On the Mechanism for Radiosensitization of Microorganisms by Sodium Chloride with Special Reference to Their DNA Repair Capacity

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Some biological aspects of radiosensitization of microorganisms by NaCl at comparatively higher concentrations were studied. Among different strains of bacteria and yeasts, the radioresistant vegetative form was particularly sensitive to NaCl present during irradiations. With *E. coli*, such radiosensitizing effect of NaCl was abolished by mutations at the *pol* and *rec* loci or by the addition of certain solutes at the time of irradiation. The effective extracellular chloride concentrations for *E. coli* B/r were not less than 0.1 M. When actinomycin D and chloramphenicol were added during reincubation of *E. coli* B/r following irradiation in the presence of NaCl, the radiation lethal effect was enhanced, the extent of which was almost independent of doses of irradiation. From these results together with the previous data, the basic mechanism for NaCl radiosensitization was discussed with special references to a possible role of Cl_2^- radical anions as attacking species and to the inhibition of *pol*- and *rec*-dependent DNA repair systems as the primary cellular damage.

It has been reported that the presence of NaCl at comparatively high concentrations during irradiation results in the enhancement of the radiation lethal effect on certain bacterial and yeast cells.^{1,2)} Concerning the radiation chemical basis of this NaCl radiosensitization, it was suggested that short-lived intermediates radiation-produced from Cl^- ions such as Cl atoms and/or Cl_2^- radical anions might be the attacking species.^{2~3)}

Other halide ions were also found to sensitize bacterial cells when irradiated in their presence, although there seemed to be some difference in mode of action as compared with Cl^- ions.²⁾ Similarly, bacterial spores and bacteriophages suspended in water or buffer were found to be sensitized to radiations by the presence of Br^- ions during irradiation, and Br_2^- radical anions formed by the reaction of OH with Br^- were implicated as damaging species.^{5~7)} Closely relating to these radiation chemical studies, biological and biochemical approaches have been made in this laboratory to draw out the whole picture of the mechanism for the radiosensitization in the presence of Cl^- ions during irradiation. One of trials along these lines is to search after the experi-

mental conditions which reduce or remove the radiosensitizing effect of NaCl added during irradiation.

In the present paper, data on the removal of NaCl radiosensitization by mutations in DNA-repair controlling genes and by the addition of certain solutes at the time of irradiation will be described together with some other biological determinations at the cellular level. The mechanism for this sensitization will also be discussed.

MATERIALS AND METHODS

Strains and culture conditions. Strains and media used in an experiment to examine the radiosensitizing effect of NaCl on different microorganisms were as follows: *E. coli* strains B, B/r, B_{s-1} and B_{s-2} (B2 broth containing 1% Kyokuto beef extract, 1% Daigo Polypepton and 0.5% NaCl at pH 7.0), *E. coli* WP2 (*try*⁻) (Haas and Doudney minimal medium supplemented with 10 mg/liter L-tryptophan),⁸⁾ *Pseudomonas fluorescens* B3-1 (BBL trypticase-soy broth), *Micrococcus radiodurans* R₁ (B broth containing 1% beef extract, 1% Polypepton, 0.5% yeast extract and 0.2% glucose at pH 7.0),⁹⁾ *Streptococcus faecium* R53 (Difco heart infusion broth),¹⁰⁾ *M. radiophilus* RBD and *Micrococcus* sp. B12 (TGY medium),^{11,12)} *Tetracoccus soyae* F-8 (1% Polypepton, 1% beef extract and 2% NaCl

at pH 7.0),¹³⁾ *Bacillus subtilis* Marburg (Schaeffer's medium),¹⁴⁾ *B. subtilis* PCI 219 (0.5% yeast extract, 0.3% Polypepton, 0.1% glucose and 0.1% NaCl at pH 7.0),¹⁵⁾ *Penicillium chrysogenum* Thom Q-176 and *Aspergillus oryzae* IFO-4296 (Czapeck's medium at pH 6.0)¹³⁾ and *Zygosaccharomyces soya* ZS-5 and *Saccharomyces cerevisiae* (YEPD medium containing 1% yeast extract, 4% Polypepton, 4% glucose at pH 5.8).¹⁾

