In such cases, cells were lysed by layering 0.05 ml of cell suspension directly onto a 0.1 ml cap of 0.1% sodium lauryl sarcosinate in 0.5 N NaOH on the alkaline sucrose gradient mentioned above. In sedimentation experiments after the reincubation in the B2 broth, the cap solution for cell lysis was supplemented with 0.01 M EDTA.⁸³ The number-average molecular weight of DNA and number of single-strand breaks per gram DNA were calculated from the sedimentation data according to the method previously described using a molecular-weight marker of T4-phage DNA.¹⁰³

DNA degradation experiments. Log-phase cells of B/r labelled with ³H-thymidine in the MM-1 as mentioned above were reincubated in the MM-1 containing 2 μ g/ml nonradioactive thymidine at 37°C for 20 min. Cells were collected and then a set of four cell suspensions was prepared as described in the paragraph of sedimentation experiments. The dose of irradiation was 28 krad. Immediately after irradiation cells were harvested and resuspended again in the chilled MM-1 containing 2 μ g/ml nonradioactive thymidine at a cell concentration of about 5×10^7 cells/ml. Following the first sampling in the cold, the incubation at 37°C was started with gentle shaking. At intervals, 0.1-ml samples were taken from each sample and spotted onto paper disk for counting radioactivity. DNA degradation experiments with P3478 and W3110 were similarly conducted except an irradiation dose of 70 krad.

Measurements of macromolecular syntheses. The syntheses of DNA, RNA and protein were studied by determining the incorporation of radioactive thymidine, uridine and amino acids, respectively, into TCA-insoluble materials. A set of four cell suspensions was prepared at a cell concentration of about 2×10^7 cells/ml as mentioned above. The radiation dose was 9.8 krad. Then, cells were collected by centrifugation, washed twice with phosphate buffer and resuspended in fresh MM-1 to the same cell concentration of 1×10^8 cells/ml by measuring OD₆₈₀. Four tenths ml of each cell suspension were added to thymidine, 400 μ g deoxyadenosine (Sigma), 200 μ g Casamino Acids (Difco) for thymidine incorporation; 2 μ Ci ³H-uridine(G) (The Radiochemical Center, 6.0 Ci/mmmole), 16 μ g nonradioactive uridine and 200 μ g Casamino Acids for uridine incorporation; and 1 μ Ci[U-¹⁴C] protein hydrolysate (The Radiochemical Center, 57 mCi/mAtom) and 200 μ g Casamino Acids for amino acid incorporation. Each reaction tube containing 2 ml of incubation mixture was transferred to 37°C and incubated with shaking for 3 to 4 hr. In an experiment to determine the initial effect during the first 20 min of reincubation, irradiation was done with doses of 1.2 and 2.8 krad (dose rate: 468 rad/min), and the cell concentration of the incubation mixture was 5.5×10^8 cells/ml. At different times of the incubation, each 0.1 ml sample was pipetted onto a paper disk, added with a drop of 2% formaldehyde and rapidly dried. After drying, the disks were assayed for TCA-insoluble radioactivity as described above. The zero-time sample was taken immediately after the addition of the cell suspension from the reaction tube held in ice-bath.

Determination of sedimentation profiles of ribosomal subunits and RNA. Log-phase cells of B/r grown to about 1×10^8 cells/ml in MM-1 were harvested and a set of four cell suspensions mentioned above was prepared at 2×10^8 cells/ml. The volume of each suspension was 250 ml and irradiation doses were ranging from 5.2 to 20.9 krad, as shown in Fig. 6. After irradiation, cells were collected, washed and resuspended in 100 ml of MM-1, and reincubated at 37°C. After 15 min, cells were pulse-labelled with ³H-uridine (0.25 μ Ci/ml, 0.25 μ g/ml) for 1 min, followed by a 15-min chase with nonradioactive uridine (250 μ g/ml) at the same temperature. Then, cultures were poured onto ice, centrifuged at 8000 rpm for 10 min. Cell pellets were washed twice with 0.01 M Tris (pH 7.4) containing 0.1 mM Mg acetate and then frozen. After addition of the equal volume of quartz powder, frozen cell pellets were carefully ground for 3 min in the cold, extracted with 1.5 ml of 0.01 M Tris (pH 7.4) containing 0.1 mM Mg acetate and 3 μ g/ml DNase. Crude extracts were spun twice for 10 min at 10,000 rpm in the cold. Each 0.2-ml portion of cell extracts obtained in this way was applied to the

