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COMBINED GENETIC EFFECTS OF CHEMICALS AND RADIATION

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INTRODUCTION

The cell itself consists of chemicals such as water, minerals, proteins, and so on. Therefore all of radiation biology is essentially the study of combined effects of chemicals and radiation. However, this presentation concerns itself specifically with chemicals introduced, like radiation, from outside the cell, and with the combined effects of these two types of agents.

When we consider environmental hazards of radiations in human being, it is important to take into account possible interactions with environmental chemicals. Similarly, the benefits of radiation could be affected, e.g., radiation killing of cancer cells could be increased by the presence of radio-sensitizing agents. Therefore, it is pertinent to examine interactions of chemicals and radiation and their effects on the cell. In this paper, our attention is focussed on genetic effects.

PATTERNS OF THE COMBINED EFFECTS OF CHEMICALS AND RADIATION

Since species of chemicals are numerous and even one chemical may possess biological effects of different types, it is necessary to understand patterns of their interactions with radiation. These aspects are summerized in Fig. 1.

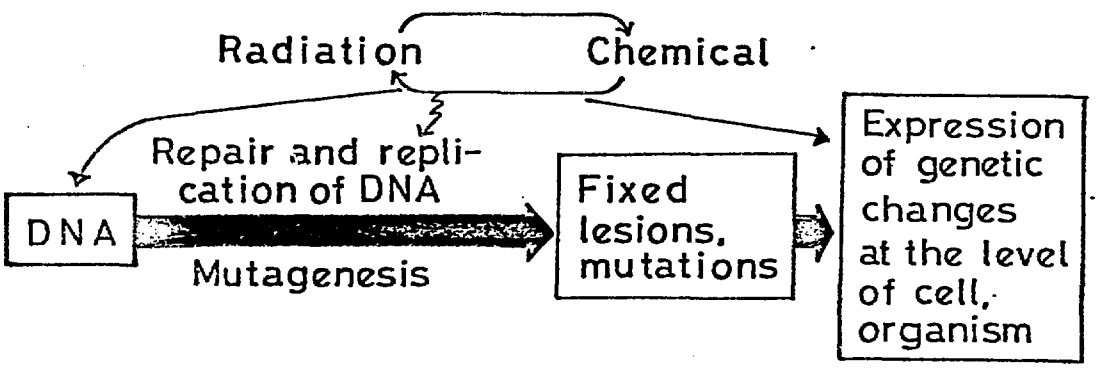


Fig. 1. Scheme of radiation chemical interactions producing genetic changes

The main interactions may be classified into the following three categories (Fig. 2).

Fig. 2. Types of combined effects

Type I

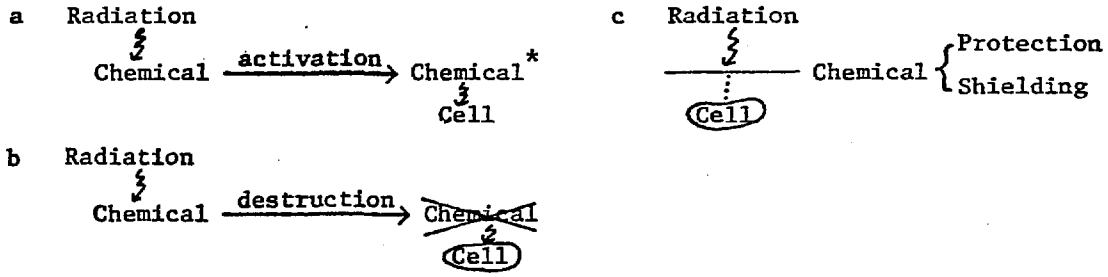
At extreme low doses:

