Evidence that Initial Ultraviolet Lethal Damage in *Escherichia coli* Strain 15 T−A−U− is Independent of Growth Phase

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SUMMARY

A study was made of the kinetics of killing by ultraviolet (u.v.) radiation (2537 Å) and subsequent photo-reactivation (with black light) of *Escherichia coli* strain 15T−A−U− (requires thymine, arginine, uracil) by using (a) logarithmic-phase cultures, (b) early-stationary-phase cultures, (c) logarithmic-phase cultures after 90 min. of incubation in the absence of arginine and uracil (−AU cultures). The stationary and −AU cultures showed the same enhanced resistance to u.v. killing. In all three cultures (1) the photo-reactivable sectors were the same, (2) the rate of photo-reactivation was the same function of u.v. dose, (3) the amount of light required for maximum photo-reactivation was a linear function of the u.v. dose. We conclude that the initial lethal lesions produced by a given u.v. dose were qualitatively and quantitatively the same in all three cultures, despite differences in u.v. survival. This implies that the stationary-phase cultures and the −AU cultures were more radiation resistant solely because they were better able to cope with the initial lethal damage. The survival curves can be satisfactorily described mathematically in terms of this model.

INTRODUCTION

Survival of irradiated logarithmic-phase bacteria can be altered by various metabolic inhibitors. For example, higher survivals may be obtained by (1) treatment with chloramphenicol before X-irradiation (Billen, 1959), (2) growth without a required pyrimidine, or amino acid, or both, before irradiation with ultraviolet (u.v.; Hanawalt, 1961) or X-rays (Billen, 1963), (3) plating on a medium containing chloramphenicol after irradiation with u.v. or X-rays (Alper & Gillies, 1960). In addition stationary-phase populations are often more resistant than logarithmic-phase populations to u.v. radiation (Durham & Wyss, 1956; Gates, 1929; Woodside, Goucher & Kocholaty, 1960) and to ionizing radiation (Stapleton, 1955). However, neither the changes in metabolic state nor the mechanisms by which such changes alter radiation-killing are understood.

We know that nucleic acid components are the principal absorbers of the u.v.
photons that kill *Escherichia coli* (Gates, 1930) and a variety of evidence strongly suggests that the initial lethal damage occurs predominantly and directly in deoxyribonucleic acid (DNA; see, for example, Wacker, 1968). Furthermore, selective inhibition, by u.v. irradiation, of DNA synthesis in *E. coli*, which has long been known (Kelner, 1953), is probably directly involved in bacterial killing (Setlow, Swenson & Carrier, 1968). Therefore it is reasonable to think that radiosensitivity changes produced by various metabolic treatments reflect changes in DNA or RNA synthesis. Polyauxotrophs of the thymine-deficient *E. coli* strain 15T− appear to be good specimens for studying the mechanisms of radiation killing, because (1) nucleic acid syntheses in these mutants can be studied in relation to other specific metabolic processes (see, for example, Maaløe & Hanawalt, 1961; Lark, Repko & Hoffman, 1963), (2) dramatic changes in radiation sensitivity of logarithmic-phase cultures of one poly-mutant, strain 15T−A−U− (requires thymine, arginine, uracil), are observed when protein and ribonucleic acid (RNA) syntheses are inhibited for various times before either u.v. irradiation (Hanawalt, 1961) or X-irradiation (Billen, 1963).

In the present paper we describe kinetics of (1) killing by far-ultraviolet (u.v.; 2537 Å) radiation of *Escherichia coli* strain 15T−A−U− in different metabolic states, and (2) the recovery from u.v. lethal damage after photo-reactivation by near-ultraviolet radiation (near u.v.). Analysis of the data indicates that pre-irradiation inhibition of protein and RNA syntheses in strain 15T−A−U− did not affect the production of initial lethal lesions by u.v. radiation. Consequently, another explanation must be found for the dramatic change in radiation sensitivity produced by this pre-irradiation treatment.