top of a 4.2-ml linear 5~20% (w/v) sucrose gradient made in 0.01 M Tris buffer containing 0.1 mM Mg acetate (pH 7.4). Gradients were centrifuged at 36,000 rpm for 180 min at 2°C in an RPS 40T-2 rotor in a Hitachi 65P ultracentrifuge. After the run, tubes were pierced at the bottom and 10-drop fractions were collected. These fractions were diluted to 1.0 ml with 0.01 M Tris buffer (pH 7.4) containing 0.1 mM Mg acetate. One tenth-ml portions of these diluents were pipetted onto paper disks for the assay of TCA-insoluble radioactivity according to the procedure described in DNA sedimentation experiments, and the remaining portions were used for the measurement of the optical density at 260 nm. For the analysis of RNA, 0.4 ml of cell extracts were added with 0.04 ml of 10% sodium dodecyl sulfate solution made in 0.01 M Tris and the mixtures were incubated at 37°C for 10 min.^{11,12} Each 0.1-ml portion of the preparations was laid on the top of a 4.2-ml gradient of the same composition as that used for the cell extract experiment and centrifuged at 36,000 rpm for 300 min at 2°C. Fractionation and determination of TCA-insoluble radioactivity were the same as described in a paragraph of DNA sedimentation experiments.

RESULTS

Production of DNA strand breaks by irradiation and their repair during reincubation

Sedimentation profiles of DNA from log-

phase cells of strain B/r were determined in alkaline sucrose gradients immediately after irradiation in the presence and absence of 1 M NaCl as well as after the reincubation at 37°C for 40 min in MM-1 medium. As can be seen in Fig. 1, the DNA peak fraction after irradiation in the presence of NaCl was found nearer to the top of the gradient than that after irradiation in the absence of the salt, *i.e.*, in the former case the DNA appeared to contain more single-strand breaks. However, there would be a possibility that DNA breaks in cells of this repair-proficient strain produced by irradiation in the absence of NaCl has been rapidly repaired to some extent in buffer even at 0°C, as described by Town *et al.*,^{8,9} judging from the broader peak in the DNA profile immediately after irradiation (Fig. 1 curve C) and irradiation temperature (5~10°C). Thus, in order to assess this possibility, the determination of DNA profiles immediately after irradiation was tried again with a polymerase-deficient strain P3478. Log-phase cells of this strain were irradiated with 14.3 krad in 0.067M phosphate buffer, pH 8.0, and at 0°C.⁸ The DNA sedimentation profiles obtained with

FIG. 1. Effects of Irradiation in the Presence of 1 M NaCl on Alkaline Sucrose Gradient Sedimentation Profiles of DNA from *E. coli* B/r Cells.

Symbols: A, unirradiated, untreated with NaCl; B and C, irradiated in NaCl-free phosphate buffer, no incubation and 40-min reincubation, respectively; D and E, irradiated in 1 M-NaCl-containing phosphate buffer, no incubation and 40-min reincubation, respectively.

Dose of irradiation: 28 krad.

FIG. 2. Alkaline Sucrose Gradient Sedimentation Profiles of DNA from *E. coli* K-12 P3478 and W3110 Cells.

Symbols: A, unirradiated, untreated with NaCl; B, unirradiated, treated with 1 M NaCl; C, irradiated in NaCl-free buffer; D, irradiated in 1 M-NaCl-containing buffer.

Irradiation: With 14.3 krad at 0°C in 0.067 M phosphate buffer (pH 8.0).

Arrows indicate region used to determine number-average mol wt.

