Induced Radiation Sensitivity with Four Radiation Protective Chemicals[1]

Many factors have been found to influence the dose-survival response of various forms of life. Among the factors studied has been the pretreatment of microorganisms and other cellular forms with various chemicals which induce an increased resistance to subsequent irradiation. Such radiation protection has been reviewed by HOLLAENDER and STAPLETON[2], PATT[3], STAPLETON[4], and others. In the course of several investigations, we have had occasion to use several of these protective chemicals on microorganisms and have encountered discrepant results from those previously reported.

The microorganisms used were *Nocardia corallina* (ATCC 4273), *Staphylococcus aureus* (O. U. culture collection), and *Escherichia coli* B/r (O. U. culture collection). *N. corallina* was grown on nutrient agar containing 1% fructose and incubated for 4 days at 29°C. *Staph. aureus* and *E. coli* B/r were grown on nutrient agar and incubated for 2 days at 37°C. Cell suspensions were prepared in saline blanks which were shaken on a vibratory shaker for 5 min and then centrifuged for 4 min at 2000 *g* to remove cell clumps. Each final suspension was examined microscopically for excessive clumping and was discarded unless it contained at least 90% single cells.

The chemicals used were C. P. glycine, pyruvic acid, sodium hydrosulfite, and phenol. Each chemical was prepared in a one molar concentration within one week prior to final dilution. The final concentration for each chemical, as determined by tolerance tests, was found to be glycine, 0·01 *M*; pyruvic acid, 0·1 *M*; sodium hydrosulfite, 0·1 *M*; and phenol, 0·01 *M*. One ml inoculum of each cell suspension was added to 5 ml of each chemical, and after 15 min contact, the organisms were centrifuged, washed, and resuspended in sterile physiological saline. The resulting suspensions were then irradiated with either X-ray or ultraviolet light.

X-irradiation was obtained from a Picker portable field unit operating at 96 kv and 4 ma using aluminium filtration. At the target distance used, the dose rate was 1000 r/min as measured with a Victoreen R meter. Cell suspensions were X-irradiated for varying times, appropriately diluted, and plated in nutrient agar. Ultraviolet irradiation was obtained from a Will Corporation germicidal lamp at a target distance of 7 inches for *N. corallina* and 14 inches for *Staph. aureus* and *E. coli* B/r. Surface plated suspensions in appropriate dilution were irradiated with the ultraviolet light.

The protective effect of pretreatment of *E. coli* B/r with the four chemicals was confirmed with both X-ray and ultraviolet light. However opposite results were obtained with *S. aureus* and *N. corallina*. Instead of displaying a protective effect, each of the four chemicals sensitized *S. aureus* to X-irradiation as shown in Figure 1. Glycine was found to have the smallest sensitization effect and pyruvic acid caused the greatest sensitization. The effect of the chemicals on the ultraviolet dose-survivor response of the organisms is given in Figure 2. With both sodium hydrosulfite and phenol treated cultures, there was no appreciable change from the rate of inactivation of the untreated culture. However both pyruvic acid and glycine sensitized *S. aureus* to ultraviolet light. Similar results using *N. corallina* were obtained with X-irradiation (Figure 3) and ultraviolet light (Figure 4).

