RELATIONSHIP BETWEEN ULTRAVIOLET SENSITIVITY AND ABILITY TO PROPAGATE ULTRAVIOLET-IRRADIATED BACTERIOPHAGE

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ABSTRACT

Hill, Ruth F. (Columbia University, New York, N.Y.). Relationship between ultraviolet sensitivity and ability to propagate ultraviolet-irradiated bacteriophage. J. Bacteriol. 88:1283-1287. 1964.—Mutants with a reduced ability to propagate ultraviolet-damaged bacteriophage T1 were isolated from Escherichia coli strain B/r. The change in this ability varied from a slight to a very marked loss; the greater the loss, the greater was the ultraviolet sensitivity of the mutant. Reduced ability to propagate irradiated T1 appears to be the more sensitive index of genetic change, because in one mutant there was no significant reduction in ultraviolet resistance. In a converse study, second-step mutants with an increased ultraviolet resistance were obtained from a mutant of E. coli B. This mutant is both unable to propagate irradiated T1 and very ultraviolet-sensitive. The increased ultraviolet resistance of the second-step mutants was not accompanied by an increased ability to propagate irradiated T1.

Mutants of Escherichia coli showing a greatly increased sensitivity to ultraviolet radiation include some which also show a greatly reduced ability to propagate ultraviolet-irradiated bacteriophage (Ellison, Feiner, and Hill, 1960). It was proposed that the simultaneous occurrence of both of these properties is the result of an inability to repair ultraviolet-induced damage to deoxyribonucleic acid (DNA). Such strains were called her− (Harm, 1963) or syn− (Rörsch, Edelman, and Cohen, 1963) or UV+ (Howard-Flanders and Theriot, 1962). A similar result was obtained with her− mutants of E. coli B (Hill and Feiner, 1964). As in the case of K-12 mutants, the bacterial survival curves for ultraviolet irradiation showed a great increase in sensitivity. However, although the survival curves for the mutants were not identical, the survival curves for irradiated T1 were the same when the different mutants were used as hosts. Apparently, mutational alteration of bacteria affecting repair of ultraviolet-damaged DNA produces unequal effects on bacterial and T1 survival.

The present paper concerns a further study of this, which shows that either the resistance to ultraviolet or the ability to repair ultraviolet-irradiated bacteriophage can be changed without a corresponding change in the other property.

MATERIALS AND METHODS

The bacterial strains employed were E. coli B, an her− mutant derived from B and designated as B−3, the ultraviolet-resistant mutant B/r, and her− derivatives of B/r. The isolation of B−3 was described (Hill and Feiner, 1964). The survival of ultraviolet-irradiated T1 is the same on B−3 as on B−1 as on the previously described ultraviolet-sensitive her− B−1 but B−3 is more ultraviolet-resistant than is B−1 (Ellison et al., 1960).

The her− mutants of B/r were obtained by a method described previously (Howard-Flanders and Theriot, 1962). After isolation, cultures were grown to the stationary phase in nutrient broth. Appropriate samples were spread on nutrient broth agar and were ultraviolet-irradiated to obtain the survival curve. The survival curve of T1 was obtained by irradiating in 2% ammonium acetate and by using 0.2 ml of the unirradiated bacterial culture for plating (Adams, 1950).

To obtain derivatives with increased ultraviolet resistance from an ultraviolet-sensitive
strain, mutant B-r was exposed to nitrofurazone. Although increased resistances to this agent and to ultraviolet appear together when some sensitive strains mutate (Szybalski and Nelson, 1954), many ultraviolet-sensitive mutants are already relatively nitrofurazone-resistant (Hill and Feiner, 1964). However, B-r is nitrofurazone-sensitive; it is inhibited by 0.25 µg/ml. By streaking on nutrient broth agar containing higher concentrations (0.5 to 2 µg/ml), several mutants showing an increased resistance to the antibiotic and to ultraviolet radiation were obtained.

The apparatus used for ultraviolet-irradiation was described previously (Hill and Rossi, 1952). The present dose rate is 8.1 ergs per mm² per sec, as measured with a dosimeter.

Results

Ten hcr- mutants of B/r were isolated. Since these were not all independent or appreciably different, complete data were obtained for only four. Figure 1 shows the survival curves obtained for ultraviolet-irradiated T₁ with the use of these mutants as hosts, compared with that for the B/r parent as host. The final slope of the T₁ survival curve was increased when the mutants were used as hosts. For mutants 10 and 7, the extent of the increase was small, whereas for mutant 4 the increase was enough to eliminate the usual break in the curve (at least to 10⁻⁴ survival).

The ultraviolet survival curves for the four mutants showed a range of increasing sensitivity, and the relative positions were the same as were the curves for irradiated T₁ (Fig. 2). However, the ultraviolet survival curve for mutant 10 was so close to that for the parent B/r that statistical analysis seemed necessary. The B/r strain had been obtained many years before the present experiments were performed, and had been maintained by serial subculture every few months. It was possible that an accumulation of genetic changes might have occurred, which would make the curve for B/r the result of curves for different types in the population. Therefore, it appeared more reasonable to compare results for mutant 10, which was a recent single-colony isolate, with results for other single colonies. Accordingly, five colonies formed by B/r cells on nutrient broth agar were picked at random,
and were grown separately in nutrient broth; determinations were made of the ultraviolet survival curves and the survival curves of irradiated T1. The data for mutant 7 were also analyzed.

