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DNA SYNTHESIS IN SENSITIVE AND RESISTANT MUTANTS OF *ESCHERICHIA COLI* B AFTER ULTRAVIOLET IRRADIATION

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SUMMARY

The kinetics of growth (cell division) and of DNA synthesis have been measured in *Escherichia coli* strains B_{s-1}, *thy*, B, *thy* and B/r, *thy* after UV irradiation (2537 Å). Using a new protocol for studying DNA-synthesis kinetics it has been shown that DNA synthesis is *not* permanently blocked by UV in the excision-deficient strain *E. coli* B_{s-1}, *thy* and that cell division proceeds without delay and at a normal rate in the survivors. In *E. coli* B/r, *thy*, DNA synthesis is stopped for various periods of time, increasing with the UV dose up to a maximum of about 0.75 generation time. After low doses of UV, synthesis resumes at near-normal rate for about 60 min and then changes to a rate proportional to the dose of UV. At higher doses, synthesis resumes at a rate proportional to the dose. Cell division is blocked in *E. coli* B/r, *thy* for about 120 min and then resumes at nearly the normal rate. The growth and DNA-synthesis kinetic data for *E. coli* B, *thy* are not as easy to interpret (because of ever-changing rates) as those for *E. coli* B_{s-1}, *thy* and B/r, *thy*, but are intermediate between these two extremes, consistent with its intermediate UV sensitivity. All 3 strains show a rapid increase in viability after UV irradiation during the first 30–60 min of growth in minimal medium as compared to zero-time plating on minimal-medium agar plates. This increase corresponds to a dose-reduction factor of 33% for *E. coli* B_{s-1}, *thy* and 69% for *E. coli* B/r, *thy*.

INTRODUCTION

UV radiation produces lesions in DNA and consequently kills bacteria (for recent reviews see refs. 5, 7, 11, 19). For a given dose of UV, the same amount of thymine dimers[14] and DNA-protein cross-links[15,18] are formed in both radiation-sensitive and radiation-resistant mutants of *E. coli* B. Since the number of lesions produced in the several strains by a given dose of UV is the same, the differences in sensitivity to killing must reside in the ability of the various strains to repair or circumvent the damage produced by the radiation. The metabolic process that appears to be the most important for the continued growth and division of a bacterium is DNA synthesis. An understanding of the effects of UV upon DNA synthesis should

Abbreviation: CFU, colony-forming units.

be helpful in understanding the mechanisms by which cells are killed by UV. Toward this end, numerous studies have been published on the effect of UV radiation upon the kinetics of the incorporation of radioactive precursors into DNA, RNA and protein[1,4,6,10,14,23].

The radioactive precursors were usually added immediately after irradiation and comparisons were made, for example, between the *apparent* rates of DNA synthesis of the control and irradiated cultures. The counts/min of radioactive precursor incorporated into acid-insoluble material per aliquot of culture were plotted as a linear function *vs.* time of growth after irradiation. With this type of graph the irradiated cultures appeared to show a slower rate of uptake of thymine relative to the unirradiated control, and the conclusion was offered that UV inhibits DNA synthesis and that the time of inhibition is dependent upon the dose of radiation. It has also been reported[23] that UV permanently inhibits DNA synthesis in the non-excising strain *E. coli* B_{s-1}.

We have previously demonstrated[21] that linear plots of counts/min *vs.* time may lead to fallacious conclusions because under these conditions the *apparent* kinetics of DNA synthesis depend upon the number of cells per ml, on the specific activity of the radioactive precursor, and on the DNA content and generation time of the cells, whereas the true kinetics of synthesis depend only upon the DNA content per cell and the generation time.

We have proposed a new protocol for studying macromolecular synthesis kinetics: *(1)* use thymine-requiring cells maintained in the presence of radioactive thymine both before and after irradiation (the incorporated radioactivity then becomes a direct measure of the amount of DNA present and is equivalent to the chemical determination of DNA); *(2)* plot the data as log counts/min *vs.* time (this type of plot eliminates the apparent dependence of synthesis kinetics on the number of cells per ml of culture); and *(3)* determine the kinetics of cell division at the same time that aliquots are taken for the determination of incorporated radioactivity (the present paper demonstrates that different conclusions are possible when the DNA-synthesis kinetic data are evaluated without the data for growth).

