Synthesis of protein and DNA in *Escherichia coli* treated with 5-bromodeoxyuridine

Resumption of DNA synthesis in *Escherichia coli* has been reported to require prior synthesis of protein when the bacteria were pretreated with a sulfur mustard[1], bis(β-dichloroethyl)sulfide, or u.v.-irradiation[2]. It has also been demonstrated that when u.v.-treated *E. coli* cells are resuspended in growth medium, inhibition of protein synthesis with chloramphenicol caused a decrease in the number of viable cells[3]. This effect was described as "chloramphenicol death". This communication reports experiments which suggest that protein synthesis is not a prerequisite for resumption of DNA synthesis in *E. coli* 15T⁻ after a prior exposure to 5-bromodeoxyuridine.

E. coli 15T⁻ was grown on a synthetic medium[4] containing 1 μg/ml thymine and 100 μg/ml BrUDR. The cells were grown with aeration for 2 h at 37°. At the end of the growth period, the viable count (measured on nutrient-agar plates after 18 h) was reduced to less than 3 % of the inoculum. The cells were harvested, washed with mineral medium, resuspended in three flasks and aerated at 37°. The time at which the cells were resuspended in fresh media corresponds to "0 min" in the figures. Flask 1 contained synthetic medium plus 1 μg/ml of thymine. Flasks 2 and 3 contained synthetic medium, 1 μg/ml thymine and 75 μg/ml chloramphenicol added at 0 min (flask 2) and after 15 min (flask 3). At intervals of 30 min, samples were withdrawn for viability, DNA (diphenylamine[5]) and RNA (orcinol[6]) determinations. Growth was followed turbidimetrically at 420 mμ in a Klett-Summerson photoelectric colorimeter. Cell count was determined with a Petroff-Hauser counter.

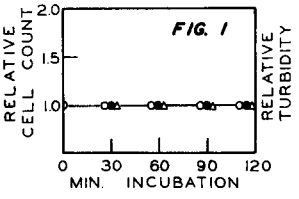
As shown in Fig. 1, no net protein synthesis (based on turbidity measurements[7]) occurred in the resuspended cells, and there was no change in cell count over a 120-min period (Fig. 1). RNA content of the cells increased by a factor of about 1.4 in the presence or absence of chloramphenicol (Fig. 2). DNA increased by a factor of 3.6 in the absence of chloramphenicol, and 2.8 in the presence of chloramphenicol (Fig. 3). This increase in DNA content was unrelated to either cell number (Fig. 1) or viability (Fig. 4). Although the pattern of cell death was variable at the three conditions (Fig. 4), in each instance the number of surviving cells at the end of 2 h was lower than at "0 time". The temporary restoration of viability produced by chloramphenicol added at "0 min" is similar to the "restoration and second death" of the same strain subjected to u.v.-radiation as described by BARNER AND COHEN[8].

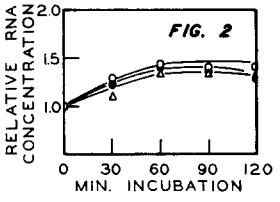
These results demonstrating continued synthesis of DNA while cell count remains constant are similar to those reported by OKAZAKI AND OKAZAKI[9]. They showed that addition of thymidine to a deoxyribose-requiring strain of *Lactobacillus acidophillus*, previously deprived of a deoxyribose source, resulted in active DNA synthesis with comparatively little increase in protein, RNA or cell number. Their results are also similar in that chloramphenicol had little effect on DNA synthesis. From similar studies, others have concluded that u.v.[2]- or mustard[1]-treated *E. coli* require protein synthesis prior to initiation of DNA synthesis.