**METHODS**

**Organisms and methods of culture.** *Escherichia coli* strain 15T−A−U− (isolated by Barner & Cohen, 1958) was obtained from Dr R. B. Setlow. Some of its physiological and genetic properties were described by Kanazir *et al.* (1959). Stocks were maintained on refrigerated nutrient agar (Difco) slopes. Test cultures were grown in a liquid minimal medium [Davis mineral salts buffer, Lederberg, 1950, containing glucose 1·0 % (w/v) as energy source; final pH 7·2] supplemented as required with thymine, 5–10 µg./ml.; arginine, 20–100 µg/ml.; or uracil, 10–20 µg./ml. Supplements are indicated as follows: fully supplemented, +T+AU; thymine only, +T−AU; arginine+uracil only, −T+AU. The amount of growth was estimated turbidimetrically at 490 mµ (E490) by using a Photovolt ‘Lumetron’ 401colorimeter. Early-stationary-phase organisms (90–120 min. after the end of exponential increase in extinction) were harvested from an overnight culture (16–18 hr at 37° in a shaker bath). For other growth phases, the overnight culture was diluted and incubated as required in the appropriate pre-warmed growth medium. To change nutrients, organisms were collected and washed on a membrane filter (Millipore, grade HA) and resuspended in the new pre-warmed medium.

The supplement concentrations used were adequate for growth; maximum growth in minimal medium (minimum lag phase; minimum generation time; maximum colony count) was obtained with the following minimum supplement concentrations: thymine, 5 µg./ml.; arginine, 20 µg./ml.; uracil, 10 µg./ml. (Figs. 1, 2). However, experiments with 14C-labelled thymine showed that, at maximum viable titre, the culture grown in thymine 5 µg./ml. had used virtually all the thymine.
U.V. damage in *E. coli* 157−A−U−

in the medium; the thymine was not exhausted when a concentration of 10 μg./ml. was used.

It was also noted that thymine at 2 μg./ml. (used by a number of workers for growth of the 157− mutants) permitted maximum exponential growth rate, but produced a slightly longer lag phase (Fig. 1) and did not give maximum viable titre, because thymineless death occurred (Fig. 2) when thymine was exhausted from the medium before the end of the logarithmic growth phase.

![Fig. 1](image1)
![Fig. 2](image2)

**Fig. 1.** Effect of thymine concentration on aerated growth of early-stationary-phase *Escherichia coli* strain 157−A−U− diluted into minimal medium (arginine: 20 μg./ml.; uracil: 10 μg./ml.; thymine: □ = 10 μg./ml., ● = 2 μg./ml., ○ = 1 μg./ml., △ = 0.5 μg./ml., ▲ = 0.1 μg./ml.).

**Fig. 2.** Effect of thymine concentration on maximum viable titre attained by aerated growth of early-stationary-phase *Escherichia coli* strain 157−A−U− diluted into minimal medium (arginine: 20 or 100 μg./ml.; uracil: 10 or 50 μg./ml.; thymine: □ = 5 or 10 μg./ml., ● = 2 μg./ml.).

The viability of bacterial cultures was assayed by spreading measured volumes (usually 0.05 or 0.1 ml.) of diluted suspensions on nutrient agar plates, and counting the colonies after incubation for 16–24 hr in a dark humidified 37° cabinet.

**Ultraviolet irradiation.** For u.v. irradiation, bacteria were collected on a membrane filter, washed with several volumes of Davis mineral salts buffer, and re-suspended and diluted in this buffer. One-ml. portions of the suspension (less than 10⁶ bacteria/ml.) were irradiated at room temperature (22–23°) in the depressions...
of a glazed ceramic spot-plate. To prevent unintentional effects by short-wavelength visible light, all u.v. irradiation experiments were done in a light-sealed laboratory illuminated by yellow fluorescent lamps (Westinghouse, F-40G0, 40 W., gold).

The u.v. source (2537 Å) was two General Electric 15 W. low-pressure mercury-vapour germicidal lamps (G15T8) in a desk-type white-enamelled reflector. Only the central 15 cm. length spans of the lamps were used. A small low-velocity exhaust fan was used to dissipate possible traces of ozone and to maintain constant temperature of the lamps.

The near-u.v. source used in photo-reactivation experiments was the central 14 cm. length spans of two General Electric 15 W. low-pressure mercury black-light lamps (F15T8-BLB; smooth spectrum 3100–4400 Å, peak at 3530 Å) in a desk-type white-enamelled reflector. Unirradiated bacterial controls exposed to the photo-reactivation source under the same conditions as for irradiated samples showed no loss of viability.