TABLE I. ANALYSIS OF ALKALINE SUCROSE GRADIENT SEDIMENTATION DATA ON DNA FROM CELLS OF *E. coli* K-12 P3478 AND W3110^a

Cell sample	Number-average molecular weight ($\times 10^7 D$)	Numbers of single breaks per gram DNA ($\times 10^{16}$)	Efficiency for single breaks (eV/single breaks)
Strain P3478			
Unirradiated, in NaCl-free buffer	9.10		
Unirradiated, in 1 M-NaCl buffer	9.21		
Irradiated with 14.3 krad in NaCl-free buffer	2.54	1.64	52
Irradiated with 14.3 krad in 1 M-NaCl buffer	2.98	1.37	65
Strain W3110			
Unirradiated, in NaCl-free buffer	12.1		
Unirradiated, in 1 M-NaCl buffer	(12.1) ^b		
Irradiated with 14.3 krad in NaCl-free buffer	3.34	1.30	69
Irradiated with 14.3 krad in 1 M-NaCl buffer	3.74	1.11	80

^a Data taken from Fig. 2.

^b Postulated to be the same as the value of cells unirradiated and untreated with NaCl.

P3478 are shown in Fig. 2, and number of radiation-induced single breaks per gram DNA and the efficiencies for single-strand breakage calculated from these data are listed in Table I. For comparison, the similar data from the sedimentation analysis with W3110 are also incorporated into Fig. 2 and Table I. By the treatment with 1 M NaCl, no significant change in DNA profile took place. An efficiency of 52 eV/single break for irradiation in the absence of NaCl calculated in this study is

in fairly good agreement with values of 59 eV/break⁽⁸⁾ and 61 eV/break⁽¹³⁾ reported by Town *et al.*, which have been obtained under the similar irradiation condition. Since the rapid buffer repair system appears to be absent in P3478, the modification of the above value of the efficiency by the DNA repair during the determination would be not significant, if any. It can be also mentioned that the initial yield of single breaks is somewhat lower in the presence of NaCl than in its absence (Fig. 2

and Table I). Thus, under the more careful experimental condition to suppress the rapid buffer repair, *i.e.*, at 0°C and in phosphate buffer, pH 8.0, it is expected to observe the same sequence of DNA peak in sedimentation profiles of the repair proficient strain. This was the case with W3110 as shown in Fig. 2 and Table I. Accordingly, the observation in Fig. 1 to show the reverse order of DNA peaks is interpreted that the radiation-damaged DNA is restored after irradiation in the absence of NaCl by the rapid buffer repair system, while it does not occur at all after irradiation in its presence. This view was evidenced by a similar observation made with a revertant from P3478 to the prototroph (data not shown). Another obvious effect of NaCl present during irradiation shown in Fig. 1 was the inhibition of the restoration of the sedimentation rate of DNA during postirradiation incubation in MM-1 at 37°C, in contrast to the complete restoration after irradiation in the absence of NaCl. Since the DNA peak position remained almost unchanged after 40 min of post irradiation incubation, it was suggested that further degradation of DNA in B/r, especially by endonuclease did not take place during reincubation following to irradiation in the presence of 1 M NaCl. Figure 3 shows the yield of DNA double-strand breaks was unaffected by the presence of 1 M NaCl during irradiation. From these results, it is concluded that (1) the presence of 1 M NaCl during irradiation does not increase the single- and double-strand breaks *in vivo*, but rather reduces the yield of single breaks, and that (2) in respect the DNA repair, both of the rapid buffer repair process requiring DNA polymerase I and the slow repair system dependent upon the growth medium are strongly inhibited after irradiation in the presence of 1 M NaCl. However, this irradiation condition appears not to inhibit the ultrafast DNA repair in buffer, since the DNA peak fraction after irradiation in the presence of 1 M NaCl under the condition inhibiting rapid buffer repair was found to shift toward the little bigger size of DNA

FIG. 3. Neutral Sucrose Gradient Sedimentation Profiles of DNA from *E. coli* B/r Cells.

Symbols: A, unirradiated; B, irradiated with 28 krad no incubation; C, irradiated with 28 krad and reincubated for 40 min at 37°C. Cell suspensions were prepared in NaCl-free phosphate buffer (○—○) and in 1M-NaCl-containing phosphate buffer (×—×).

as compared with that after mild heating and subsequent irradiation.