each agent (radiation or chemical)
must act INDEPENDENTLY

The effects are ADDITIVE

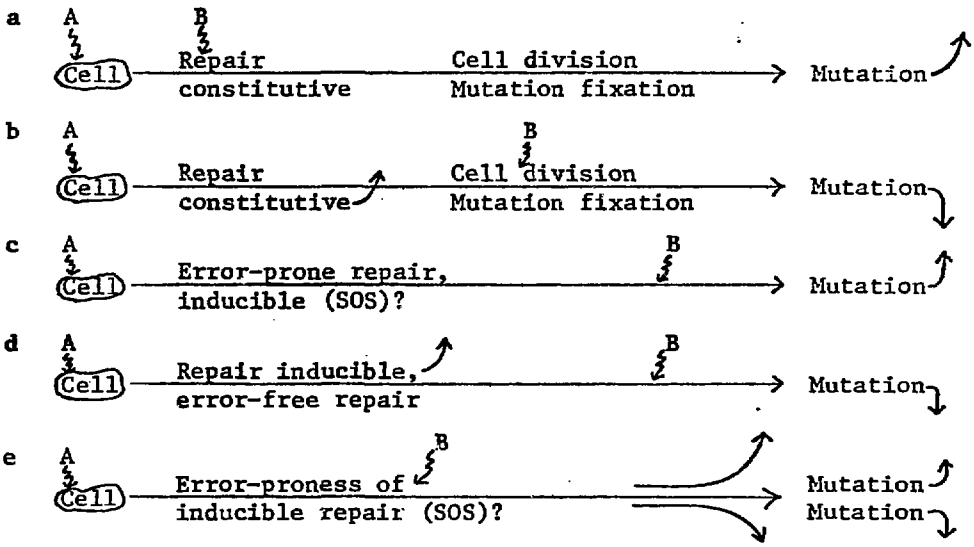
Types II

Interactions outside of the cell



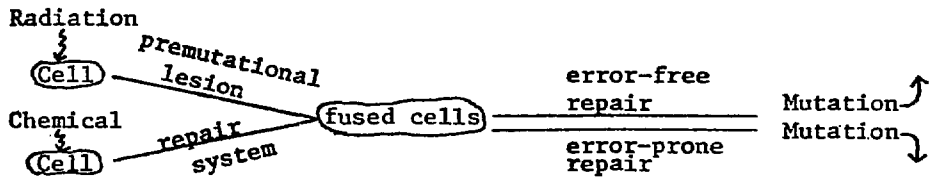
Types III

Interactions indirectly in the cell



Type IV

Interactions via two different cells



1] When doses of both chemicals and radiations are very low, no interaction is expected among them and their effects on the cell must be independent and arithmetically additional.

2] Interactions of chemicals and radiation outside of the cell.

a: Radiosensitization (or photosensitization) often involves radiation modification of chemicals producing DNA-damaging factors.

b: When chemicals are destroyed by radiations, the chemical effects are reduced or eliminated.

c: Radiations (especially light) are shielded by certain chemicals, acting as radio-protectors.

3] Interactions indirectly via the cell.

a: When an agent A (radiation or chemical) affects DNA as a mutagen and an other agent B (mostly chemical) affects the cellular DNA-repair machineries, the mutation induction by the former is enhanced by reducing the repair capacity.

b: When agent A affects DNA as a mutagen, mutation inducibility is lowered by inhibiting cell divisions with agent B, since this prolongs the period during which DNA repair can occur (such as excision repair in UV-exposed cells).

c: If a mutagenic agent A induces an error-prone repair enzyme, mutation inducibility by second agent, B, is generally enhanced,

d: If a chemical mutagen A induces an error-proof repair enzyme, the DNA damage produced by a second agent, B, is repaired more correctly; mutation inducibility is lowered.

e: Certain chemical agents may serve as either error-proofing or error-promoting factors. Induced mutability will be either declined or enhanced.

4] Interaction via two different cells

When agent A affects the genetic material of one cell, and agent B affects the repair system of another cell with which the first cell will fuse (as in the case of germcells of sexually reproducing organisms), the mutagenic action of agent A can be modified.

a. If agent B inhibits error-free repair processes, the mutation frequency will be enhanced.

b. If agent B inhibits error-prone repair process, the mutation frequency will be reduced.

EXAMPLES

In the course of our studies on mutation induction by chemicals and radiations, we encountered interaction phenomena of different types. Though analysis of the mechanisms involved are not complete, we describe below the typical data and their interpretations.

