SYNTHESIS OF DNA IN X-IRRADIATED ESCHERICHIA COLI B

B. Miletić, Ž. Kućan, and Lj. Zajec
Department of Radiobiology, Institute "Ruđer Bošković"
Zagreb, Yugoslavia

Received March 10, 1961

The synthesis of DNA is temporarily inhibited in E. coli B irradiated with U.V. light (Kelner 1953, Kanazir and Errera 1955). We tried to find out what was the effect of X-irradiation on DNA in the same bacteria. Doudney (1956) has shown that X-irradiation inhibits the synthesis of DNA in E. coli B/r, but the bacteria were treated with cysteamine before irradiation. When our study was still in progress a paper by Stuy (1960) came out. His results agree to a great extent with the results given in the present paper. We found a degradation of DNA in E. coli B irradiated with X-rays. However, we showed that different results are obtained by using different methods of chemical fractionation.

E. coli B was grown in a glucose containing mineral medium. Log-phase cultures (5×10^8 bacteria per ml) were cooled to $+4^{\circ}C$ and irradiated in the same nutritive medium.

The growth of the bacteria irradiated with 16 000 r in relation to the controls is somewhat slowed down. RNA synthesis takes place in the irradiated cells without delay and at the same rate as growth. Great changes of DNA synthesis are observed after high doses of X-rays. In this case the observed biosynthetic patterns depend considerably on the method of chemical fractionation. By the extraction of DNA by the procedures of Schmidt-Tannhäuser-Schneider (Volkin and Cohn 1954) or Ogur-Rosen (1950), the samples of the cell-suspensions taken immediately after irradiation contain

about 20% less DNA than the nonirradiated control. When the chemical fractionation is performed by Burton's (1956) method the results differ considerably from those previously mentioned. In most experiments the DNA content of the bacteria in the samples taken immediately after irradiation does not differ from the nonirradiated control in this case. The degradation of DNA takes place only during incubation at 37°C, the extent and duration of the degradation process depending on the dose. For that reason in many experiments the chemical fractionation of the irradiated cultures was determined simultaneously by the procedure of Schmidt-Tannhäuser-Schneider and by Burton's method. A typical result is shown in Fig. 1. The picture of the behaviour of DNA in the irradiated bacteria, as analysed by the methods mentioned above, shows a marked difference during the first fifteen to twenty minutes of incubation after irradiation. After that time interval of incubation the values of the DNA content are equal by both methods.

It is interesting to note that the final degradation of DNA, measured by Burton's method, is smaller than the degradation determined by the method of Schmidt-Tannhäuser-Schneider, or Ogur-Rosen. The extraction of nucleic acids by the methods of Schmidt-Tannhäuser-Schneider or Ogur-Rosen are preceded by rather a long washing of the bacteria at +4°C. Burton's procedure is much shorter, since the bacteria are not washed but perchloric acid in a final concentration of 2.5% is added immediately to the sample for analysis. The curves of DNA synthesis during the incubation after irradiation, obtained by the mentioned methods of measuring the DNA content, could be explained assuming that in the samples analysed immediately after irradiation by the procedures of Schmidt-Tannhäuser-Schneider, or Ogur-Rosen, the degradation of DNA occurs during the washing of the bacteria and the extraction of nucleic acids. The process of DNA synthesis

