Effect of Heat and Plating Medium on Survival of
Escherichia coli after Treatment with
Radiomimetic Chemicals

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ABSTRACT

ZAMPIERI, ANTONIO (Palo Alto Medical Research Foundation, Palo Alto, Calif.), AND JOSEPH GREENBERG. Effect of heat and plating medium on survival of Escherichia coli after treatment with radiomimetic chemicals. J. Bacteriol. 89:931–936. 1965.—Survival of Escherichia coli strain S and its radiation-resistant mutant R4, after treatment with mitomycin C, azaserine, nitrogen mustard, 1-methyl-3-nitro-1-nitrosoguanidine, nitrofurazone, and proflavine was studied. With all agents except proflavine, R4 was more resistant than was S. Survival of strain S was greater on minimal glucose-salts medium than on Penassay (Difco), and greater on the latter than on Tryptone (Difco) agar; survival of S was greater when posttreatment incubation temperature was 45 C than when it was 37 C. Post-treatment plating medium or temperature had no effect on survival of R4. Visible light did not affect survival of S or R4. The survival curves of R4 were exponential; those of S exhibited decreasing sensitivity with time of exposure. With proflavine, photoactivation by visible light was demonstrable, but there was no difference in survival between S and R4. Survival of either strain was not affected by post-treatment plating medium or incubation temperature.

When used to treat microorganisms, radiomimetic chemicals share a number of biological properties with ultraviolet light, such as cross-resistance, mutagenesis, filament formation, induction of lysogenic phages, inactivation of transforming deoxyribonucleic acid (DNA), and, with some chemicals, inhibition of DNA synthesis (reviewed by Zampieri and Greenberg, 1964; Terawaki and Greenberg, 1964).

One biological property of ultraviolet radiation is that its lethal effect can be reduced by postirradiation growth conditions such as the presence in the plating medium of nitrogenous organic substances (Roberts and Aldous, 1949), elevated temperature of incubation (Anderson, 1951), and illumination with visible light (Kelner, 1949). Recovery in the presence of nitrogenous organic matter (plating-medium effects, pme) and elevated temperature of incubation (heat reactivation, hr) is observed when a radiation-sensitive strain such as Escherichia coli B, but not when a radiation-resistant strain such as B/r, is used. Photoreactivation (pr) is observed with both radiation-sensitive and -resistant strains.

Some of the lethal effects of the radiomimetic chemicals, nitrous acid (Zampieri and Greenberg, 1964) and mitomycin C (Winkler, 1962), against radiosensitive strains of E. coli were found to be subject to pme, hr, or both. There has been no evidence for pr after treatment with radiomimetic chemicals. It was, therefore, of interest to determine whether the lethal effects of other kinds of radiomimetic agents, such as mono- and bis-functional alkylating agents, nitrofurazone, and proflavine, were subject to pme, hr, or pr, and if these recovery phenomena were restricted to a radiosensitive strain of E. coli, such as strain S.

It will be shown that lethal damage caused by none of the radiomimetic chemicals tested was photoreactivable. However, lethal damage to the radiation-sensitive strain E. coli S (a derivative of strain B), but not to its radiation-resistant mutant R4, was subject to pme and hr when such damage was caused by nitrogen mustard, 1-methyl-3-nitro-1-nitrosoguanidine, azaserine, nitrofurazone, mitomycin C, and nitrous acid. Damage caused by proflavine, on the other hand, was subject to neither pme nor hr.

MATERIALS AND METHODS

Bacterial strains. The strains of bacteria, their source, derivation, and properties, as well as the chemicals and media used, and most of the methods, have been described in detail (Woody-Karrer and Greenberg, 1963).

To test for the sensitivity to the chemicals,
bacteria were grown overnight in peptone broth, washed twice in buffered saline, and diluted to 10⁵ bacteria per milliliter of buffer and saline containing the compound. Mitomycin C, nitrofurazone, azaserine, 1-methyl-3-nitro-1-nitrosoguanidine, and nitrogen mustard were tested in phosphate-buffered saline (pH 6.8); nitrous acid in acetate buffer (pH 4.2); and proflavine in tri(hydroxymethyl)aminomethane (Tris) buffer (pH 7.8); 1 ml of the preparation in a 50-ml flask was incubated in a shaking water bath at 37 C and sampled at intervals. The samples, appropriately diluted, were plated in duplicate on minimal glucose-salts (M9), Tryptone, and Penassay (Difco) agar, and incubated at 37 C for 18 to 24 hr. The terminal sample was plated in quadruplicate on Tryptone agar; two of the plates were incubated at 37 C and two at 45 C.