DNA degradation during postirradiation incubation

The effect of the presence of NaCl during irradiation on DNA degradation in B/r during postirradiation incubation is shown in Fig. 4a. All incubation mixtures were not added with 1 M NaCl. In the case of no pretreatment with NaCl, decrease in TAC-insoluble radioactivity was not observed with unirradiated cells, while about 25% loss was seen after 120 min of reincubation from cells irradiated with 28 krad. On the other hand, the different kinetics of DNA degradation was found when cells treated with NaCl. The treatment with

FIG. 4. DNA Degradation during Postirradiation Incubation in *E. coli* Cells.

Strains and doses of irradiation: (a), B/r, 28 krad; (b), P3478, 70 krad; (c), W3110, 70 krad.

Symbols: A~D, see the legend of Fig. 2.

1 M NaCl prior to postirradiation incubation* induced some DNA degradation even if cells were not irradiated, but irradiation with 28 krad in the presence of NaCl did not enhance DNA degradation during postirradiation incubation. Thus, the time course curve of the loss of TCA-insoluble radioactivity from cells irradiated with 1 M NaCl is identical with that for NaCl-treated and unirradiated cells (Fig. 4a). The same effect of NaCl on DNA degradation was found with a *pol*⁻ mutant P3478 as well as its progenitor W3110 (Fig. 4b and c). As will be described elsewhere, NaCl present during irradiation enhanced the radiolethality in the latter strain similarly to B/r, but not in the former strain. Subsequently, it is concluded that there is no correlation between DNA degradation and NaCl radiosensitization.

Macromolecular syntheses

The kinetics of DNA, RNA and protein syntheses by log-phase cells of B/r treated with four different procedures as described in METHOD AND MATERIALS were measured. Figure 5 shows that the macromolecular syntheses were completely inhibited for 240

min after 8 krad in the presence of 1 M NaCl, while it proceeded in cultures of cells unirradiated or irradiated with the same dose in the absence of NaCl, although it was delayed to some extent by irradiation and the treatment with NaCl. In another experiment made at a lower NaCl concentration, macromolecular syntheses by cells irradiated with 9.8 krad in the presence of 0.2 M NaCl took place at the same time after about 150 min of incubation. However, some difference in initial effect of the presence of 1 M NaCl during irradiation with 1.2 and 2.8 krad on macromolecular syntheses was observed with the extent of inhibition. As indicated in Table II, protein synthesis was most severely inhibited as compared with DNA and RNA syntheses after 20 min of incubation.

Sedimentation properties of ribosomal subunits and their RNA

In a previous study,¹⁾ sedimentation profiles of ribosomal subunits in *E. coli* B/r demonstrated that after irradiation with 28 krad the formation of 50S and 30S subunits is severely inhibited by irradiation in the presence of 1 M NaCl, while it is unaffected by irradiation in its absence. Immediately after irradiation in the presence of 1 M NaCl, no formation of these

* NaCl was removed from cell suspensions by centrifugation just before postirradiation incubation.

FIG. 5. Syntheses of DNA (a), RNA (b), and Protein (c) in *E. coli* B/r Cells during Postirradiation Incubation.

Symbols: A~D, see the legend of Fig. 2.

Dose of irradiation: 8.0 krad.

TABLE II. MACROMOLECULAR SYNTHESES IN *E. coli* B/r CELLS DURING THE FIRST 20 MIN OF POSTIRRADIATION INCUBATION

Samples	Dose of irradiation (krad)	Relative radioactivity incorporated into TCA-insoluble fraction (%)		
		DNA	RNA	Protein
Control-1 (unirradiated, in NaCl-free buffer)	0	100 ^a	100	100
Control-2 (unirradiated, in 1 M-NaCl buffer)	0	24.5 (100) ^b	13.7 (100)	26.3 (100)
Irradiated in NaCl-free buffer	1.2	100	159	96.3
	2.8	91.2	139	93.4
Irradiation in 1 M-NaCl buffer	1.2	22.4 (91.4)	14.7 (107)	19.2 (73.0)
	2.8	10.2 (41.7)	7.15 (52.1)	6.25 (23.8)

^a % Control-1 of cpm value in TCA-insoluble fraction.

