REVERSION OF THE EFFECTS OF RADIATION ON LYSOGENIC BACTERIA

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Received April 25, 1963

An early indication that the effects of radiation on lysogenic bacteria can be reversed by the medium used after irradiation, is found in a paper by Weigle and Delbrück (1951). By plating the bacteria immediately after irradiation, they could produce a reversal of the induction in a small fraction of the organisms.

The present paper describes the reversing effect of some amino acids on irradiated lysogenic bacteria.

The following strains of E. coli K12 were used: a lysogenic strain 169(λ), requiring thiamine, threonine and leucine for growth; strain C600- a non-lysogenic derivative of strain 169(λ); strain W3693, which requires uracil for growth, and a lysogenic derivative of this strain, strain W3693(λ). The minimal medium for the growth of strains 169(λ) and C600, was described before (Ben-Gurion, 1962). The same medium was used for the growth of strains W3693 and W3693(λ), except that leucine and threonine were omitted and uracil (20γ/ml) added instead.

The irradiation of the bacteria and the induction procedure have been carried out, as described elsewhere (Ben-Gurion, 1963). The viable counts were carried out on minimal media supplemented with 2% bacto agar(Difco).

When the two lysogenic strains had been grown in minimal media, washed and irradiated with U.V. for 1 minute and then diluted again into their respective minimal media, with and without added cysteine (2.5γ/ml), there was a marked difference in the titer of phages produced by the irradiated

bacteria, as can be seen from Figure 1. When the bacteria had grown before irradiation in minimal media supplemented with cysteine (2.5γ/ml), the production of phage was the same, whether cysteine was added after irradiation or not.

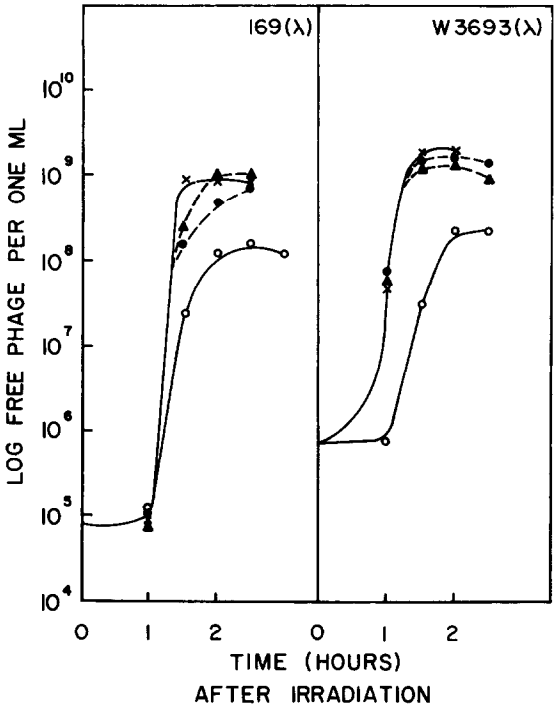

Fig 1 presents experiments of induction with and without cysteine.

Effects of cysteine on induction by U.V.

×———×cells grown before and after induction in minimal media.
○———○cells grown before induction in minimal media, and after induction in minimal media supplemented with cysteine (2.5γ/ml).
●- - - - -●cells grown before and after induction in minimal media supplemented with cysteine (2.5γ/ml). ▲- - - - -▲cells grown before induction in minimal media supplemented with cysteine, and after induction in minimal media without cysteine.

In order to determine whether the decrease in phage production by irradiated bacteria, caused by the addition of cysteine, was due to a decrease in the number of induced bacteria or to a reduction in the burst size, the number of induced bacteria was measured after incubation of the irradiated cells with and without cystein for 40 minutes, as described

previously (Ben-Gurion, 1962). A typical experiment showed that cells of strain 169(λ), irradiated for one minute, produced 1.8×10^7 induced cells per 1 ml, and only 2.7×10^6/ml after incubation with cysteine. Strain W3693(λ), in a similar experiment, showed a reduction from 1.3×10^7 induced cells/ml to 3.5×10^6/ml. The loss of infectious centers could be due to abortive induction, to reversion of the induction or to both. In order to determine the cause for the loss of infectious centers, viable counts of the irradiated bacteria were carried out (Table 1).

