

HEAT REACTIVATION OF ULTRAVIOLET-INACTIVATED BACTERIA¹

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Various microorganisms and bacteriophages that have been inactivated or rendered nonviable by ultraviolet irradiation can be reactivated to a very high degree by exposure to visible light (Kelner, 1949*a,b*; Dulbecco, 1949; Novick and Szilard, 1949; Johnson, Flagler, and Blum, 1950). Kelner investigated the photoreactivation of four species of microorganisms including *Escherichia coli*, strain B/r.

In the study of the photoreactivation phenomenon, *E. coli* strain B, as well as strain B/r, was used. The former reactivates with visible light as does the latter, but in addition a similar reactivation of B cells can be accomplished by heat in the absence of visible light (Anderson, 1949*a,b*; Stein and Meutzner, 1950). The present paper is a more extended report on this phenomenon.

METHODS

The cultures used in these studies, strain B and its radiation-resistant mutant B/r, were grown for 16 to 18 hours at 37 C under aeration, in standard Difco nutrient broth. Suspensions to be irradiated were prepared by dilution of the culture into a non-ultraviolet-absorbing buffer to give cell concentrations of 1 to 2×10^7 organisms per ml. Irradiation was carried out using 5-ml portions contained in a 50-ml rotating quartz flask.

Two 15-watt G.E. germicidal lamps served as a source of ultraviolet. The lamps were horizontally mounted in a ventilated housing with their centers directly over a 2.5-inch-square opening through which the irradiation was directed upon the quartz flask. The flask was adjusted at a distance to irradiate the suspension at a rate of approximately 700 ergs per second per cm².

After irradiation, samples were removed from the flask and appropriately diluted in sterile buffer solution. Five-hundredths ml of the final suspension were placed on the surface of each of four nutrient agar plates and spread with a glass spreader. As a standard procedure all plates were preincubated overnight, removed just prior to use, and returned to the incubator immediately after having been spread. All seeded plates were placed in a single layer on the incubator shelves to ensure a minimum time to reach temperature equilibrium and to obtain uniform temperature for all plates at any incubation temperature. The seeded plates were incubated at 30, 37, and 40 C in the dark, or in medium light intensity at 37 C. Plates at 30 C were incubated for 48 to 72 hours, whereas

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plates at 37 and 40 C were incubated for 24 to 48 hours because of the more rapid colony growth rate at the higher temperatures.

EXPERIMENTAL RESULTS

Survival ratios obtained with strain B/r, in which the plates seeded with irradiated cells were not exposed to visible light but incubated at 37 C in the dark,

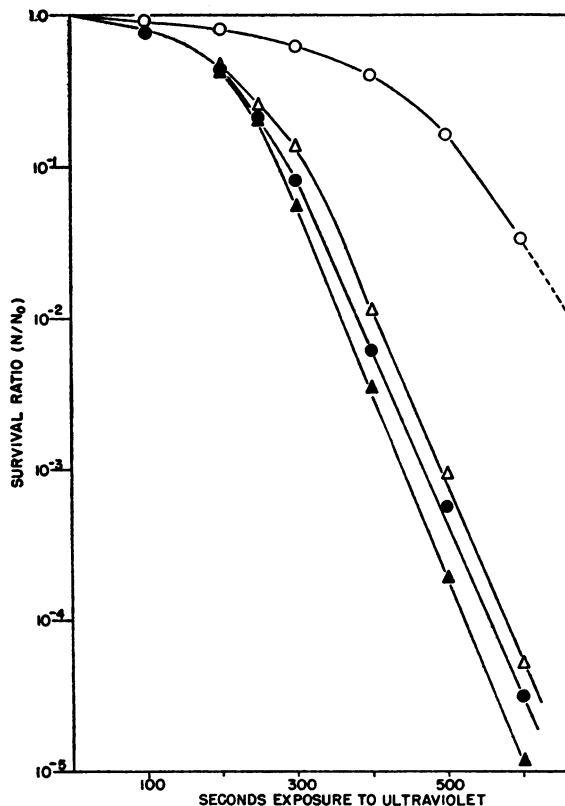


Figure 1. Reactivation of B/r. Survival curves of strain B/r obtained with dark incubation at 30, 37, and 40 C and incubation at 37 C in visible light of medium intensity. Each point is the average of four aliquot plates. ○, 37 C light; △, 40 C dark; ●, 37 C dark; ▲, 30 C dark.

give curves of the so-called multiple-hit type as shown in figure 1. Survival ratios obtained from plates that were exposed to medium intensities of visible light at 37 C for 2 hours, prior to dark incubation at 37 C, give curves which are similar to the dark curves but which show a 500- to 1,000-fold increase over the dark survivors at the higher ultraviolet exposures.