^b % Control-2 of cpm value in TCA-insoluble fraction.

subunits was found and after 15 min of postirradiation incubation the formation of 50S subunits was greatly delayed. In this study, with the more careful experimental procedure to minimize the dissociation of 50S by the NaCl treatment, the determinations of the dose effect on the syntheses of RNA species were made at 15 min after irradiation in the presence and absence of 1 M NaCl. Sedimentation profiles obtained are shown in Figs. 6 and 7. In cells 1-min pulse labelled with ³H-uridine at a time of 15-min postirradiation incubation and then chased for 15 min, the ³H radioactivity incorporated into ribosomal sub-

units and ribosomal RNA clearly decreased with increasing doses of irradiation in the presence of 1 M NaCl, but it was almost unchanged with cells irradiated in its absence. Under the experimental condition in the present study, some delay of the formation of 50S subunits was observed after irradiation in the presence of 1 M NaCl as reported in cases of irradiation either in the absence¹⁴⁾ or presence³⁾ of 1 M NaCl. Values of cpm and specific radioactivity (cpm per unit OD₂₆₀) of peaks of 50S and 30S subunits once increased at 5.2 krad and then remarkably decreased with increasing doses, a little more rapidly in

50S peak than in 30S peak. Besides 50S and 30S subparticles, some amounts of ribosomal subunits having smaller sedimentation values were detected in extracts from cells irradiated with 5.2 and 10.4 krad in the presence of 1 M NaCl. But such subunits in the abnormal size were not found at higher doses, where the formation of 50S and 30S subunits was greatly inhibited. On the other hand, the syntheses of

rRNAs appeared to proceed with normal molecular sizes, 23S and 16S. In comparison with cell survival (Fig. 6a), it was noted that with 10.4 krad or high doses, which clearly enhance the radiolethality in the presence of NaCl, the inhibition of 50S and 30S formation as well as rRNA synthesis became severer in parallel with the decrease in surviving fraction (Figs. 6 and 7). According to the data pre-

FIG. 6. Sedimentation Profiles of Cell Extracts from *E. coli* B/r Cells Pulse-labelled with ^3H -Uridine and Survival Curves.

Symbols: A, unirradiated, no treatment with NaCl; B, irradiated in the absence of NaCl with 20.9 krad; D~G, irradiated in the presence of 1 M NaCl with 5.2, 10.4, 15.7 and 20.9 krad, respectively.

Cell survival: open circle, in NaCl-free; closed circle, in 1 M-NaCl-containing buffer.

FIG. 7. Sedimentation Profiles of RNA Isolated from the Extracts of *E. coli* B/r. Symbols: A~F, corresponding to each shown in the legend of Fig. 6.

TABLE III. RADIOACTIVITY IN RIBOSOMAL SUBUNITS^a

Samples No. (symbols in Fig. 6)	Dose of irradiation (krad)	cpm at peak of		Specific activity (cpm/OD ₂₆₀ /cm) of	
		50S	30S	50S	30S
Suspended in NaCl-free buffer					
1 (A)	0	7938	5200	6963	6551
2 (B)	20.9	7440	5613	7294	7796
Suspended in 1 M-NaCl buffer					
3 (C)	0	7801	5151	7092	6698
4 (D)	5.2	10933	7372	14017	11702
5 (E)	10.4	4144	2423	9938	8843
6 (F)	15.7	203	244	541	904
7 (G)	20.9	149	124	382	428

^a The values in this table were read and calculated from data shown in Fig. 6.

sented by Fujita and Miyakoshi,¹⁴⁾ decrease in the peak heights of both 23S and 16S RNAs was not more than 50% of the value of the unirradiated control when cells were irradiated with 200 krad in the absence of NaCl. In this study, no significant difference in RNA-peak height was found between extracts from the

unirradiated control and after 20.9 krad under the same condition. On the other hand, the peak height of 23S RNA was reduced to 2.8% of the control value by irradiation in the presence of 1 M NaCl. In the case of 16S RNA it decreased to 3.5%. Thus, it is noted that the inhibition of rRNA synthesis was more remarkable when cells were irradiated in the presence of NaCl than in its absence.