Cells were grown to the log phase at 30° or 37°C with shaking, harvested, washed with 0.067 M phosphate buffer at pH 7.0 for bacterial strains and at pH 6.0 for yeast and resuspended in the same buffer. Spores of *B. subtilis* were harvested from a 2-day-old culture grown at 37°C, purified by the SDS-lysozyme treatment,¹⁴⁾ and resuspended in distilled water. Fungal spores were collected from the 1-week-old culture on broth agar slant and the suspensions made in 0.067 M phosphate buffer at pH 6.0 were subjected to irradiation after filtration.

In order to investigate the effects of mutations affecting DNA repair on NaCl radiosensitization, the following derivatives of *E. coli* K-12 were employed: P3478 (*polA1*), W3110 (*pol+*), AB2500 (*uvrA6*), AB2487 (*recA13*), AB2480 (*recA13 uvrA6*), AB2499 (*uvrB5*) and AB2498 (*uvrC34*) (kindly provided by Prof. H. S. Kaplan and Dr. J. C. Miller); AB1899 (*lon*⁻) and AB1157 (*pol+ rec+*) (by Dr. H. I. Adler); AB1886 (*uvrA6*) (by Prof. P. Haward-Flanders); AB2470

(*recB21*), JC1553 (*recA1*) and NH4033 (*recC22*) (by Dr. M. Oishi). These strains were incubated in B2 broth at 37°C with shaking. If necessary, 5 µg/ml thymine was supplemented. Log-phase cells harvested by centrifugation were suspended in 0.067 M phosphate buffer at pH 7.0 to be subjected to irradiation.

Irradiation and determination of survivors. Cell suspensions were irradiated by ⁶⁰Co gamma rays with air bubbling. Dose rate was about 2.1 krad/min for most strains, 490 rad/min for radiosensitive mutants of *E. coli* and 4.9 krad/min for extremely radioresistant strains such as *M. radiodurans*. Cell concentrations of the suspensions were less than 5 × 10⁸ cells/ml. After irradiation cell survival was determined by counting the number of visible colonies developed on nutrient agar plates.

Reagents. Amino acids and chloramphenicol were kindly provided by Ajinomoto Co., Ltd. and Sankyo Co., Ltd., respectively. Actinomycin D was purchased from Sigma Chemical Co.

RESULTS

Effects of 1 M NaCl added during irradiation on radiosensitivities of different microorganisms

The results of experiments to examine radio-

TABLE I. RADIOSENSITIZING EFFECTS OF NaCl ADDED DURING IRRADIATION ON DIFFERENT MICROORGANISMS

Strain	Temp. of incubation (°C)	LD ₁₀ ^a (krad)	Radiosensitizing effect of 1 M NaCl ^b	Source ^c
<i>E. coli</i> B/r	37	29.5	††	T. Alper
<i>E. coli</i> B	37	10.3	+	T. Alper
<i>E. coli</i> B _{s-1}	37	4.2	—	R. F. Hill
<i>E. coli</i> B _{s-2}	37	4.4	—	R. F. Hill
<i>E. coli</i> 2-7 (IAM1182)	37	15.3	††	IAM
<i>E. coli</i> WP2 try ⁻	37	12.6	+	B. A. Bridges
<i>Pseudomonas fluorescens</i> B3-1	30	5.7	±	H. Ito
<i>Micrococcus radiodurans</i> R ₁	30	965	††	A. W. Anderson
<i>Micrococcus radiophilus</i> RBD	30	665	††	N. F. Lewis
<i>Micrococcus</i> sp. B12	30	960	††	This laboratory
<i>Streptococcus faecium</i> R53	30	170	††	C. F. Niven (Jr.)
<i>Tetracoccus soyae</i> F-8	30	42	+	S. Omata
<i>B. subtilis</i> marburg 168	37 (vegetative cells)	13.4	+	This laboratory
"	(spores)	184	—	"
<i>B. subtilis</i> PCI219	37 (spores)	250	—	IAM
<i>Penicillium chrysogenum</i> Thom Q-176	30 (spores)	40	—	G. Nakamura
<i>Aspergillus oryzae</i> IFO-4296	30 (spores)	34	—	G. Nakamura
<i>Zygosaccharomyces soya</i> ZS-5	30	90	††	IAM
<i>Saccharomyces cerevisiae</i> H-336	30	300	††	IAM

