Potentialiation by Halogen Compounds of the Lethal Action of γ -Radiation on Spores of *Bacillus cereus*

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SUMMARY

Spores of *Bacillus cereus* were inactivated by γ -radiation more effectively in the presence than in the absence of a variety of halogen compounds. Where homologous series of compounds were used, activity decreased in the order $I > Br > Cl$ and F . Activity did not depend upon affinity of the compounds for thiol groups. Potassium iodate was the most active compound tested. Iodate and iodoacetamide (IAM), and to a lesser extent iodide, were most effective at low pH values, but potentiation by 3,5-diiodo-L-tyrosine was hardly affected by pH value. Survivor curves of spores irradiated in water plus IAM or iodate were convex, suggesting that potentiation increased with increase in γ -radiation dose. The compounds tested were all more effective potentiators of radiation inactivation of spores in water than in sodium phosphate buffers or in complex medium, and potentiation was greater with low than with high spore concentrations. Histidine antagonized the potentiation of radiation inactivation by iodate, iodide and iodoacetamide. Potentiation decreased with decrease in temperature, and was not shown at -15° . The results suggest that potentiation resulted from toxicity to spores of halogen-free radicals, and to a lesser extent free halogen, formed by reaction of the compounds with the radiolytic products formed from water during irradiation.

INTRODUCTION

Iodoacetamide (IAM) potentiated the lethal action of ionizing radiation for some vegetative bacteria, including the remarkably radiation-resistant *Micrococcus radiodurans* (Dean & Alexander, 1962), by a mechanism which probably depended on formation of free radicals from the IAM (Dewey & Michael, 1965). Other halogen compounds (e.g. iodoacetic acid (Bridges, 1962), iodide (Dean & Alexander, 1965), other halides (Matsuyama, Namiki & Okazawa, 1967)), were also capable of potentiating the lethal action of ionizing radiation for vegetative bacteria, though little work of this nature has been done with bacterial spores. Apart from interest in the mechanisms of action, there are practical reasons for studying such potentiators. For instance, radiation preservation of foodstuffs and other perishable materials would become a more practical possibility if the necessary doses could be diminished: selective sensitization of malignant mammalian cells to radiation could be an important aid to therapy. These reasons, and paucity of data concerning the mechanism of spore resistance to ionizing radiation, prompted this study of some compounds found to potentiate the γ -radiation inactivation of spores.

METHODS

Spores. *Bacillus cereus* strain PX was grown for 3 days at 30° on the surface of agar containing potato extract (Difco, 0.4 %, w/v), yeast extract (Difco, 0.4 %, w/v) and glucose (0.25 %, w/v), pH 7.3. The free spores were washed off the agar, cleaned by centrifuging six times from distilled water and stored in water at a concentration of about 20 mg. equiv. dry wt./ml. at 4°.

Chemicals were 'Analar' grade or were the purest obtainable from British Drug Houses Ltd, Poole, Dorset, or Koch-Light Laboratories Ltd, Colnbrook, Buckinghamshire. Thyroid hormone analogues were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Viable counts. Samples (1 ml.) were washed out of ampoules into 9 ml. amounts of L-cysteine solution (10 mM) then further decimally diluted in sterile distilled water. The cysteine was included to quench the thiol reagents used in some experiments and prevent them being carried over into the poured plates of yeast glucose agar used for viable counts. Colonies were counted after incubation of plates for 2 days at 37°.

Radiation was from a ⁶⁰Co source which irradiated samples at a dose rate of about 0.8 Mrad/h. at about 20° unless otherwise stated. Samples (1 ml.) for irradiation were sealed in glass ampoules so that, with the high doses used, irradiation was essentially anoxic over the range studied. Control samples bubbled with N₂ or sealed *in vacuo* were used to ensure that residual O₂ did not contribute to the observed effects of potentiators. Samples were held on ice when not being irradiated, and were used within about 3 h. of the termination of irradiation. Unirradiated controls were always included in experiments and showed no fall in viable count unless stated in Results.