The results of this analysis showed that the T1 survival curves obtained with mutants 10 and 7 as hosts, and the ultraviolet survival curve for mutant 7, were significantly different from the curves obtained with the five control colonies (Table 1). However, the ultraviolet survival curve for mutant 10 was not significantly different. Evidently, this mutant had a reduced ability to repair ultraviolet-irradiated T1 without a significant change in ultraviolet resistance.

The reverse situation was found for the case of derivatives of the hcr- ultraviolet-sensitive B-r strain. Here, the acquisition of increased ultraviolet resistance left the hcr property unchanged (Fig. 3 and 4).

**DISCUSSION**

Recent investigations of ultraviolet-induced effects in bacteria and phages led to the following picture. A major type of damage is the intrastrand thymine dimer in DNA (Beukers and Berends, 1960; Setlow and Setlow, 1962). The dimer is believed to stop DNA synthesis (Setlow, Swenson, and Carrier, 1963). However, repair is possible either through photoreversal (Setlow and Setlow, 1963) or through a dark process which involves excision of the dimer from DNA (Boyce and Howard-Flanders, 1964; Setlow and Carrier, 1964). Defective ability to accomplish dark repair is responsible for the increased ultraviolet sensitivity and reduced ability to propagate ultraviolet-irradiated phage, manifested by hcr- mutants (Howard-Flanders and Theriot, 1962; Harm, 1963).

The present findings are not necessarily incompatible with this picture if two things are considered. First, the rate of dark repair may by an important factor in bacterial and phage survival. Second, repair of ultraviolet-damaged phage can only occur inside the cell, i.e., in the presence of bacterial DNA, whereas repair of ultraviolet-damaged bacterial DNA does not involve this complication. There are reports that

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**TABLE 1. Statistical comparison of B/r mutants 7 and 10 with control colonies of B/r**

<table>
<thead>
<tr>
<th>Ultraviolet irradiation of</th>
<th>Ultraviolet dose</th>
<th>Culture*</th>
<th>Mean survival</th>
<th>(Mean survival $\pm$ $\sigma$)†</th>
<th>d/$\sigma$</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>80 sec</td>
<td>Controls</td>
<td>10.4</td>
<td>8.6-12.2</td>
<td>1.3</td>
<td>0.003</td>
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<tr>
<td></td>
<td></td>
<td>No. 10</td>
<td>8.0</td>
<td>4.4-11.6</td>
<td>4.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 7</td>
<td>3.1</td>
<td>1.6-4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Controls</td>
<td>2.2</td>
<td>1.8-2.6</td>
<td>1.0</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 10</td>
<td>1.8</td>
<td>0.90-2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 7</td>
<td>0.50</td>
<td>0.25-0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>Controls</td>
<td>0.37</td>
<td>0.24-0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 10</td>
<td>0.27</td>
<td>0.13-0.41</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 7</td>
<td>0.053</td>
<td>0.030-0.076</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>Controls</td>
<td>0.048</td>
<td>0.024-0.072</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 10</td>
<td>0.035</td>
<td>0.028-0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 7</td>
<td>0.0056</td>
<td>0.0013-0.0099</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>80</td>
<td>Controls</td>
<td>3.85</td>
<td>3.60-4.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. 10</td>
<td>0.79</td>
<td>0.59-0.99</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>No. 7</td>
<td>0.53</td>
<td>0.49-0.56</td>
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</tbody>
</table>

* Five control colonies were each tested in two experiments; the two mutants were tested in four experiments each. All platings were in duplicate.

† Symbols: $\sigma$ = standard deviation; d = difference between mean survivals of controls and mutant or between mean survivals of T1 as measured with controls and with mutant; P = probability that d is due to chance.
T1 infection does not disrupt bacterial chromatin, and that ultraviolet-irradiated T1 is not an efficient bacterial killing agent (Luria and Delbrück, 1942; Luria and Human, 1950). Thus, it seems possible that in T1 infection there may be a competitive situation between the replication of phage and of bacterial DNA. If the phage DNA has been damaged, the necessity for its repair may increase the chance that replication of bacterial DNA and, hence, cell survival will ensue. Possibly, unreplicated phage DNA is eliminated by the cell. The behavior of the B/r mutant 10 can then be understood as being due to a slight reduction in the rate of repair, so that the chance of bacterial survival after infection with irradiated T1 is increased, but the reduction is not enough to affect the survival of the cell itself after irradiation. In the case of the other her- mutants of B/r, the reduced rate of repair would be enough to affect both situations.

The reverse finding, that the ultraviolet resistance of the her- mutant of B could be increased by another mutational change without a concomitant increase in ability to reproduce irradiated T1, can be explained in similar fashion. In this case, the rate of repair would be sufficiently increased to increase bacterial resistance, but would still be too small to allow damaged T1 to succeed in the competition with the replication of undamaged bacterial DNA.

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**Literature Cited**