^a Determined in the absence of NaCl.

^b ††, very effective; +, effective; —, ineffective.

^c The authors acknowledge the kind supplies strains by the investigators listed in the Table.

FIG. 1. Survival of Different Bacterial Cells Exposed to ^{60}Co Gamma Rays in the Presence (closed symbols) and Absence (open symbols) of 1 M NaCl.

A, *E. coli* B/r; B, *E. coli* B₈₋₁; C, *Micrococcus radiodurans* R₁; D, *Streptococcus faecium* R53; E, *Tetracoccus soyae* F-8; F, *Bacillus subtilis* Marburg 168 (real line, vegetative cells; broken line, spores).

sensitizing effects of 1 M NaCl with different microorganisms are summarized in Table I. Some examples of survival curves in the presence and absence of 1 M NaCl are illustrated in Fig. 1. The radiosensitizing effect of NaCl was observed with the vegetative form, but not with bacterial and fungal spores. In a case of *B. subtilis* Marburg 168, log-phase cells were sensitive to the radiosensitizing effect of NaCl, although not so remarkable perhaps because of the chain formation by this strain. Spores were insensitive to the effect of NaCl (Fig. 1F). Of vegetative bacteria, remarkable radiosensitizing effect of NaCl was observed with the extremely radioresistant group such as *M. radiodurans* R₁,¹⁶⁾ *Strep. faecium* R53,¹⁰⁾ *M. radiophilus* RBD¹¹⁾ and *Micrococcus* sp. B12,¹²⁾ where the characteristic shoulder of their survival curves was greatly diminished. Among *E. coli* strains, B/r and 2-7 (IAM1182) which are comparatively resistant to radiation increase their radiosensitivities in the presence of 1 M NaCl. In contrast, radiosensitive strains B₈₋₁ and B₈₋₂ were not sensitized by the presence of NaCl. With *M. radiodurans*,^{17,18)} *Micrococcus* sp. B12¹²⁾ and *E. coli* B/r,¹⁹⁾ it was

evidenced by the sedimentation analysis that these bacterial strains are endowed with the efficient DNA repair capacity. Thus, it is concluded that the effect of NaCl radiosensitization is confined to DNA-repair proficient strains in the vegetative form. It is also noted that a halophilic bacterium, *Tetracoccus soyae* F-8, is sensitized by the presence of 1 M NaCl during irradiation (Fig. 1E).

Effect of mutations of genes governing the DNA repair on NaCl radiosensitization

As mentioned above, NaCl radiosensitization was observed with DNA-repair proficient strains. Subsequently, it would be expected that some DNA-repair-deficient mutants are insensitive to NaCl radiosensitization. Survival curves of *pol*, *rec* and *uvr* mutants mostly derived from *E. coli* K-12 and their progenitor strains were determined in the presence and absence of 1 M NaCl. A *lon*⁻ strain AB1899 was also tested. The results obtained are shown in Fig. 2. Progenitor strains AB1157 and W3110 were remarkably sensitized by the presence of NaCl. Strains AB2500 (*uvrA6*), AB1886 (*uvrA6*) (data not shown), AB2499

FIG. 2. Survival Curves of *E. coli* K-12 Derivatives in the Presence (closed symbols) and Absence (open symbols) of 1 M NaCl.

