A POSSIBLE EXPLANATION OF CHLORAMPHENICOL DEATH IN IRRADIATED ESCHERICHIA COLI B/r

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Alper (1963) observed that when Escherichia coli strain B/r was incubat with chloramphenicol immediately after exposure to X-rays or to ultraviole light there was enhanced inactivation of the colony forming ability of the irradiated population. This phenomenon was also observed in several stra of UV-irradiated E. coli by Okagaki (1960), who termed it chloramphenicol death.

During an investigation of the action of chloramphenicol on E. coli B/r grown in a defined medium containing sulphanilamide (Kaplan et al., 1962; Gillies, 1966) we have observed that the decrease in survival of the X-irradiated bacteria was more extensive when they were treated with chloramphenicol in a liquid medium than when they were incubated on solid medium containing chloramphenicol. A similar observation was reported recently by Billen & Jorgensen (1964) who found that the survival of X-irradiated E. coli 15 $T^-A^-U^-$ was decreased when the bacteria were incubated in a liquid medium deprived of thymine, arginine and uracil but nc when they were treated in the same manner on solid medium.

Experiments are described in this paper which indicate a possible reaso for chloramphenicol induced inactivation of irradiated E. coli B/r and why it is more apparent in liquid than on solid incubation medium.

Escherichia coli B/r was grown for 18 hours in a defined medium containing sulphanilamide and thymine. The bacteria were washed in M/15 phosphate buffer (pH 7.0) and resuspended in buffer to give a concentration of approximately 5×10^7 organisms/ml. The suspension was equilibrated with oxygen and irradiated with 30 Krads of X-rays (230 Kev; 15 mA; no added filtration; dose rate 2 Krads/min) to give a surviving fraction of 10^{9}

After irradiation a sample of the bacterial suspension, to be incubated in liquid medium, was centrifuged, the supernatant removed, and the bacteria suspended in the same volume of warm defined nutrient medium (Forage & Gillies, 1964) containing chloramphenicol (5μg/ml). The liquid culture was incubated with aeration at 37°. Samples were removed at appropriate intervals, diluted onto plates of nutrient medium which were incubated overnight to obtain viable cell counts. For incubation on solid medium another sample of the irradiated suspension was diluted and dispensed onto pieces of cellophan (Alper & Gillies, 1958) lying on the surface of warm plates of nutrient medium of the same composition as that of the liquid medium and containing chloramphenicol. The plates were incubated at 37° and at required intervals bacteria on their cellophan carriers were transferred to fresh plates of nutrient medium containing no chloramphenicol and incubated overnight for viable colony counts. The same treatments were applied to samples of unirradiated E. coli B/r.

The additional killing effect of incubating irradiated E. coli B/r with nutrient medium containing chloramphenicol is illustrated in Fig. 1. It can be seen that the decrease in survival was much more marked when the bacteria were incubated in liquid medium than when they were incubated on cellophan on solid medium. We suggest that one explanation of these results is that some factor required to maintain survival of the irradiated cells diffuses out of the bacteria and that it is made apparent in the presence of chloramphenicol. It is likely that the rate of loss of substances will be maximum in liquid medium, but on solid medium and particularly when the bacteria are incubated on cellophan these substances may adhere to, or remain in the near vicinity of, the cells and thereby the loss of compounds from the bacteria will be decreased. This will result in loss of a smaller amount of the vital factor from the bacteria incubated on cellophan and thus more cells will survive than would in liquid medium. The fact that the rate of inactivation of the irradiated E. coli B/r slowed down after about 2 hours incubation with chloramphenicol may be an indication that the rate of loss of the vital factor from the cells slows down with time. An estimate of the rates at which diffusion of the proposed factor from the cells does decrease on cellophan and in liquid medium was obtained by observing the effect on cell survival of transferring cells from solid medium containing

chloramphenicol to liquid medium at intervals after the start of incubation and in the reverse manner by transferring bacteria from chloramphenicol-containing liquid medium to cellophan lying on solid medium at intervals. The results of these transfers are also shown in Fig. 1. Transfer of the irradiated bacteria from cellophan to liquid medium and in the reverse direction during the first 30 mins. of incubation did not alter the level of survival from that of bacteria incubated throughout that time on cellophan, suggesting that relatively little loss of the factor from the cells had occurred during that time. Incubation of the bacteria on cellophan for at least 1 hour before transfer to liquid medium resulted in a marked additiona

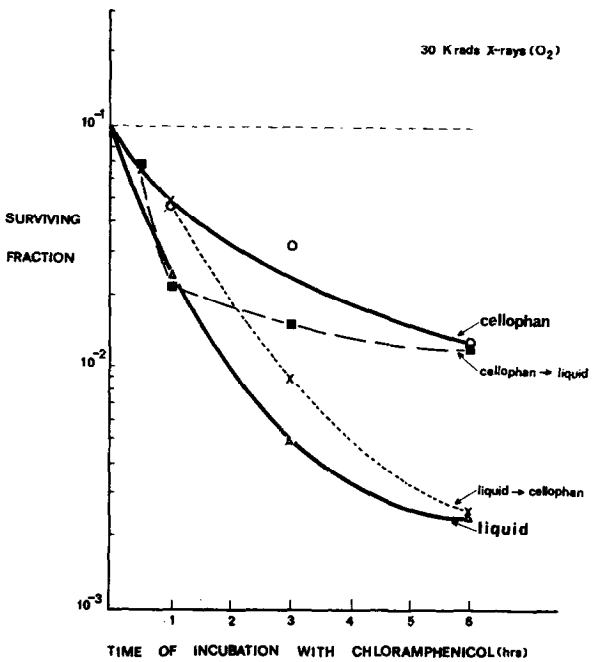

FIG. 1. Survival of aerobically X-irradiated Escherichia coli B/r during incubation with nutrient medium containing chloramphenicol (CMP) either in liquid conditions or on cellophan lying on solid agar.

