The Protection of Bacteria against Radiation Effects

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The lethal and mutagenic effects of ultraviolet radiations on bacteria have been known for many years, but the mechanism or mechanisms by which radiations induce mutations or kill cells remain unknown. Ample evidence is available to show that neither of these effects can be attributed to a direct genetic change, although nongenetic material may be affected, which in turn effects a genetic change. Possibly the same common factor is responsible for both effects, their expression being influenced by some other means.

One approach to this problem is to find a substance (*s*) which offers protection to bacteria against the deleterious effects of radiation and, by a relation of its role in cell metabolism, to determine where the primary change may occur. To date the protective action of many substances against radiation damage (*1–3*) has not been proved to be either a true physiological protection or simple physical absorption. It is obvious that such a distinction is of the utmost importance in this work.

To determine this, thrice-washed 12-hr broth cultures of *Escherichia coli*, strain B/r (*4*), were appropriately suspended at pH 7.0, standardized with an Evelyn photometer using a #660 filter to give a 60% transmission reading in a 1:10 dilution, and divided into three parts, as follows:

a) Irradiation controls: Washed cells suspended in the *M*-9-I buffered solution of Anderson (*5*) and irradiated to give the irradiation control curve.

b) Test suspensions: Substances to be tested for protective ability made up in *M*-9-I buffer in three concentrations. Washed cells were suspended in these solutions, standardized, and used to determine whether the test substance offered any protection to the cells when in contact with them during irradiation.

c) Protected suspensions: The test solutions, instead of being in contact with the bacteria were placed in a quartz cell above the standardized bacterial suspension (prepared as in [*a*]) to determine whether the lethal and mutagenic rays are absorbed by the test substance, thus giving a physical protection to the bacteria.

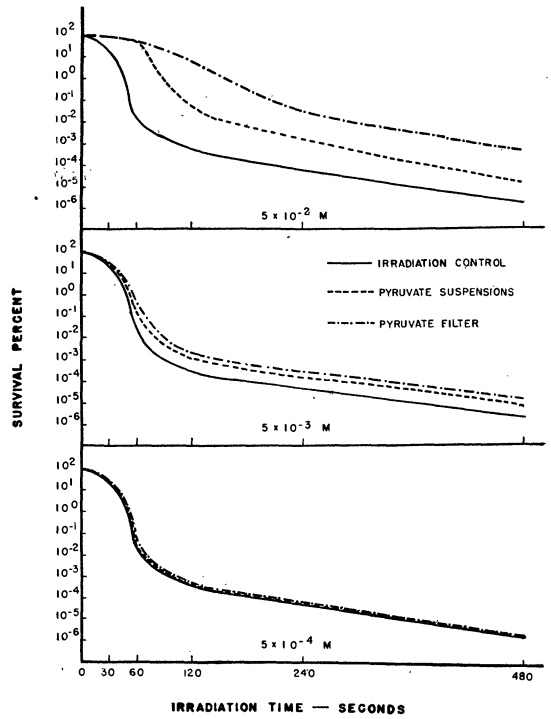

FIG. 1. Effect of sodium pyruvate on *E. coli* B/r during ultraviolet irradiation (2537 A).

The source of irradiation was a 15-w G-E germicidal lamp estimated to deliver 95% of its energy in the 2537 A line. Samples were irradiated in open Petri dishes held in a mechanical shaker, the frequency and amplitude of which were of the order to produce standing waves. This procedure insures an adequate exposure of all cells to the ultraviolet rays. Samples of 10 ml volume were held at a distance of 37 cm from the light source and irradiated for 0, 30, 60, 120, 240, and 480 sec. All experiments were carried out in a darkened room as a precaution against the photoreactivation phenomenon (*6, 7*). Survival was determined by the drop plate method (*8*), using nutrient agar plates. The mutation studied was that of color

TABLE 1

APPARENT PROTECTION AND ABSORPTION OF SODIUM PYRUVATE AGAINST THE MUTAGENIC EFFECTS OF ULTRAVIOLET LIGHT (2537 A) ON *E. Coli* B/r

Irradiation time (sec)	Mutation (%)						
	Irradiation control	Pyruvate suspensions			Pyruvate filter		
		$5\times10^{-2}M$	$5\times10^{-3}M$	$5\times10^{-4}M$	$5\times10^{-2}M$	$5\times10^{-3}M$	$5\times10^{-4}M$
0	0.00	0.00	0.23	0.00	0.20	0.00	0.10
30	9.20	1.02	8.40	9.64	0.70	5.55	8.53
60	10.27	4.00	9.94	10.61	2.24	7.60	9.15
120	12.93	10.96	11.01	12.60	8.92	10.41	12.90
240	12.14	10.67	11.28	12.00	8.61	11.44	12.50
480	13.41	10.60	11.69	13.20	10.78	11.29	12.90

