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# EFFECT OF X-RAYS ON BACTERIAL RIBOSOMES

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SUMMARY

Crude extracts from starved, non-irradiated and irradiated *Escherichia coli* cells were collected in 3 mM phosphate buffer containing 0.01 M $Mg^{2+}$. The radiated cells were found to contain diminished amounts of free RNA and 88-S ribosomes. Starved cells also showed similar effects. At the various doses used, up to 90 krads, it was observed that 70-S particles were not affected, whereas the amount of 88-S particles was greatly diminished. It was concluded that the enzyme system that controls the conversion of 70-S to 88-S particles was more susceptible to radiation damage.

## INTRODUCTION

There is a growing evidence that the ribonucleoprotein particles of the cell are the centres of protein synthesis[1-3], though not all of them are active in the process[4]. Attempts to study the effects of ionising radiation on such ribosomal centres have been made by BILLEN AND VOLKIN[5], who reported that due to irradiation of *E. coli* by X-rays, the ribosomal particles with a sedimentation coefficient of 40 S were disrupted. It is now established that in cell-free extract of *E. coli* there are four types of ribosomes of sedimentation coefficients 30 S, 50 S, 70 S and 100 S. The 70-S particle is formed of one 30-S and one 50-S particle, while the 100-S particle is a dimer of 70-S ribosomes[7,8].

BILLEN AND VOLKIN[5] used a dose of the order of 120 krads and no study on the effect of variation of doses was reported. But small doses of the order of a few krads are found to affect cell in a variety of ways, such as by inhibiting the division[9], decreasing the rate of nucleic acid synthesis[10], *etc.* It was therefore considered worthwhile to attempt to study the effect of various doses of radiation on the ribosomal distributions of *E. coli*. This communication presents results of sedimentation analyses of the extracts from X-irradiated *E. coli* immediately after exposure and after a period of incubation of the exposed cells in nutrient medium.

## MATERIALS AND METHODS

*Specimen and growth medium*: *E. coli* cells for inoculation were obtained from 12-h old Morton–Engley (M–E) slants (Tryptone, 2%; NaCl, 0.5%; glucose, 0.5%; potassium dihydrogen phosphate, 0.25%; yeast extract, 0.2%; and agar, 2%). Inoculation was given in M–E medium (composition same as above, but excluding agar).

*Growth*: For the two types of experiments, cells were grown for about 45 min and 2.5 h, respectively. The cells were then collected in M–E medium by centrifugation at concentrations of the order of $10^7$ cells/ml and $10^{11}$ cells/ml, respectively.

*Source of irradiation and the method of exposure*: The source of irradiation was a continuously operated X-ray machine run at 80 kV. The dose rate at the point of irradiation measured with a Victoreen r-meter was $2 \cdot 10^3$ R/min. The cells at the concentrations mentioned above were divided into two equal parts, one part of which was irradiated to the desired dose in thin-walled test tubes while the other part which was in every way similar to the former except for radiation was kept as control.

The general scheme of the experiment is shown in Table I.

TABLE I

GENERAL SCHEME OF THE EXPERIMENT

| *Condition of the cell during exposure* | *Dose of radiation (krads)* (1) | *Amount of medium in which the cells were allowed to grow (ml)* (2) | *Time after which the growth was diluted to 1 l and penicillin added (h)* (3) | *Time required for formation of protoplasts (h)* (4) |
|---|---|---|---|---|
| Logarithmically growing cells in M–E medium at a concentration of the order of $10^7$ cells/ml. Total volume 5 ml. | 0 | 500 | 2.5 | 2 |
| | 24 | | 4.0 | |
| | 30 | | 4.0 | |
| | 60 | | 4.5 | |
| Logarithmically growing cells in M–E medium at a concentration of the order of $10^{11}$ cells/ml. Total volume 5 ml. | 0 | 500 | 0 | 2 |
| | 40 | | | |
| | 60 | | | |
| | 80 | | | |
| | 90 | | | |

*Formation of protoplasts and collection of crude cell-free extract*: The irradiated cells and their controls were separately mixed with about 500 ml of the growth media and were allowed to grow for a definite period as given in Table I, Column 4. 500 ml of fresh M–E medium containing 4 % sucrose and 0.2 % $MgSO_4$ were then mixed up with the above cell suspensions and $2 \cdot 10^5$ I.U. of penicillin added. Protoplast formation was complete after 2 h (ref. 10). The protoplasts were then collected by centrifugation. The crude cell extract in all cases was obtained by lysing the protoplasts formed with 3 mM phosphate buffer containing 0.01 M $Mg^{2+}$ at pH 7.0. This crude extract containing the bulk of RNA, mostly in the form of ribonucleoprotein particles, was freed from cell debris and other impurities by repeated centrifugation.