Another feature that has not been adequately considered in previous studies on the effect of UV radiation upon DNA-synthesis kinetics is the fact that after irradiation a very heterogeneous population of cells exists. One can postulate the presence of two major classes of cells after irradiation: *(1)* colony formers synthesizing DNA at normal, delayed, or reduced rates; and *(2)* non-colony formers synthesizing DNA at normal, delayed, or reduced rates. The kinetics of DNA synthesis of an irradiated population of cells will, therefore, be some combination of the kinetics of the various populations of cells present after irradiation. This stresses the fact that knowledge of the growth characteristics of the irradiated cultures is absolutely necessary if the chemical data for the incorporation of precursors into DNA is to be properly understood.

The purpose of this paper is to reinvestigate the effect of UV radiation on DNA synthesis and growth in *E. coli* B_{s-1}, *thy*, B, *thy* and B/r, *thy* strains which differ in sensitivity to killing (when plated on minimal-agar plates) by as much as a factor of 75. The present data were gathered and plotted so that the kinetics of DNA synthesis may be directly compared with the kinetics of growth. Pyrimidine dimers do *not* permanently inhibit DNA synthesis in the sensitive strain *E. coli* B_{s-1},

thy. DNA synthesis in the resistant strain *E. coli* B/r, *thy* is *not* delayed longer than one generation time although cell division may be delayed for up to about 3 generation times.

MATERIALS AND METHODS

Escherichia coli strains B_{s-1}, *thy* and B/r, *thy* were obtained from Dr. D. FREIFELDER (Brandeis University) and strain B, *thy* (W-4516) was obtained from Dr. E. LEDERBERG (Stanford University). Cells were grown in a shaking water bath at 37° on a salts–glucose medium[8] supplemented with [2-^{14}C]thymine (2 μg/ml; 0.167 μC/μg, Calbiochem.). When they had entered log-phase growth and reached a density of about $8 \cdot 10^7$ cells/ml, strains B_{s-1}, *thy* and B/r, *thy* were harvested by centrifugation, suspended at the appropriate cell density (cited in the illustrations) in minimal medium *minus* glucose and thymine and irradiated at 2537 Å as previously described[20]. *E. coli* strain B, *thy* was diluted to the appropriate cell density before irradiation with medium *minus* glucose and thymine. The irradiated cells were then returned to a flask at 37° containing glucose and [2-^{14}C]thymine. Aliquots of this culture, in a Gyrotory water bath (New Brunswick Scientific Co.) at 37°, were taken at various times for the determination of viable counts and for the measurement of [2-^{14}C]thymine incorporation.

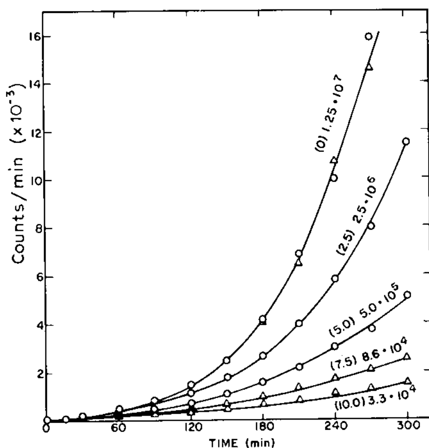
For determining the number of CFU present, aliquots were spread on agar plates made with the same medium used for the incorporation studies except for the substitution of non-radioactive thymine. The aliquots taken for measurement of radioactivity were diluted 1:2 with 4% formaldehyde to stop metabolism. Aliquots (0.1 ml) of the formaldehyde-killed cells were placed on 2.3-cm Whatman 3MM discs and dried under heat lamps. The discs were then washed 3 times in bulk in 5% trichloroacetic acid and 2 times in water and once in ethanol and dried. Their radioactivity content was determined in a liquid scintillation counter as previously described[16].