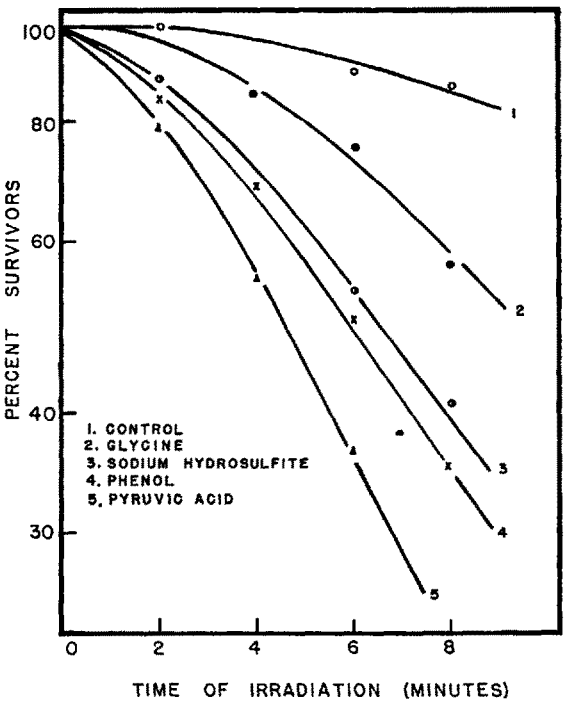

Fig. 1.—The effect of four 'protective' chemicals on the X-ray response of *Staphylococcus aureus*

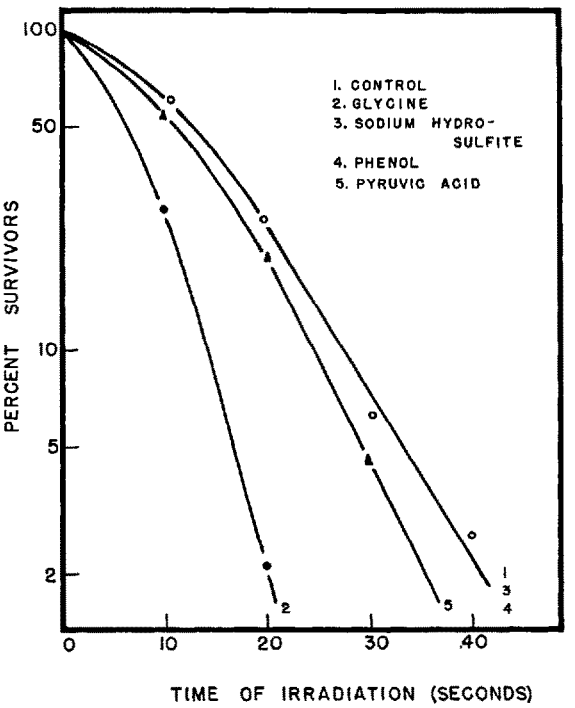

Fig. 2.—The effect of four 'protective' chemicals on the ultraviolet response of *Staphylococcus aureus*

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[2] A. HOLLAENDER and G. E. STAPLETON, Physiol. Rev. *33*, 77 (1953).

[3] H. M. PATT, Physiol. Rev. *33*, 35 (1953).

[4] G. E. STAPLETON, Ann. N. Y. Acad. Sci. *59*, 604 (1955).

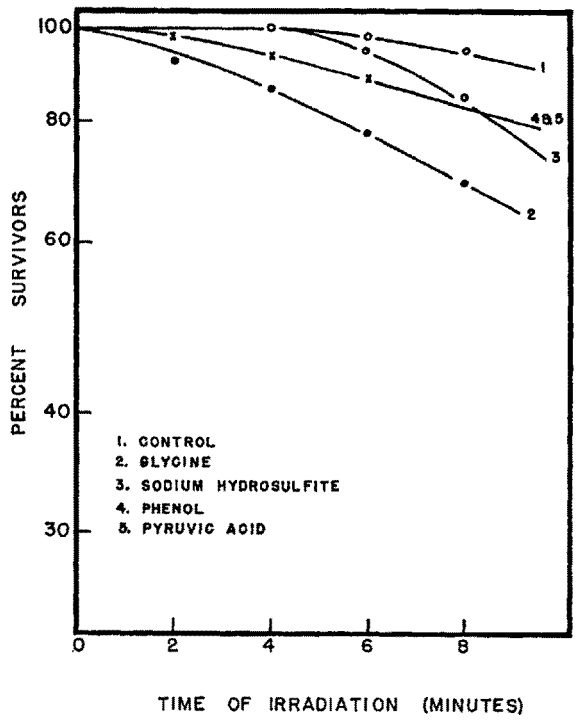

Fig. 3.—The effect of four 'protective' chemicals on the X-ray response of *Nocardia corallina*