These differences in the requirement of protein synthesis for initiation of DNA duplication may be due to differences in cell-protein damage produced by mustards,

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; BrUDR, 5-bromodeoxyuridine.

u.v.-light, deoxyribose limitation and BrUDR. It has been established that BrUDR inhibits DNA synthesis by limiting utilization of thymidylate[10], or it may be incorporated in place of thymidine[11] into DNA. No other effects of this compound are known. Similarly, limitation of deoxyribose would be expected to interfere primarily with DNA synthesis. Alkylating agents and u.v.-irradiation are known to produce changes in both DNA and protein structure[12]. Thus it is reasonable to expect that BrUDR or deoxyribose limitation produce a relatively specific effect on cellular DNA





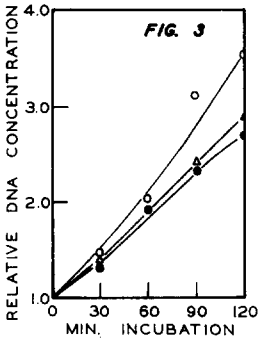
o — O CLMP; ● — CLMP at "o min"; △ — CLMP at "15 min".

Fig. 1. Effect of chloramphenicol (clmp) on cell count and turbidity of *E. coli* 15T⁻ after BrUDR treatment. Cells were grown for 2 h in the presence of 100 μg/ml BrUDR and then resuspended in synthetic medium plus 1 μg/ml thymine at "o min". 75 μg/ml chloramphenicol was added at times indicated.

$$\text{Relative turbidity or cell count} = \frac{\text{turbidity or cell count at } t \text{ min after resuspension}}{\text{turbidity or cell count at the time of resuspension}}$$

Fig. 2. Effect of chloramphenicol on RNA in *E. coli* 15T⁻ after BrUDR treatment. Cells were grown in the presence of 100 μg/ml BrUDR for 2 h and then resuspended in synthetic medium plus 1 μg/ml thymine at "o min". 75 μg/ml chloramphenicol was added at times indicated.

$$\text{Relative concentration of RNA} = \frac{\text{concentration at } t \text{ min after resuspension}}{\text{concentration at the time of resuspension}}$$



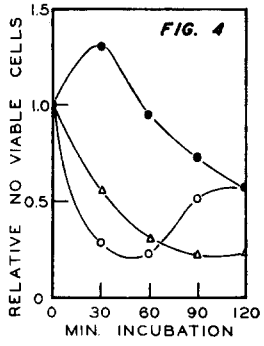


Fig. 3. Effect of chloramphenicol on DNA synthesis in *E. coli* 15T⁻ after BrUDR treatment. Cells were grown in the presence of 100 μg/ml BrUDR for 2 h, and then resuspended in synthetic medium plus 1 μg/ml thymine at "o min". 75 μg/ml chloramphenicol was added at times indicated

$$\text{Relative concentration of DNA} = \frac{\text{concentration at } t \text{ min after resuspension}}{\text{concentration at the time of resuspension}}$$

Fig. 4. Effect of chloramphenicol on viability of *E. coli* 15T⁻ after BrUDR treatment. Cells were grown in the presence of 100 μg/ml BrUDR for 2 h, and then resuspended in synthetic medium plus 1 μg/ml thymine at "o min". 75 μg/ml chloramphenicol was added at times indicated.

$$\text{Relative number of viable cells} = \frac{\text{viable cells at } t \text{ min after resuspension}}{\text{viable cells at the time of resuspension}}$$

whereas u.v.-light or mustards cause additional damage to functional proteins to the extent that renewal is necessary prior to continued DNA synthesis.

Although BrUDR is known to produce a primary effect on DNA, there is a continued synthesis of DNA in BrUDR-treated *E. coli* which serves no apparent purpose for survival or cell division. This useless DNA synthesis without concomitant cell division suggests that the inability of *E. coli* to survive in growth medium after exposure to high concentrations of BrUDR is due primarily to loss of DNA function rather than failure to produce DNA.

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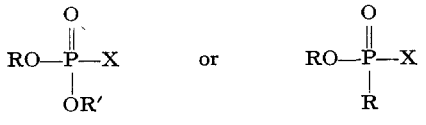
Electronic structure and activity of organophosphorus inhibitors of esterases

The group of substances known as the "nerve gases" and which may be represented by the general formulas



where X is –F, –CN or $-O.C_6H_4 \cdot NO_2$ and R and R′ are alkyl groups (and where the O's may sometimes be replaced by N or S) are highly specific inhibitors of enzymes which possess esterase activity, particularly cholinesterase and acetylcholinesterase[1]. Their mode of action bears striking similarity to the first stage of the process of enzymic hydrolysis of the biochemical substrates themselves: the substituted