To test for the effect of visible light, one of two flasks prepared as above was covered with aluminum foil, and the other was uncovered. Both were incubated in the presence of two 20-w General Electric white fluorescent lamps placed 12 cm above the sample flask. This was sufficient illumination to permit, in 1 hr of exposure, a fivefold recovery of ultraviolet-irradiated cultures.

RESULTS
In Fig. 1 through 7 is shown the survival of E. coli strain S and its radioresistant mutant type R₄ after treatment with test chemical agents and plating on Tryptone, Penassay, and M9 medium. Table 1 shows the effect of visible light on survival during treatment with test compounds and the effect of posttreatment incubation temperature. Since the results with mitomycin C, azaserine, 1-methyl-3-nitro-1-nitrosoguanidine, nitrogen mustard, nitrous acid, and nitrofurazone are quite similar, these will be described together. R₄ was more resistant than was strain S, and exhibited no plating-medium effect or heat reactivation. On the other hand, there were more surviving colonies of strain S on Tryptone medium incubated at 45 C than there were at 37 C. There were more survivors of strain S on Penassay than on Tryptone medium, and more on M9 than on either Penassay or Tryptone. However, with none of the chemicals was survival of strain S, even on M9, equal to that of R₄. The survival curves of R₄ treated with each chemical was exponential over the period of treatment. The survival curves of strain S, in general, were similar to the survival curve of strain S after ultraviolet irradiation; i.e., they had a constantly changing slope, indicating increased resistance with time of exposure. Neither strain S nor strain R₄ exhibited
any difference in survival, whether illuminated by visible light or not.

For reference purposes, survival after penicillin and streptomycin treatment of strains S and R₄ is also shown in Table 1. With neither strain was there any evidence of heat-reactivation or (data not shown) plating-medium effect after treatment with these antibiotics.

Survival of strain S after an ultraviolet radiation dose of 230 ergs/mm² was: on Tryptone medium, 10⁻²; on Penassay, 2.9 × 10⁻²; on M9, 8.0 × 10⁻². Incubation in buffer at 37°C in the dark for 1 hr resulted in no increased survival; with visible light there were four to five times as many survivors after 1 hr at 37°C.

Tryptone medium differs from Penassay in having sodium chloride and sodium citrate added to it and in containing Tryptone instead of peptone. In Table 2 is shown the survival of E. coli S after treatment with nitrosoguanidine and plating on various permutations of ingredients in Penassay and Tryptone medium. When Tryptone medium was prepared without citrate, or when Tryptone was replaced by peptone, no significant change in survival was observed. When sodium chloride was omitted from Tryptone medium, survival approached that on Penassay. However, when sodium chloride alone or together with sodium citrate was added to Penassay medium, survival was not decreased from that observed with Penassay medium itself.

Because the results with proflavine differed from those with other chemicals tested, they are treated separately. As seen in Fig. 7, there was a marked photoreactivation in visible light with both strains S and R₄. However, there was no difference in sensitivity between strains S and R₄; neither strain exhibited any plating-medium effect, and neither exhibited heat reactivation (Table 1).

**DISCUSSION**

From the data presented, it is apparent that lethal damage resulting from all of the radiomimetic agents tested, except proflavine, is subject to pme and hr. However, none of the lethal damage was photoreactivable. Recovery phenomena have been attributed to enzymes which repair chemical damage to DNA. Photoreactivating enzyme (Setlow and Setlow, 1962) is known to split the thymine dimers in DNA, the predominant lethal chemical change attributed to
ultraviolet irradiation. Since none of the radiomimetic chemicals is known to cause the formation of thymine dimers, it is not surprising that no photoreactivation of chemically induced damage was observed. By the same token, photoreactivating enzyme would appear to be quite specific in its repair mechanism, namely, splitting of thymine dimers.

Other recovery enzymes are known to operate in the absence of visible light, and these also repair ultraviolet radiation-induced lesions. One of these, not very well characterized, is associated with host-cell reactivation (hcr) and liquid-holding reactivation. This enzyme, present in radiation-resistant E. coli, but absent in certain mutants hypersensitive to radiation, is known to excise thymine dimers, together with adjacent nucleotides, from radiation-injured DNA (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964). The specificity of this enzyme for excising thymine dimers is not known. However, it has been observed that radiation-hypersensitive or hcr-less mutants of E. coli usually display increased sensitivity to radiomimetic chemicals (Greenberg and Woody-Karrer, 1963). This suggests that the hcr enzyme is not specific for the chemical lesions it can correct.

