REACTIVATION OF IRRADIATED ESCHERICHIA COLI B BY RIBONUCLEASE

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Addition of ribonuclease to an actively growing culture of Escherichia coli B which has been exposed to ionizing radiation (Cobalt 60 γ-rays) results in a substantial increase in survivors. This paper discusses conditions under which this phenomenon has been found to take place.

Experimental conditions. Well aerated log phase cultures of E. coli B in nutrient broth which have reached a steady state of growth are irradiated directly in this medium while growing at 37°C. After irradiation the cultures are washed and resuspended in fresh nutrient broth or synthetic Roberts C medium (Roberts et al. 1955). At this point the culture is usually split into two parts. A solution of RNAase (Worthington) is added to one part and the number of viable cells is followed during incubation by periodically taking aliquots which are plated on nutrient agar after suitable dilution through cold (7°C) nutrient broth.

Reactivation with RNAase. Figure 1 shows the course of reactivation caused by the addition of RNAase in a typical experiment. A culture of E. coli B was grown and irradiated with a dose of 40Kr. in nutrient broth. One half of the culture was washed and resuspended in fresh nutrient broth, the other half in Roberts C medium. During subsequent incubation, 40 μg/ml of RNAase was added to one half of each culture. The amount of reactivation reached a maximum within about ten minutes, and in this experiment, fluctuated around values of about ten times the survivors in the control cultures. The smaller reactivation curve in the control cultures, to which

the RNAase reactivation is additive, occurs when dilutions for plating are made through a cold medium of low salt concentration, such as nutrient broth. Direct cell counts made with a Petroff-Hausser bacteria counter do not show a measurable change in cell numbers during these experiments.

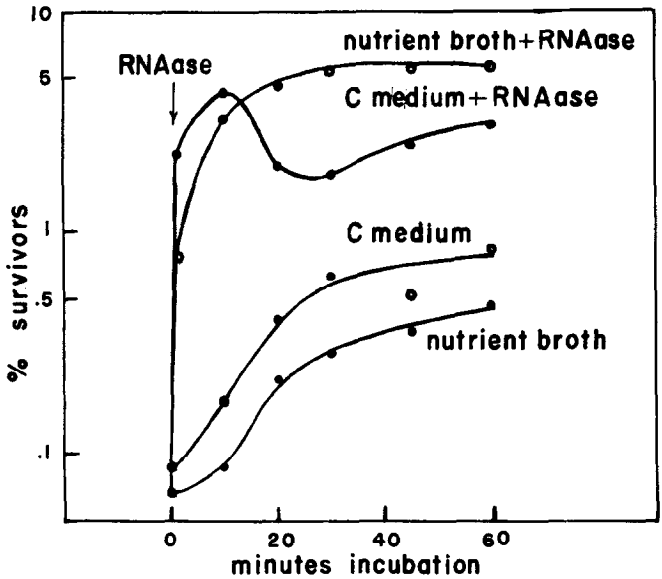


FIG. 1

Figure 2a shows the amount of reactivation occurring after one minute incubation with RNAase in the medium (nutrient broth) as a function of enzyme concentration. With a radiation dose of 40 Kr. and a cell concentration of 5×10^7/ml., maximum reactivation was reached with a concentration of 10µg/ml RNAase. The reactivation is radiation dose dependent. A plot of the ratio of survivors of RNAase treated cultures to untreated controls as a function of radiation dose is shown in Figure 2b.

Protamine sulfate has been used by Otaka et al. (1962) to protect messenger RNA from degradation by RNAase in vitro. Reactivation of E. coli B by RNAase is prevented by addition of protamine sulfate (200 µg/ml) immediately before addition of RNAase to the culture.

Nutritional conditions necessary for reactivation with RNAase. In experiments where shifts between complete (nutrient broth) and synthetic

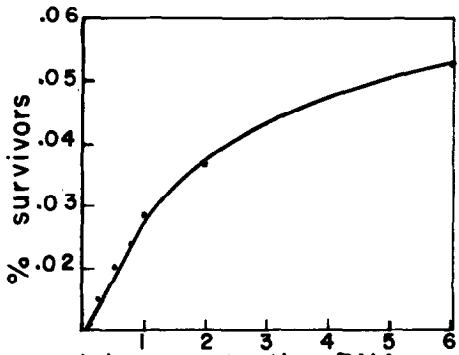
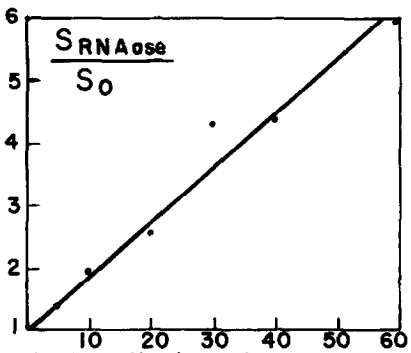