RESULTS

(a) *E. coli* B_{s-1}, *thy*

DNA-synthesis kinetics. Data for the uptake of [^{14}C]thymine by prelabeled cultures of *E. coli* B_{s-1}, *thy* are shown in Fig. 1 as a linear plot (counts/min at time zero set to zero) and in Fig. 2 as a log plot *versus* time after different doses of UV radiation (2537 Å). We have previously discussed the difficulty of arriving at conclusions from kinetic data plotted as linear functions of counts/min *vs.* time since the slopes are dependent in part upon the cell number[21]. Fig. 1 is included here for comparison with Fig. 2 as further illustration of these difficulties. The slopes of the curves in Fig. 2 are not dependent upon cell number and can be directly compared with the kinetics for growth shown in Fig. 3.

Even in the heavily-irradiated cultures (10 erg/mm^2; surviving fraction $2.6 \cdot 10^{-3}$), DNA synthesis proceeds at nearly the normal rate for about the first 15 min after irradiation (Fig. 2). The rate then progressively decreases until about 120 min after irradiation, at which time a new linear rate is established which is maintained for at least the next 3 h. The control curve begins to change slope after 4 h due to



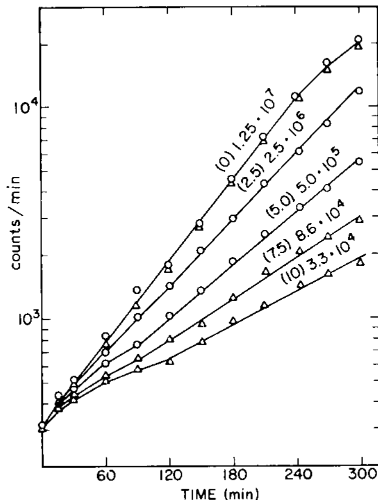


Fig. 1. The uptake of [^{14}C]thymine by cultures of *E. coli* B_{s-1}, *thy* after various doses of UV (2537 Å). The experimental procedures are described in the text. The exposure dose of UV in erg/mm^2 is shown in parentheses for each curve. The numbers following each set of parentheses indicate the number of CFU present at time zero after irradiation when plated on minimal-medium agar plates. The different symbols indicate different experiments. The data represent the radioactivity present in the cells from 0.05 ml of culture.

Fig. 2. The uptake of [^{14}C]thymine by prelabeled cultures of *E. coli* B_{s-1}, *thy* after various doses of UV (2537 Å). These data are taken from the experiment shown in Fig. 1 (linear plot) but are presented here in the more appropriate semi-log plot.

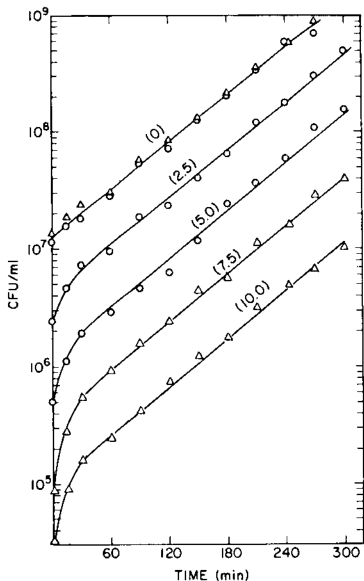


Fig. 3. The growth rate of control and irradiated cultures of *E. coli* B_{s-1}, *thy*. At the time aliquots were taken for the determination of [^{14}C]thymine incorporation (Fig. 2), aliquots were taken for plating on minimal-medium agar plates. These latter results are plotted here as CFU/ml *vs.* time after irradiation. The different symbols indicate different experiments.

saturation of the culture. UV radiation obviously does not permanently stop DNA synthesis in *E. coli* B_{s-1}, *thy*. Within experimental error the final slopes of the incorporation curves in Fig. 2 all extrapolate back to the initial point at time zero. This implies that the total population of cells, whether capable of forming colonies or not, are synthesizing DNA. We will evaluate the slopes of these lines below, however, as a combination of two slopes, one positive (normal rate of synthesis) and one negative (contribution of dead cells for various periods of time).