RESULTS

*Potentiation of the lethal effect of γ -radiation on *Bacillus cereus* spores by various chemicals*

Table 1 summarizes the effects of a number of chemicals on the destruction of *Bacillus cereus* spores by γ -radiation. The following points are of interest. (1) A number of chemicals which potentiated the lethal action of radiation on spores in water failed to do so effectively in buffer (see particularly iodoacetic acid, iodoacetamide, bromide and iodide, bromate and iodate). (2) Numerous bromine and iodine compounds were potentiators, but the thiol reactants *p*-chloromercuribenzoic acid (*p*CMB) and *N*-ethylmaleimide (NEM) were not. (3) Where series of halogen compounds were used, the iodine derivatives were more effective than the bromine, and the chlorine and fluorine derivatives were relatively ineffective (see chloro-, bromo- and iodoacetic acids; fluoride, chloride, bromide and iodide; chlorate, bromate and iodate). (4) Benzoic acids with *o*-, *m*- or *p*-iodine substituents were about equally effective. (5) Tyrosine had little effect on the radiation inactivation, but iodine-containing derivatives of it, and related thyroid hormone analogues were moderately active, especially L-thyroxine in water. (6) Iodate was the most effective potentiator found.

The strong antagonism by sodium phosphate buffer of the potentiation caused by IAM was obvious when radiation dose: survivor curves were studied (Fig. 1, 2). Potentiation caused by iodide and iodate was much less affected by the buffer. When spores were irradiated in a rich medium (heart infusion broth) the potentiation was

largely lost, apart from a slight increase in slope of the survivor curve when iodate was present (Fig. 3). It was also noticed that the amount of potentiation differed with different spore crops, and was clearly greater with fresh well-cleaned spores than with poorly cleaned suspensions or suspensions that had been stored for long periods.

Fig. 4 shows that the inactivation of spores even at low concentrations was only slightly influenced by the thiol reactants *p*CMB and NEM at doses up to 1 Mrad, whereas IAM significantly increased the inactivation rate.

Table 1. *Inactivation by γ-radiation of spores of Bacillus cereus suspended in different reagents*

Reagents were used at a concentration of 1 mM except iodobenzoic acid at 0.1 mM. Spores (1.5×10^8 /ml.) were suspended in sodium phosphate solution (10 mM; pH 7.0) or in water as indicated in the table. The γ-radiation dose was 0.6 Mrad. *N*-Bromosuccinimide (1 mM) killed unirradiated control spores in sodium phosphate (4.1 % survivors) during the experimental period. The other reagents did not affect viability of unirradiated spores.

Reagent	Spores (%) surviving irradiation in	
	Phosphate solution	Water
Control (no addition)	2.28	2.87
Acetamide	2.84	—
Chloroacetic acid	1.27	0.54
Trichloroacetic acid	1.88	—
Bromoacetic acid	2.36	0.085
Iodoacetic acid	1.25	0.036
Iodoacetamide	1.41	0.0001
L-Tyrosine	1.24	1.61
DL-Thyronine	1.04	0.57
L-Thyroxine	1.04	0.045
3,5,3'-Triiodo-L-thyronine	2.38	0.29
3,5-Diiodo-L-tyrosine	0.38	0.42
<i>o</i> -Iodobenzoic acid	2.24	0.34
<i>m</i> -Iodobenzoic acid	1.88	0.52
<i>p</i> -Iodobenzoic acid	1.65	0.49
<i>p</i> -Chloromercuribenzoic acid	1.04	1.23
<i>N</i> -Ethylmaleimide	0.58	1.40
<i>N</i> -Bromosuccinimide	0.0065	< 0.00001
Potassium fluoride	—	2.13
Potassium chloride	—	2.83
Sodium bromide	0.45	0.034
Potassium iodide	0.23	0.068
Potassium chlorate	4.30	2.56
Sodium bromate	2.70	0.024
Potassium iodate	0.012	< 0.00001

The effect of concentration of three of the most effective potentiators in water is shown in Table 2. *N*-Bromosuccinimide was not as toxic alone as it was in buffer (see Table 1). Table 2 emphasizes that iodate was more effective than bromate and that concentrations as low as 0.01 mM significantly potentiated the lethal action of radiation.