*Sedimentation analysis*: Analytical ultracentrifugation of the cell-free extracts from control and irradiated cells was carried out with a model E, Spinco ultracentrifuge at 39460 revolutions/min, equipped with Schlieren optical system, using the standard 12-mm cell. All experiments were carried out in 3 mM phosphate buffer containing 0.01 M $Mg^{2+}$ at pH 7.0.

## RESULTS

Ultracentrifugal analysis of the cell-free extract obtained from irradiated *E. coli* showed schlieren patterns similar to those reported by other workers for extracts from non-irradiated *E. coli*[6,12]. Two major ribonucleoprotein peaks with sedimentation

coefficients of 70 S and 88 S, besides the soluble-protein peak (with a sedimentation coefficient of 5 S) were observed. Three other peaks with sedimentation coefficients 30 S, 50 S, and 104 S were also noted. Another peak with a sedimentation coefficient of 23 S has been observed in all experiments. This peak is supposed to be due to free RNA[4].

All values of sedimentation coefficients reported here are with respect to water at 20°. The concentration dependence of sedimentation coefficients had been studied and is represented in Fig. 1 for the 70-S, 88-S and 104-S components. However, they have been rounded off for convenience of discussion.

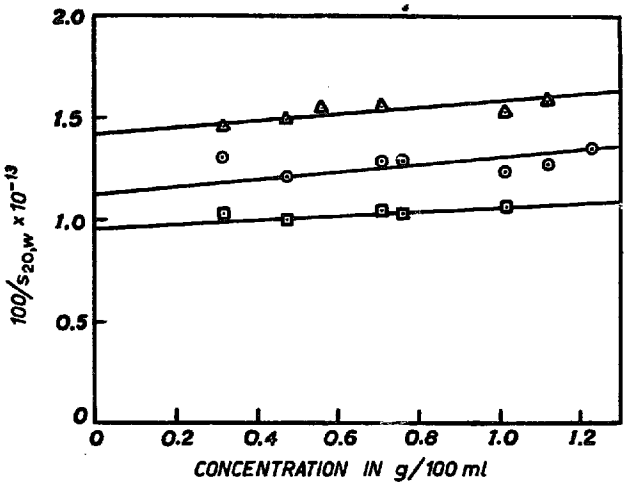


Fig. 1. Concentration dependence of sedimentation coefficient for the 70-S (△—△), 88-S (⊙—⊙) and 104-S (⊡—⊡) components.

Figs. 2a, 2b, 3a and 3b show the optical patterns for the extracts from *E. coli* studied under different conditions. Fig. 2a represents the pattern due to the extract from non-irradiated growing *E. coli*, whereas Fig. 2b represents that due to extracts from cells exposed to 80 krads and subsequently grown for periods as shown in Table I, Column 4. Figs. 3a and 3b are the optical patterns obtained with extracts from cells which were suspended at a concentration of $10^{11}$ cells/ml for 45 min and exposed

