Influence of Chloramphenicol on Irradiated Bacteria

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An increase in the survival number was observed when UV-irradiated *E. coli* B cells were held in presence of chloramphenicol [1]. On the other hand under identical condition a decrease in the survival level was reported after UV-exposure of some other strains of *E. coli* by the same author. These apparently contradictory findings led us to study the response of X- and UV-irradiated bacteria to later treatment with chloramphenicol. The bacteria used were *E. coli* 15 T A U-bar, requiring thymine, arginine and uracil for its growth. The cells were routinely grown in nutrient broth.

An overnight culture was diluted 50-fold and the growth was continued at 37 °C water-bath shaker with continuous shaking. The cells were collected at logarithmic phase of growth and washed free of all nutrients with tris buffer. Cells were then suspended in warmed tris-glucose media supplemented with thymine [2]. X-irradiation was done in cold in a watch glass from an X-ray machine run at 80 kV, 9 mA. The dose rate at the position and condition of irradiation as measured from $FeSO_4$-dosimetry was 6 krads per min. Irradiation with UV-light was carried out with a 15 W G.E. germicidal lamp. The dose rate was 7.5 erg/mm²/sec as determined from ultraviolet actinometry. Catalase was used in the incubation media at a concentration of $(2-4)\,\mu$g per ml and chloramphenicol (CM) at a concentration of 100 μg per ml. The viable number of bacteria was determined by spreading technique on nutrient agar plates. The visible colonies developed on the plates after 18 hours of incubation at 37 °C were counted for viable number.

The influence of chloramphenicol and catalase on the survival of variously X-irradiated bacteria is shown in Fig. 1. Their effect on the unirradiated control cells is also shown in the same figure. It may be seen from the uppermost horizontal line of the figure that the presence of chloramphenicol, catalase or of both did not influence in any way the unirradiated bacterial number. The number surviving remained constant at 100% level for the four hours of experiment. However, when the cells were progressively exposed to higher doses of X-rays, incubation at 37 °C showed that there was a decrease in number upto a period of half an hour in cases of all doses of X-rays. The decrease in X-ray survivors due to X-irradiation depended upon the dose of X-ray. Interesting results were obtained when the cells variously exposed to X-ray were incubated in buffer containing chloramphenicol (100 μg/ml). The decrease in X-ray survivors due to the incubation only in buffer was further enhanced by the incubation of X-irradiated bacteria in presence of chloramphenicol. Thus at a particular dose of X-ray, survival number of irradiated cells after one hour incubation in buffer only is higher than the survival obtained by incubation of the irradiated cells in presence of chloramphenicol for the same period. This enhancement of the death of X-irradiated bacteria by the treatment of chloramphenicol is directly related to the dose of irradiation. The chloramphenicol-induced death could not be checked by using catalase in the incubation medium.

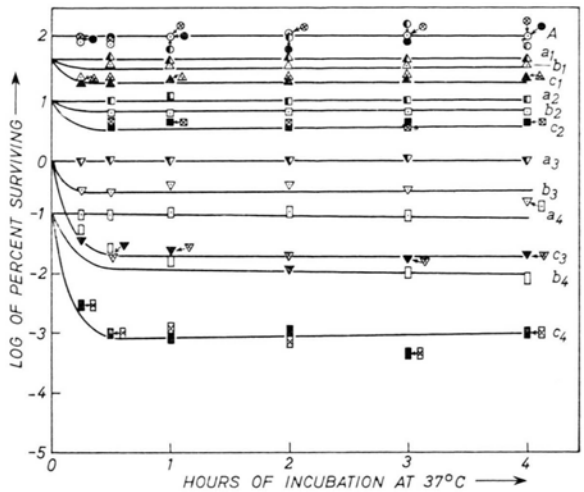

Fig. 1. Survival of bacteria: Curve A; Unirradiated and incubated at 37 °C in phosphate buffer containing: ⊙: CM (0) + catalase (O); ◐: CM (100 μg/ml) + catalase (0); ●: CM (0) + catalase (2 μg/ml). ⊗: CM (100 μg/ml) + catalase (2 μg/ml); Curves a_1, a_2, a_3, a_4: Irradiated to 6, 12, 18, 24 Krads of X-rays respectively and incubated in P.B. + catalase (2 μg/ml) + CM (0); Curves b_1, b_2, b_3, b_4: Irradiated to 6, 12, 18, 24 Krads respectively and incubated in P.B. + CM (0) + catalase (0). Curves c_1, c_2, c_3, c_4: Irradiated to 6, 12, 18, 24 Krads and incubated in P.B. + CM (100 μg/ml) + catalase (0) shown by ▲, ◪, ▽, ▮ respectively and incubated in P.B. + CM (100 μg/ml) + catalase (2 μg/ml) shown by ⧋, ⊠, ▽, ⊠ respectively.