Incident dose rates were measured with a home-made meter (described by Jagger, 1961), and found to be 9 ergs mm⁻² sec⁻¹ at 60 cm. from the u.v. source and 94 ergs mm⁻² sec⁻¹ at 10 cm. from the photo-reactivation source.

RESULTS

Survival after ultraviolet irradiation

Fig. 3 shows u.v. survival data from thirteen experiments (three doses per experiment) for: (1) logarithmic-phase cultures; (2) early-stationary-phase cultures; (3) logarithmic-phase cultures grown for 90 min. in +T – AU medium (hereafter referred to as ‘–AU cultures’). The curves shown in Fig. 3 are plots of an equation whose form and derivation are presented in the Appendix to this paper. For the present, it is only necessary to note that the equation involves the following three constants and no other constants:

α is the negative of the slope at high u.v. doses. Regression lines drawn to the experimental data (which include about twice as many points as are shown in Fig. 3) give the value $\alpha = 0.0199 \pm 0.0076$ for the logarithmic-phase data, and $0.0196 \pm 0.0087$ for the early-stationary-phase and – AU data combined. The final slopes are therefore identical within experimental error.

$M + 1$ is the ordinate intercept of the final slope. It has the value 5 for the logarithmic data and the value 273 for the early-stationary and – AU data combined.

$\tau$ describes the rate at which the curves at low doses approach the final slope. It has the value $\tau = 0.0183 \pm 0.0013$ for the logarithmic data and $0.0173 \pm 0.0003$ for the early-stationary and – AU data combined. Therefore, these portions of the curves are similar (in the geometric sense) for all three cultures.

The fact that two of the three constants needed to draw these curves were invariant for logarithmic, early-stationary, and – AU cultures suggests that the three cultures were identical in some aspects of their photo-sensitivity. The derivation of the survival curve is based upon $\alpha$ being a function only of initial lethal damage, while $M + 1$ and $\tau$ are related to repair events. The constancy of $\alpha$ therefore suggests that the initial lethal lesions were the same in all three cultures, while the higher extrapolation number ($M + 1$) of the early-stationary and – AU curves is consistent with greater repair occurring in these cultures.
Photo-reactivable sector

The results of a typical series of photo-reactivation experiments are shown in Fig. 4. Maximum photo-reactivation after each u.v. dose was taken as the survival corresponding to the highest point on the respective photo-reactivation curves (arrows in Fig. 4). Similar determinations were made for each of the three cultures.

Survival after maximum photo-reactivation (light survival) was plotted against the u.v. dose for each culture. The results of thirteen experiments with a logarithmic-phase culture are shown in Fig. 5. For -AU and early-stationary-phase cultures, the u.v. and light survivals were the same and therefore data were combined. Results of eighteen experiments are shown in Fig. 6.
The fraction of the u.v. dose which is apparently eliminated by photo-reactivation is called the photo-reactivable sector, a concept introduced by Dulbecco (1950). The numerical value of the photo-reactivable sector is

\[ 1 - \frac{\text{u.v. dose at a given survival}}{\text{u.v. dose giving same survival after maximum photo-reactivation}}. \]

The applications and significance of this concept were reviewed by Jagger (1958).

The photo-reactivable sector for each culture was calculated from the u.v. survival curves (see Fig. 3), and tested for regression on dose. There was no regression. Consequently, the respective photo-reactivable sectors are constant at all doses. Table 1 shows the photo-reactivable sectors determined in forty-six experiments. Sectors were estimated from the data pertaining to each culture, as well as from lumping the stationary and \(-\)AU data (subtotal) and from lumping all the data (total). The photo-reactivation data were analysed for variance within and among the photo-reactivable sectors of the various cultures using the F-statistic method (Snedecor, 1956). The means were not different at the 1% level.
of significance, showing that the photo-reactivable sectors were the same for all three cultures. This means that the spectrum of initial damages, as measured by photo-reactivability, was the same in the sensitive as in the resistant cultures. Thus, the initial lethal lesions were, in at least this respect, qualitatively the same in logarithmic-phase, −AU, and early-stationary-phase cultures.