Fig. 1

Fig. 2

Fig. 1. Dose:survivor curves of spores of *Bacillus cereus* γ -irradiated in sodium phosphate solution (10 mM; pH 7.0), to which was added: iodoacetamide (1 mM) (●), potassium iodide (1 mM) (□), potassium iodate (1 mM) (△), control, no addition (○). Arrows indicate that counts were below the level shown by the symbol.

Fig. 2. Dose:survivor curves of spores of *Bacillus cereus* γ -irradiated in water instead of buffer as in Fig. 1. Symbols as in Fig. 1.

Effect of iodoacetamide and spore concentration on inactivation of spores with γ -radiation

The effect of iodoacetamide (IAM) present during irradiation on the shape of radiation dose:survivor curves of *Bacillus cereus* PX spores varied according to the spore concentration. This is shown in Fig. 5(a-c), in which initial spore concentrations of 1.5×10^{10} , 1.5×10^8 and 1.5×10^6 per ml. were used respectively. Spores in the most concentrated suspension were hardly influenced by even 10 mM IAM (Fig. 5a), whereas spores at lower concentrations were clearly more readily killed when IAM was present (Fig. 5b, c). Even 0.1 mM IAM strongly increased the radiation kill when the spore concentration was only 1.5×10^6 per ml. (Fig. 5c). The apparent synergism of IAM and radiation depended more on the radiation dose than on the IAM concentration over the range studied. In addition to steepening the survivor curves, IAM caused them to become convex (Fig. 5b, c). This suggested that the apparent synergism of IAM with γ -radiation depended on some function which increased with increase in radiation dose. The same pattern of response was seen when potassium iodate was used as potentiator.

Absence of spore inactivation by 'carried over' iodoacetamide and iodate

The possibility existed that the increased slopes of survivor curves in Fig. 5(b, c) were due to inhibition of colony formation caused by potentiator carried over with the diluted spore suspensions into the plates used for counting. To investigate this,

Fig. 3

Fig. 4

Fig. 3. Dose:survivor curves of spores of *Bacillus cereus* γ -irradiated in heart infusion broth to which was added (1 mM) iodoacetamide (●), potassium iodide (□), potassium iodate (Δ), control, no addition (○).

Fig. 4. Dose:survivor curves of spores of *Bacillus cereus* γ -irradiated in water containing reagents for thiol groups. The concentration of spores was 1.5×10^8 /ml. ●, Iodoacetamide (1 mM); □, *p*-chloromercuribenzoic acid (1 mM, pH value to 7.0 with NaOH); Δ, *N*-ethylmaleimide (1 mM); ○, control (no addition). Arrow indicates that the counts were below the level shown by the symbol.

Table 2. *Effect of concentrations of potassium iodate, sodium bromate and N-bromosuccinimide on inactivation by γ -radiation of spores of Bacillus cereus*

Spores (1.5×10^8 ml.) were suspended in water or in aqueous solutions of the reagents for irradiation. Unirradiated control suspensions retained full viability in the presence of the reagents. The γ -radiation dose was 0.6 Mrad.

Reagent	Concentration (mM)	Spores surviving radiation (%)
Control (no addition)	—	2.87
Potassium iodate	0.01	0.85
	0.1	0.087
	1.0	< 0.00001
Sodium bromate	0.01	2.94
	0.1	2.43
	1.0	0.024
<i>N</i> -Bromosuccinimide	0.01	1.24
	0.1	0.84
	1.0	< 0.00001

control spores and spores which had been γ -irradiated at a dose of 0.6 Mrad were incorporated into yeast glucose agar plates containing different concentrations of IAM or KIO_3 . The numbers of colonies which grew when the plates were incubated (Table 3) showed that these potentiators in the plates were not inhibitory to control or irradiated spores as long as their concentrations did not exceed about $10\ \mu\text{M}$ IAM or $1\ \text{mM}$ KIO_3 . As these concentrations were not exceeded in any of the plates during the experiments described, it was concluded that the observed synergism of these compounds with γ -radiation was not due to carry-over.

