EFFECT OF CHLORAMPHENICOL ON THE BIOSYNTHESIS OF DNA IN X-IRRADIATED ESCHERICHIA COLI B

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Doudney (1956) has found that X-irradiation inhibits the synthesis of DNA in E. coli B/r pretreated with cysteamine. In an earlier work (Miletić et al. 1961) we demonstrated that small doses of X-irradiation have no measurable effects on the synthesis of DNA in E. coli B, but higher doses result in a partial degradation of DNA. Stuy (1960) observed the same with E. coli B/r. As we observed different patterns of DNA degradation immediately after higher doses of X-rays, depending on the method of chemical fractionation, that led us to a hypothesis which postulates that during the first 20 minutes after irradiation a degradation of a part of DNA takes place, the remaining portion replicating without a measurable delay. To verify whether there still exists an inhibition of DNA synthesis for a very short period immediately after X-irradiation, we added chloramphenicol to our cultures. This antibiotic is known to inhibit the resynthesis of DNA after the temporary inhibition of its synthesis by U.V.-light /Harold and Ziporin (1958), Drakulić and Errera (1959) and Doudney (1959)/.

E. coli B was grown in a mineral medium with glucose. When the bacterial cultures attained a concentration of about 5×10^8 cells per ml, they were chilled and portions of the culture irradiated with various doses of X-rays. To half of the irradiated suspension chloramphenicol was added (10 μg/ml

final concentration). The chemical fractionation and the determination of DNA were performed according to Burton's (1956) method.

Growth in both the control and irradiated culture stops soon in the presence of chloramphenicol. The synthesis of RNA continues for a considerably longer period. Both growth and RNA synthesis are significantly more inhibited in the 16 000 r irradiated culture than in the control.

Fig. 1 shows the synthesis of DNA in bacteria irradiated with 16 000 r and irradiated and treated with chloramphenicol. Considerably more DNA is degraded in the first 10 minutes after irradiation in the presence of chloramphenicol than in its absence, and the degradation lasts for some 20-30 minutes longer. Then the degradation in the irradiated chloramphenicol-treated culture stops, but no synthesis of DNA takes place afterwards. At the same time, the synthesis of DNA in the nonirradiated-chloramphenicol-treated culture comes to its end as well. In the irradiated and chloramphenicol-treated cultures approximately 50% of the total DNA is degraded.

Small doses of X-irradiation (1000 r 50% survival) have no measurable effect on DNA synthesis. In this case the addition of chloramphenicol does not effect the degradation of DNA or the inhibition of its biosynthesis.

From these results we could postulate that higher doses of X-irradiation also effect an inhibition of DNA synthesis, which is, unlike the inhibition of DNA synthesis observed with U.V.-light, very quickly repaired. But the situation seems to be more complicated with X-rays, because they simultaneously initiate some processes which result in the degradation of DNA (perhaps an activation of DNase?). If chloramphenicol is added, and consequently the biosynthesis of proteins inhibited in the irradiated cultures, presumably

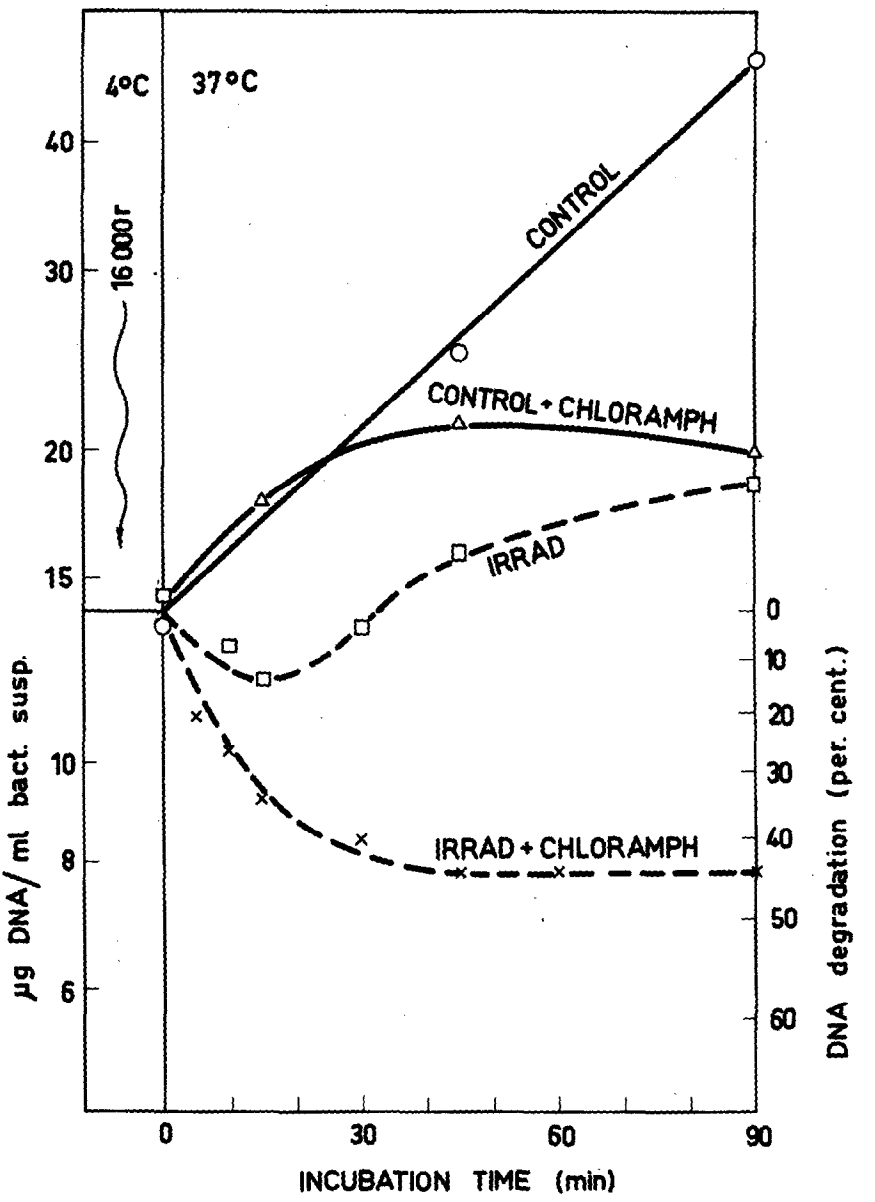

Fig. 1 Synthesis of DNA in Escherichia coli B exposed to 16 000 r (survival 7×10^{-4}) treated with chloramphenicol immediately after irradiation.

only the degradative processes take place, so they are stressed and prolonged.

As mentioned before the above results are observed only with high doses of X-rays (about 10^{-3} survival, or less). With 1000 r (50% survival) there is no measurable effect on DNA synthesis, and the addition of chloramphenicol also has no effect. Billen (1960) studying the biosynthesis of DNA in E. coli B/r irradiated with 10 000 r did not observe any degradation, but only some slowing down of the biosynthesis of DNA. In his experiments the addition of chloramphenicol was also without effect on DNA synthesis. Thus his results seem to be comparable to our own with E. coli B irradiated with 1000 r. The surviving fractions of the bacterial populations are of the same order of magnitude in both cases.

It could be thus tentatively concluded that with the dose range of X-rays we studied, disturbances of DNA metabolism would possibly depend on the same mechanism which also determines the radiosensitivity of the cells, and not on the number of ionizations produced in the DNA molecule itself.

It seems quite likely that both the degradation of DNA and the effect of chloramphenicol on DNA metabolism after X-irradiation depend on the degree to which the mechanism of DNA biosynthesis is damaged.

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