1] Photodynamic mutation induction by fluorescein dyes

One of the most detailed studies on photodynamic genetic actions has been done with psoralen (1). Similar effects are found by us with fluorescein dyes. Phloxine was the first chemical whose mutagenicity was detected by means of the Bacillus subtilis rec-assay, a DNA-repair test procedure for assaying DNA-damaging agents (2, 3). It was later noted that the DNA-damaging capacity of phloxine increased very much by exposing it to white light (Fig. 3). Yoshikawa and his collaborators (4) studied 10 different fluorescein dyes and concluded that most of them did not damage DNA without light. However, some of them, such as erythrosine, phloxine B and rose bengal showed marked DNA-damaging effects.

2] Radiosensitization with iodine compounds, DNA repair and mutation

The radiosensitization phenomena were first discovered some twenty years ago using iodine compounds, such as iodoacetic acid, iodoacetamide, or alkali halides (5-8). Since then, we have been interested in elucidating the mechanisms involved.

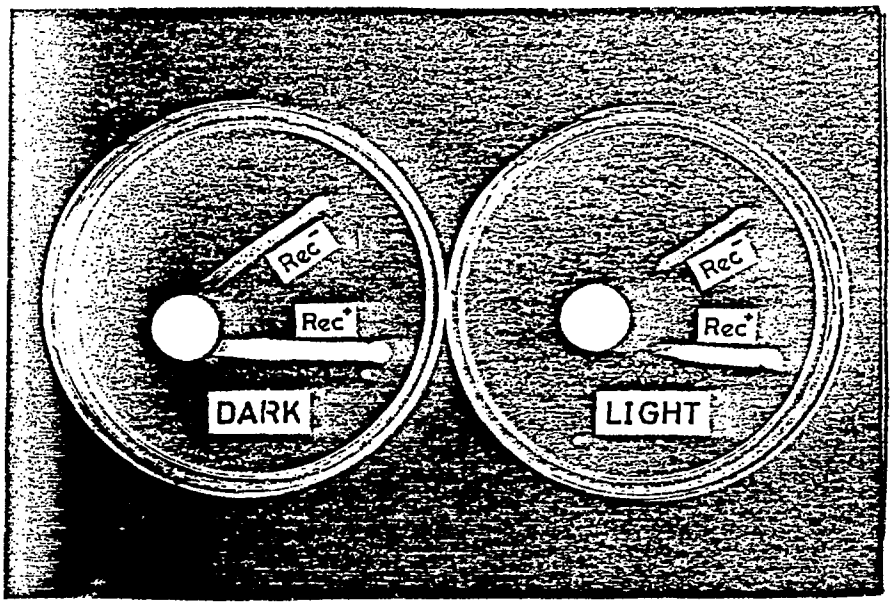


Fig. 3. Photodynamic effects of phloxine. DNA-damaging-capacities are shown by means of the Bacillus subtilis Rec-assay.

Our past studies showed that several halogenated purines or pyrimidines, such as 5-iodouracil, 5-iodocytosine, 5-iodouridine, 5-iodo-2'-deoxyuridine, 5-iodo-2'-deoxycytidine, 6-iodo-purine, and 6-chloropurine, are strong radiosensitizers, and that these compounds, dissolved in phsophate buffer solution at pH 5.4, produced by gamma-irradiation products toxic on B. subtilis or E. coli cells which are relatively stable at 0°C but are labile by heating or by treatment with SH-agents (9-13). Similar effects were also observed with iodoacetic acid, iodoacetamide or alkali halides. Involvement of such radiation-induced toxic products in iodine-radiosensitization was shown not only with bacteria but also with cultured mammalian cells in vitro (14-17).

Two hypothesis have been examined so far. In the first, radiation induced toxic products might inactivate repair enzyme(s) involved in DNA-repair in gamma-irradiated cells. In the second, the toxic products may damage essential proteins that are non-enzymatic and bound to the cell membrane.

