Effect of Combined Action of Ionizing Radiation and Fluoride Ion
on Lethality of Microorganisms*

NAMIKI, Mitsuo**

(Received, Sept. 19, 1966)

ABSTRACT

Enhancement of radiolethality of E. coli was observed by KF during irradiation. The shape of survival curve of exponential type was not altered by KF, and it was only effective at pH below 6.0 and not so influenced by oxygen and additional SH compound. Pre- or post-irradiation treatment with KF did not exert any changes in viability of cells. Referring to this enhancement, effect of fluoride ion on water radiolysis was investigated, and these results were discussed in comparison with the cases of other halide ions.

INTRODUCTION

In addition to the modification of the biological effects of radiation by oxygen\(^1\)\(^-\)\(^4\), an appreciable number of works have recently been undertaken about the enhancement of radiolethality of microorganisms in combination with chemical reagents such as nitric oxide\(^5\)\(^-\)\(^6\), N-ethylmaleimide\(^7\)\(^-\)\(^8\), phenylmercuric acetate\(^9\) and p-hydroxymercuric benzoate\(^11\), iodoacetic acid\(^8,10\) and its amide\(^12\), vitamin K\(_2\) and its analogues\(^13\)\(^-\)\(^16\), and halogenated pyrimidines\(^17\)\(^-\)\(^18\).

Concerning the modification of radiation effects on biological and chemical systems by addition of inorganic and organic halogen compounds, a serial studies

* The major part of this report has been presented at the 6th Annual Meeting of the Japan Radiation Research Society, 17th July, 1964.

** Radiobiology Research Group, The Institute of Physical and Chemical Research, Bunkyo-ku, Tokyo. Present address: Department of Agriculture Chemistry, Faculty of Agriculture, Nagoya Univ., Chikusa-ku, Nagoya, Japan.
have been reported\textsuperscript{19–20} by the author's research group, and it was demonstrated that the presence of alkali halides during irradiation of microbial cells brought about a remarkable enhancement of radiolethality, and various factors affecting on the enhancing action were investigated.

However, these studies regarding alkali halides were confined only to the effects of chloride, bromide and iodide as a halogen ion, and little attention has been paid to the role of fluoride compound in the effects of radiation on biological and chemical systems, except for fluoroadenosine\textsuperscript{18}.

The present investigation was undertaken to see the effect of fluoride ion on radiolethality of some microorganisms, and the characteristic properties found in its action will be presented and discussed in relation to the enhancing effects of other halide ions.

\textbf{MATERIALS AND METHODS}

\textit{Microorganisms}

\textit{Escherichia coli} 2–7 was used mainly as a test microorganism, which was supplied by the Institute of Applied Microbiology, The University of Tokyo. \textit{Escherichia coli} B/r was also used in some experiments, which was supplied by courtesy of Dr. A. Bridges.

\textit{Irradiation}

Irradiation was carried out with a cobalt-60 γ-radiation source and a dose rate mainly used was $4 \times 10^4$ or $1.5 \times 10^5$ R/hr. The temperature during irradiation was kept at 4–10°C. In the experiments with oxygen free condition, the cell suspensions were bubbled with purified nitrogen over 15 minutes prior to irradiation.

\textit{Microbiological procedures}

The procedures used in this study were essentially the same as those described in the cases of studies on the effect of other halides\textsuperscript{24–25}.

The \textit{E. coli} 2–7 was grown with shaking at 30°C in medium containing 1% peptone, 1% meat extract and 0.5% NaCl, at pH 7.0. After incubation for 18 hours, the cells were harvested and washed twice with phosphate buffer by centrifugation, and the cell suspensions of about $10^8$ cells/ml were made with 0.067 M phosphate buffer of a given pH.

The cell suspensions were mixed with the reagent solution immediately before irradiation and then irradiated with the above conditions.