A, W3110 (*pol*⁺ *rec*⁺ *uvr*⁺); B, AB1157 (*pol*⁺ *rec*⁺ *uvr*⁺); C, AB2500 (*uvrA6*); D, AB2499 (*uvrB5*); E, AB2498 (*uvrC34*); F, AB1899 (*lon*⁻); G, P3478 (*polA1*); H, AB2487 (*recA13*); I, JC1553 (*recA1*); J, AB2470 (*recB21*); K, NH4033 (*recC22*); L, AB2480 (*recA13 uvrA6*).

(*uvrB5*) and AB2498 (*uvrC34*) as well as AB1899 (*lon*⁻) were sensitized to some extent. In contrast, radiosensitivities of P3478 (*polA1*), AB2487 (*recA13*), JC1553 (*recA1*), AB2470 (*recB21*), NH4033 (*recC22*) and AB2480 (*recA13 uvrA6*) were not affected at all by the addition of 1 M NaCl during irradiation. These facts indicate that the mechanism for NaCl radiosensitization involves the inhibition of DNA-repair process controlled by *pol* and *rec* genes.

Effects of solutes added during irradiation on NaCl radiosensitization

If the solute in test would react with Cl₂⁻ and/or Cl atoms which are assumed to be the attaching species, it would be expected to protect bacterial cells against the radiosensitizing action of NaCl. Effects of various solutes on dose multiplying factors (DMF) of 1 M NaCl for 1% survival were determined with *E. coli* B/r. The results obtained are indicated in Table II. An example of survival curves determined with the effect of L-tryptophan is shown in Fig. 3.

Under the experimental condition of this study, the DMF value of 1 M NaCl in the absence of the solute varied from run to run

TABLE II. EFFECTS OF SOLUTES ADDED DURING IRRADIATION ON NaCl RADIOSENSITIZATION OF *E. coli* B/r

Solute	Concentration (M)	DMF of 1 M NaCl ^a		Relative DMF ^b
		in the presence of solute	in the absence of solute	
Cystamine	6.7×10^{-5}	1.77	6.29	0.28
	1×10^{-2}	1.39	6.29	0.22
2-Mercaptoethanol	6.7×10^{-5}	2.14	8.41	0.25
L-Tryptophan	6.7×10^{-5}	2.63	7.24	0.36
	1×10^{-3}	1.73	7.24	0.24
L-Histidine	6.7×10^{-5}	2.63	7.24	0.36
	1×10^{-3}	1.65	7.24	0.23
Glycine	6.7×10^{-5}	3.31	6.38	0.52
	1×10^{-4}	2.72	6.38	0.43
Dimethylamine	1×10^{-1}	5.19	8.41	0.62
Ethanol	6.7×10^{-5}	6.58	5.13	1.28
Formate	6.7×10^{-5}	4.35	5.13	0.85
Nitrofurazone	1×10^{-4}	1.21	8.41	1.44

^a Calculated for 1% survival

$$\left(= \frac{1\% \text{ Survival dose in the absence of NaCl}}{1\% \text{ Survival dose in the presence of 1M NaCl}} \right)$$

^b See text.

in a range of 5.13 to 8.41. The effects of added solutes are represented in terms of the relative DMF defined as follows:

FIG. 3. Effect of L-Tryptophan Added during Irradiation on NaCl Radiosensitization (*E. coli* B/r log-phase cells).

A, Control; B, 1 M NaCl; C, 6.7×10^{-5} M L-tryptophan; D, 1×10^{-3} M L-tryptophan; E, 1 M NaCl + 6.7×10^{-5} M L-tryptophan; F, 1 M NaCl + 1×10^{-3} M L-tryptophan.