Aliquot plates incubated at 30 and 40 C in the dark give survival curves that closely parallel those obtained at 37 C in the dark. A maximum difference in survival of 3- to 4-fold is obtained, between the 30 and 40 C incubation, at the longer exposures to ultraviolet. This agrees with the value found by Kelner

(1949*b*) for the effect of temperature on the photoreactivation of strain B/r during the period of illumination.

Figure 2 presents the data obtained in a similar experiment with the parent strain B. A single-hit type of survival curve is obtained in the dark at 37 C with this organism in contrast to the multiple-hit survival curve obtained with B/r under similar conditions.

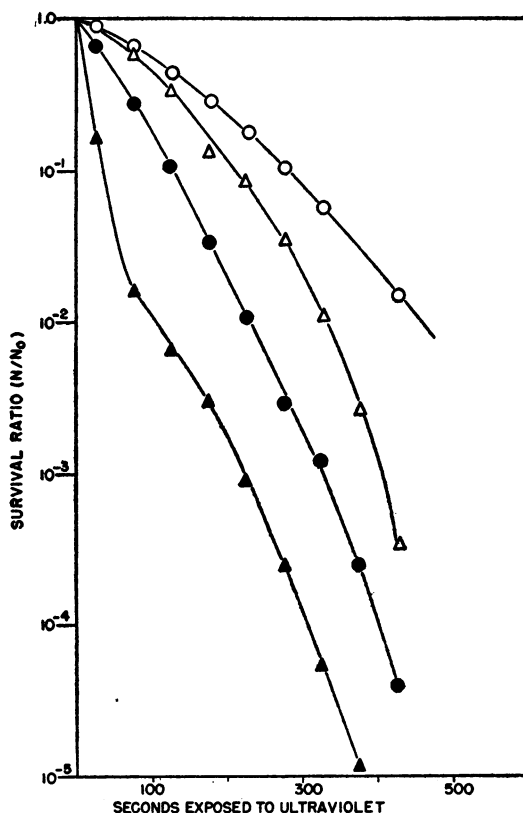


Figure 2. Reactivation of B. Survival curves of strain B obtained with dark incubation at 30, 37, and 40 C and incubation at 37 C in visible light of medium intensity. Each point is the average of four aliquot plates. ○, 37 C light; △, 40 C dark; ●, 37 C dark; ▲, 30 C dark.

A striking increase in survival ratio is also obtained with B when plates seeded with inactivated cells are exposed to visible light. In this particular experiment a 450-fold increase in survivors was obtained at the longer exposures to ultraviolet.

The temperatures at which plates seeded with ultraviolet-inactivated B organisms are incubated in the dark have pronounced effects on their survival. This is in sharp contrast to the results obtained with the B/r strain. The same temperature increment with B cells resulted, in this particular experiment, in a 300-fold increase in survivors. Thus it is seen that a 10-degree rise in incubation

temperature, between 30 and 40 C, produces a reactivation of ultraviolet-inactivated B organisms of the same order of magnitude as does photoreactivation.

The difference in the ability of strains B and B/r to respond to heat reactivation is shown in a more obvious manner in table 1. The reactivation effect of both heat and visible light is expressed in terms of dose-reduction ratios (see Kelner, 1949b). For photoreactivation the dose-reduction ratio for any given survival is the ratio of the ultraviolet exposure of light-treated cells to that ultraviolet exposure of dark cells which gives an equal survival. For heat reactivation the dose-reduction ratio for any given survival is the ratio of the ultraviolet exposure of cells incubated at 40 C to the ultraviolet exposure of cells incubated at 30 C which produces the same level of survival. These ratios were obtained graphically from figures 1 and 2.

TABLE 1

Comparison of dose-reduction ratios for photoreactivation and heat reactivation of strains B and B/r

PERCENTAGE SURVIVORS	B		B/r	
	D_L/D_D at 37 C	D_{40c}/D_{30c}	D_L/D_D at 37 C	D_{40c}/D_{30c}
50	2.64	9.00	2.00	ca. 1.00
10	2.20	6.17	1.84	1.14
5	2.12	5.52	1.80	1.14
1	ca. 2.00	3.17	ca. 1.75	1.12
0.5		2.47		1.12
0.1		1.83		1.11

The dose-reduction ratios for photoreactivation (D_L/D_D) for any given survival level is the ratio of the ultraviolet exposure (D_L) of light-treated cells to that ultraviolet exposure (D_D) of dark-treated cells which gives an equal survival. The dose-reduction ratios for heat reactivation (D_{40}/D_{30}) for any survival level is the ratio of the ultraviolet exposure (D_{40}) of cells incubated at 40 C to the ultraviolet exposure (D_{30}) of cells incubated at 30 C which produces the same level of survival.

