Division Delay Induced in Escherichia coli by Near-Ultraviolet Radiation

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Beams of near-ultraviolet radiation at several principal emission lines of a mercury arc were isolated with a grating monochromator and directed upon cell suspensions. During subsequent incubation at room temperature in Nutrient Broth, the population was studied by removing samples and obtaining cell numbers and cell size distributions with an electronic cell counter. Division delay without lethality was observed. The shapes of the dose-response curves for induction, the doses of near-ultraviolet radiation required, and the action spectrum for division delay were found to be similar to those for growth delay (in broth) and for photoprotection. These findings indicate that all three effects, division delay, growth delay, and photoprotection, are induced by a common type of critical event. Changes in cell size distribution in the culture during incubation in Nutrient Broth after near-ultraviolet irradiation are very similar for control and irradiated populations, although these changes occur at a much later time in the irradiated population. This indicates that, in Nutrient Broth, the population recovers completely from the inhibition of growth and division, thus justifying use of the term “delay,” and suggesting that the damage is nongenetic.

Growth and division are two of the most fundamental characteristics of living cells. Consequently, the mode of action of any agent that produces specific effects on growth or division is of general interest in cell biology. This subject has recently become of especial interest in the field of radiation action. Some years ago, Alper and Gillies (2) suggested that the ability of bacteria to recover from lethal effects of treatment with ultraviolet or ionizing radiation was closely associated with their ability to grow afterwards; suboptimal conditions of growth generally led to higher survival. Adler and Hardigree (1) have now been able to form a basis for prediction of conditions that will enhance the ability of bacteria to survive ionizing radiation, given advance knowledge of whether the radiation will affect primarily growth mechanisms or division mechanisms.

How might growth inhibition lead to higher survival? It is now generally agreed that bacteria possess internal enzymatic systems for repair of radiation damage (11, 12, 13), which are especially effective for damage induced by far-ultraviolet (1,900 to 3,000 Å) radiation (“far UV” or “UV”). For UV-induced mutation, these systems often behave as if they operate at a limiting rate, and they are effectively turned off either by deoxyribonucleic acid (DNA) synthesis at the mutated locus (4) or by generalized protein synthesis (14). These repair systems probably operate in the same fashion for UV lethal damage (see, e.g., 5). Consequently, any delay in growth or division, or in both, immediately after far-UV irradiation may imply a concurrent delay in protein or DNA synthesis, or in both, and thus lead to higher survival by permitting more repair of DNA to take place.

It has been observed by Jagger, Wise, and Stafford (10) that irradiation of Escherichia coli B with near-ultraviolet (3,000 to 3,800 Å) radiation (near UV) induces a delay in growth and division after the cells have been placed in nutrient medium. The action spectrum for growth delay of cells placed in nutrient broth after near-UV irradiation (“growth delay [broth]”) was not significantly different from the action spectrum for photoprotection, a phenomenon in which irradiation with near UV partially protects the cells from subsequent killing by far-UV radiation (for review, see 7). On the basis of this and other similarities, they proposed that the essential action of a photoprotection treatment was the in-

1 Based in part on a dissertation submitted by S. L. Phillips in partial fulfillment of requirements for the M.S. degree, The Pennsylvania State University.
duction of a delay in growth or division, or in both. This delay might operate by permitting, after far-UV irradiation, more extensive action of intracellular recovery systems.

The present paper describes the course of near-UV-induced division delay (broth). Cell division was measured with an electronic cell counter. The action spectrum was obtained and found to be similar to those for growth delay (broth) and for photoprotection. Observations were also made on the distribution of cell sizes in the near-UV irradiated population, in an effort to reach a further understanding of the events involved in near-UV induction of growth and division delays.

The only earlier work of which we are aware on near-UV-induced division delay in bacteria is that of Hollaender (6), who showed, among other things, that the effect exists for E. coli in broth and is a direct function of radiation dose. The present paper may be considered an extension of both this work and that of Jagger et al. (10).