In our studies on enzymatic mechanisms of repair of DNA damage induced by ionizing radiations (18-23), we isolated, from B. subtilis cells, PA (primer activating) enzymes that enhance priming activity for DNA-polymerase 1 (Micrococcus luteus) of gamma-ray irradiated DNA (Colicine El plasmides). We isolated an endonuclease and exonuclease. These enzymes are supposed to be involved in either excision of damaged bases or cleasing of "dirty" termi in repair pathways of DNA damage induced by ionizing radiations (Fig. 4). We tested if the radiation-induced toxic products might specifically inactivate the above gamma-ray specific repair enzymes. The answer is so far negative.

The alternate hypothesis is supported by the following experimental results. Genetic variant clones resistant to iodine-radiosensitization isolated from cultured cells of Chinese hamster were found to possess modified cell membrane as suggested by reduced aggregate forming ability in rotation culture (16).

It is noted that induced mutability by gamma-irradiation is reduced under iodine-radiosensitization conditions either in bacteria or in mammalian cultured cells (Fig. 5). We suppose that potentially mutant cells may possess DNA altera-

tions that are specifically sensitive to the radio-sensitization treatment.

TABLE I

Radio-sensitizing capacities and readiation-induced toxicities of halogenated bases and their derivatives. Cells of Bacillus subtilis Marbourg were (a) resuspended in phosphate buffer solutions containing each of reagents and exposed to gamma-ray (10 kR) from a ^{137}Cs source at a dose rate of 40 kR/hr or (b) each reagent buffer solution was first exposed to 10 kR gamma-ray at 0°C combined with bacteria and kept at 0°C for 10 min. See (10) for the details.

Reagent (1 mM)	(a) Survival fraction (%) after simultaneous treatment with γ-ray (10 kR) and drug		(b) Survival fraction(%) after treatment by irradiated (10 kR) drug solution	
	pH 7.0	pH 5.4	pH 7.0	pH 5.4
None	12.9	12.9	96.6	75.8
5-iodouracil	0.41	0.000011	17.2	0.000025
5-iodocytosine	0.52	0.010	21.9	0.000033
5-iodouridine	0.0035	0.0064	1.5	0.00037
5-iodo-2'-deoxyuridine	0.12	0.0011	3.1	0.00032
5-iodo-2'-deoxycytidine	0.0080	0.000028	3.1	0.000021
6-iodopurine	0.01	0.00040	1.5	0.000033
6-chloropurine	3.4	0.00058	10.9	0.000025

3] Antimutagenic action of cobaltous chloride

Presence of cobaltous chloride ($CoCl_2$) in plating media reduced very markedly nitrosoguanidine-induced reverse mutations in Escherichia coli B/r WP2 try (24). We then found that similar cobalt effects exist in gamma-ray-induced mutations (Fig. 6).

Different hypotheses exist to explain the above findings. In the first, the metal compound may act as an error-proofing factor in mutagenesis involving SOS repair functions that are supposed to be error-prone (25). This hypothesis is being examined in the in vitro system in our laboratory in which the rate of incorporation of mismatched bases into AT-polymer is measured in the presence or in the absence of cobaltous chloride. In an alternate hypothesis, we suppose that cobaltous chloride is acting in selection of mutants. Since we found that some Try^+ revertant clones are sensitive to the metal compound, it is probable that cobaltous chloride is acting only in selection of the metal-resistant clones. The metal sensitivity of revertants may be explained by supposing that gamma-irradiation

induces mutations very efficiently primarily at a mutator site. Subsequently, the mutator function acts on the try or its suppressor site. This hypothesis may be supported in different ways. For example, UV-induction of mutations in E. coli involves a nonchromosomal factor (17).