Fig. 5. Influence of iodoacetamide on inactivation of different concentrations of spores of *Bacillus cereus* by γ -radiation. Spore concentrations were (a) $1.5 \times 10^{10}/\text{ml.}$, (b) $1.5 \times 10^8/\text{ml.}$, (c) $1.5 \times 10^6/\text{ml.}$ Concentrations of iodoacetamide present during irradiation of the aqueous spore suspensions were nil ($\circ$), $100\ \mu\text{M}$ ($\bullet$), $1\ \text{mM}$ ($\triangle$), $10\ \text{mM}$ ($\square$).

Table 3. *Effect of iodoacetamide in the medium on viability of control and γ -irradiated spores of Bacillus cereus*

Poured plates were made from yeast glucose agar containing different concentrations of iodoacetamide or potassium iodate and about 150 viable spores per plate. The irradiated spores had been 98% inactivated by a dose of 0.6 Mrad.

Concentration of potentiator in the medium	Colony count (%) following incubation in medium containing			
	Iodoacetamide		Potassium iodate	
	Unirradiated spores	Irradiated spores	Unirradiated spores	Irradiated spores
$10\ \mu\text{M}$	84.3	104	113	104
$100\ \mu\text{M}$	70.8	18.8	123	96
$1\ \text{mM}$	0	0	119	64.5
$10\ \text{mM}$	0	0	0	0

Role of leakage products in protecting high spore concentrations from γ -radiation plus IAM

Since irradiated spores are known to leak material including calcium, dipicolinic acid, amino acids and peptides (Farkas & Kiss, 1965) it was possible that high spore concentrations were less susceptible to radiation plus IAM because of leakage of protective substances. To test this, spores of *Bacillus cereus* at a concentration of 1.5×10^{10} /ml. were first irradiated in water at 0.6 Mrad and then centrifuged. Fresh spores at the lower concentration of 1.5×10^8 /ml. were then suspended in the supernatant from the irradiated thick suspension and irradiated at 0.6 Mrad in the presence or absence of 1 mM IAM. Table 4 shows that the supernatant from the irradiated dense suspension protected the less-dense suspension from the lethal effect of radiation plus IAM. (The low overall recovery from samples containing supernatant was due to some germination (about 70 %) which occurred during preparation.)

Table 4. *Protective effect of leakage products on the lethal action of γ -radiation plus iodoacetamide on spores of *Bacillus cereus**

Spores (1.5×10^8 /ml.) were suspended in water or in supernatant fluid from an irradiated (0.6 Mrad) dense spore suspension (1.5×10^{10} /ml.). The γ -radiation dose was 0.6 Mrad. Iodoacetamide concentration was 1 mM.

Suspending fluid	Spores (%) surviving irradiation	
	In absence of IAM	In presence of IAM
Water	1.04	0.032
Supernatant from irradiated dense suspension	0.47	0.470

Effect of pH value on effectiveness of the potentiators

The three potentiators iodoacetamide, iodide and iodate were all more effective at low than at high pH values (Table 5), though the relative effect of pH value was different for each reagent and effectiveness of the thyroid hormone analogue 3,5-diiodo-L-tyrosine was hardly pH-dependent. Control spores were more radiation-sensitive at low than at high pH. When spores were irradiated with iodide or iodate in a rich medium in place of buffers, the increased effect at low pH values was lost (Table 6). Indeed, the potentiators were now less effective at low than at high pH values, though this was partly due to germination (resulting in enhanced radiosensitivity) of spores in the rich medium at higher pH values during the experiment.

Antagonism of potentiators by histidine: role of iodine and long-lived radiation products

To quench any iodine, and probably also any iodine-based free radicals formed during irradiation, histidine was included with some samples (Schramm, 1964). The results (Table 7) showed that histidine indeed antagonized action of the three potentiators tested, suggesting involvement of iodine or halogen-based free radicals.

Iodine itself killed spores in the absence of radiation during a similar time-temperature regime to that undergone by the irradiated spores (Table 8) and, like irradiation in the presence of some of the potentiators (Table 5), was more lethal at low than at high pH value.