Growth kinetics (cell division). The data for the kinetics of growth during the time the samples were taken for the incorporation studies are given in Fig. 3. The final growth rates (beyond about the first 30 min) are all parallel with the control, regardless of the dose of UV. During the first 30 min, there is a rapid increase in viable counts of the irradiated cultures. The amount of this increase is a function of the dose of UV. A survival curve deduced from the extrapolation of the final slopes of the individual-growth curves back to time zero gives a line whose slope is 1.33 compared to the slope for the survival curve determined by plating for viable counts at time zero. Thus, if the cells are not immediately plated but are maintained in growth medium, they show an increase in viability compared to zero-time plating. This difference in viability is not a consequence of the cells being more fragile to spreading on plates during the first 30 min after irradiation since the same results were obtained when the aliquots of cells were pipetted onto the agar plates but were not spread with a glass spreader. The use of Oxoid Agar-Agar #3 instead of Difco Agar (both made up in minimal medium) reduced this effect (*i.e.*, a higher survival was observed at time zero) but did not abolish it. Some chemical(s) present in the less pure agar (Difco) must inhibit the recovery exhibited by these cells in minimal medium. This recovery is not observed if the cells are grown and plated on yeast extract. The survival of cells grown in minimal medium is considerably less at time zero if plated on yeast-extract plates as compared to minimal-medium plates. A minimal-medium effect on the survival of *E. coli* B_{s-1} has been briefly mentioned by WITKIN[24]. Minimal-media recovery in *E. coli* K-12 has been correlated with a recovery process independent of excision-repair and dependent upon certain recombination genes[3].

Correlation of growth and DNA-synthesis kinetics. In attempting to correlate the kinetics of growth and DNA synthesis for irradiated *E. coli* B_{s-1}, *thy*, one is faced with the obvious dilemma that the growth and DNA synthesis of the irradiated cultures cannot continue at their divergent rates without the ultimate death of the cultures (compare Figs. 2 and 3). At some time beyond 5 h, the growth curves for the irradiated cells must either decrease to keep in step with DNA synthesis or DNA synthesis must increase to keep pace with growth.

An experiment to investigate these alternatives is shown in Fig. 4. This was essentially a repeat of the experiment described in Fig. 2 except that the DNA-synthesis kinetics were followed for 10 h instead of 5. The culture was diluted 1:20 in fresh medium every 210 min to maintain the culture in exponential growth. The results in Fig. 4 have been corrected for these dilutions. The control curve is a straight line from time zero as expected. The line for the irradiated culture (1.5 erg/mm^2; surviving fraction 0.55) curves from time zero until about 460 min of growth, at which time the curve straightens out and assumes the same linear kinetics as the control culture. If one extrapolates this line (parallel with the control curve) back