**Materials and Methods**

The bacterium used was E. coli strain B, originally obtained from M. Demerec. Cultures were grown overnight at room temperature with aeration in Nutrient Broth (Difco). They were then diluted 1:100 into fresh broth, and cell division was followed by use of a Coulter cell counter, with a 30-μ aperture. The cell counter was used with the gain trim set at 6, the gain switch set at 6, the aperture current set at 4, and the threshold set at 6 (for determination of cell size distribution, where the threshold was raised in 8-unit intervals). For counting, cells were diluted to a concentration of approximately 8 × 10^8 cells/ml in 0.9% NaCl. Doubling time in the logarithmic phase was about 45 min. When the bacteria had reached a concentration of 1.5 × 10^8 to 1.8 × 10^8 cells/ml, they were collected on a membrane filter (0.45-μ pore size; Millipore Corp., Bedford, Mass.), and washed and resuspended at the same concentration in 0.067 M sodium-potassium phosphate buffer (pH 6.8). Such suspensions of logarithmic-phase cells were exposed to the radiation from a monochromator and were stirred with a magnetic flea during irradiation. Control cells were held during the irradiation period in a test tube, with stirring.

After irradiation, suspensions of irradiated or control cells were diluted 1:10 into broth, and their division was followed on a Coulter counter. Frequency distributions of cell size were made for cultures irradiated at 3,341 A, and parallel microscopic observations were made by use of a Unitron phase microscope. Viable plate counts taken during incubation of control and irradiated cell populations were 90 ± 5% of Coulter counts. These were determined by dilution in phosphate buffer and plating on Nutrient Agar (Difco), with incubation at 37 C. Irradiation at 3,130 A caused roughly 10% killing at the highest doses, but other wavelengths were nonlethal at the doses used.

Cell suspensions were irradiated in a rectangular quartz spectrophotometer cuvette (optical depth, 1.0 cm) in the focal plane beyond the exit slit of a Bausch & Lomb grating monochromator, no. 33-86-45-49 (10 × 10 cm grating with 1,200 lines per mm). The monochromator was illuminated by a 500-w high-pressure mercury arc lamp (Philips SP-500) with quartz window. Possible stray far-UV radiation was minimized by placing at the exit slit a polyester film (DuPont Mylar) that is virtually opaque below 3,000 A. Wavelengths used are shown in Table 1. Band widths were 100 A. Dose rates were measured with a circular bismuth-silver thermopile (Eppley no. 5382) connected to a portable galvanometer (Leeds & Northrup, no. 2430-a). The detection system was calibrated with a National Bureau of Standards standard carbon-filament lamp. Dose rates were measured immediately before and after each run.

Table 1 shows average dose rates used in experiments contributing to the action spectrum for division delay. The dose rate at 3,130 A is unusually low because the Mylar filter is partially effective at this wavelength. No effort was made to control dose rate, since (i) photoprotection shows no dependence (7), and growth delay only a slight dependence (10), on dose rate, and (ii) the dose rates used are low compared, for example, to those that produce important saturation effects in photoactivation (9).

All operations, except incubation of plated bacteria, were carried out at room temperature (26 ± 1 C).

**Results**

**Kinetics of cell division**. Figure 1 shows the course of cell division in a population irradiated at 3,341 A. In the controls, exponential division was resumed only after about 1.5 hr of incubation (estimated by extrapolation of final slope to level of original titer). This delay was caused by the holding in phosphate buffer. It reached its full extent after an average of only 20 min in buffer, then remained constant (with no change in curve shape) up to at least 3 hr in buffer. Controls were held for the average irradiation time in a given run. Some of the low-dose points took less than 20 min (compare Fig. 2 and second column, Table 1), and in these runs the controls were generally held longer than 20 min, since several doses were given in a single run (see below). Nevertheless, there is no evidence from the kinetics of division delay (Fig. 2) of unusual behavior at these low doses.

Irradiated cells (Fig. 1) underwent a dose-dependent division delay in addition to the control division delay. Otherwise, kinetics were of the same form as for controls.

The cell-division curves (Fig. 1) for all cultures become exponential or nearly so after a 10-fold increase in cell number. The "division-delay factor" is defined as the ratio t_d/τ0, where τ0 is the time required for a 10-fold increase in the
number of cells in the control culture, and $t_x$ is the time required for a 10-fold increase in the number of cells in a culture irradiated with dose $x$ (see Fig. 1).

Figure 2 shows the reciprocal of the division-delay factor as a function of radiation dose for four wavelengths. Each point represents a complete growth curve. (The experiment of Fig. 1 would therefore provide three points at 3,341 A.) Three to five separate experiments were done at each wavelength. Straight lines are fitted to the points (excluding zero-dose points) by linear regression analysis, giving lines that pass through the origin (within experimental error) for all wavelengths except 3,656 A, for which the intercept is 1.13.

The shapes of the dose-response curves for induction of division delay (broth), represented by the curves of Fig. 2, are not significantly different from those observed by Jagger et al. (10) for growth delay (broth) and those observed by Jagger and Stafford (8) for photoprotection.