The manner in which hr and pme operate is not known. It would appear that under conditions of rapid growth or DNA synthesis, the lethal damage is converted to an irreparable form before the repair mechanism of the cell can operate. A difficulty presents itself in attempting to explain the differences in sensitivity to chemicals and radiation between E. coli S and its radiation-resistant mutant R4, or between B and B/r. Strain B has dark recovery enzymes (Boyce and Howard-Flanders, 1964) and is able, given proper conditions, or, perhaps, time, to repair both radiation and, as shown in this report, chemically induced lesions. Since the amount of lethal damage is the net effect of irreparable fixation of injury and repair prior to fixation, the difference between strains S and R4 or B and B/r could be attributed to differences in the rate of either process. It should be feasible, with the methods of Setlow and Carrier (1964), to determine whether the rate of dark repair is significantly different in strains S and R4.

Proflavine is exceptional among radiomimetic

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**Fig. 5.** Survival of Escherichia coli strain S and its radioresistant mutant R4 exposed to nitrofurazone. Symbols: circles, plated on Tryptone agar; squares, plated on Penassay agar; triangles, plated on M9 agar.

**Fig. 6.** Survival of Escherichia coli strain S and its radioresistant mutant R4 exposed to nitrosoguanidine. Symbols: circles, plated on Tryptone agar; squares, plated on Penassay agar; triangles, plated on M9 agar.
agents in that with it no recovery phenomena were demonstrable, even in E. coli S. Furthermore, it was not even possible to demonstrate by the methods used in these experiments that strain R₄ was more resistant than was S. It has been shown (Woody-Karrer and Greenberg, 1964) with the gradient plate method that radiation-resistant mutants of E. coli S are cross-resistant with proflavine. Furthermore, most of the survivors of proflavine treatment, when the chemical is incorporated in Tryptone agar, are radiation-resistant.

A possible explanation of these inconsistencies may be that the radiation-sensitive and -resistant strains differ in their sensitivity to proflavine only

![Table 2. Survival of Escherichia coli S after treatment with nitrosoguanine (5.0 µg/ml) in phosphate-buffered saline (pH 6.8) for 60 min](http://jb.asm.org/)

<table>
<thead>
<tr>
<th>Medium</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone agar</td>
<td>7 × 10⁻³</td>
</tr>
<tr>
<td>Minus sodium citrate</td>
<td>9 × 10⁻³</td>
</tr>
<tr>
<td>Minus Tryptone (plus peptone)</td>
<td>7 × 10⁻³</td>
</tr>
<tr>
<td>Minus sodium chloride</td>
<td>2 × 10⁻³</td>
</tr>
<tr>
<td>Penassay agar</td>
<td>3 × 10⁻²</td>
</tr>
<tr>
<td>Plus sodium chloride*</td>
<td>2.8 × 10⁻²</td>
</tr>
<tr>
<td>Plus sodium chloride* and citrate†</td>
<td>2.9 × 10⁻²</td>
</tr>
</tbody>
</table>

* 0.8%.
† 0.2%.

![Figure 7. Survival of Escherichia coli strain S and its radioresistant mutant R₄ exposed to proflavine. Symbols: circles, plated on Tryptone agar; squares, plated on Penassay agar; triangles, plated on M9 agar.](http://jb.asm.org/)

**TABLE 1. Effect of temperature of incubation on Escherichia coli S and its radioresistant mutant R₄ after treatment with chemical agents**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival on Tryptone* agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent</td>
<td>Dose†</td>
</tr>
<tr>
<td></td>
<td>µg/ml</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>0.1</td>
</tr>
<tr>
<td>Azaserine</td>
<td>1.0</td>
</tr>
<tr>
<td>Nitrosoquinidine</td>
<td>15</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>50</td>
</tr>
<tr>
<td>Nitrous acid</td>
<td>340</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>10</td>
</tr>
<tr>
<td>Proflavine</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin</td>
<td>50</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>50</td>
</tr>
</tbody>
</table>

* Average of 4 to 5 experiments.
† Exposed 60 min at 37 C in 0.02 M phosphate-buffered saline (pH 6.8); except proflavine, tris(hydroxymethyl)aminomethane buffer 0.08 M (pH 7.8); and nitrous acid, acetate buffer 0.1 M (pH 4.2). Nitrofurazone exposed 120 min; proflavine and nitrous acid, 10 min.
if exposed to proflavine under conditions similar to those which favor the fixation of chemical or radiological damage, i.e., in media with high organic nitrogen content. This seems plausible in view of the postulated mechanism of the lethal effects of proflavine (Lerman, 1963), namely, that proflavine intercalates itself between adjacent bases in DNA, causing errors in replication. We have found (unpublished data) that, when exposed to the chemical in a medium high in organic nitrogen, E. coli S was significantly more sensitive than was R4. However, even under these conditions, hr and pme could not be demonstrated in either strain. This suggests that dark-repair enzymes cannot repair the lethal damage caused by proflavine, which would not be surprising if the lethal damage were an error in replication. However, it suggests also that strains S and R4 might differ not in the rate of repair but in the rate of fixation of lethal damage.

Acknowledgments

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Literature Cited