Aqueous solutions of IAM, KI and KIO₃ (1 mM) irradiated alone at 0.6 Mrad were not toxic when inoculated with *Bacillus cereus* spores (1.5×10^8 /ml.) and incubated for up to 24 h. at room temperature, suggesting that any major toxic irradiation products were short-lived.

Table 5. *Influence of pH value on destruction of spores of Bacillus cereus by γ -radiation in the presence of iodine compounds in buffers*

Spores (1.5×10^8 ml.) were suspended in sodium phosphate solutions (10 mM) with or without the reagents and pH values adjusted with HCl or NaOH. The radiation dose was 0.6 Mrad.

Reagent	Spores (%) surviving radiation in buffer at			
	pH 3.0	pH 5.0	pH 7.0	pH 9.0
Control (no addition)	0.300	1.440	3.940	5.800
Iodoacetamide (1 mM)	$< 10^{-4}$	0.0004	0.170	0.650
Potassium iodide (1 mM)	$< 10^{-4}$	0.025	0.100	0.079
Potassium iodate (1 mM)		$< 10^{-4}$	$< 10^{-4}$	0.00076
Potassium iodate (0.1 mM)	0.016	0.071	0.069	0.046
3,5-Diiodo-L-tyrosine (1 mM)	0.450	0.590	0.460	0.220

Table 6. *Influence of pH value on destruction of spores of Bacillus cereus by γ -radiation in the presence of potassium iodide or iodate in a rich medium*

Spores (1.5×10^8 /ml.) were suspended in heart infusion broth adjusted to the pH values shown with HCl and containing KI or KIO₃ at 1 mM. The radiation dose was 0.6 Mrad.

Reagent	Spores (%) surviving radiation in broth at			
	pH 4.0	pH 5.0	pH 6.0	pH 7.0
Control (no addition)	4.23	8.00	0.54	0.120
Potassium iodide	4.65	5.20	0.79	0.040
Potassium iodate	2.30	3.62	0.23	0.007

Table 7. *Antagonism by histidine of the potentiating action of iodine compounds on inactivation of Bacillus cereus spores by γ -radiation*

Spores (1.5×10^8 /ml.) were suspended in water containing the reagents $\pm$ histidine (10 mM). The radiation dose was 0.6 Mrad.

Reagent and concentration	Histidine present or absent	Spores (%) surviving radiation
Control (no addition)	Absent	2.800
	Present	2.600
Iodoacetamide (1 mM)	Absent	0.0001
	Present	1.570
Potassium iodide (1 mM)	Absent	0.068
	Present	1.000
Potassium iodate (0.1 mM)	Absent	0.085
	Present	0.900

Potentialiation of radiation inactivation at different temperatures

Spores of *Bacillus cereus* in water were inactivated by γ -irradiation more or less similarly at -15° , 2° and 30° . However, when spores were irradiated in potassium iodate solutions, temperature had a much greater effect on inactivation. The potentiating action of iodate was completely suppressed in the frozen samples at -15° (Fig. 6a), and measurably less intense at 2° than at 30° (Fig. 6b, c).

Fig. 6. Influence of temperature on inactivation of spores of *Bacillus cereus* by γ -radiation in the presence of potassium iodate. Temperatures during irradiation were (a) -15° , (b) 2° , (c) 30° . The aqueous spore suspensions contained about 10^8 spores/ml. and either no addition (O), or potassium iodate at $100\ \mu\text{M}$ (●) or $1\ \text{mM}$ (Δ). Arrows indicate no recovery at that level of count.