\[ f(\text{Phtr}) = 1 - \frac{P_x - S}{P_\infty - S} \]

(where \( P_x \) = viable bacteria after dose \( X \) of photo-reactivating light; \( P_\infty \) = viable bacteria after maximum photo-reactivation; \( S \) = viable bacteria after zero dose of photo-reactivating light), is plotted logarithmically against photo-reactivation dose. The negative of the slope of the resulting curve expresses the rate at which photo-reactivable bacteria are being photo-reactivated. Different shapes of the photo-reactivation rate curve are obtained for different U.V. dose ranges (see Fig. 7). This behaviour is not understood. To our knowledge, experiments of this type have not been attempted previously, which reflects the surprising paucity of kinetic data.

**Fig. 7.** Photo-reactivability of ultraviolet-irradiated bacteria of *E. coli* strain 15 \( T^{-A^{-}U^{-}} \) in different growth states. The negative of the slope of the curve at any point describes the rate at which photo-reactivable cells are being photo-reactivated. Each point represents the average value of \( f(\text{Phtr}) \) for a given culture in a given U.V. dose range (numbers on curves, in ergs mm.\(^{-2}\)). Key: \( \bigcirc \) = logarithmic phase \((+T + AU)\); \( \triangle \) = early-stationary-phase \((+T + AU)\); \( \blacksquare \) = −AU (90 min. \(+T - AU\)).

**Fig. 8.** Dose of photo-reactivating light giving maximum photo-reactivation plotted against ultraviolet dose. Calculated average regression line is shown. Key: \( \bigcirc \) = logarithmic phase \((+T + AU)\); \( \triangle \) = early-stationary-phase \((+T + AU)\); \( \blacksquare \) = −AU (90 min. \(+T - AU\)).
on cellular photo-reactivation. For all three cultures, the photo-reactivation rate curves (Fig. 7) were identical for the same u.v. dose. Since photo-reactivation rate is a function of (1) the number of photo-reactivable lesions/cell, and (2) the efficiency of the photo-reactivation system, these data indicate that the amount of photo-reactivable damage might be the same in the sensitive as in the resistant cultures (see Conclusions). The regression of maximum photo-reactivation dose was linear for each culture. The average regression line is shown in Fig. 8, which also shows that, except at very low u.v. doses, about 700 incident photons of photo-reactivating light were required to reverse the damage done by each incident photon of u.v. radiation.

The positive sign of the Y-intercept of the regression line in Fig. 8 (which suggests that photo-reactivation at very low doses requires more light than might be expected) might not be significant. The 95% confidence interval of the Y-intercept, 0.088 ± 0.224 erg mm.⁻² × 10⁻⁶, shows the true intercept could be zero or a small negative number. The probability that the sign of the Y-intercept is positive is borne out by recent experiments of ours (unpublished) which showed that the amount of light required for photo-reactivation in strain b₄₋₁ (Dₛ of 1 erg mm⁻²) was not 1% but rather 20% of that required for photo-reactivation at the same survival value of strain b (Dₛ of about 100 ergs mm⁻²).

Conclusions

The shapes of the u.v. survival curves for logarithmic-phase, -AU, and early-stationary-phase cultures of Escherichia coli strain 15⁻T⁻A⁻U⁻ (Fig. 8) are consistent with the idea that the initial u.v. lesions are alike in all three cultures. The constancy of the photo-reactivable sectors (Figs. 5, 6) show that the initial lethal lesions were, as reflected by photo-reactivability, qualitatively the same at all u.v. doses in all three cultures. That the course of photo-reactivation was the same at any u.v. dose (curve shapes in Fig. 7) adds to the evidence that the initial lesions were qualitatively the same in all three cultures. The photo-reactivation rate was the same function of u.v. dose in all three cultures (Fig. 7), and the number of photons required for maximum photo-reactivation was the same linear function of u.v. dose in all three cultures (Fig. 8).

