DEOXYRIBONUCLEIC ACID INSTABILITY IN THE PRESENCE OF CHLORAMPHENICOL IN ULTRAVIOLET-LIGHT-EXPOSED BACTERIA*

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It has been demonstrated that the ultraviolet light (UV) induced lag in deoxyribonucleic acid (DNA) synthesis in bacteria (Kelner, 1953) is overcome through the synthesis of ribonucleic acid (RNA) and protein (Harold and Ziporin, 1958; Doudney, 1959; Drakulic and Errera, 1959). In studies of the effects of chloramphenicol (chl) on induced mutation and on nucleic acid synthesis in "synchronized" cultures of Escherichia coli exposed to UV, Doudney and Haas (1960a; 1960b) demonstrated that a relation exists between the amount of postirradiation RNA synthesized at the time of chl addition and the relative rate of DNA synthesis in the presence of chl. The maximum rate of DNA synthesis in the presence of chl occurs when the amount of RNA has just doubled in the culture at the time of chl addition. These studies of the relation of RNA formation to DNA synthesis have been extended to log phase cultures of E. coli strain B/r, where similar results are observed (Doudney, unpublished data). In the course of these studies it was observed that, when chl is added immediately following UV-exposure, a marked decline in the amount of DNA takes place with incubation in chl. This chl-promoted instability of DNA after UV-exposure appears not

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to be related directly to the UV-induced lag in DNA synthesis; however it may be related to such phenomena as the failure of nucleic acid precursor synthesis in UV-exposed cells in the presence of chl (Drakulic et al., 1961) and chl-promoted death of UV-exposed bacteria (Okagaki, 1960).

RESULTS

If chl is added immediately after radiation exposure, a progressive decrease in the amount of DNA (as measured by the Burton reaction) is observed, which begins after 15 minutes incubation. At 150 minutes incubation, the amount of DNA is decreased by eighty percent (Table 1). Though protein synthesis is blocked by the addition of chl, no decrease in amount of protein is observed with incubation for 150 minutes, indicating that the cells remain intact. Addition of chl after 15 minutes incubation following exposure produces a decline in amount of DNA, which begins after 15 minutes incubation in chl, to about the same level. If chl is added after 30 minutes incubation following UV-exposure, no decrease in amount of DNA is observed; synthesis of DNA is initiated after 60 minutes incubation.

RNA synthesis takes place but at a reduced rate in UV-exposed cells in the presence of chl added immediately following UV-exposure (Table 1). RNA synthesis proceeds for 45 minutes and then ceases; there is no decrease in amount of RNA in the culture with subsequent incubation in chl. If chl is added after 15 minutes incubation, synthesis of RNA in the next 15 minutes in not appreciably affected; however, after this period of incubation the rate of synthesis declines and synthesis eventually ceases. No effect of chl on RNA synthesis

TABLE 1

Effect of chloramphenicol on the amount of RNA and DNA in UV-exposed cultures of Escherichia coli strain B/r, with post-irradiation incubation.

Time of chloramphenicol addition**	Min. postirradiation incubation* 15	30	45	60	75	105	150
	Relative amount DNA***						
0	0.92	0.79	0.72	0.58	0.45	0.20	0.18
15	1.02	1.02	0.88	0.78	0.65	0.40	0.22
30	1.02	1.04	1.04	1.01	1.18	1.48	1.73
	Relative amount RNA***						
0	1.12	1.22	1.35	1.31	1.33	1.35	1.35
15	1.25	1.45	1.60	1.66	1.66	1.61	1.60
30	1.28	1.48	1.77	2.03	2.41	-	-

*Minimal (salts-glucose) medium is inoculated with a 7 hr liquid culture of Escherichia coli strain B/r to an optical density of 0.08 at 700 mμ measured in optical tubes with a 1.8 cm lightpath in a Bausch and Lomb type 33-29-40 colorimeter. The culture is incubated at 37°C with vigorous aeration until an optical density of 0.43 is reached. The culture is chilled in an ice bath. Twenty ml samples in large petri dishes, are exposed with mechanical stirring, to 30 sec UV light at 30 cm from a Gates "Raymaster" mercury vapor lamp. The culture is rapidly rewarmed to 37°C and incubation, with aeration, continued. **After the indicated minutes of incubation following UV-exposure chloramphenicol is added to a concentration of 20 μg per ml. ***DNA: 1=37 μg per 5 ml sample of cell suspension based on a purified salmon sperm DNA standard. RNA: 1=180 μg per 5 ml sample of cell suspension based on a purified yeast RNA standard. Five ml culture samples, taken at appropriate intervals during the postirradiation incubation period for analysis of RNA, DNA and protein, are chilled, spun down in the cold and resuspended in cold 0.5 N perchloric acid. The suspension is spun again to obtain a precipitate containing the nucleic acids and protein. Nucleic acids are hydrolyzed by incubation in hot perchloric acid for 50 minutes at 70°C (Ogur and Rosen, 1950). Analysis for DNA is that of Burton (1956). For RNA determination the ultraviolet adsorption at 260 mμ and 290 mμ is determined (Visser and Chargaff, 1950) and the amount of DNA, as determined by the Burton analysis of the same sample, is then subtracted with correction for extinction coefficients. Protein is determined by the Folin method.

is observed for at least 90 minutes, when the antibiotic is added after 30 minutes following UV-exposure.

DISCUSSION

The effect of chl on RNA synthesis may be related to the chl-promoted decline in amount of DNA in the cell. On this basis, the capacity to form RNA (or its precursors) may depend on the functional integrity of the cellular DNA. Failure of cellular synthesis of acid soluble 260 mμ absorbing material in UV-exposed cells in the presence of chl (Drakulic et al., 1961) may be explained on this basis. Sensitivity of these syntheses to chl is observed during a comparable time period to the sensitivity of the cellular DNA to chl following UV-exposure (Doudney, unpublished data). Furthermore, the effect of chl on synthesis of acid soluble 260 mμ absorbing material parallels the effect of chl on RNA synthesis. Similarly, chl-promoted lethality following UV-exposure (Okagaki, 1960) may be related to the sensitivity of the DNA to chl. Cells of E. coli strain B/r are sensitive to chl for approximately 20-30 minutes following UV-exposure (Doudney, unpublished data), which is the same period of time that DNA remains sensitive to chl.

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