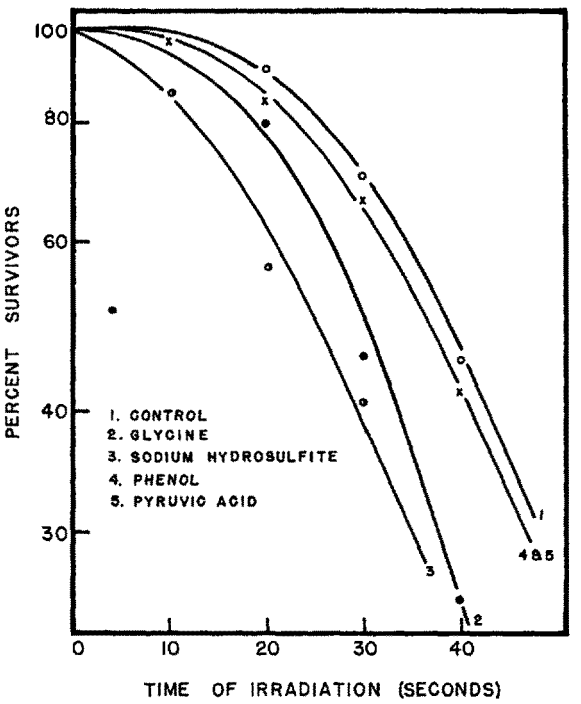

Fig. 4.—The effect of four 'protective' chemicals on the ultraviolet response of *Nocardia corallina*

According to PATT[3], the radiation protective effect of pretreatment of cell suspensions with various chemicals supports the premise that many of the biological effects of radiation are indirect. This mainly involves the transfer of energy from radiation activated water molecules to essential biological components. Many of the protective effects could thus be explained on the basis of both the treatment chemical and some biological component competing for active radicals which are formed in the irradiated water. However such simple competition cannot be used as an explanation in all cases, and therefore more complex energy transfer schemes have been postulated. It does not appear probable that competition for active radicals should be operative in *E. coli* B/r and not in *S. aureus* and *N. corallina*.

STAPLETON[4] suggested that the lethal action of ionizing radiations on bacterial cells could be due to a radiation initiated chain reaction that results in the ultimate destruction of a large number of biologically important molecules. The terminal result of the chain reaction in the case reported here would be the inhibition of cell division, which is the cellular function which was used as an index of radiation effect. The inhibition of cell division could be caused by any one of many cellular alterations, so no single terminal action of the radiation was detected.

There is accumulated evidence that some radiation damage has a delayed effect, and some radiation damage may be repaired by the cell[5,6]. Since a so-called protective chemical may impart increased radiation resistance to one genus and radiation sensitivity in another, it is conceivable that the chemical alters the normal metabolism of the cell to such an extent as to enhance or impair the radiation recovery ability of the cell. If such is the case, it would appear that a study of the protective action of various chemicals on cells of different metabolic types might yield better insight into the mechanisms involved in the protective action.

A second explanation of the discrepant results reported here is that an oxygen effect is the determining cause. Each of the cells studied may have different internal availability of oxygen which is altered in the chemically changed metabolic pattern of the cell. If so, again the study of metabolic types could aid in a better understanding of protective action.

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Résumé

L'action protectrice de la glycine, de l'acide pyruvique, du phénol, et de la soude hydrosulfite contre les dommages causés à l'*Escherichia coli* par l'irradiation aux rayons X ou bien aux rayons ultraviolets a été confirmée. Toutefois, il a été constaté que ces corps chimiques rendent les cellules de *Staphylococcus aureus* et de *Nocardia corallina* plus sensibles à l'action de ces deux radiations.

[5] A. HOLLAENDER (Ed.), *Radiation Biology*, Vol. I. *High Energy Radiation* (McGraw Hill, New York).

[6] A. HOLLAENDER (Ed.), *Radiation Biology*, Vol. II. *Ultraviolet and Related Radiations* (McGraw Hill, New York).