BIOSYNTHESIS OF ACID-SOLUBLE NUCLEIC ACID PRECURSORS IN ULTRA VIOLET-IRRADIATED AND CHLORAMPHENICOL-TREATED *ESCHERICHIA COLI* B

M. DRAKULIĆ, S. ŠMIT AND S. STAVRIĆ

Department of Radiobiology, Institute "Rudjer Bošković", Zagreb (Yugoslavia)

(Received June 20th, 1960)

SUMMARY

In cultures of *E. coli* B, in which both DNA and protein synthesis are permanently inhibited by u.v.-irradiation, followed immediately by the addition of chloramphenicol, very large losses of intracellular nucleic acid precursors were observed during incubation after irradiation. If the antibiotic is added 35 min after irradiation, which affects only the permanent inhibition of protein synthesis, but does not prevent the resuming of DNA synthesis after the temporary inhibition induced by u.v.-irradiation, no such losses can be observed.

The possible explanations of the above results are discussed.

INTRODUCTION

It has been demonstrated (HAROLD AND ZIPORIN[2]; DRAKULIĆ AND ERRERA[1]) that the addition of chloramphenicol immediately after u.v.-irradiation permanently inhibits DNA synthesis in *Escherichia coli* B.

In view of the fact that the temporary inhibition of DNA synthesis which takes place in *E. coli* B by u.v.-irradiation alone is accompanied by an accumulation of DNA derivatives (KANAZIR AND ERRERA[3]), we were interested to know whether we could observe a similar phenomenon in u.v.-irradiated and chloramphenicol-treated cells.

MATERIALS AND METHODS

E. coli B was maintained and cultivated as described earlier (DRAKULIĆ AND ERRERA[1]).

Absorbancy measurements were made with a Beckman model DU spectrophotometer, in 10 mm depth cuvettes at a wavelength of 660 mμ.

Chemical fractionations of the bacteria were carried out by the somewhat modified OGUR-ROSEN procedure (OGUR AND ROSEN[5]). 10–20 ml of bacterial suspension were centrifuged, washed with cold physiological saline solution, and delipidated twice with 5 ml ethanol–ether (3:1) by stirring with glass rods in an ice bath for 20 min each time.

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

The acid-soluble nucleic acid precursors were extracted with 2 ml of 2 % perchloric acid at 4° by stirring with glass rods during 20 min. After centrifugation the precipitate was washed once more with 2 % perchloric acid, and the extract and the wash were pooled. RNA was extracted with 10 % perchloric acid in the refrigerator during 30–40 h.

The nucleic acid precursors and RNA were determined by their absorption maxima at 260 mμ, as measured with a Beckman model DU spectrophotometer, in 10-mm depth quartz cuvettes. The amount of RNA was calculated on the basis of the fact that an extinction of 1.00 corresponds to 35,5 μg. In some experiments RNA was also determined by the orcinol reaction (MEJBAUM[4]).

The u.v.-spectra of the media were also determined with a Beckman model DU spectrophotometer, in 10-mm depth quartz cuvettes.

Irradiation was performed on 55-ml aliquots of bacterial suspension in 13,5-cm diameter Petri dishes stirred with the hand, at a distance of 100 cm from a Mazda low-pressure mercury lamp, giving approx. 540 ergs/mm²/min of 253,7 mμ u.v.-light.

EXPERIMENTS AND RESULTS

A log phase culture of *E. coli* B at an absorbancy of 0.100–0.200 was divided into two parts, one of which was irradiated with a total dose of 900 ergs u.v./mm², the other being kept as a control.

We performed two types of experiments with these cultures.

Experiment I

Immediately after irradiation the control and the irradiated cultures were divided into two parts. To one part of each, a solution of chloramphenicol was added to a final concentration of 10 μg/ml.

Thus we worked, as we did in the experiments described in an earlier paper (DRAKULIĆ AND ERRERA[1]), with four suspensions:

(a) control culture
(b) irradiated culture
(c) control culture plus chloramphenicol
(d) irradiated culture plus chloramphenicol

These cultures were reincubated after irradiation at 37° with aeration by bubbling air through the suspensions. Samples for absorbancy measurements and chemical analysis were taken at the time intervals, shown in Fig. 1, which shows the synthesis of nucleic acid precursors, and in Fig. 2, which shows growth and RNA synthesis.