Table 1

The effect of cysteine added after irradiation on the survival of lysogenic cells

strain used	irradiation time (min)	viable count immediately after irrad.	after one hour incubation in minimal medium	after one hour incubation in minimal medium+ cystein
169(λ)	0	1.6×10^7		
	1	3.0×10^6	2.4×10^6	6.8×10^6
	2	8.0×10^5	4.5×10^5	2.8×10^6
	3	1.0×10^5	3.7×10^4	7.7×10^5
W3693(λ)	0	2.9×10^7		
	1	7.5×10^6	1.2×10^6	9.6×10^6
	2	9.2×10^5	6.2×10^4	3.9×10^6
	3	5.4×10^4	7.2×10^3	2.5×10^5

The experiments were repeated using thiolhistidine instead of cysteine, but no effect on the induction or on the survival of the irradiated lysogenic bacteria was observed.

Table 2 summarizes experiments of irradiated non-lysogenic bacteria incubated after irradiation with and without cysteine.

The reversion of the effects of radiation by cysteine is limited to the lysogenic strains only.

Table 2

The effect of cysteine added after irradiation on the survival of non-lysogenic bacteria

strain used	irradiation time (min)	viable count		
		immediately after irradiation	after one hour incubation	
			in minimal media	in minimal media + cysteine
C600	0	2.5×10^7		
	3	3.3×10^6	3.0×10^6	2.9×10^6
	4.5	2.5×10^5	2.9×10^5	1.2×10^5
W3693	0	2.0×10^7		
	2	6.0×10^6	6.0×10^6	4.0×10^6
	3	6.0×10^5	6.0×10^5	1.5×10^5
	4	2.5×10^5	2.8×10^5	1.2×10^5

When other amino acids were added together with cysteine to the irradiated lysogenic bacteria, no single amino acid, at a concentration of 1×10^{-3} M, reversed the cystein effect completely. When a mixture of all the 17 amino acids tested (histidine, proline, tyrosine, phenylalanine, lysine, arginine, glutamic acid, aspartic acid, threonine, leucine, valine, isoleucine, serine, tryptophan, glycine, methionine and alanine, at a concentration of 4×10^{-4} M each) was added together with cysteine, the effect of the later was completely reversed.

Serine and valine, at a concentration of 40 γ/ml, had a similar effect on the survival of the irradiated bacteria as cysteine. Their action was also reversed by the addition of other amino acids.

Since the effect of these three amino acids could be reversed by the addition of other amino acids, it seemed that their activity was due to some interference with the availibility of other amino acids, for some time after the irradiation. Experiments with chloramphenicol at a dose of 100 γ/ml added after irradiation for a short time (12 min), demonstrated that this treatment increased the survival of irradiated lysogenic bacteria,

while it did not increase the survival of the non-lysogenic bacteria. Table 3 summerizes the results.

Table 3

The effect of chloramphenicol (100γ/ml) on the survival of irradiated bacteria

strain used	irradiation time (min)	viable count after 1 hour incubation in minimal medium	viable count after 12 min. with chloram. in minimal medium than 48 min. without chloram.
169(λ)	3	8.0×10^4	2.7×10^5
C600	3	2.4×10^6	1.3×10^6
	4.5	4.0×10^5	1.4×10^5
W3693(λ)	2	1.8×10^5	5.3×10^5
W3693	2	5.0×10^6	2.8×10^6
	4	3.0×10^5	1.8×10^5

These observations suggest that if immediately after irradiation protein synthesis is disturbed, this disturbance can reverse the process of induction, caused by ultra violet light. Since ultra violet light interfers with DNA synthesis (Kelner, 1953), it is possible that the synthesis of proteins that proceeds (Hanawalt and Setlow, 1960) without parallel DNA synthesis results in some imbalance which is the cause of induction. If protein synthesis is inhibited at the same time at which DNA synthesis is arrested, no such imbalance and, therefore, no induction will occur.

The author wishes to acknoledge the valuable technical assistance of Miss K. Schajevitz.

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