These findings (that photo-reactivation kinetics in all cultures were a function only of u.v. dose, and were independent of the survival obtained in the various cultures) can be accounted for only in two ways: (1) the photo-reactivation system was equally efficient in all cultures and the amount of photo-reactivable damage was the same in all three cultures; or (2) the photo-reactivation system had different efficiencies in the three cultures, but this was exactly matched by inverse differences in the amount of u.v. damage. With respect to the second possibility, one would hardly expect the numbers of lesions produced and the photo-reactivation efficiencies to differ greatly, if, as suggested above, the lesions were qualitatively the same in all three cultures. Furthermore, such an exact balance between number of lesions produced and photo-reactivation efficiency seems extremely unlikely. We therefore reject the second possibility on the grounds of a priori improbability, and conclude that the efficiency of photo-reactivation was the same in all three cultures. This would mean that the data of Figs. 7 and 8 reflect the production of a constant number of photo-reactivable lethal lesions for a given u.v. dose in all three cultures.
Since the photo-reactivable sectors were the same in all three cultures, it follows that, if the number of photo-reactivable lesions were constant for a given u.v. dose, then the number of non-photo-reactivable lesions was also constant. In other words, in all three cultures the initial u.v. lesions appeared to be quantitatively identical, and the photo-reactivable lesions quantitatively and qualitatively identical. It seems likely, therefore, that the initial u.v. lethal damage was the same in all three cultures. The data also showed that \(-\text{AU}\) and early-stationary-phase cultures were photo-biologically indistinguishable. This is discussed in the following paper (Ginsberg & Jagger, 1965), where it is concluded that the \(-\text{AU}\) culture is, in fact, an early-stationary-phase culture.

**DISCUSSION**

The greater radiation resistance of the stationary-phase cultures might be related to the presence of 'spare' genetic material, since inactivation of cells having 'extra copies' of a gene might require extra hits. Stapleton (1955), for example, suggested that the higher resistance of *Escherichia coli* B/r to X- and gamma-rays at different phases of the growth cycle might be correlated with the number of nuclear bodies/cell. Billen (1959) observed that the X-ray resistance of logarithmic-phase *E. coli* 15T\(^-\) was increased when the organisms were incubated with chloramphenicol (CAP), before irradiation under which conditions RNA and DNA syntheses continued while protein synthesis was inhibited. He found that the X-ray resistance did not change when thymine was withheld during the pre-treatment with CAP. Under these conditions, RNA synthesis continued but DNA and protein syntheses were inhibited. Billen concluded that the surplus DNA was probably responsible for the enhanced resistance during CAP treatment, and he cited data of other workers that are in agreement with this interpretation.

On the other hand, Gillies & Alper (1960) reported that in strains B or B/r of *Escherichia coli*, the DNA content at stationary-phase (where both strains are usually more resistant) was lower than in logarithmic phase.

Our own control data for *Escherichia coli* strain 15T\(^-\)A\(^-\)U\(^-\) showed that the amount of DNA per total number of bacteria was not significantly different in logarithmic-phase and stationary-phase cultures, and that if any difference did exist, there was slightly less DNA/organism in the stationary-phase cultures (Ginsberg, 1968; and unpublished data). Finally, differences in multi-cellularity and multi-nuclearity observed among cultures of *E. coli* strain 15T\(^-\)A\(^-\)U\(^-\) in different growth phases did not correlate with radiation sensitivity (Ginsberg, 1968). Consequently, we think it unlikely that the difference in radiation survival between cultures of the same strain irradiated in different growth states can be accounted for on the basis of differences in amounts of genetic material. A great deal of evidence (Boyce & Howard-Flanders, 1964; Harm, 1963; Setlow & Carrier, 1964) showed that metabolic repair of u.v. lethal damage can occur in bacteria. This suggests that differences in radiation survival of cultures in different growth phases or metabolic states might reflect primarily differences in the ability of the cells to cope with the initial damage, rather than differences in the damage itself. Our data indicate that, in at least one system, the initial u.v. lethal damage does not differ at all, and that, consequently, differences in survival reflect solely the ability of the cells to cope with this damage.
Setlow et al. (1963) reported that equal numbers of thymine dimers were initially produced by the same u.v. dose in two strains of *Escherichia coli* (B/r and B*-J) which differed in their sensitivity to killing by a factor of several hundred. Though a different comparison (different strains) from ours (different states of one strain), their results and ours together support a possible general conclusion, namely, that most differences in radiation sensitivity of bacteria reflect differences in ability to cope with the initial lethal damage rather than differences in the damage itself.