The synthesis of nucleic acid precursors in the control culture was slowed down less than the growth was. In the irradiated culture the addition of chloramphenicol immediately after irradiation did not change the synthetic pattern of the precursors for the first 40 min; but after that period a large diminution of the u.v.-absorption in the 2 % perchloric acid extract for some 3 h or more was observed.

Fig. 2 shows that the addition of chloramphenicol slows down RNA synthesis considerably in both the control and the irradiated suspensions after some 40 min. However, a permanent inhibition of RNA synthesis was never observed in our experiments.

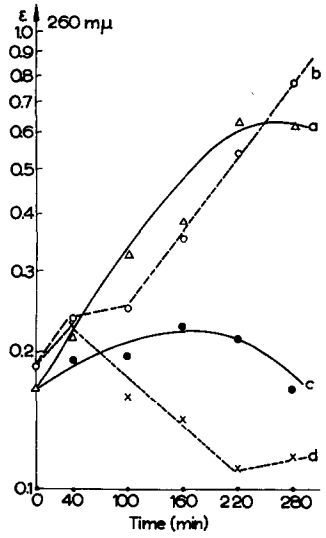

Fig. 1. Synthesis of acid-soluble nucleic acid precursors in four cultures of *E. coli* B: a = control; b = irradiated; c = control plus chloramphenicol added at o min; d = irradiated plus chloramphenicol added at o min.

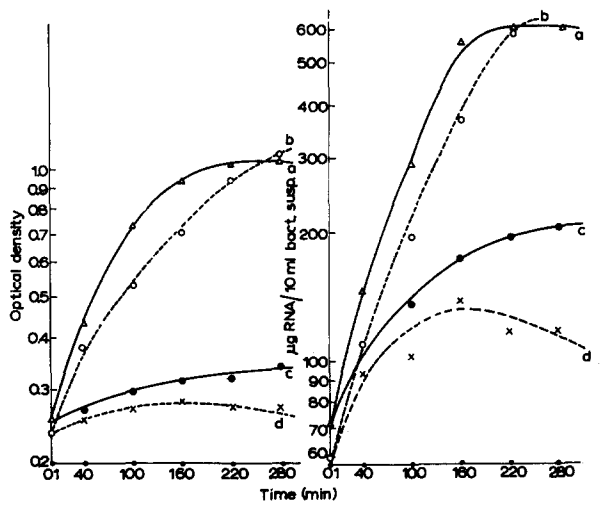

Fig. 2. Growth (left) and RNA synthesis (right) in four cultures of *E. coli* B: a = control; b = irradiated; c = control plus chloramphenicol added at o min; d = irradiated plus chloramphenicol added at o min.

Experiment II

This type of experiment was designed to find out whether the addition of chloramphenicol 35 min after irradiation is also accompanied by a loss of intracellular nucleic acid precursors.

As Fig. 3 shows, no degradation of the acid-soluble precursors was observed under these conditions in the control culture (curve c_1), or in the irradiated culture (curve d_1).

To find out whether the loss of u.v.-absorption at 260 mμ in curve d of Fig. 1 is due to leakage of the precursors through the cellular membrane, we measured the u.v.-spectra of the media in which the following cell suspensions were grown:

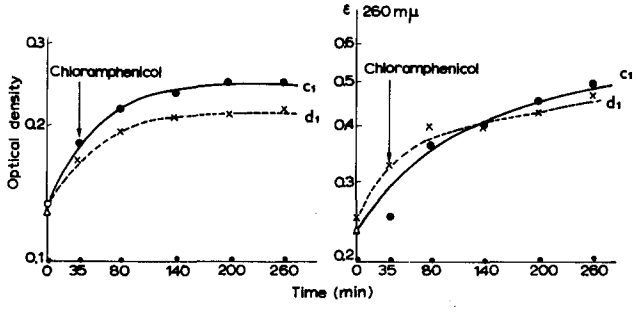

Fig. 3. Growth (left) and the synthesis of acid-soluble nucleic acid precursors (right) in a control culture (c_1) and an irradiated culture (d_1) of *E. coli* B. To both cultures chloramphenicol was added 35 min after irradiation.

(1) c = control suspension with chloramphenicol added at 0 min after irradiation.

(2) c_1 = control suspension to which chloramphenicol was added 35 min after irradiation.

(3) d = irradiated suspension to which chloramphenicol was added at 0 min after irradiation.