After irradiation, they were treated with appropriate decimal dilution with phosphate buffer followed by incubation on nutrient agar to count the viable cell colonies. Surviving fractions were determined in comparison with the survivor of the control treated with the same manner excepting irradiation. \textit{E. coli} B/r was incubated and tested under the similar experimental conditions.

Reagents of Guaranteed Grade and a thrice distilled water were used throughout the experiments.
RESULTS

Survival curve and oxygen effect in irradiation with fluoride ion

The survival curves for *E. coli* 2-7 irradiated with or without the presence of 0.01 M potassium fluoride are shown in Fig. 1. This cells gave a survival curve of exponential type in phosphate buffer. As apparently observed the radiolethality was increased by the presence of 0.01 M potassium fluoride when tested at pH 5.0, whereas no increase was found at pH 7.5.

The shape of survival curve of exponential type was not altered by the irradiation with fluoride ions both in the presence and absence of oxygen, and this fact is to be noted as compared with the cases of other halide ions. The enhancement with fluoride ions could be observed either in aerobic or anaerobic conditions with almost the same extent in dose modifying factor, about 1.5; that meant there was no oxygen effect in fluoride action.

![Graph showing survival fraction vs. γ-ray dose](image_url)

**Fig. 1.** Effect of potassium fluoride on the radiolethality of *E. coli* irradiated in air or in nitrogen (at pH 5.0 or 7.5 in 0.067 N phosphate buffer)

Circle: KF free control. Triangle: KF 0.01 N at pH 5.0. Square: KF 0.01 N at pH 7.5.

In the above plots, open and solid illustrate respectively "in air" and "in nitrogen".
As shown in Fig. 2, essentially the same results about the effect of fluoride ion were obtained in the case of E. coli B/r.

Effects of pH and reagent concentration.

From the result presented above, the enhancing action of fluoride ion appeared to be influenced by pH during irradiation, therefore, the surviving fractions of E. coli 2–7 irradiated at different pH in phosphate buffer were determined and pre-

Fig. 2. Effect of potassium fluoride on the radiolethality of E. coli B/r. (in 0.067 M phosphate buffer, pH 5.0 at 4°C).
Circle: KF free control, Triangle: KF 0.1 M. In the above plots, open and solid illustrate respectively “in air” and “in nitrogen”.

Fig. 3. Effect of potassium fluoride on the radiolethality of E. coli 2–7. Effect of pH during irradiation.
○: KF free control, △: KF 0.01 M in 0.067 M phosphate buffer. Irradiation: 2.4×10⁴ rads in air at 10–15°C. (I) irradiated, (U) unirradiated.
sented in Fig. 3. It was clearly demonstrated that the effect of fluoride ion was strongly depend upon pH during irradiation and the increase in radiolethality could only be obtained at below pH 6.0.

The effect of concentration of fluoride ion on the enhancement was investigated at pH 5.0 and 7.5, and the results are given in Fig. 4. As indicated with dotted line, no appreciable per se toxicity of potassium fluoride was observed under the same conditions except for irradiation even at high concentration as 0.5 M of KF. With irradiation at pH 7.5, there was no change in surviving fraction whatever by the presence of fluoride ions even at 0.5 M, while at pH 5.0, the increase in lethality could be observed at concentration less than 0.01 M of KF, although the increase was not so remarkable above 0.01 M.

Effect of cysteine on the action of fluoride ion.

In order to investigate the modification by the presence of additional compound, the effect of fluoride ions on radiolethality of *E. coli* was tested in combination with cysteine of various concentrations. Fig. 5 shows that the action of fluoride ions was not affected by the presence of cysteine up to 0.001 M and above which the surviving fraction was moderately increased with the increase in cysteine concentration, thus it was modified as that in the case of halide free control under similar way.

Influence of cell concentration at the time of irradiation.

As described previously\(^{23}\), the enhancement of radiolethality by 1.0 M sodium

![Diagram](https://example.com/diagram.png)

**Fig. 4.** Effect of potassium fluoride on the radiolethality of *E. coli* 2-7. KF concentration during irradiation (in 0.067 M phosphate buffer, pH 5.0 and 7.5).