DISCUSSION

The present study has demonstrated several inhibitory effects of irradiation in the presence of NaCl on cellular functions associated with critical macromolecular components, such as the prevention of DNA repair, DNA degradation, macromolecular syntheses and RNA species formation. Among these alterations, the inhibition of DNA degradation after irradiation in the presence of NaCl was not involved in the primary mechanism for NaCl

radiosensitization, since this effect is observed not only with DNA-repair proficient strains B/r and W3110 but also with a *pol*⁻ mutant P3478 which is not sensitized by the presence of NaCl during irradiation, as described in the preceding paper.¹⁾ In respect to the inhibition of RNA-species formation, no accumulation of rRNA smaller than the normal 23S and 16S RNAs was found in cells irradiated in the presence of NaCl, while the amount of rRNA synthesized was remarkably reduced with increasing doses in the high dose region. These findings can be interpreted by a view that, in cells irradiated in the presence of NaCl, RNA syntheses is initiated only when damages on template DNA are less than a certain limit. The same view was described by Fujita and Miyakoshi¹⁴⁾ on the mechanism by which DNA damages produced on *in vivo* irradiation in the absence of NaCl brought about the inhibition of syntheses of RNA in *E. coli* cells. In addition, RNA and protein syntheses were not found to start before the initiation of DNA synthesis. Since the normal and repair DNA replication in irradiated cells are considered to be in competition,¹⁵⁾ it is plausible that the inhibition of DNA repair is of primary importance in the mechanism for NaCl radiosensitization. This view is supported by the following observations reported in the preceding paper.¹⁾ Some DNA-repair deficient mutants were not sensitive to the action of NaCl, as seen with *pol*⁻ and *rec*⁻ strains as well as Bs-1. The effects of chloramphenicol and actinomycin D to inhibit protein and DNA-dependent RNA syntheses added during reincubation following irradiation in the presence of NaCl were almost independent of radiation doses, suggesting that the inhibition of these macromolecular syntheses may be not involved in the primary process of NaCl radiosensitization.

As shown in Fig. 1, the rejoining of DNA-strand breakage during postirradiation incubation was profoundly inhibited by the presence of NaCl during irradiation. In this figure it can be seen that rapid buffer repair and growth medium-dependent repair are blocked after

such irradiation. The ultrafast DNA repair appears not to be inhibited by irradiation in the presence of NaCl, since the DNA from cells irradiated in the presence of NaCl sediments somewhat faster than that from cells subjected to mild heating, followed by irradiation in the absence of the salt with the same dose (data not shown). Inhibition of DNA repair may result in the inhibition of the concomitant syntheses of RNA species and proteins, eventually leading to the inhibition of cell growth. However, it has been reported that RNA and protein syntheses may be involved in the DNA repair process.^{16,17)} Subsequently, it would be also likely that damages in rRNA and protein synthesizing units caused by irradiation in the presence of NaCl, which have been observed in this study, bring about the inhibition of DNA repair. Another important aspect of the study on NaCl radiosensitization is its radiation chemistry which will be discussed elsewhere.

In contrast to the results obtained in this study, it was previously mentioned that lesion caused by irradiation in the presence of NaCl increases DNA-strand breakage, while the rejoining of broken pieces of DNA is not inhibited.³⁾ These contradictory observations were due to the involvement of rapid buffer repair in DNA sedimentation analysis and to the too high cell concentration at the time of irradiation. It has been noted that irradiation in NaCl-radiosensitization study should be done with cell-concentrations of about 5×10^8 cells/ml or lower, since cellular damages are significantly diminished with the higher cell concentrations during irradiation in the presence of NaCl. The recent findings obtained by the more careful sedimentation procedure and irradiation at cell concentration lower than 2×10^8 cells/ml have reversed our previous observations as mentioned in this study.

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