FIG. 2a



2b

(Roberts C) media have been made after irradiation and prior to RNAase addition, it has been found that reactivation occurs to an appreciable extent only in those cases in which the culture has been grown in complete medium before irradiation, or has had at least a short period of incubation in complete medium after irradiation but prior to the addition of RNAase. Cultures grown before and after irradiation in synthetic media are not reactivated by RNAase, but they show a higher survival than cultures exposed to complete media. It appears that RNAase is able to restore the additional damage to viability which growth in a complete medium causes in irradiated E. coli B. This is illustrated in Table 1, where the data from a typical experiment show that nutrient broth cultures of E. coli B, although much more sensitive to radiation than minimal salts cultures, can be brought to survivals in the range of the latter by treatment with RNAase.

Synthetic capacity of irradiated E. coli B incubated with RNAase. Although a radiation dose of 40Kr. reduces the viability of E. coli B to the order of 0.01% to 0.1% survivors, capacity for synthesis of new protein is by comparison inhibited only moderately (less than 50%). Addition of RNAase in concentrations sufficient for maximum reactivation in broth cultures does not measurably reduce the rate of increase in O.D. or cell protein (Biuret) until approximately 80 to 100 minutes after irradiation.

Further incubation in the presence of RNAase does cause a leveling off of protein synthesis, and direct microscope measurements made on elongated cells show that after a dose of 40Kr., the RNAase treated cells grow to only about one-half the final length of the control cells. The rate of formation of the induced enzyme β-galactosidase by a culture grown and irradiated in nutrient broth and resuspended in a minimal-lactose medium is slightly higher in the presence of RNAase than in controls. This might suggest some inhibitory effect of the RNAase on a repressor substance, but attempts to produce evidence of synthesis of β-galactosidase in cultures in the absence of inducer by treatment with RNAase have so far failed.

Table 1

Survival of E. coli B grown to a density of 5×10^7 cells/ml in Roberts C minimal glucose medium or nutrient broth prior to irradiation of 40Kr. and resuspended after irradiation in the medium indicated. After a 15 minute incubation period at 37°C in the presence or absence of 40μg/ml RNAase, aliquots were plated on the agar medium shown.

Pre-irradiation incubation medium	Post-irradiation incubation medium	Agar plating medium	Survivors/ml x 10^4 RNAase added at start of post-irradiation incubation	
			0	40μg/ml
Glucose min.	Nutrient broth	Nutrient	1.5	4.2
		Glucose min.	13.0	32.0
		Lactose min.	9.5	20.0
	Glucose min.	Nutrient	8.8	5.0
		Glucose min.	15.0	14.0
		Lactose min.	18.0	45.0
	Lactose min.	Nutrient	30.0	25.0
		Glucose min.	51.0	47.0
		Lactose min.	48.0	40.0
Nutrient broth	Nutrient broth	Nutrient	0.68	27.0
	Glucose min.	Nutrient	1.3	34.0

Effect of RNAase treatment on cellular RNA. Determination, by the orcinol reaction, of the total amount of RNA in irradiated cultures of E. coli B incubated in RNAase for 5 minutes did not reveal any difference in content from non-enzyme treated controls. This result and the evidence for

undiminished growth rate and induced enzyme formation of the cells, indicated that RNAase treatment was not preventing formation of new functional RNA or breaking down old RNA to a point where a significant amount of degradation products would leak out of the cells.

In order to investigate possible intermediate changes in the RNA of the reactivated cultures, density gradient centrifugal analyses of the phenol purified RNA are being carried out at present using essentially the method of Hayashi and Spiegelman (1961). Initial experiments of this nature indicate that the principal effect of the RNAase treatment is to break down the 23S and 16S ribosomal fraction of the previously existing RNA to molecules which sediment in the general region of 4S to 12S.

These results indicate that the ribosomal RNA may be the fraction of the cell RNA in which radiation damage causes the most lethal effect, and that the breaking down of this RNA to smaller pieces rescues many of these cells. The data in Table 1, showing broth incubated cells having lower survival but being capable of reactivation by RNAase, also lend support to this hypothesis. As Kjeldgaard (1961) has shown, cells grown in broth contain a higher proportion of their dry weight in ribosomal RNA than cells grown in glucose salts medium. His data also show that when cells are shifted from salts medium to broth there is an initial high rate of ribosomal RNA synthesis. This would explain why a post-irradiation shift from glucose salts medium to broth decreases survival of E. coli B, since radiation damaged RNA precursors would be preferentially going into ribosomal RNA.

Ultracentrifugational analysis of RNA in reactivated cultures is being continued and radioactive pulse labeling of the RNA will be carried out in order to follow the fate of the degraded RNA in the bacteria.

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