Cell concentration: \(3.4 \times 10^5\) cells/ml in 0.067 M phosphate buffer. Irradiation: \(\gamma\)-ray dose, \(2.4 \times 10^4\) rads at 4-10°C.
chloride could be observed only in the cell concentration below $10^7$ cells/ml, and above which the presence of sodium chloride rather manifested a protection against radiation lethal effect. Therefore, the effect of 0.01 M potassium fluoride was investigated at different cell concentrations and the surviving fractions were illustrated in Fig. 6. The results indicated that the effect of fluoride ion was slightly weakened with increasing cell concentration, but the modification ran almost parallel to the case of halide free control, it thus meant that the net enhancement with KF might be independent of the cell concentration in a preparation.
Effect of pre- or post-treatment with potassium fluoride on radiolethality of E. coli.

To determine whether the increased cell death is due only to the combined action of radiation and fluoride ions during irradiation, following experiments on the effect of pre- or post-irradiation treatment with potassium fluoride were under-

Fig. 7. Effect of pretreatment with potassium fluoride on radiolethality of E. coli 2–7. The cells were pretreated with 0.01 M KF at 4°C or 30°C for 5 or 40 minutes. After centrifugation followed by resuspension in 0.067 M phosphate buffer, pH 5.0, the cells were irradiated with $2.4 \times 10^4$ rads.

Fig. 8. Effect of irradiated potassium fluoride on the viability of E. coli. The 1:1 mixture of unirradiated cell suspension and KF solution was incubated at pH 7.5 or 8.0 and at 4°C or 37°C for 40 minutes. U: KF 0.1 M unirradiated (final concentration)

1-a: KF 0.1 M irradiated with $1.2 \times 10^4$ rads.
1-b: KF 0.1 M irradiated with $6.0 \times 10^3$ rads.
1-c: KF 0.1 M irradiated with $2.0 \times 10^4$ rads.
taken. Experimental conditions such as time, temperature, pH, and reagent concentration were determined in reference to the conditions used in the experiments which were done to evaluate the effect of the reagent during irradiation, that is, 0.01 M potassium fluoride, pH 5.0, 4°C and 2.4×10⁴ rad were used as a standard condition.

In the experiments to investigate the pretreatment effect, the cells were contacted with 0.01 M KF at 4°C or 30°C for 5 or 40 minutes, and after centrifugation followed by resuspension in KF free buffer, pH 5.0, they were irradiated with 2.4×10⁴ rads. The results presented in Fig. 7 demonstrate that the radiosensitivity of cells was unaffected by the pretreatment of KF even they were contacted at high temperature, 30°C.

Table 1. Effect of potassium fluoride on the viability of irradiated E.coli.

<table>
<thead>
<tr>
<th>(U) cells (control)</th>
<th>(I) cells + KF free</th>
<th>+ KF 0.1 M</th>
<th>+ KF 0.01 M</th>
<th>+ (I) KF 0.1 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable counts/ml</td>
<td>2.1×10⁷</td>
<td>2.7×10⁶</td>
<td>3.3×10⁶</td>
<td>3.6×10⁶</td>
</tr>
</tbody>
</table>

(U) Unirradiated.
(I) Irradiated with 2.4×10⁴ rads at pH 5.0 in air. The mixture of cell suspension and KF solution was incubated at 30°C for 40 minutes.

Effect of Potassium Fluoride on \(G(H_2O_2)\)-value with \(\gamma\)-irradiation of Water.

Fig. 9. Effect of potassium fluoride on \(G(H_2O_2)\)-value with \(\gamma\)-irradiation of water. KF in the thrice distilled water, pH 5.5. Irradiation: 2.27×10⁴ rads.
EFFECT OF COMBINED ACTION OF IONIZING RADIATION AND FLUORIDE ION ON LETHALITY OF MICROORGANISMS

The effect of irradiated potassium fluoride solution on viability of unirradiated cells was tested using 0.1 M KF immediately after irradiation whose dosages were far higher than that used in the experiments described previously. It was shown in Fig. 8 that the irradiated KF solution did not exert any effect on cell viability under different conditions in time and temperature of incubation.