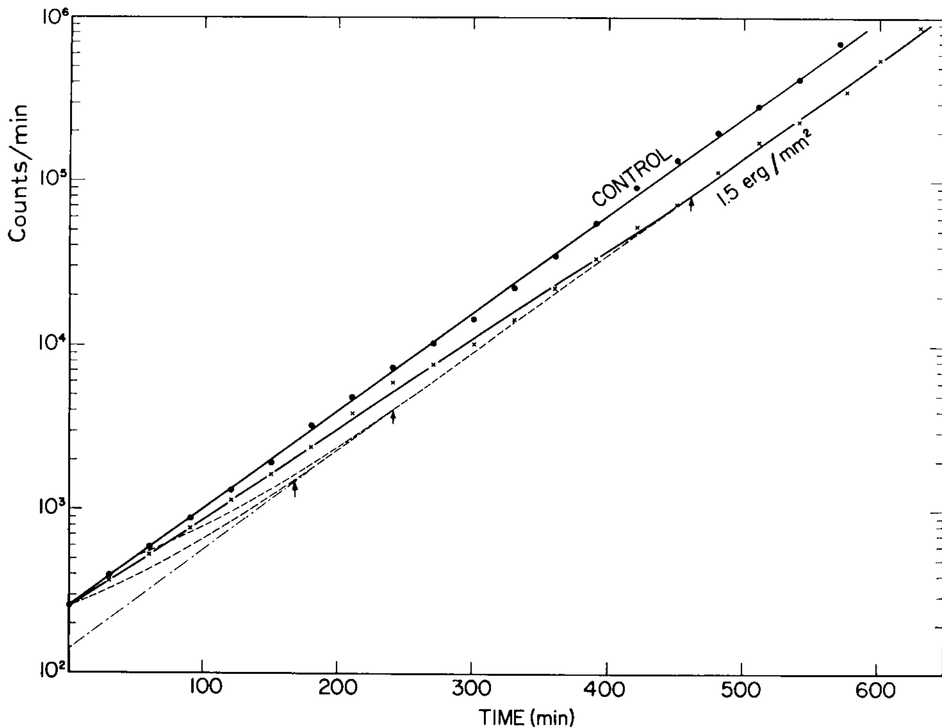


Fig. 4. The uptake of [^{14}C]thymine by control and irradiated (1.5 erg/mm^2) cultures of *E. coli* B_{s-1}, *thy*. This is a repeat of the experiment in Fig. 2 but run for 600 min. The cultures were diluted 1:20 in fresh medium every 210 min. The results have been corrected for these dilutions. The arrows indicate the time when the several curves become parallel to the control curve. The dashed line starting at time zero from the same point as the control represents the incorporation kinetics expected if the non-colony forming cells synthesized no DNA. The dashed line above represents the synthesis kinetics expected if all cells synthesized normally for one generation and then the non-colony forming cells stopped synthesizing DNA. The experimental results fall outside both these lines, indicating neither model is satisfactory. See text for further discussion.

TABLE I

CORRELATION OF VIABLE COUNTS DETERMINED BY PLATING AND BY SPECIFIC ACTIVITY OF [^{14}C]-THYMINE LABELED CELLS

	Control	*1.5 erg/mm²*
Viable cells at time zero (T_0)	$1.2 \cdot 10^7$ cells/ml	$6.6 \cdot 10^6$ cells/ml
Counts/min/ml washed cells at T_0	$5.2 \cdot 10^3$ counts/min/ml (259 counts/min/0.05 ml)[a]	--
Specific activity of cells	$4.3 \cdot 10^{-4}$ counts/min/cell	—
Counts/min for irradiated culture extrapolated to T_0	--	$1.5 \cdot 10^2$ counts/min/0.05 ml[a] ($3.0 \cdot 10^3$ counts/min/ml)
Number of cells equivalent to extrapolated counts/min at T_0	--	$7.0 \cdot 10^6$ cells/ml

[a] Data taken from Fig. 4.

to time zero, it should give the number of counts equal to the number of surviving cells. As seen in Table I, there is a close agreement between the viable counts determined by direct plating ($6.6 \cdot 10^6$ cells/ml) and those deduced by extrapolating the [^{14}C]thymine incorporation data ($7.0 \cdot 10^6$ cells/ml).

The increase in rate of DNA synthesis (at about 460 min) to equal that of the control is not interpreted as a sudden change in kinetics, but rather as an expression that the contribution to synthesis by the non-colony forming cells has finally been diluted out by the contribution of the viable cells. The difference in incorporation between the extrapolated line and the experimental points is due to residual synthesis in the non-colony forming cells.

The dashed line starting at time zero from the same point as the control represents the incorporation kinetics expected if the non-colony forming cells synthesized no DNA. The dashed line above this represents the synthesis kinetics expected if all cells synthesized normally for one generation and then non-colony forming cells stopped synthesizing DNA. The experimental results fall outside both these lines, indicating that neither model is satisfactory. These two lines constitute the extreme cases of a previous model offered to explain the DNA-synthesis kinetics in UV-irradiated *E. coli* B_{s-1} (ref. 23). Since the experimental results do not agree with the prediction of either of these models, it suggests that DNA synthesis continues for many "generation times" in the non-colony forming cells.

The survival curve for *E. coli* B_{s-1}, *thy* is compared with those for strains B, *thy* and B/r, *thy* in Fig. 5.