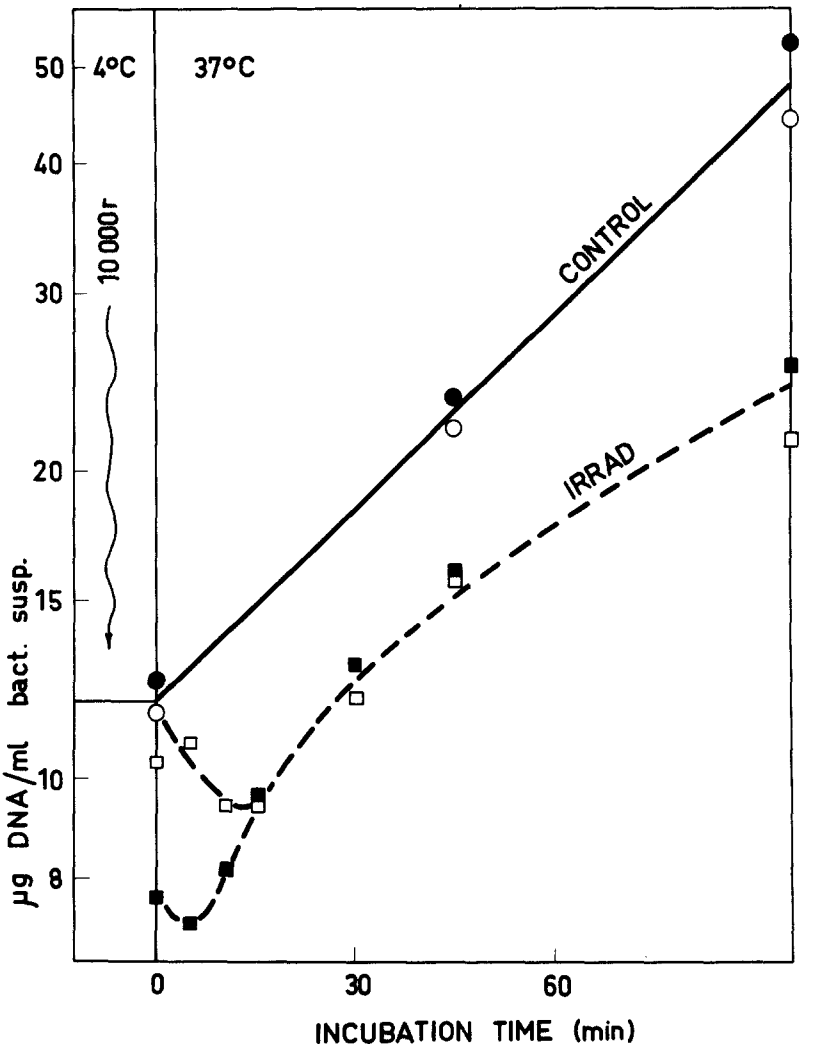

Fig. 1 Synthesis of DNA in Escherichia coli B exposed to 16 000 r (survival 7×10^{-3}). Chemical fractionation by the method of Schmidt-Tannhäuser-Schneider: ● = control; ■ = irradiated. Chemical fractionation by the method of Burton: ○ = control; □ = irradiated.

would however occur only during the incubation and would start immediately without delay at 37°C. The curve of the DNA content, as obtained by Burton's method, would in fact be the resultant of the two processes occurring in the irradiated cells simultaneously: one being degradative, the other synthetic. Stuy (1959) also arrived at the conclusion that the degradation and synthesis of DNA in U.V.-irradiated bacteria occurred simultaneously, but he was not able to confirm this conclusion by biochemical methods.

With low X-ray doses (1000 r, survival 50%) the picture is essentially different. Both the growth and synthesis of RNA, as well as DNA synthesis are almost identical as in the nonirradiated control cultures.

By irradiating E. coli B/r with a dose of 10 000 r, Billen (1960) has not found any essential alteration in DNA synthesis. Stuy has found an appreciable degradation of DNA in E. coli B/r irradiated with a dose of 48 000 r (survival 2.6×10^{-3}). A comparison of the results obtained with E. coli B/r and our own results obtained with E. coli B shows that the results would agree provided that the survival, and not the dose, were taken as a basis. Taking into account that the DNA content is equal in the log-growing E. coli B and in B/r (Harold and Ziporin 1958, Gillies and Alper 1960), it may be tentatively postulated that the degradation of DNA in E. coli B is not the consequence of a direct effect of X-rays on DNA.

The most important conclusion drawn from these experiments is that with lower X-rays doses the irradiation produces no visible effect on DNA synthesis. With higher doses the degradation of a part of DNA takes place, the other part of DNA being replicated probably without a considerable delay.

References

Billen, D. (1960) J. Bacteriol. 80, 86

Burton, K. (1956) Biochem. J. 62, 315

Doudney, C.O. (1956) J. Bacteriol. 72, 488

Gillies, N.E. and Alper, T. (1960) Biochim. Biophys. Acta 43, 182

Harold, F.M. and Ziporin, Z.Z. (1958) Biochim. Biophys. Acta 28, 482

Kanazir, D. and Errera, M. (1955) Biochim. Biophys. Acta 16, 477

Kelner, A. (1953) J. Bacteriol. 65, 252

Ogur, M. and Rosen, G. (1950) Arch. Biochem. Biophys. 25, 262

Stuy, J.H. (1959) J. Bacteriol. 78, 49

Stuy, J.H. (1960) J. Bacteriol. 79, 707

Volkin, E. and Cohn, W.E. (1954) Methods of Biochemical Analysis Vol. I, David Glick, Ed. Interscience Publishers Inc. pp. 287