Table 1 shows the susceptibility of irradiated cells to toxic effect of potassium fluoride, in this case too the cell viability was not changed by irradiation. _Effect of potassium fluoride on G(H₂O₂) in radiolysis of water._

For reference to consider the mechanism of the effect of fluoride ions on radiolethality, _G(H₂O₂)_ values in the radiolysis of different concentrations of KF solution were determined. As can be seen in Fig. 9, no change in the yield of _H₂O₂_ was observed by the presence of potassium fluoride up to 1.0 M.

DISCUSSION

As shown in Fig. 1, the presence of potassium fluoride during irradiation apparently brings about the enhancement of radiolethality of _E. coli_ cells. The facts that no change in viability of irradiated or unirradiated cells was found by pre- or post-irradiation treatment with KF might eliminate the possibility of involvement of the following events; the treatment with KF prior to irradiation raises the sensitivity of cells to radiation, the irradiation of cells brings about increased susceptibility to toxic effect of KF, or the formation of some long-lived bactericidal species in irradiated KF solution. Thus, it can be to say that the enhancing effect may be resulted from combined action of radiation and potassium fluoride at the time of irradiation.

In the case of this enhancement, contribution of the action of cations in alkali halides could be excluded from the fact reported previously that the addition of various kinds of sodium and potassium salts such as _K₂SO₄_, _Na₂SO₄_, _KClO₄_, and _NaNO₃_, to irradiating cell suspension provided more or less protective effect at higher concentration. Therefore, the enhancement with potassium fluoride might be due to the action of fluoride ion as in the cases of other alkali halides such as _NaCl_, _KBr_ and _KI_.

However, the synergistic effect of fluoride ion on radiolethality exhibited some characteristic properties essentially different from those of other halide ions.

One of its features could be pointed out in the shape of the dose-survival curves obtained by the presence of alkali halide. As presented previously, the exponential type of survival curve of _E. coli_ irradiated in phosphate buffer was modified to a sigmoidal and/or cumulative type by the presence of chloride, bromide and iodide ions. While in the case of fluoride ion, the survival curve with increased cell death is still of an exponential type. This fact suggests that the enhancement of radiolethality by fluoride ion is caused by a different type of mechanism.

Characteristic points in the fluoride action are also found in modification by
oxygen or other additives. In most of the reagents known as a sensitizer or enhancer for radiation lethal effect on cells, the action is more or less modified by the presence of oxygen. The enhancing effect in general appears prominently in anaerobic condition, and in some cases, the effect is considerably weakened or completely abolished by the presence of oxygen\textsuperscript{8,15}, though such modification depends somewhat on the nature of microorganism.

As to the enhancement by alkali halides, it was observed that bromide and iodide ions are less effective in aerated suspension than in nitrogen system\textsuperscript{24,30}, while in the case of fluoride, as shown in Fig. 1, the enhancing action is not so altered by the presence of oxygen and gives a similar extent of DMF (dose modifying factor).

Modification of sensitizing action by additional compounds, especially by the presence of biological substances such as sulfhydryl compounds, amino acid and protein, appears to be an important factor to consider the mechanism of enhancing action and through which to elucidate a mode of lethal action of radiation on living cells. The modification may also be considered to be one of the essential factors to evaluate the enhancing reagent from the viewpoints of utilization in the fields of food irradiation and radiotherapy.