From Fig. 2, it can be seen that, at 3,341 A, the dose required for 60% survival of division ability is about $2.37 \times 10^4$ photons mm$^{-2}$, which is 141,000 ergs mm$^{-2}$. This compares with values reported by Jagger et al. (10) for 60% survival of growth ability and of ability to be photoprotected of about 230,000 ergs mm$^{-2}$ and 200,000 ergs mm$^{-2}$, respectively. Recent dosimetry indicates that these values are about 50% too high (because of neglect of internal reflection by the walls of the cuvette) and should be about 150,000 and 130,000 ergs mm$^{-2}$, respectively. Therefore, radiation of wavelength 3,341 A is as effective in producing division delay (broth) as it is in producing growth delay (broth) or photoprotection.

Action spectrum for division delay. The lines in Fig. 2 may be thought of as “survival curves” of division ability, and the negative of the slope of any one line is related to the efficiency of the associated wavelength for production of division delay. We use as the measure of division delay at each wavelength the inverse of the dose (in photons mm$^{-2}$) required to reduce $t_0/t_x$ to 0.50. This corresponds to a “division-delay factor” of 2.0. These numbers, normalized to 100 at 3,341 A, form an action spectrum, and are shown in Table 1, where they are compared with numbers derived in the same manner for growth delay (broth) and for photoprotection.

The definition of the division-delay factor, as illustrated in Fig. 1, is not unique. Therefore, it was of interest to measure division delay by another method. One can extrapolate the line representing exponential growth downward until it intersects a horizontal line representing the initial concentration of cells immediately after irradiation. The times from onset of postirradiation incubation to these intersections may now be called “$t_0$” for the controls and “$t_x$” for the irradiated cells. In this manner, “survival curves” for division delay ($\log$ of $t_0/t_x$ versus dose) were again constructed. The action spectrum determined in this way was not significantly different from that determined by the former method.

It is seen from Table 1 that the action spectrum for division delay is very similar to those for growth delay and photoprotection, showing the same shape and the same peak wavelength. Nevertheless, certain small differences, although sometimes not statistically significant in our experiments, may well reflect real differences. For example, we have never found photoprotection from killing in E. coli B at 4,047 A, although we usually find slight division and growth delays. At 3,130 A, the action for division delay is higher.
than for the other two effects. This difference may be real. Division delay is produced with higher efficiency than growth delay in the far-UV region (3), and photoprotection is not induced by far UV. Therefore, the higher effectiveness for division delay at 3,130 Å may reflect the onset at this wavelength of behavior generally associated with wavelengths below 3,000 Å.

In summary, the data of Fig. 2 and of Table 1 show that, for division delay (broth), the shapes of the dose-response curves for induction, the doses of near UV required, and the action spectrum are very similar, if not identical, to those for growth delay (broth) and photoprotection.

**Cell size distribution**. In two experiments, bacterial size distributions were determined at various incubation times after 3,341 Å irradiation. Doses were used (ca. 180,000 erg mm$^{-2}$) which caused a division-delay factor of approximately 2.0. Data for a control and irradiated sample are shown in Fig. 3. These data, plus microscopic observations, show that changes in frequency distribution during the incubation period are very similar (although they occur at different times) in control and irradiated populations. The frequency distributions for both populations have the following characteristics: (i) at the outset, there is a high proportion of small cells produced by storage in phosphate buffer; (ii) a rapid shift in the maximum then occurs from the first to the second discriminator setting (this distribution is typical during the division-delay period); (iii) a further shift to increased cell size occurs as the cells recover their ability to divide (more marked, only in the experiment shown, in the control than in the irradiated population (compare plots 3 and 4 of control with 16 and 17 of irradiated)); and (iv) distributions shift back to the form of (i) as the cells reach late log phase ($4 \times 10^8$ to $7 \times 10^9$ cells per ml).

At 3,341 Å and longer wavelengths, more than 95% of the irradiated cells survive. It is therefore evident that the cell counter is measuring a division delay (implying subsequent recovery of division ability) among almost all cells in the population, and not just continued rapid division in a small fraction of the population. It is apparent that either some cells of the population escape the division delay or division is not totally blocked, since, even at the highest doses used, the cell number still increases slowly from time-zero (Fig. 1).