(4) d_1 = irradiated suspension to which chloramphenicol was added 35 min after irradiation.

Fig. 4 shows that the extinctions at 260 mμ of the media in which cultures d and d_1 were grown differ only slightly.

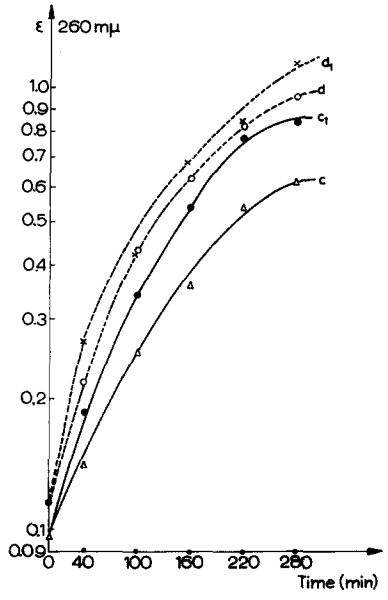

Fig. 4. Extinction at 260 mμ of the media in which four cultures of *E. coli* B have been grown: c = control plus chloramphenicol added at 0 min; d = irradiated plus chloramphenicol added at 0 min; c_1 = control plus chloramphenicol added at 35 min; d_1 = irradiated plus chloramphenicol added at 35 min.

DISCUSSION

The experiments just described indicate that a very significant loss of acid-soluble u.v.-absorption is observed in extracts from u.v.-irradiated *E. coli* B if chloramphenicol is added to the cell suspension immediately after irradiation. This phenomenon is, however, not observed if the antibiotic is added 35 min later.

Several explanations of these results could be imagined:

(1) The loss of acid-soluble u.v.-absorption might be the consequence of the leakage of nucleic acid precursors out of the cells. The u.v.-absorption of the media (Fig. 4) does not, however, confirm this assumption.

(2) The loss could be attributed to an exhaustion of the nucleic acid precursors pool, because RNA synthesis seems to be slowed down less by the addition of chloramphenicol (Fig. 2) to the irradiated cells than the synthesis of the precursors (Fig. 1).

But this assumption does not explain why the phenomenon was observed only when chloramphenicol was added immediately after irradiation, and this phenomenon does not happen if the antibiotic is added 35 min later.

(3) It is also possible that there is a feed-back, *i.e.* a repression of some of the purine, and/or pyrimidine-building enzymes, due to the permanent inhibition of DNA synthesis. But the losses of the precursors always observed in our experiments were so large that we do not think that they could be explained by enzyme repression.

(4) The essential difference between the two irradiated cultures to which chloramphenicol is added immediately or 35 min after u.v.-treatment, is that, in the first instance, when a loss of acid-soluble material occurs (Fig. 1), both protein and DNA synthesis are permanently inhibited, while in the second instance, in which no such loss occurs (Fig. 3), only protein synthesis is permanently inhibited. It is conceivable that u.v.-irradiation damages certain fractions of DNA, which are, in some way, connected with the biosynthesis of enzymes and that these are important for the building up of purine and pyrimidine bases. If the synthesis of new DNA molecules is prevented as happens, for example, when bacteria are irradiated and chloramphenicol is added immediately after irradiation, some enzymes which synthetize precursor molecules might be used up after a certain period of time, and might not be reformed. If the catabolism of the bases is affected less under these conditions, the catabolic–anabolic equilibrium would be disturbed, and a degradation of the precursors would be observed as the consequence of the disequilibrium.

If this assumption were true, it would mean that the cells contain only very limited amounts of some of the enzymes necessary for the synthesis of the nucleic acid precursors. These enzymes could represent "pace-makers", limiting the metabolic activities of normal cells.

REFERENCES

[1] M. DRAKULIĆ AND M. ERRERA, *Biochim. Biophys. Acta*, 31 (1959) 459.
[2] F. M. HAROLD AND Z. Z. ZIPORIN, *Biochim. Biophys. Acta*, 29 (1958) 439.
[3] D. KANAZIR AND M. ERRERA, *Proc. 1st Internat. Photobiol. Congress, Amsterdam*, 1954, p. 162.
[4] W. MEJBAUM, *Z. physiol. Chem.*, 258 (1939) 117.
[5] M. OGUR AND G. ROSEN, *Arch. Biochem. Biophys.*, 25 (1950) 262.