An appreciable number of the enhancing reagent reported are known well as a compound highly reactive with SH groups; that is NEM, HMB, iodoacetic acid, vitamin K\textsubscript{5} and its analogous naphthol compounds, and quinones. It is especially evident not only with these compounds but also with other enhancing reagents that the enhancement of radiolethality is greatly weakened or cancelled by the additional sulfhydryl compound or other biological substances.\textsuperscript{14}

In the cases of other halide ions, particularly chloride ion, modification of the enhancing effect of halide ions by additional cysteine was characteristic, that is, small amount of cysteine not merely cancelled the effect of halide ions, on the contrary, provoked the protective action of the halide ions\textsuperscript{30}.

In this respect, the behavior of fluoride ion against modifying action of cysteine (SH compound) is to be noted that, as shown in the case of E. coli (Fig. 5), the enhancement was not appreciably affected by additional cysteine except at higher cysteine concentration at which per se protective effect was observed. This result may provide another evidence to suggest that the effect of fluoride ion is provoked by the mechanism different from that proposed in the cases of other halide ions.

Concerning the mechanism of the effect of other halide ions, it was postulated\textsuperscript{30,24} that the enhancement of radiolethality might involve additional damages in some cellular components by the action of halogen free radicals formed through the electron transfer reaction between halide ion and OH radical. This scheme is based on the experimental results about the effects of alkali halides on various radiation actions such as H\textsubscript{2}O\textsubscript{2} formation with water radiolysis,\textsuperscript{29} radiolysis of some amino acids and nucleic acid bases, inactivation of some enzymes and bacteriophages, denaturation of DNA\textsuperscript{30}, and inactivation and mutation induction of
EFFECT OF COMBINED ACTION OF IONIZING RADIATION AND FLUORIDE ION ON LETHALITY OF MICROORGANISMS

microbial cells.\textsuperscript{30})

In respect to such radiation chemical and biochemical effects of halide ions, the mode of fluoride action was essentially different from other halide ions. In consideration of the thermodynamic date relating to radical-ion transfer of the type $\text{OH}^+ + \text{X}^- \rightarrow \text{OH}^- + \text{X}^+$,\textsuperscript{31}) it seems inconceivable because of its high endothermic reaction that fluoride ion could react with OH radical according to this type of reaction and forms fluorine radical as in the cases of other halide ions. In fact, in the experiments about the effects of potassium fluoride on radiolysis of water shown in Fig. 9, and moreover on radiation inactivation of some enzymes in aqueous system (unpublished date), fluoride ion did not provide any modification expectable as a result of such radical scavenging reaction.

In the possible mechanisms discussed about the chemical enhancement of radiolethality, it was assumed especially in the case of sulphydryl binding reagents that the effect of reagent under radiation action might be associated in any way with their particular reactivity for native or potential sulphydryl groups or sulfur radicals in cellular substances essential in vital processes. This idea may not be applicable to the effect of fluoride ion because of the absence of positive evidences for such reactivity in the experimental results presented above.

On the basis of above viewpoints, a reasonable explanation about the mechanism whereby the enhancement occurred could not be made under present conditions.

As it was known that mode of sensitizing action was considerably changed by difference in species or strain of microorganism used, additional investigations on the action of fluoride ion with different types of microorganisms will be undertaken to extend informations about the effect and to get some clue to its mechanism. It is to be noted that the preliminary experiments carried out on radiolethality of Micrococcus radiodurans provided a similar enhancement by fluoride ion, although in that case the effect was considerably abolished by the addition of a small amount of cysteine (unpublished results). Further works from other aspects will be done to explain this characteristic effect of fluoride ion.

ACKNOWLEDGMENT

The author wishes to express his thanks to Dr. A. Matsuyama, Dr. T. Kada and Dr. Y. Okazawa, for their advices and discussion, and also to Misses U. Ito, M. Shikata and Mr. A. Taki for their excellent technical assistance.

The research was supported in part by grant from the IAEA Reserch Contract, No. 213/RB.

REFERENCES


EFFECT OF COMBINED ACTION OF IONIZING RADIATION AND FLUORIDE ION ON LETHALITY OF MICROORGANISMS