**TABLE 1. Action spectra for division delay (broth), growth delay (broth), and photoprotection from killing in Escherichia coli B**

<table>
<thead>
<tr>
<th>Wavelength (Å)</th>
<th>Avg dose rate (ergs mm$^{-2}$ sec$^{-1}$)</th>
<th>Division delay $\delta$</th>
<th>Growth delay $\delta$</th>
<th>Photoprotection $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,130</td>
<td>14</td>
<td>70 $\pm$ 4</td>
<td>100 $\pm$ 8</td>
<td>53</td>
</tr>
<tr>
<td>3,341</td>
<td>85</td>
<td>20.3 $\pm$ .5</td>
<td>14</td>
<td>2.3</td>
</tr>
<tr>
<td>3,656</td>
<td>340</td>
<td>2.1 $\pm$ .2</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>4,047</td>
<td>179</td>
<td>10</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* For these experiments on division delay only.

* Relative efficiency per incident quantum (see text). Errors shown are derived from standard errors of slope and intercept.

* From Table 1, Jagger et al. (10).

* Corrected for killing [from Table 1, Jagger et al. (10)].
FIG. 3. Growth and division behavior in Nutrient Broth at 26 C at various times after irradiation with $1.8 \times 10^6$ ergs mm$^{-2}$ at 3,341 A. (a) Total cell count, determined with Coulter counter, as a function of time. Circles are controls; squares are irradiated cells. (b) Cell size distributions at various times after irradiation, for controls (top row) and irradiated population (bottom row). Each vertical bar shows the percentage of cells counted at one Coulter counter discriminator setting minus the percentage counted at the next lower setting. Constant differences in discriminator setting (8 units) were used. The numbers on the plots indicate the points on the curves of (a) from which the samples were taken.

DISCUSSION

The shapes of the dose-response curves for induction, the doses of near UV required, and the action spectra for near-UV induction are almost identical for division delay (broth), growth delay (broth), and photoprotection. Considering the experimental errors involved in determination of all three of the action spectra, the only significant difference among them (see Table 1) is a slight induction of both division delay and growth delay at 4,047 A, but no photoprotection of E. coli at this wavelength. [However, photoprotection in Pseudomonas aeruginosa does occur at 4,047 A (see 8)].

These findings indicate that all three effects are induced by a common type of critical event. This is probably damage to the respiratory system (see 7). Some of these effects may be the causes of some of the others. For example, Jagger et al. (10) suggested that induction of a growth or division delay, or both, may be causative for photoprotection. It is clear that cells that have undergone the extensive growth delay produced by a typical photoprotection treatment must also experience a delay in (or lowered rate of) both protein and DNA synthesis, either of which might lead to greater repair of UV damage in DNA.

In the present experiments, the changes in cell-length distribution undergone by the irradiated culture are not significantly different from those undergone by the control culture. Consequently, not only the data on kinetics, doses, and action spectra, but also the data on cell size distribution, all contribute to the conclusion that, for cells placed in nutrient broth after near-UV irradiation, growth and division are delayed to the same extent. Furthermore, the fact that, after general resumption of cell division, cell size distribution in the irradiated population is similar to that of the controls indicates that the daughter cells are by this criterion normal in their division pattern. This indicates that, within experimental error, the irradiated population eventually recovers completely from the initial division delay, a finding consistent with the hypothesis that the effects observed here are caused by a nongenetic damage (probably to the respiratory system).

Earlier microscopic observations (10) have shown that cells irradiated by near UV and then plated on Nutrient Agar develop as follows: (i) initially, no growth occurs; (ii) when growth resumes, some cells form very long "filaments" (cells exhibiting growth without division), whose relative numbers greatly exceed those in the control population; and (iii) eventually, many of these filaments divide at some point along their length, producing cells of normal appearance that go on to form colonies. If such extensive filament formation occurred in the present experiments, it would be expected to alter the cell size distribution to a far greater extent than is evident from Fig. 3b. If one makes the usual assumption...
that pulse heights obtained with the Coulter counter reflect cell volume, then the average cell in the populations of Fig. 3 is increasing not more than threefold in length, and there are very few cells that are many times (e.g., 10 times) longer. These interpretations are borne out by microscopic observations, in which the numbers of filaments and the extent of filament formation are seen to be quite low, and are not different from those of controls.

The observations of Jagger et al. (10) not only were made on cells on solid media but were made at 37°C, rather than at 26°C as in the present experiments. The relative contributions of these two factors to the observed differences is not known.

These findings do not permit us to associate either growth delay or division delay more closely than the other with photoprotection. Most photoprotection experiments involve plating on Nutrient Agar very shortly after near-UV treatment. Comparative studies of photoprotection in liquid and on solid media are clearly in order, since the relationships between growth and division differ under the two conditions.

Acknowledgments

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