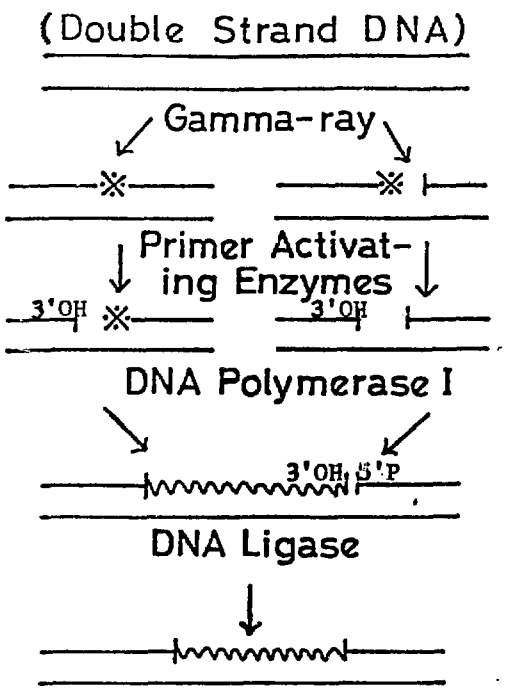


Fig. 4. Tentative scheme of processes of DNA-damage induced by γ-irradiation.

—— * —— DNA damage not resulting in chain scission

—— * |—— DNA damage resulting in chain scission

Newly synthesized region

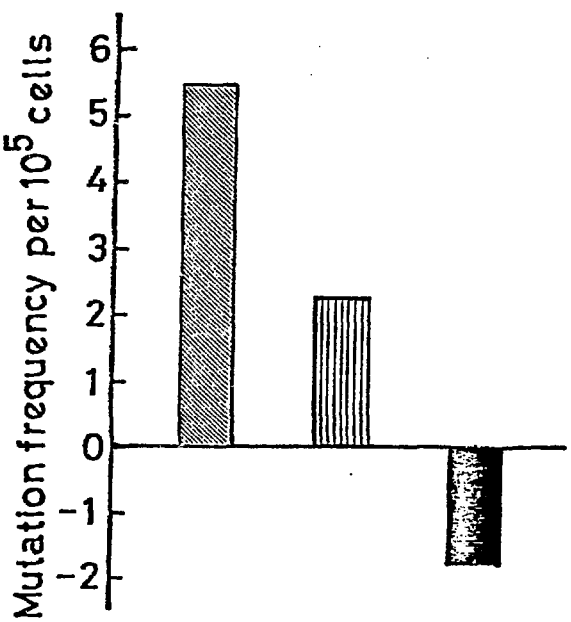


Fig. 5. Effect of γ-ray induced toxic products on γ-ray induction of 8-azaguanine resistant ($8AG^R$) mutations in Chinese hamster V79 cells. 1 x 10^5 cells were plated per dish and incubated for 24 hours. After washing with glucose-free Tyrode's solution they were exposed to γ-ray (400 R) from a ^{137}Cs source (6.4 kR/hr), treated with γ-ray irradiated 5 IU solution (3 mM, 10 kR, 0°C) for 20 min. at room temperature and incubated for 6 days for expression. $8AG^R$ colonies were scored by the replate methods.