Relative DMF

$$\text{Relative DMF} = \frac{\text{DMF of NaCl in the presence of the solute}}{\text{DMF of NaCl in the absence of the solute}}$$

The more effective solute for the removal of NaCl radiosensitization gives the smaller value of relative DMF. As can be seen in Table II, thiol compounds and amino acids such as tryptophan and histidine which react with Cl_2^- radical anions remove the radiosensitizing effect of NaCl. However, glycine which was found not to react with Cl_2^- anions²⁰⁾ was also effective for the removal of NaCl radiosensitization, although it was a little less effective than tryptophan and histidine. Dimethylamine, a charge scavenger, was not so effective. Hydrogen-donating substances such as ethanol and formate and an electron-affinic radiosensitizer nitrofurazone exhibited almost no effect on the enhancement of radiosensitizing effect of NaCl.

Dependence of radiosensitizing effect of NaCl upon its concentrations

If NaCl radiosensitization were due to the action of Cl_2^- anion radicals and/or Cl atoms, their formation would require the chloride concentrations sufficient for the spur reaction.^{3,21)} Subsequently, the relationship between extracellular chloride concentration dur-

FIG. 4. Effects of Concentration of NaCl on Its Radiosensitizing Action (*E. coli* B/r log-phase cells).

a, Survival curves at different NaCl concentrations (A, control; B, 0.1 M; C, 0.2 M; D, 0.3 M; E, 0.4 M; F, 0.5 M; G, 0.6 M; H, 0.7 M; I, 0.8 M; J, 0.9 M; K, 1.0 M).

b, Relationship between NaCl concentration and the extent of radiosensitizing effect (A, represented by DMF; B, by % inhibition, see text).

ing irradiation and the extent of its radiosensitizing effect was determined with *E. coli* B/r and the result is shown in Fig. 4. Two curves represented by different parameters similarly show that NaCl is effective for radiosensitization at concentrations of 0.1 M or higher and the chloride concentration-radiosensitizing effect curve reaches the plateau at about 0.6 M.

Effects of inhibitors of RNA and protein syntheses added during postirradiation incubation on NaCl radiosensitization

The inhibition of RNA and protein synthesis during postirradiation incubation was found to reduce the survival of DNA-repair-proficient strains of *E. coli* and *M. radiodurans* by blocking the repair of radiation-produced DNA-strand breaks.^{22,23} In connection with this finding, effects of actinomycin D and chloramphenicol added during postirradiation incubation on survival of *E. coli* B/r log-phase cells were determined to examine the correlation between NaCl radiosensitization and the inhibition of RNA and protein syntheses.

In Fig. 5, curves F and H represent the dose-effect relation being found when cells are irradiated in 1 M NaCl-containing phosphate buffer, harvested by centrifugation and then reincubated for 2 hr in B2 broth containing either actinomycin D or chloramphenicol, respectively, at the constant concentration. The toxic effects of these two metabolic inhibitors to decrease cell survival were almost independent of radiation dose, *i.e.*, they exhibited the "additive" effect. If they interfere with the same process required for the cell viability as that damaged by radiosensitizing action of NaCl, the additional decrease in cell survival by the addition of these metabolic inhibitors should not be so significant as compared with that by the presence of only NaCl during irradiation. Then, with increasing doses, curves F (irradiated with NaCl and reincubated with actinomycin D) and H (irradiated with NaCl and reincubated with chloramphenicol) in Fig. 5 should become closer to curve D which is the survival curve for cells irradiated with NaCl and reincubated without any inhibitor. However, it was not

FIG. 5. Effects of RNA and Protein Syntheses Inhibitors Added during Post-irradiation Incubation on NaCl Radiosensitization of *E. coli* B/r.

A, C, E and G; irradiated in NaCl-free phosphate buffer.

B, D, F and H; irradiated in 1 M NaCl containing phosphate buffer.

a: immediately after irradiation.

b: after 2-hr postirradiation incubation in B2 broth at 37°C (C & D, no additive; E & F, 34 μ g/ml actinomycin D; G & H, 18 μ g/ml chloramphenicol.)

the case as shown in Fig. 5.

