Incidence and Characteristics of Beta Radiation Survivors (*Escherichia coli*)

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Received for publication January 12, 1956

It has been recognized that when a population of bacterial cells is exposed to ionizing radiation in arithmetically progressive doses, the number of survivors decreases geometrically until the entire population is destroyed. This exponential survival pattern was first observed with x-rays and later with other ionizing particles such as electron, proton, and so forth (Lea, 1946; Bacq and Alexander, 1955).

Lea (1936) found that when *Bacillus mesentericus* spores, *Escherichia coli* and *Staphylococcus aureus* were irradiated by alpha and beta particles from polonium and radium respectively, the numbers surviving diminished exponentially as a function of the dose. More recently, bacteria, bacterial spores, yeasts, fungi and viruses have been exposed to high voltage electrons and in all cases similar exponential survival patterns were demonstrated (Dunn *et al.*, 1948; Katznelson *et al.*, 1952; Edwards *et al.*, 1954; Bellamy and Lawton, 1954; Koh *et al.*, 1956; Bridges *et al.*, 1956; Pepper *et al.*, 1956; and Fluke and Pollard, 1955).

The question arose as to why a few cells in a microbial population could withstand a radiation dose which killed[1] the majority of the population. Two explanations were considered. One was the target theory, which acknowledges that not every cell will receive a lethal dosage; the other was the possibility of spontaneously occurring resistant mutants. The present study was made in order to determine whether the radiation survivors were spontaneously occurring resistant mutants. In addition, the biochemical characteristics of radiation survivors were studied with respect to their catalase activities and nutritional requirements.

MATERIALS AND METHODS

The test organism was a strain of *Escherichia coli* which had been maintained in our laboratory for several years. The organism was grown in nutrient broth for 18 to 24 hr at 37 C. The bacterial cells were washed twice with distilled water and after the final centrifugation were resuspended in distilled water to the original volume. One-tenth-ml aliquots of washed bacterial cells, in serially diluted suspensions, were streaked onto 2 per cent Bacto agar plates to provide a thin film. The plates were then exposed to beta rays generated by a 2 million electron volt Van de Graaff accelerator.[2] The radiation dosages varied from 10,000 to 70,000 reps (Roentgen equivalent physical unit).

After irradiation, a thin layer of nutrient agar was poured over the surface. Radiation survivors formed colonies between the two agar layers after 48-hr incubation at 37 C. The numbers of organisms in the inoculum were determined on nonirradiated plates similarly prepared. Sixteen survivors were isolated from plates exposed to the maximum substerilizing dose. These were identified as R (resistant) strains. By exposing R strains to maximum substerilizing doses, nine RR strains were obtained. The resistance of the parent strain to cathode rays was compared with the resistance of R and RR strains exposed to equal radiation dosages. To detect catalase activity, sufficient 3 per cent hydrogen peroxide solution U.S.P.[3] was added to 20-hr cultures of the test organisms on nutrient agar slants to submerge the agar. The presence of bubbling, indicative of release of oxygen, was presumptive evidence of catalase activity.

TABLE 1. *Minimal medium* for Escherichia coli*

KH_2PO_4	2.0 g
$(NH_4)_2HPO_4$	2.0 g
NaCl	4.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g
Glucose	2.0 g
Distilled water	1,000 ml

pH 7.2.

Glucose was aseptically added after autoclaving.

* D. T. Smith and D. S. Martin. 1948. *Zinsser's Textbook of Bacteriology*, 9th. ed., p. 47. Appleton-Century-Crofts, Inc., N. Y.

In order to determine whether the radiation survivors required nutritional factors different from those of the parent strain, the survivors were grown in a simple synthetic medium (table 1) which was known to be adequate for growth of the parents.

[1] Inability of the organisms to reproduce under these conditions of test.

[2] Built by the High Voltage Engineering Corp., Cambridge, Mass., and installed at our laboratory.

[3] Merck & Co., Inc., Rahway, N. J.