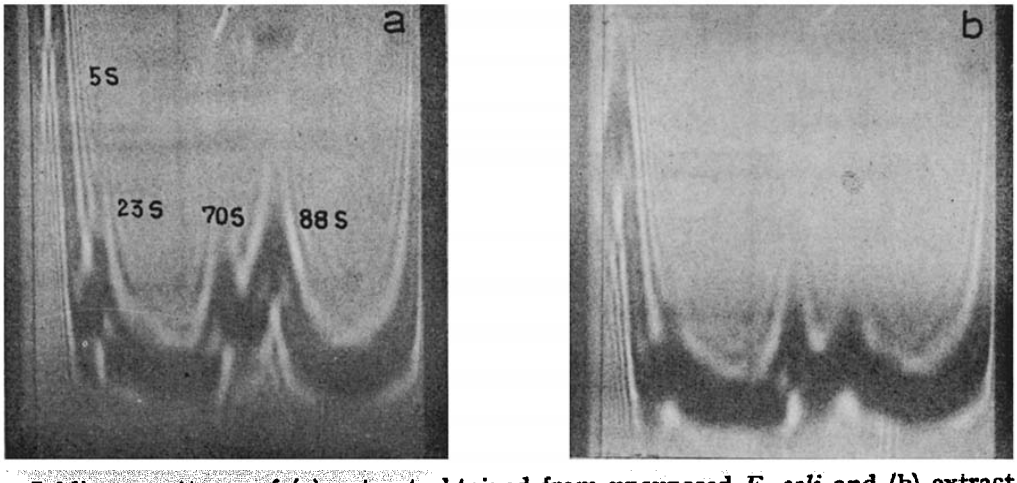


Fig. 2. Schlieren patterns of (a) extract obtained from unexposed *E. coli* and (b) extract from *E. coli* exposed to 80 krads and subsequently grown for 4 h. Both patterns were taken 8 min after the ultracentrifuge had reached 39460 revolutions/min.

to 24 krads at the same cells concentration, respectively. In both cases the cells were immediately subjected to penicillin treatment for formation of protoplasts. The general nature of the patterns is similar in these figures. However, the most interesting point to be noted is that at comparable concentrations of the extracts, the ratio of the amount of 88-S ribosome, to that of the 70-S ribosome as judged from the area of Gaussian patterns is greater than unity for Fig. 2a, less than unity for Figs. 2b, 3a and 3b.

Another interesting observation is that the peak due to free RNA though quite prominent in Figs. 2a and 3a was found to diminish in Fig. 2b, whereas in Fig. 3b it was almost absent. Figs. 2a and 2b, 3a and 3b are at comparable extract concentrations.

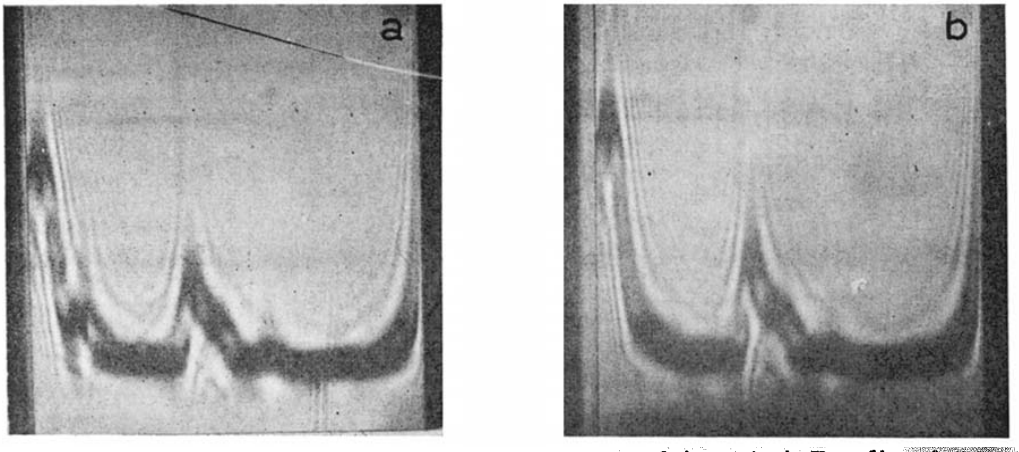


Fig. 3. Schlieren patterns of (a) the extract from unexposed (starving) *E. coli* and (b) extract from *E. coli* exposed to 24 krads and immediately subjected to penicillin treatment. Both patterns were taken 12 min after the ultracentrifuge had reached 39460 revolutions/min.

## DISCUSSION

The variation in the ratio of 88-S to 70-S ribosomes under different conditions of the cell indicates the physiological change in the state of the cell since the ribosome content is characteristic of the physiological state of the cell, both in its magnitude and in the relative quantities of the various components present. Figs. 2a and 2b, 3a and 3b were obtained under different conditions of the cells. In the former case the cells were actively growing during irradiation and penicillin was added only after a definite period of growth after irradiation, whereas in the latter case the cells were virtually starved during irradiation because at the concentration of $10^{11}$ cells/ml cell division was not possible and that penicillin was added immediately after irradiation. The result was that in the former case the cells seemed to show a sort of recovery resulting in a greater concentration of free RNA (23-S peak) compared to the latter cases at comparable concentrations.