Table 8. *Lethality of iodine for spores of Bacillus cereus at different pH values*

Buffers were sodium phosphate (10 mM) with pH values adjusted as for the radiation experiments (see Table 5). Iodine was dissolved in KI solution ($2\times$ molarity of I_2). Spores ($1.5\times 10^8/\text{ml.}$) were exposed to the reagents in sealed ampoules at room temperature for the indicated times before dilution for viable counts.

pH value	Iodine concentration	Survivors (%) following incubation in iodine solution for:		
		1 h.	4 h.	24 h.
3.0	500 μM	0.17	0.0024	$< 10^{-4}$
	50 μM	85.0	48.5	6.65
5.0	500 μM	0.063	0.0079	$< 10^{-4}$
	50 μM	104	121	101
7.0	500 μM	0.16	0.024	$< 10^{-4}$
	50 μM	103	116	111
9.0	500 μM	0.24	0.021	$< 10^{-4}$
	50 μM	124	102	73.5

*Effect of γ -radiation plus potentiators on spores other than *Bacillus cereus* PX*

Spores of *Bacillus coagulans*, *B. subtilis*, *B. polymyxa* and *B. cereus* strain T were all killed more readily by γ -radiation when in the presence of IAM and KIO₃, though the effectiveness of the potentiators differed quantitatively according to the spores used.

DISCUSSION

A variety of halogen compounds could potentiate the lethal action of γ -radiation for spores of *Bacillus cereus* and, in addition to toxic chemicals like IAM, relatively innocuous compounds like L-thyroxine, diiodo-L-tyrosine, iodide and iodate could act as potentiators. In homologous series potentiator activity decreased in the order I > Br > Cl and F, the latter two being essentially inactive. The potentiation was probably due mostly to halogen or halogen-free radicals rather than thiol reactivity of some of the reagents, because the two reagents for thiols, *p*CMB and NEM, did not potentiate action of radiation on spores whereas iodide and bromide, which one would not expect to react with thiols, did potentiate. In contrast, certain radiation-resistant vegetative bacteria could be inactivated readily by ionizing radiation in the presence of reagents for thiols (Bridges, 1968). For example, *N*-ethylmaleimide was a potentiator for the radiation inactivation of *Escherichia coli* and a *Pseudomonas* species (Bridges, 1961, 1962), as was *p*-hydroxymercuribenzoate for *Micrococcus radiodurans*, *E. coli* and *Sarcina lutea* (Bruce & Malchman, 1965). Further study suggested that thiol reactivity was not the sole attribute of effective potentiators (Barnes, Ashwood-Smith & Bridges, 1969; Mullenger & Ormerod, 1969). These reagents may in fact act by being particularly efficient electron acceptors, reacting with short-lived hydrated electrons to form negatively charged radicals which are longer-lived and more able to damage vegetative bacteria (Adams & Dewey, 1963; Adams & Cooke, 1969); however, such radicals, at least from NEM and *p*CMB, were negligibly lethal for spores.

Quenching by histidine, which would react with iodine (Schramm, 1964) but not with free IAM, supports the hypothesis that potentiation of the radiation inactivation of spores was caused by halogen-free radicals. This hypothesis is also compatible with the observed convex shape of the survivor curves of spores irradiated in water plus IAM or iodate, which indicated an effect which was cumulative with radiation dose. Loss of potentiator activity at -15° strongly implicated free radical intermediates. The low content of organic matter in fresh well-cleaned spore suspensions probably accounted for their greater sensitivity to potentiators than old less-clean suspensions. The effect of pH value was interesting in that *potentiation* was greatest at low pH values when no rich medium was present, but in the presence of medium the potentiator was more effectively *antagonized* at low pH value. Presumably the toxic radiation product(s) reacted more effectively at low than at high pH values with either medium constituents ('antagonism') or with the spores ('potentiation'). The reason for antagonism of potentiators by sodium phosphate buffers is not clear, because antagonism did not depend on pH value alone.

Iodine alone killed spores during the same periods of time involved in the radiation experiments (< 3 h.). Could it be that free iodine formed by radiolysis partly caused the observed potentiation? Antagonism of the potentiation by histidine is compatible