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APPENDIX

*Derivation.* We here derive an expression that describes the survival curves obtained in this study (see Fig. 3). When these curves are plotted as the logarithm of surviving fraction against u.v. dose, they have the following characteristics: (a) at low doses a constant negative slope, (b) at high doses a constant negative slope steeper than the initial slope, (c) in different cultures different initial slopes but the same final slopes. We assume that (1) a culture that experienced no intra-cellular recovery from lethal lesions would show a single-hit survival curve, (2) all deviations from this simple curve must then reflect recovery events, but (3) these recovery events rapidly diminish in importance at high doses, so that the final slopes of all the curves are the same and reflect the initial sensitivity (without recovery) of individual targets in the cell.

The surviving fraction, *S*, after dose *D* can be represented as the sum of (a) the fraction of the total population that has suffered no lethal damage (*e^−aD*), and (b) the fraction of the total population that has suffered lethal damage (*1−e^−aD*) but has a probability, *P*, of subsequent recovery. That is,

\[ S = e^{-aD} + P(1 - e^{-aD}). \]  

It would be desirable at this point to break down fraction (b) of this population into component subpopulations, each of which sustained a given number of hits and which has a fixed probability of recovery. However, we may avoid the mathematical complexities of such analysis by following the empirical approach outlined below.

Since *e^−aD* is the fraction of the total population that has escaped initial lethal damage, *e^−aD|S* is the fraction of the surviving population that has escaped initial damage. Therefore, the fraction of the surviving population that has sustained initial damage (but subsequently recovered) is *1−(e^−aD)/S*. This fraction for any
dose can be determined from the data, since \( \alpha \) is the final slope of the curve (see assumption (8) above). Plotting of this fraction against dose for our data reveals a curve that rises exponentially from zero and approaches a plateau at high doses (the plateau beginning essentially at the point where the slope of the survival curve becomes constant). Expressing this mathematically,

\[
1 - \frac{e^{-\alpha D}}{S} = A(1 - e^{-rD}),
\]

where \( A \) is a constant representing the maximum value of the function, and \( r \) is a constant related to the rate at which the function rises. Solving equation (2) for \( S \) gives

\[
S = \frac{e^{-\alpha D}}{(1 - A) + A e^{-rD}}.
\]

Let \( A/(1 - A) = M \). Then, substituting and rearranging,

\[
S = \frac{(M + 1) e^{-\alpha D}}{1 + Me^{-rD}}.
\]

Equation (3) may now be substituted back in equation (1) to obtain an expression for the probability, \( P \).

In order to get an expression for the slope of the survival curve, we may take the logarithm of equation (3), and then the derivative with respect to dose

\[
\ln S = \ln (M + 1) - \alpha D - \ln (1 + Me^{-rD}),
\]

\[
\frac{d (\ln S)}{dD} = -\alpha + \frac{Mr e^{-rD}}{1 + Me^{-rD}}.
\]

**Discussion.** At high doses, the denominator of equation (3) approaches unity, and \( S \) approaches \((M + 1) e^{-\alpha D}\). Thus, on a semilogarithmic plot, the constant \(-\alpha\) is the slope of the survival curve at high doses, and the constant \(M + 1\) is the Y-intercept of the extrapolated slope. With these two constants thus determined, one can then calculate the value of \( r \) from equation (3).

From equation (4), it can also be seen that, as the dose becomes very high the slope approaches \(-\alpha\). At zero dose, the slope becomes \(-\alpha + Mr/(1 + M)\). Now, if \( M \) is small, the slope at zero dose approaches \(-\alpha\). Thus, for small \( M \), the curve approaches single-hit form (if \( M \) is zero, the curve is single-hit). For large \( M \), the slope at zero dose, \( m_o \), approaches \(-\alpha + r\). Thus, there is a minimum initial negative slope obtainable, regardless of the value of \( M \), and this minimum is determined by \( r \), which can also be seen to represent the difference between the minimum initial slope and the final slope (i.e. \( r = (m_o)_{\text{min}} - (-\alpha)\)).