From the table it can be seen that ultraviolet-inactivated cells of B/r show practically no heat reactivation effect, the dose-reduction ratio remaining essentially at unity over the range of survival levels shown. On the other hand, strain B exhibits a very striking degree of heat reactivation, especially at the higher survival levels. The dissimilarity of the 30 and 40 C survival curves of B is reflected in the change in the value of the dose-reduction ratios at the different survival levels.

Despite the very great difference in the ability of the B and B/r strains to respond to heat reactivation, their response to photoreactivation is quite similar, as may be seen by a comparison of their dose-reduction ratios for this method of reactivation.

Although the dose-reduction values for the heat reactivation of strain B vary widely with the percentage of survivors, it is clear that the ratios obtained are of the same order of magnitude as those found by Kelner (1949b) and by Novick and Szilard (1949) for photoreactivation of the B/r strain.

In an attempt to determine whether or not heat reactivation of ultraviolet-inactivated organisms is a general phenomenon, eight additional strains of *E. coli* and three haploid and four diploid strains of *Saccharomyces cerevisiae* were tested. None of the yeast strains and but one other coli strain exhibited a response to the heat treatment, over a 10-degree temperature increment, greater than that found for the B/r strain. The second coli strain to show reactivation is a smooth variant of strain B and is serologically related to it. Survival curves of this strain show that it reactivates in a manner similar to that of, and to approximately the same degree as, strain B.

X-ray-inactivated cells of B and B/r are not reactivated by exposure to high intensities of visible light. Heat reactivation tests with X-ray-inactivated cells showed the survival ratio of strain B/r to be essentially unaffected. However, cells of B with a survival ratio of 10^{-5} at an incubation temperature of 30 C showed a 13-fold increase in survival ratio on aliquot plates placed at 40 C.

DISCUSSION

Although the experimental methods and units of expression of reactivation differ from those reported by Stein and Meutzner (1950), there is essential agreement with their findings for the heat reactivation of the B strain of *E. coli*.

From the experiments of Stein and Meutzner it is not possible to determine unequivocally that the heat reactivation effect is due exclusively to the heat reactivation of the ultraviolet-inactivated cells themselves. In their experiments the cells were exposed to ultraviolet radiation on the surface of nutrient agar plates. These plates were then incubated at various temperatures, and reactivation quotients were determined for each temperature increment. Therefore the possibility of the production of a growth-inhibitory substance by the ultraviolet irradiation of the organic medium, with its subsequent decomposition by incubation at elevated temperatures, is not excluded.

That the effect is on the cells themselves rather than on the medium is made probable by the experimental methods employed in the studies reported in the present paper. In all experiments the cells were irradiated in a liquid non-ultraviolet-absorbing medium that was diluted prior to plating the irradiated cells on nutrient agar. Parallel assays at different dilutions of the irradiated suspension showed the assay to be independent of the amount of the irradiated medium placed on the nutrient plates.

The degree of heat reactivation obtained for the two strains of coli in which this phenomenon was found to occur is of the same order of magnitude as that reported by Kelner (1949b) and Novick and Szilard (1949) for the photoreactivation of the B/r strain of coli.

It was found that cells of the B/r strain of coli inactivated by X-rays do not show photoreactivation or heat reactivation. X-ray-inactivated cells of the B strain show no photoreactivation and but a slight degree of heat reactivation. This is another indication that the mechanisms by which biological damage is accomplished are different for these two types of radiation.

Heat reactivation may not be as general a phenomenon as photoreaction.

This is indicated by the fact that, of ten coli and seven yeast strains tested, only two strains of coli were found capable of heat reactivation.

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SUMMARY

Ultraviolet-inactivated cells of several strains of *Escherichia coli* and *Saccharomyces cerevisiae* have been tested for their ability to be reactivated as a result of heat treatment after irradiation. The magnitude of reactivation obtained, with a 10-degree difference in incubation temperature, for two strains of *E. coli* approximates that obtained by photoreactivation. The heat-reactivation phenomenon does not occur to any appreciable extent in X-ray-inactivated B/r cells and only slightly in B cells. Heat reactivation may not be as general a phenomenon as photoreactivation.

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