with the toxic agent being free iodine. A comparison of Tables 5 and 8, however, shows that the lethal effect of the most effective potentiators (see, for example, 0.1 mM-KIO₃ in Table 5) was too great to be solely accounted for by the equivalent amount of iodine, even assuming stoichiometric yield of free halogen (50 μ M, see Table 8) from the potentiator during irradiation. Furthermore, irradiation of potentiator solutions at their effective concentrations did not make them subsequently toxic to spores, as one would have expected had sufficient stable iodine molecules been formed. The results therefore suggested that some further radiation product was involved in killing spores, most probably a halogen-containing free radical such as was proposed by Dewey & Michael (1965) to explain potentiation of the radiation inactivation of *Micrococcus radiodurans* and other vegetative bacteria by IAM. Indeed, study of the effect of free radical reactants on potentiation of radiation inactivation of *M. sodonensis* by iodide suggested that the responsible radicals were I $\cdot$ and/or I₂ $\cdot^-$ (Mullenger, Singh, Ormerod & Dean, 1967), formed by reaction of I $^-$ ions with OH radicals produced by radiolysis of water (Sworski, 1955; Dainton & Peterson, 1962).

The enhancing effect of halides and halogenophenols on the lethal action of ionizing radiation for vegetative bacteria and yeasts was studied extensively by Namiki and Matsuyama and their colleagues. Halogenophenols were effective in the decreasing order I > Br > Cl, as in this study, and monosubstituent positions in the order *p*- > *m*- > *o*- (Matsuyama, Namiki, Okazawa & Kaneko, 1963). In contrast, we found *o*-, *m*- and *p*-iodobenzoic acids to be about equally effective as potentiators with spores. Their data suggested that death of *Escherichia coli* irradiated plus halogenophenols resulted largely from indirect action, including toxic free radicals and phenols formed by radiolysis (Namiki, Matsuyama, Okazawa & Kaneko, 1963). Relative resistance of spores to phenols may explain responses different from those obtained with vegetative cells. High concentrations of NaCl (e.g. 10 %, w/v) enhanced the lethality of γ -radiation for *E. coli* (Matsuyama, Okazawa, Namiki & Sumuki, 1960) partly, but not solely, because of increased sensitivity of irradiated cells to high concentrations of NaCl compared with unirradiated cells (Okazawa, Namiki, Yamashita & Matsuyama, 1960). Study of other halides showed that iodide and bromide were more effective than chloride as potentiators of the radiation inactivation of *E. coli* (Namiki, Okazawa & Matsuyama, 1961) and iodate was a particularly effective potentiator (Kada, 1969): as with spores, the halides acted most effectively at low pH values and, it was postulated, probably via the agency of reactive free radicals.

Potentiation of radiation inactivation of such dormant forms as spores is certainly distinct from the potentiation caused by halogenated pyrimidines and related compounds in actively growing bacterial or mammalian cells (Vermund & Gollin, 1968). In such instances potentiation depends on incorporation of the metabolite into cell macromolecules. It is likely that in spores irradiated in the presence of diverse iodine and bromine compounds the lethal damage is more directly inactivation of enzyme(s) critical in spore germination (Gould, 1971), rather than damage to deoxyribonucleic acid or repair processes such as may result from ionizing radiation alone. The sites of lethal damage in similarly irradiated vegetative bacteria are not yet known with certainty.