DISCUSSION

In this study, the radiosensitizing effect of NaCl was observed with the radioresistant vegetative forms, but not with radiosensitive strains. The former group of radioresistant strains has been found to be endowed with the efficient DNA-repair capacity. With DNA-repair-proficient *E. coli* strains W3110, AB1157 as well as B/r, the remarkable radiosensitizing effect of NaCl was determined. In contrast, mutations in certain genes controlling the DNA repair process in *E. coli* clearly affected the effectiveness of NaCl during irradiation. As seen in Fig. 3, the *pol* and *rec* mutations completely remove the effect of NaCl. In addition, it must be noted that the above DNA-repair-proficient strains were similarly sensitized to radiation by the presence of 1 M NaCl near to the level of sensitive mutants P3478 (*pol*⁻) and AB2487 (*rec*⁻) (Fig. 3). These results indicate that NaCl radiosensitization is closely related with the DNA repair inhibition and that in the case of *E. coli* it results from the inhibition of *pol*- and *rec*-dependent repair of DNA.

The addition of some solute during irradiation was also found to abolish the radiosensitizing effect of NaCl. The additives highly or considerably reactive with Cl₂⁻ radical anions such as thiol compounds, tryptophan and histidine were very effective to abolish the radiosensitizing effect of NaCl.^{4,20)} This supports a view that an attacking species in NaCl radiosensitization is Cl₂⁻ radical anions. However, glycine was also found to protect *E. coli* B/r cells to some extent against the radiosensitizing action of NaCl, although it was much less reactive with Cl₂⁻ anions. In this case, added glycine might be effective not as a competitors, but through other mode of action—probably as a protector for some vital component against the damaging action of Cl₂⁻ anions. Further investigations are still required to establish a view on the attacking species in NaCl radiosensitization. The reac-

tion process to form Cl₂⁻ radical anions has been postulated as follows:^{2,4)}

In neutral solution, reaction (3) may take place in a spur. Thus, if the attacking species in NaCl radiosensitization were Cl atoms or Cl₂⁻ anions, spur reaction (3) should occur even at the minimum intracellular chloride concentration effective for NaCl radiosensitization.* The minimum extracellular chloride concentration was determined to be about 0.1 M, as shown in Fig. 2. The intracellular chloride concentration in this case is estimated to be ~0.03 M in average, based on an experiment using ³⁶Cl-NaCl (unpublished data). Since the half-life value of reaction (3) is estimated to be ~10⁻⁸ sec for 0.03 M of the chloride concentration as $K_{\text{OH}+\text{Cl}^-} = 4 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ in 0.8 N H₂SO₄,²⁴⁾ the Cl₂⁻ formation by spur reactions is possible at this intracellular chloride concentration. If there were heterogenous chloride distribution in bacterial cells, the possibility of occurring reaction (3) would increase. These considerations support a view that the attacking species may be Cl₂⁻ radical anions and/or Cl atoms.

The effect of actinomycin D and chloramphenicol added after irradiation in the presence of NaCl may give us another information on the action mechanism of radiosensitizing NaCl. Some reports^{22,23,26,27)} described the involvement of certain RNA and protein synthesis in the DNA repair process in *E. coli* as well as in *M. radiodurans*. The results obtained in this study indicate that effects of actinomycin D and chloramphenicol, which are the inhibitor of DNA-dependent RNA synthesis and protein synthesis, respectively, were "additive" for the radiosensitizing action of NaCl over the radiation dose range studied, that is, independent of doses of irradiation in

* It has been described that NaCl radiosensitization is due to the short-lived species formed from Cl⁻ ions, but not from Na⁺ ions.²⁵⁾

the presence of NaCl. This fact suggests that the inhibition of RNA and protein syntheses due to these metabolic inhibitors may not be involved in the primary process of NaCl radiosensitization. Some other mechanism might be operative in the inhibition of DNA repair by irradiation in the presence of NaCl. Further investigation on cellular damage due to NaCl added during irradiation are required for the elucidation of the basic mechanism for NaCl radiosensitization.