Equation (3) provides a satisfactory description of the data, both qualitatively (see above) and quantitatively (see text discussion of Fig. 3). The fact that two of the three constants are invariant for the three different cultures studied encourages us to believe that the fit of equation (3) to the data may be more than fortuitous, and that, consequently, it may be possible to arrive at reasonable interpretations of the constants (see below).

Equation (3) takes into account the radiation response of the population at both high and low doses without requiring assumptions of different kinds of initial damage. Also, it isolates mathematically the events involved in (a) induction of initial lesions, and (b) recovery from these lesions. It can readily be extended to the
case where the survival curve in the absence of recovery is of the multi-target type, instead of the single-hit type assumed here.

Haynes (1964) proposed an equation with constants rather similar to those in our equation, but he expresses the survival as a single exponential function, which includes all survivors. His approach is thus basically different from ours, in which the undamaged survivors and the damaged-but-recovered survivors are treated separately, recovery factors being applied only to the latter fraction.

**Interpretation.** The fact that equation (3) is derived in a reasonable way and provides a good description of the experimental data permits us to put a physical interpretation on the constants. The experimental curves bear a resemblance to multi-target curves, in that the final slopes are invariant. In multi-target analysis, the Y-intercept of the extrapolate of the final slope is the number of targets that must be inactivated in order to inactivate the cell. It therefore seems likely that the Y-intercept of the extrapolate of the final slope for our curves \((M+1)\) is related to a critical target number. We suggest that \(M\) represents the average number of lesions that can be repaired in the time available for repair. Roughly speaking, cells that have sustained fewer than \(M\) lesions will probably recover, while those sustaining \(M+1\) or more lesions probably will not recover. The maximum number of lesions that a cell can repair might be the product of \((a)\) rate of repair, and \((b)\) time to a repair-limiting point (e.g. onset of DNA synthesis). We have observed (unpublished data) a delay of about 60 min. in the incorporation of radioactive thymine into stationary-phase *Escherichia coli* strain 15T-A-U- subcultured in liquid medium. In parallel experiments, a logarithmic culture reached normal incorporation rate within 5 min. If the stationary culture \((M = 272)\) repairs 272 lesions/organisms in 60 min., then the repair rate must be about 4·5 lesions/min. Assuming the same repair rate for a logarithmic culture \((M = 4)\), the time to the repair-limiting point would be about a minute, which is consistent with the observed kinetics of resumption of DNA synthesis. Thus, the data are consistent with the interpretation that the difference in the survival curves for logarithmic and stationary cultures reflects, not a difference in the rate of repair, but a difference in the time available for repair.

We assumed at the outset that the constant \(a\) represents the final slopes of the curves. We suppose that, in the region where the curve approaches closely to this final slope, one begins to see the fraction of the population that sustained too many hits to be repaired within the time available for repair. Since a single unrepaired hit remaining in a cell will inactivate it, this fraction of the population dies with single-hit kinetics. That the final slopes are invariant suggests that the target cross-section is invariant with dose or mode of culture.

Equation (2) expresses the experimentally supported concept that ‘the fraction of survivors that sustains initial damage and subsequently recovers’ begins at zero, for zero dose, rises exponentially at low doses, and approaches a plateau at high doses. The constant \(r\) expresses the rate of this exponential rise, and is thus a measure of the degree of curvature of the survival curve as it makes the transition from the initial slope to the final slope. It can therefore be considered a measure of the rate of repair processes. That this constant is the same for logarithmic and stationary cultures in our system suggests that repair machinery is the same in both cultures, further supporting our suggestion that the essential difference in the response of the two cultures rests simply in the time available for repair.
Finally, we may note (see discussion of equation (4)) that, for a given repair rate ($r$), there appears to be a minimum initial negative slope obtainable, regardless of the value of $M$ (which, in our interpretation, means regardless of the time available for repair). This suggests that some lesions are inherently irreparable by existing intracellular machinery. If this were not the case, then, for cultures that have a long time available for recovery, we would expect the survival curves to be simply of the multi-target type, with zero initial slope. That such survival curves are rarely seen for ultraviolet inactivation suggests that ultraviolet usually produces a significant fraction of damage irreparable by normal cell processes.

REFERENCES