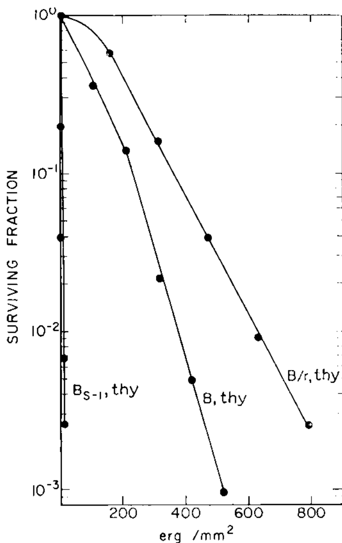
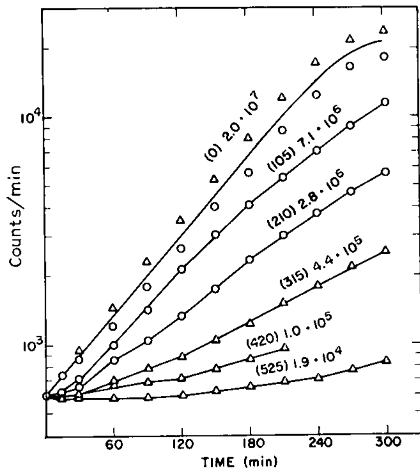


Fig. 5. Survival curves for UV-irradiated (2537 Å) *E. coli* B_{s-1}, *thy*, B, *thy* and B/r, *thy*, when grown in minimal medium and plated on minimal-medium agar plates. Data are from the zero-time plating results shown in Figs. 3, 7 and 9.

(b) E. coli B, thy

E. coli B, *thy* does not show an abrupt cessation of DNA synthesis after UV irradiation (Fig. 6) as does *E. coli* B/r, *thy*, nor does it continue at nearly the normal rate at early times as does *E. coli* B_{s-1}, *thy*, but rather shows a response intermediate between these two extremes. After 105 erg/mm^2 (surviving fraction: 0.35) there is a 30-min period of reduced synthesis and then a normal rate of synthesis until 120 min. Then a rate of synthesis less than that for the control is established that persists

to the end of the experiment (300 min). After 210 erg/mm² (surviving fraction: 0.14) the culture shows a marked decrease in DNA-synthesis rate (but not a cessation) for 30 min and then increases to a new rate that is less than the normal rate. A similar response is shown after higher doses of UV.



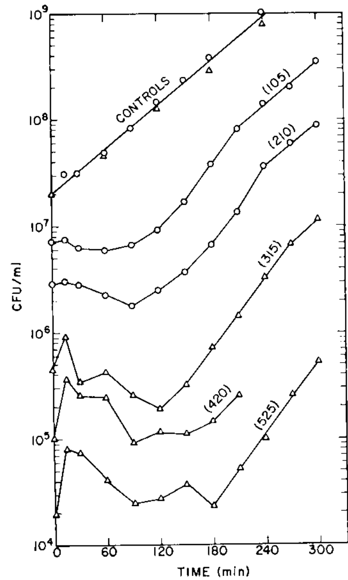


Fig. 6. The uptake of [¹⁴C]thymine by prelabeled cultures of *E. coli* B, *thy* after various doses of UV (2537 Å). The experimental procedures are described in the text. The exposure dose of UV in erg/mm² is shown in parentheses for each curve. The numbers following each set of parentheses indicate the number of CFU present at time zero after irradiation when plated on minimal-medium agar plates. The different symbols indicate different experiments. The data represent the radioactivity present in the cells from 0.05 ml of culture.

Fig. 7. The growth rate of control and irradiated cultures of *E. coli* B, *thy*. At the time aliquots were taken for the determination of [¹⁴C]thymine incorporation (Fig. 6), aliquots were taken for plating on minimal-medium agar plates. These latter results are plotted here as CFU/ml *vs.* time after irradiation. The different symbols indicate different experiments.

After irradiation the number of CFU increased for the first 10–15 min, followed by a general loss in viability, followed by an increase in CFU and finally a return to the control rate of growth (for 105 and 210 erg/mm², resp.; Fig. 7). Growth in minimal medium (compared to immediate plating on minimal-medium agar) appears to favor the recovery of a certain population of cells but kills another population.