γ-ray-induced

γ-ray-induced and treated with non-irradiated 5IU

γ-ray-induced and treated with irradiated 5IU

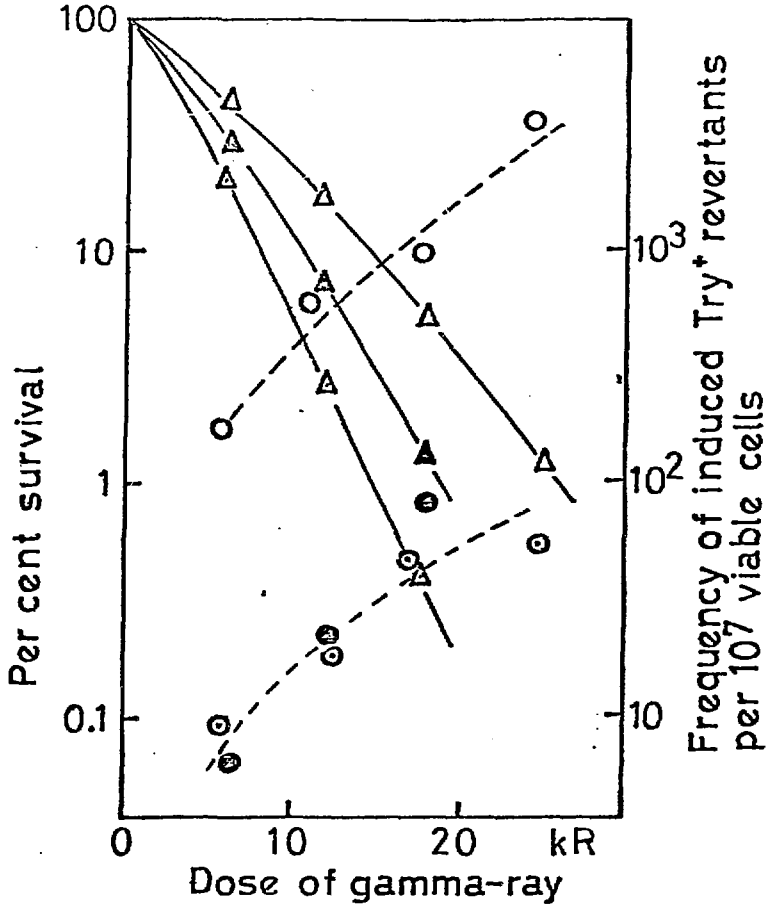


Fig. 6. Effect of cobaltous chloride on δ-ray induction of mutations in Escherichia coli WP2 B/r try. Bacteria grown overnight in broth were washed, exposed to δ-ray from a ^{137}Cs source at 0°C and plated after different dilutions on semi-enriched minimal agar containing cobaltous chloride and incubated for 2 days at 37°C. Experimental procedures are similar to those described in the (24) except irradiation.

Survivals

Δ——Δ $CoCl_2 \cdot 6H_2O$ 0 μg/ml

Δ——Δ " 15 μg/ml

Δ——Δ " 20 μg/ml

Mutations

O——O " 0 μg/ml

●——● " 15 μg/ml

⊙——⊙ " 20 μg/ml

4] Alteration of mutation frequency radiation-induced in the male by chemical treatment of the female

When Drosophila males (mature spermatozoa) were X-irradiated and mated to caffeine-treated females, the frequency of certain chromosomal mutations (dominant lethals and sex-chromosome losses) induced in the paternal genome was greater than when the irradiated males were mated to females that had not received caffeine (28). When the endpoint scored was translocations (formed by "misrepair"), the effect was in the opposite direction (29). These results were interpreted as caffeine-inhibition of a repair system present in the oocyte with which the irradiated sperm combines. Further evidence for such a postfertilization repair system comes from the finding that the genetic constitution of untreated females has a major influence on mutation rates obtained by mutagenizing a constant strain of males. This has been shown for the mouse (30) as well as for Drosophila (31).

In the mouse, experiments combining radiation to one sex with chemicals to the other are, to date, only in early stages. Males were X-irradiated (spermatozoa and spermatids sampled), and females (mature oocytes) treated with caffeine. When the endpoint was dominant lethals, the level of X-ray effect induced in the male genome was independent of caffeine treatment of the female (32). However, when the endpoint was sex-chromosome-loss, and a different strain of females was used, caffeine potentiation was statistically significant at the 5 % level (Russell et al. 1978 and unpublished), paralleling the Drosophila results.

CONCLUSIONS

Interactions of chemicals and radiation are complex and there may exist other unexpected patterns that are not mentioned above. We show some examples. "Photodynamic mutation induction by fluorescein dyes" (C1) and "Radiosensitization with iodine compounds" are classified as "Interactions of chemicals and radiation outside of the cell". On the other hand, the "Antimutagenic effects of cobaltous chloride" is concerned with events taking place in cells that had already been exposed to a mutagenic agent. It is likely that the action of a mutagenic agent is not direct and that cellular functions, such as mutators or repair systems, are involved in the mutagenesis initiated by the agent. Such cellular functions can be affected by a second agent. In sexually reproducing organisms, the two agents can also act on separate cells (♂ and ♀ germcells) which subsequently fuse. Interaction effects of all types will be useful in future research in shedding light on the main pathway of mutagenesis.

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