RESULTS AND DISCUSSION

Resistances of survivors. From data obtained with *Escherichia coli* (parent strain), one R strain (R/16) and one RR strain (RR/1), the survival curves shown in figure 1 were drawn. The R/16 strain was obtained from one isolate of the parent strain which had survived a dose of 50,000 reps and the RR/1 strain derived from an R strain that had survived 60,000 reps. The number of cells in the initial population is shown at the beginning of each curve. The three survival curves are practically identical. The killing dose, 70,000 reps for the parent strain *E. coli*, was sufficient to destroy radiation survivors.

When smaller numbers of cells were subjected to irradiation, lower doses were required to kill all strains, as shown in figure 2. It can be seen that the slopes of the curves for the parent strain are similar irrespective of the concentration of cells irradiated. This same relationship was observed for the R/16 and RR/1 strains.

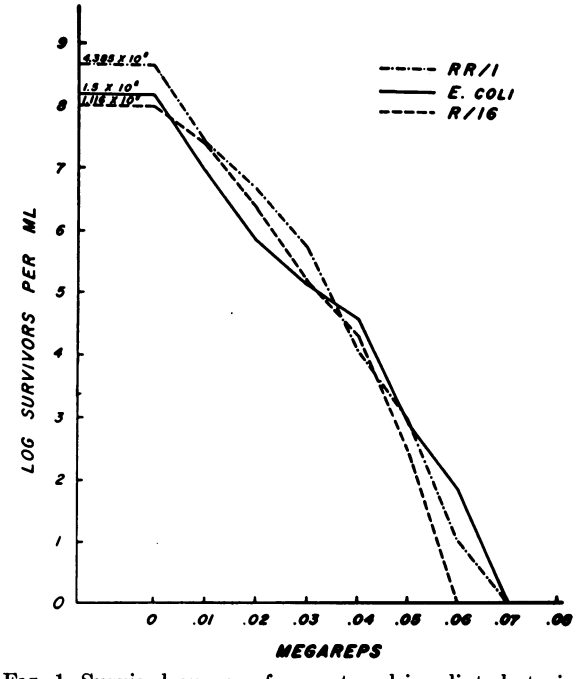

FIG. 1. Survival curves of parent and irradiated strains of *Escherichia coli*. The R/16 strain originally survived 0.05 megarep and the RR/1 strain 0.06 megarep.

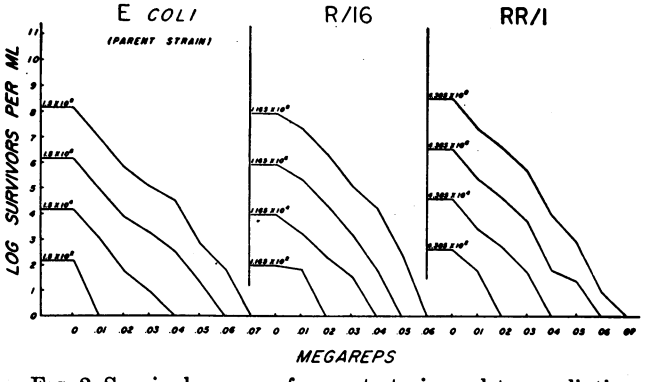

FIG. 2. Survival curves of parent strain and two radiation-resistant strains of *Escherichia coli* plotted to show differences in doses required when concentrations of cells are decreased.

Additional studies with 15 R strains and 8 RR strains gave similar results. Since no RR strain was more resistant than the parent strain, it was assumed that there were no induced resistant mutants.

Biochemical characteristics. The *E. coli* (parent strain) and the irradiated strains R/16 and R/1 isolates all exhibited catalase activity.

Growth of the R/16 and R/1 strains, in the synthetic medium employed, did not differ from that of the *E. coli* parent strain.

These observations differ significantly from results obtained with drug-resistant (Bryson and Demerec, 1950) and U.V. resistant (Witkin, 1946) mutants.

ACKNOWLEDGMENTS

The technical assistance rendered by Mr. John R. Tekowitz and by the Department of Pharmaceutical Chemistry is acknowledged.

SUMMARY

It was found that isolates of *Escherichia coli* which resisted the high radiation dosages employed were not spontaneously occurring mutants. These survivors were identical to the parent in their susceptibility to radiation, catalase activity, and nutritional requirements. Electron beam irradiation did not appear to induce radiation-resistant mutants in this strain of *Escherichia coli*.

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