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REFERENCES

- 1) A. Matsuyama, Y. Okazawa, M. Namiki and Y. Sumiki, *J. Radiat. Res.*, **1-2**, 98 (1960).
- 2) A. Matsuyama, M. Namiki and Y. Okazawa, *Radiat. Res.*, **30**, 687 (1967).
- 3) A. Matsuyama and M. Namiki, *Agric. Biol. Chem.*, **29**, 593 (1965).
- 4) Su-Jen Shih Chen, S. Arai and A. Matsuyama, *Sci. Pap. Inst. Phys. Chem. Res.*, **68**, 21 (1974).
- 5) M. Simic, E. L. Powers, M. Centilli and M. Cross, *Int. J. Radiat. Biol.*, **25**, 43 (1974).
- 6) J. L. Redpath and D. L. Dewey, *ibid.*, **25**, 189 (1974).
- 7) J. L. Redpath, *ibid.*, **27**, 493 (1975).
- 8) F. L. Haas and C. O. Doudney, *Proc. Natl. Acad. Sci. U.S.A.*, **43**, 871 (1957).
- 9) Y. Okazawa and A. Matsuyama, *Agric. Biol. Chem.*, **31**, 1505 (1967).
- 10) A. Matsuyama, M. J. Thornley and M. Ingram, *J. Appl. Bacteriol.*, **27**, 110 (1964).
- 11) N. F. Lewis, *J. Gen. Microbiol.*, **66**, 29 (1971).
- 12) A. Matsuyama, Y. Okazawa, H. Arai and M. Mifune, *Sci. Pap. Inst. Phys. Chem. Res.*, **67**, 35 (1973).
- 13) T. Ueno, M. Kawai and S. Omata, *J. Ferment. Technol.*, **39**, 383 (1961).
- 14) F. Yatagai, T. Takahashi, Y. Kitajima and A. Matsuyama, *J. Radiat. Res.*, **15**, 90 (1974).
- 15) A. Matsuyama, Y. Okazawa and M. Namiki, *Agric. Biol. Chem.*, **25**, 367 (1961).
- 16) A. W. Anderson, H. C. Nordan, F. R. Cain, G. Parrish and D. Duggan, *Food Technol.*, **10**, 575 (1956).
- 17) S. Kitayama and A. Matsuyama, *Biochem. Biophys. Res. Commun.*, **33**, 418 (1968).
- 18) A. D. Burrell, P. Feldschreiber and C. J. Dean, *Biochim. Biophys. Acta*, **247**, 38 (1971).
- 19) R. A. McGrath and R. W. Williams, *Nature*, **212**, 534 (1966).
- 20) G. E. Adams, J. E. Aldrick, R. H. Bisby, R. B. Cundall, J. L. Redpath and R. L. Willson, *Radiat. Res.*, **49**, 278 (1972).
- 21) M. Anbar and J. K. Thomas, *J. Phys. Chem.*, **68**, 3829 (1964).
- 22) S. Kitayama and A. Matsuyama, *Agric. Biol. Chem.*, **35**, 644 (1971).
- 23) A. K. Ganesan and K. C. Smith, *J. Bacteriol.*, **111**, 575 (1972).
- 24) M. Burton and K. C. Kurien, *J. Phys. Chem.*, **63**, 899 (1959).
- 25) M. Namiki, Y. Okazawa and A. Matsuyama, *Agric. Biol. Chem.*, **25**, 108 (1961).
- 26) W. Wehrli and M. Stachelin, *Bacteriol. Rev.*, **35**, 290 (1971).
- 27) C. K. K. Nair and D. S. Pradhan, *Int. J. Radiat. Biol.*, **28**, 385 (1975).