TABLE 2

APPARENT PROTECTION AND ABSORPTION OF SEVERAL SUBSTANCES AGAINST THE LETHAL EFFECTS OF ULTRAVIOLET LIGHT (2537 A) ON *E. coli* B/r

Irradiation time (sec)	Irradiation control	Tryptophan ($5\times10^{-3}M$)	Sodium thioglycollate ($1\times10^{-1}M$)	Cysteine HCl ($2.5\times10^{-2}M$)	Glutathione ($1\times10^{-3}M$)
		A, Test suspensions—survival (%)			
0	100	100	100	100	100
30	44.18	95.50	91.03	81.06	52.08
60	5.8×10^{-1}	88.33	74.25	28.52	2.7×10^{-1}
120	1.4×10^{-3}	47.88	38.46	7.9×10^{-2}	1.6×10^{-2}
240	5.5×10^{-5}	5.99	5.38	2.4×10^{-4}	7.7×10^{-5}
480	2.8×10^{-6}	2.8×10^{-1}	1.4×10^{-2}	7.4×10^{-5}	3.7×10^{-6}
		B, Protected suspensions—survival (%)			
0	100	100	100	100	100
30	44.18	98.86	98.71	88.89	77.27
60	5.8×10	94.06	90.71	77.88	7.72
120	1.4×10^{-3}	86.73	79.73	4.7×10^{-1}	2.1×10^{-1}
240	5.5×10^{-5}	45.52	73.99	2.3×10^{-2}	5.9×10^{-4}
480	2.8×10^{-6}	6.94	45.86	1.0×10^{-3}	1.0×10^{-5}

response on mannitol-tetrazolium agar (*9*). The following substances were tested for their protective ability, each being employed in three concentrations.

Sodium pyruvate (Nutritional Biochemicals Corp.)
5×10^{-2}, 5×10^{-3}, $5\times10^{-4}M$

DL-Tryptophan (General Biochemicals, Inc.)
5×10^{-3}, 5×10^{-4}, $5\times10^{-5}M$

L-Cysteine HCl (Coleman, Bell Co.)
2.5×10^{-2}, 2.5×10^{-3}, $2.5\times10^{-4}M$

Sodium thioglycollate (Baltimore Biological Laboratory)
1×10^{-1}, 1×10^{-2}, 1×10^{-3}M

Glutathione (Eastman Kodak Company)
1×10^{-3}, 1×10^{-4}, $1\times10^{-5}M$

Increasing doses of ultraviolet were administered to successive aliquots of the cell suspensions. Survival and mutation percentages for each dose were determined from the colonies that developed on plates after 24-hr incubation. All the substances tested appeared to offer protection to the bacteria when the cells were suspended in them during irradiation. This apparent protection was shown to be due, however, to a physical absorption of the lethal and mutagenic rays by the test substance when the rays were passed through it in the quartz cell prior to being directed on a standard cell suspension.

All substances tested behaved in a similar manner, but their "protective" ability varied in degree. The results of one of several experiments are given in full, that using sodium pyruvate (Fig. 1 and Table 1). The results of the other test substances in one concentration only, are summarized in Table 2.

The five substances employed in these experiments are quite dissimilar in structure; however, they all show fairly strong absorption in the 2537 A line. The substances were run through a Beckman photospectrometer and their extinction coefficients, 1%, 1 cm, before and after 15-min irradiation determined. The log of these values is given in Table 3.

The results of these experiments indicate the necessity of discriminating between a true physiological protection and a simple physical absorption of the lethal and mutagenic rays. Any attempt to formulate a theory of the mechanism of radiation effects on cells or of its prophylaxis, on the basis of results obtained using "protective" substances, must take this into consideration.

TABLE 3

EXTINCTION COEFFICIENTS AT 2537 A FOR SAMPLES OF TEST SUBSTANCES BEFORE AND AFTER ULTRAVIOLET IRRADIATION

Test substance	Concentration	Log $E^{1\%}_{1\text{ cm}}$	
		Unirradiated	Irradiated
DL-Tryptophan	$1\times10^{-4}M$	1.38	1.20
Sodium pyruvate	$1\times10^{-3}M$	1.05	—
Sodium thioglycollate	$1\times10^{-3}M$	1.53	1.34
Cysteine hydrochloride	$2.5\times10^{-3}M$	1.16	1.28
Glutathione	$1\times10^{-3}M$	1.25	1.30

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