The active centre of protein synthesis is believed to be the 70-S ribosomes[4]. It is supposed that the transformation of 70-S to 88-S ribosome is intimately associated with the growth of the cell and the release of newly synthesized protein from the ribosomal templates. The 70-S and 88-S forms are believed to exist in equilibrium with each other[12]. The decrease in the 88-S to 70-S ratio due to irradiation indicates a hampering in the protein synthesis of the cell. This hampering of protein synthesis

is also evident with the starved cells (Fig. 3a). Thus radiation and starvation seem to produce the same final effect though the mechanism of production might be different in the two cases.

BILLEN AND VOLKIN[5] reported a marked diminution in the 70-S peak due to irradiation to a dose of 120 krads. In the present study much lower doses have been used, which showed that the 88-S ribosomes were first affected and that 70-S particles might be affected at still higher doses.

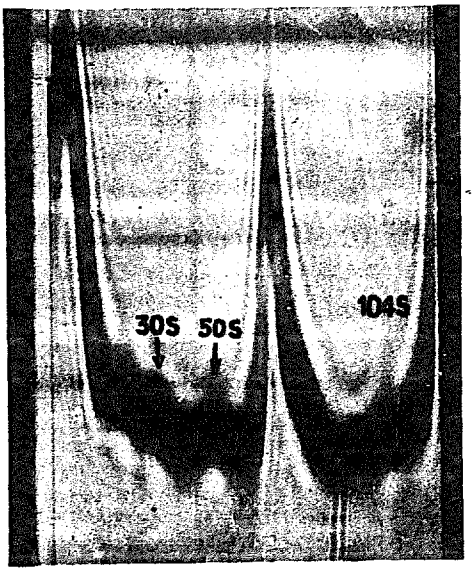


Fig. 4. Schlieren pattern of extract obtained from *E. coli* exposed to 90 krads and immediately subjected to penicillin treatment. The pattern was taken 12 min after the ultracentrifuge had reached 39460 revolutions/min.

In a series of present experiments when the cells were exposed to 90 krads and immediately subjected to penicillin treatment, the 88-S component was completely absent. This dose seems to be the optimum dose at which the 88-S component is completely disrupted with a consequent increase in the 70-S component. However, the result is markedly different (Fig. 2b) if the cells are allowed to recover their ability to synthesize protein during the latent period which may run up to as much as 3 h at the maximum dose used.

The results of the present study conclusively prove that it is the enzyme system that controls the conversion of 70-S to 88-S particles which is most susceptible to radiation damage.

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REFERENCES

1 K. MCQUILLEN, *Progr. Biophys. Biophys. Chem.*, 12 (1962) 69.
2 H. M. DINTZIS, H. BORSOOK AND J. VINOGRAD, *Microsomal Particles and Protein Synthesis*, Pergamon Press, New York, 1958, p. 95.

[3] J. W. LITTLEFIELD AND E. B. KELLER, *J. Biol. Chem.*, 224 (1957) 13.
[4] G. L. BROWN, *Brit. Med. Bull.*, 18 (1962) 10.
[5] D. BILLEN AND E. VOLKIN, *J. Bacteriol.*, 67 (1954) 191.
[6] A. TISSIERES, J. D. WATSON, D. SCHLESSINGER AND B. R. HOLLINGWORTH, *J. Mol. Biol.*, 1 (1959) 221.
[7] C. E. HALL AND H. S. SLAYTER, *J. Mol. Biol.*, 1 (1959) 329.
[8] H. E. HUXLEY AND G. ZUBAY, *J. Mol. Biol.*, 2 (1960) 10.
[9] S. B. BHATTACHARJEE, *Radiation Res.*, 14 (1961) 50.
[10] S. B. BHATTACHARJEE, M. SARKAR AND N. N. DAS GUPTA, *Biochim. Biophys. Acta*, 66 (1963) 123.
[11] J. LEDERBERG, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 574.
[12] E. BOLTON, R. BRITTEN, D. COWIE, B. MCCARTTY, K. MCQUILLEN AND R. ROBERTS, *Ann. Rept. Biophys. Section Carnegie Inst. Wash.*, (1958–59) 266.