To transfer bacteria from incubation on cellophan to liquid medium, a piece of cellophan was removed from the nutrient agar at the required time and shaken in liquid medium for 4 mins. to resuspend the bacteria. The liquid medium was then diluted and pipetted onto nutrient agar to obtain the viable colony count. To transfer from liquid medium to cellophan, bacteria in liquid medium were dispensed onto pieces of cellophan lying on nutrient agar, containing no CMP, and incubation continued to obtain the viable colony count.

decrease in survival, beyond that caused by incubation on cellophan only, but incubation for a period longer than about 2 hours on cellophan before transfer had little further effect. It is presumed that this response is caused by the rapid removal of the vital factor, which had accumulated around the bacteria lying on cellophan, when the cells were shaken in liquid medium; the slowing down in the rate of diffusion is evidenced by the sharp levelling off in the effect on survival when incubation on cellophan is continued beyond 2 hours. Conversely the effect of transferring bacteria from liquid to cellophan medium indicates that the postulated factor diffuses from the cells in liquid medium for at least 6 hours after the start of incubation.

If our hypothesis is correct then chloramphenicol death in E. coli B/r should be prevented, or at least its effects reduced, by treating the irradiated bacteria with the medium in which a suspension of irradiated or unirradiated E. coli B/r have been incubated, because the presence of

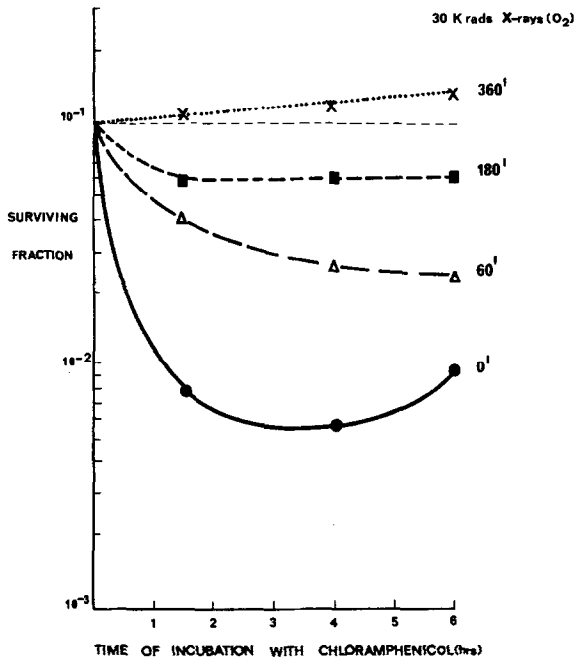

FIG. 2. The effect on survival of incubating aerobically X-irradiated Escherichia coli B/r in 2 ml. of the supernatant fractions derived from thick suspensions of E. coli B/r which had been incubated, for the time intervals (in min.) shown on the right hand side of the figure, in liquid medium containing CMP.

the postulated vital factor in the medium should reduce its net rate of diffusion out of the bacteria. To test this, X-irradiated E. coli B/r were incubated in the cell-free supernatant fraction prepared by centrifuging a thick suspension (2.0×10^9 cells/ml) of E. coli B/r which had been incubate previously for various time intervals in nutrient medium containing chloramphenicol. Viable colony counts were made at intervals. It can be seen, from Fig. 2, that the longer the thick cell suspension had been incubated with the chloramphenicol medium then the more effective was the supernatant obtained from it in preventing chloramphenicol death when added to X-irradiated test E. coli B/r. The supernatant fraction was equally effective whether it was prepared from an irradiated or an unirradiated thick cell suspension. These findings certainly suggest that a factor which diffuses progressively from the bacteria is capable of maintaining survival of X-irradiated E. coli B/r, incubated in medium containing chloramphenicol. This factor was heat labile, being inactivated by heating at 75° for 20 mins. in a water bath.

Billen & Jorgensen (1964) thought that the decrease in survival of X-irradiated E. coli 15 $T^-A^-U^-$ which they observed when the cells were incubated in liquid medium deficient in thymine, arginine and uracil might be due to the release of a toxic substance from the killed cells, but they were unable to substantiate this hypothesis by direct experiment. Our proposed mechanism invoking the release of a factor required for the survival of a proportion of the irradiated cells seems to be more likely, at least in the case of E. coli B/r. The sensitivity of the factor to heat suggests that it may be a protein and therefore possibly an enzyme. That enzymes can diffuse out of bacteria was demonstrated recently by Hamilton and Steele (1966) who reported that a large amount of DNAase activity is released from X-irradiated Micrococcus radiodurans and they believe that this may represent damage to the repair system which requires initial excision of damaged DNA in this strain. Also Adler, Fisher, Hardigree and Stapleton (1966) have reported that a factor which diffuses out of E. coli B/r and which promotes cell division and recovery of an X-irradiated strain of E. coli K12 appears to be partially enzymatic in nature. We are engaged at present in attempts to characterise the factor which prevents chloramphenicol death in E. coli B/r.

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