Fig. 2 shows how survival of UV-irradiated bacteria was influenced by incubation in presence of chloramphenicol (100 μg/ml). As seen from Fig. 2, the initial bacterial number surviving a dose of UV-light decreases rapidly and reached a saturation in about half an hour of incubation in buffer. The survival curves obtained by holding UV-irradiated cells in presence or absence of chloramphenicol were coincident. Moreover, catalase in the incubation medium could not stop the death of UV-irradiated bacteria.

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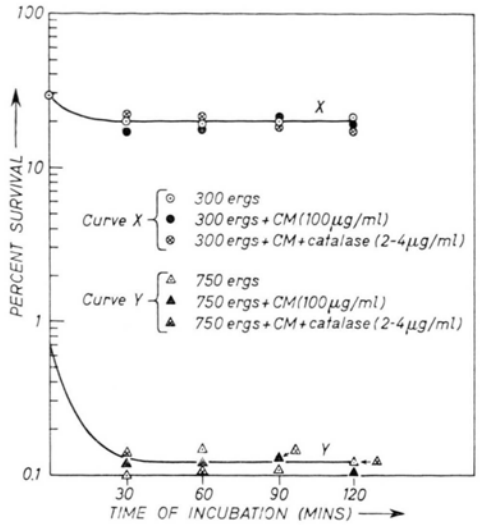

Fig. 2. Survival curves after 300 erg (X) and 750 erg (Y) of UV: ⊙, △, UV-irradiated bacteria incubated only in buffer. ●, ▲, UV-irradiated bacteria incubated in buffer + chloramphenicol. ⊗, ⧋, UV-irradiated bacteria incubated in buffer + chloramphenicol + catalase.

To see whether there is any macromolecular release from irradiated bacteria under the experimental conditions specified above, irradiated and non-irradiated bacteria were incubated in presence of chloramphenicol, catalase or of both, for half an hour. Supernatants obtained from both the samples of bacteria by centrifugation were supplemented with appropriate growth requirement including glucose except the following in different cases:

1. thymine, 2. thymine and uracil, and 3. thymine, uracil and arginine. — Turbidimetry at 450 mμ of cells grown overnight at 37 °C showed that in no case supernatant from unirradiated cells supported growth, whereas supernatant from X-irradiated cells showed a heavy growth in the case of 1. and 2., but not in the case 3. This observation was not found to be true for UV-irradiated bacteria. The X-ray and UV-dose used in such experiments were 24 Krads and 750 erg/mm² respectively.

The results of the present experiment showed that the bacteria that were normally insensitive to the action of chloramphenicol with respect to their colony forming ability became sensitive on being exposed to X-rays. Thus instead of an increase in the number of X-ray survivors as has been observed in the case of UV-irradiated *E. coli* B cells [1], a decrease in the number was found to take place. This result corroborated the finding of OKAGAKI [1] with other strains of *E. coli* in the sense that by incubating the UV-irradiated cells in presence of chloramphenicol he observed a decrease in survival. Our results with UV-irradiated cells pointed out that chloramphenicol used in the incubation medium did not influence the survival obtained by holding UV-irradiated bacteria only in buffer. These results are in contrast with the finding of OKAGAKI [1].

X-irradiated cells on incubation in buffer at 37 °C showed a gradual decrease in survival number due to peroxide formation [3]. This effect could be checked by catalase as had been observed in this experiment also. However, the presence of catalase could check the decrease in survival number, neither of X-irradiated bacteria incubated in presence of chloramphenicol, nor of UV-irradiated bacteria incubated in presence or absence of the antibiotic. This indicated that the death of X-irradiated bacteria due to the presence of chloramphenicol and the death of UV-irradiated cells in its presence or absence were not a manifestation of peroxide action.

The results on the release from irradiated bacteria pointed out that the X-irradiated bacteria under various experimental conditions released in the incubation media some macromolecules of the nature of thymine or uracil. This was untrue for UV-irradiated bacteria.

The cause why X-ray induced death in bacteria was enhanced by chloramphenicol, while UV-irradiated population remained uninfluenced by it is not clear.

Wee thank Prof. N. N. DAS GUPTA, Palit Professor of Physics, University of Calcutta, for his interest in the work.

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