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REFERENCES

- ADAMS, G. E. & COOKE, M. S. (1969). Electron-affinic sensitization. I. A structural basis for chemical radiosensitizers in bacteria. *International Journal of Radiation Biology* **15**, 457-471.
- ADAMS, G. E. & DEWEY, D. L. (1963). Hydrated electrons and radiobiological sensitization. *Biochemical & Biophysical Research Communications* **12**, 473-477.
- BARNES, J. H., ASHWOOD-SMITH, M. J. & BRIDGES, B. A. (1969). Radiosensitization of bacterial cells by carbonyl compounds. *International Journal of Radiation Biology* **15**, 285-288.
- BRIDGES, B. A. (1961). The effect of *N*-ethylmaleimide on the radiation sensitivity of bacteria. *Journal of General Microbiology* **29**, 467-472.
- BRIDGES, B. A. (1962). The chemical sensitization of *Pseudomonas* species to ionizing radiation. *Radiation Research* **16**, 232-242.
- BRIDGES, B. A. (1968). Sensitization of organisms to radiation by sulphhydryl-binding agents. *Advances in Radiation Biology* **3**, 70-94.
- BRUCE, A. K. & MALCHMAN, W. H. (1965). Radiation sensitization of *Micrococcus radiodurans*, *Sarcina lutea*, and *Escherichia coli* by *p*-hydroxymercuribenzoate. *Radiation Research* **24**, 473-481.
- DAINTON, F. S. & PETERSON, D. B. (1962). Forms of H and OH produced in the radiolysis of aqueous systems. *Proceedings of the Royal Society A* **267**, 443-463.
- DEAN, C. J. & ALEXANDER, P. (1962). Sensitization of radioresistant bacteria to X-rays by iodoacetamide. *Nature, London* **196**, 1324-1326.
- DEAN, C. J. & ALEXANDER, P. (1965). The sensitization of bacteria to X-rays by iodoacetamide and related compounds. *Progress in Biochemical Pharmacology* **1**, 46-51.
- DEWEY, D. L. & MICHAEL, B. D. (1965). The mechanism of radiosensitization by iodoacetamide. *Biochemical & Biophysical Research Communications* **21**, 392-396.
- FARKAS, J. & KISS, I. (1965). Observations on biochemical changes in irradiated spores of *Bacillus cereus*. *Acta biologica Academiae scientiarum hungaricae* **12**, 15-21.
- GOULD, G. W. (1970). Mechanism of the inhibition of germination of bacterial spores by γ -irradiation in the presence of iodoacetamide and iodate. *Journal of General Microbiology* **64**, 301-309.
- KADA, T. (1969). Radiosensitization by potassium iodate and related compounds. *International Journal of Radiation Biology* **15**, 271-274.
- MATSUYAMA, A., NAMIKI, M. & OKAZAWA, Y. (1967). Alkali halides as agents enhancing the lethal effect of ionizing radiations on micro-organisms. *Radiation Research* **30**, 687-701.
- MATSUYAMA, A., NAMIKI, M., OKAZAWA, Y. & KANEKO, I. (1963). Synergistic actions of halogenophenols on radiation inactivation of micro-organisms. *Agricultural & Biological Chemistry* **27**, 349-357.
- MATSUYAMA, A., OKAZAWA, Y., NAMIKI, M. & SUMUKI, Y. (1960). Enhancement of radiation lethal effect on micro-organisms by sodium chloride treatment during irradiation. *Journal of Radiation Research* **1-2**, 98-106.
- MULLENGER, L. & ORMEROD, M. G. (1969). The radiosensitization of *Micrococcus sodonensis* by *N*-ethyl maleimide. *International Journal of Radiation Biology* **15**, 259-269.
- MULLENGER, L., SINGH, B. B., ORMEROD, M. G. & DEAN, C. J. (1967). Chemical study of the radiosensitization of *Micrococcus sodonensis* by iodine compounds. *Nature, London* **216**, 372-374.
- NAMIKI, M., MATSUYAMA, A., OKAZAWA, Y. & KANEKO, I. (1963). Some aspects of synergistic actions of halogenophenols on radiation lethal effect. *Agricultural & Biological Chemistry* **27**, 359-365.
- NAMIKI, M., OKAZAWA, Y. & MATSUYAMA, A. (1961). Combined effects of radiation and inorganic reagents during irradiation on radiosensitivity of bacterial cells. *Agricultural & Biological Chemistry* **25**, 108-114.
- OKAZAWA, Y., NAMIKI, M., YAMASHITA, S. & MATSUYAMA, A. (1960). Enhancement of overall lethal effect of ionizing radiations on micro-organisms by sodium chloride. *Bulletin of the Agricultural Chemistry Society of Japan* **24**, 235-242.
- SCHRAMM, M. (1964). Unmasking of sulphhydryl groups in pancreatic α -amylase. *Biochemistry* **3**, 1231-1234.
- SWORSKI, T. J. (1955). Yields of hydrogen peroxide in the decomposition of water by cobalt γ -radiation. II. Effect of chloride ion. *Radiation Research* **2**, 26-32.
- VERMUND, H. & GOLLIN, F. F. (1968). Mechanisms of action of radiotherapy and chemotherapeutic adjuvants. A review. *Cancer* **21**, 58-76.