INDUCTION OF COLICIN SYNTHESIS IN Escherichia coli BY X-RAY IRRADIATION

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X-ray irradiation increases the production of colicins in strains of Escherichia coli CA-23 (D) and, to a lesser degree, strains CA-18 (B), but has no effect on strains CA-7 (V), CA-53 (I), or Bacillus freundi (A). Colicin D appears in the medium after 35-40 min and its concentration in the medium reaches a maximum 120-150 min after irradiation. The number of "lacunae" and the colicin concentration are functions of the dose of irradiation. However, the final effect of induction depends on the composition of the medium in which irradiation and subsequent incubation are carried out.

In 1952, Jacob and co-workers [6] showed that irradiation of a colicinogenic culture with ultraviolet rays stimulates colicin synthesis in the culture. A similar inducing effect can be obtained by treatment of a colicinogenic culture by various physical and chemical agents [1,3,5,7].

In this investigation the effect of x-ray irradiation on colicin synthesis was studied in various strains of Escherichia coli.

EXPERIMENTAL METHOD

Experiments were carried out on 5 colicinogenic strains of E. coli CA-7 (V), CA-18 (B), CA-23 (D), CA-53 (I), and Bacillus freundi (A), and on strain E. coli J,* sensitive to all colicins, as indicator.

A culture in the phage of exponential growth was centrifuged and resuspended in half the initial volume of broth or synthetic glucose–salt medium M9, pH 6.8-7.2. The density of the bacterial suspension varied from $6 \cdot 10^1$ to $8 \cdot 10^7$ bacterial cells/ml. The suspension obtained was poured in volumes of 2 ml into specially made plastic cells. The thickness of the layer of liquid irradiated was 0.5-0.7 cm. The culture was kept for 30 min at 4° before irradiation. Irradiation was carried out on the RUM-7 x-ray apparatus (50 kV, 15 mA, filter Al 0.1 mm, distance from radiation source 5 cm, dose rate 4 kR/min). The duration of irradiation was 15, 19, 120, 150, 225, and 450 sec (doses of 1000, 6000, 8000, 10,000, 15,000, and 30,000 R, respectively).

The number of cells producing colicin – the number of "lacunae" [8] – and the survival rate of the culture were determined immediately, and the concentration of colicin in the medium [4] was determined 2-3 h after irradiation. For determination of the latent period of colicin synthesis, the culture was irradiated in a dose of 10,000 R.

EXPERIMENTAL RESULTS

Of the 5 colicinogenic strains used, a definite induction effect as a result of x-ray irradiation was observed with only 2 strains: CA-23 (D) and, to a lesser degree, CA-18 (B). For this reason, the subsequent experiments were carried out with strain CA-23 (D).

The results given in Fig. 1 show that colicin synthesis began after 35-40 min and reached its maximum after 120-150 min of incubation at 37° after irradiation. The latent period of colicin synthesis was independent of the medium in which irradiation was carried out. From analysis of the curves of colicin concentration in the medium and the number of "lacunae" (Figs. 2 and 3), it can be concluded that these values

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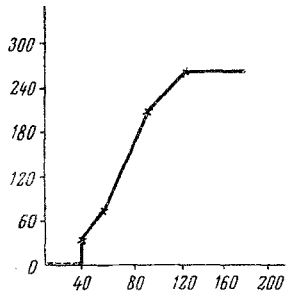

Fig. 1. Concentration of colicin in medium as a function of incubation time at 37° after irradiation. Abscissa, time of incubation (in min); ordinate, concentration of colicin (in conventional units).

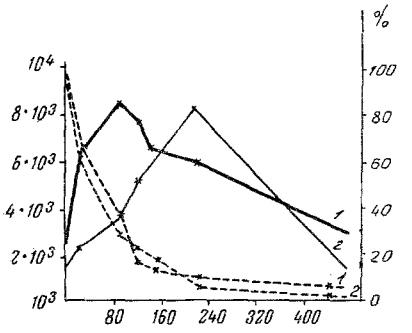

Fig. 2. Number of "lacunae" in population (continuous line) and survival rate of culture (broken line) after irradiation in broth (1) and in glucose-salt medium (2). Abscissa, time of irradiation (in sec); ordinate on the left, number of "lacunae" on the right, survival rate of culture (in %).

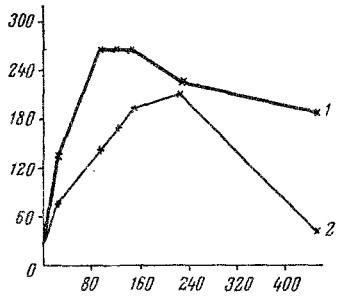

Fig. 3. Concentration of colicin in medium as a function of time or irradiation. 1) Irradiation and incubation in broth; 2) irradiation and incubation in synthetic medium. Abscissa, time of irradiation (in sec); ordinate, colicin concentration (in conventional units).

are functions of the dose of irradiation. However, broth as the medium for irradiation led to a more rapid and more intensive increase in the colicin concentration in the medium and in the number of "lacunae" in the population than synthetic medium. The process reached a maximum after a much shorter period of irradiation. The reason for this was probably some difference in the action of the medium. However, it is not yet clear whether this was the result of an increase in the concentration of secondary products in the broth after irradiation which could themselves bring about an induction effect [5], or whether it was due to a decrease in sensitivity of the culture to x-ray irradiation during growth in the synthetic medium. Meanwhile, the fact that the value of the maximum for the number of "lacunae" in the population was relatively constant for different conditions of irradiation suggests that the number of cells in the population capable of induction by x-ray irradiation itself is a constant value.

The curves of the colicin concentration in the medium after irradiation under different conditions are similar in character to the curves of the number of "lacunae" in an irradiated population. However, the colicin concentration in the medium after irradiation and further incubation in broth reached a higher maximum than after irradiation in synthetic medium. Hence, on the basis of these results it can be postulated that irradiation and subsequent incubation for 3 h in nutrient broth lead to an increase in the quantity of colicin produced by one induced cell.

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