(c) E. coli B/r, thy

DNA-synthesis kinetics. The data for the uptake of [¹⁴C]thymine by prelabeled cultures of *E. coli* B/r, *thy* (Fig. 8) clearly indicate that there is a temporary cessation of DNA synthesis after UV irradiation. At doses up to about 315 erg/mm² (12% survival) the time of cessation of DNA synthesis is a function of the dose of radiation. Even after higher doses, however, the cessation of DNA synthesis does not persist for longer than about 30 min (0.75 generation time).

After doses up to about 315 erg/mm², DNA synthesis resumes at essentially the normal rate for about 60 min and then changes to a new rate that appears

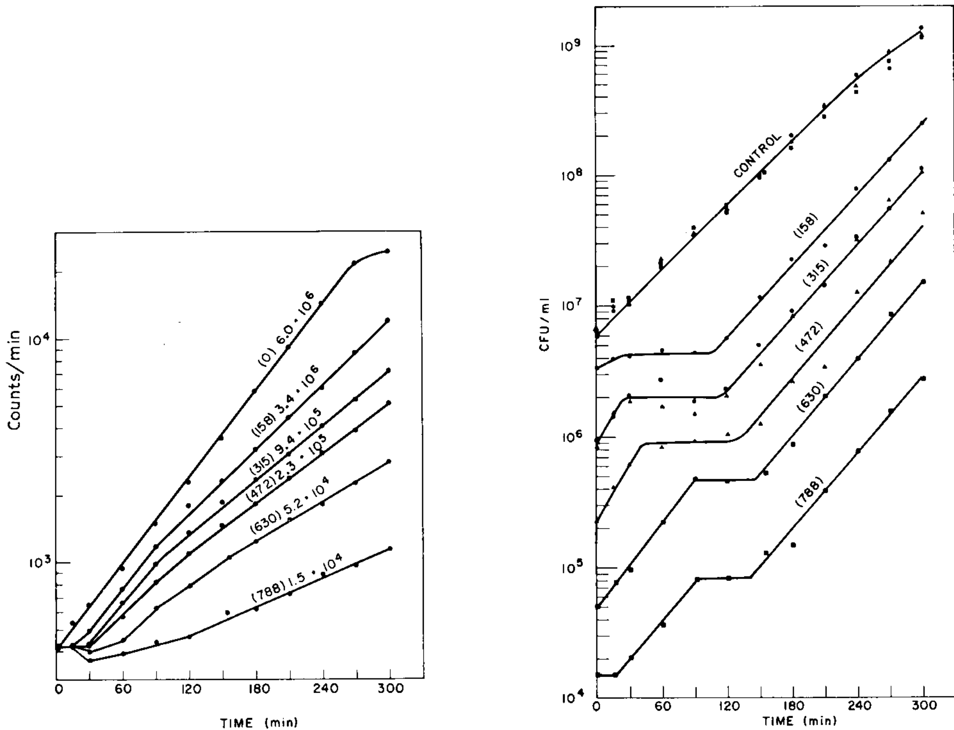


Fig. 8. The uptake of [^{14}C]thymine by prelabeled cultures of *E. coli* B/r, *thy* after various doses of UV (2537 Å). The experimental procedures are described in the text. The exposure dose of UV in erg/mm² is shown in parentheses for each curve. The numbers following each set of parentheses indicate the number of CFU present at time zero after irradiation when plated on minimal-medium agar plates. The results are from 3 experiments. The control and 315-erg/mm² values have been averaged. The separate experiments are delineated in Fig. 9. The data represent the radioactivity present in the cells from 0.05 ml of culture.

Fig. 9. The growth rate of control and irradiated cultures of *E. coli* B/r, *thy*. At the time aliquots were taken for the determination of [^{14}C]thymine incorporation (Fig. 8), aliquots were taken for plating on minimal-medium agar plates. These latter results are plotted here as CFU/ml *vs.* time after irradiation. The different symbols indicate different experiments.

proportional to the dose of UV. The final slopes extrapolate back to the zero-time point. This extrapolation to the zero-time point implies that the total population of cells, whether capable of forming colonies or not, is synthesizing DNA during this time. At doses higher than about 630 erg/mm² (surviving fraction: $9.0 \cdot 10^{-3}$) the amount of degradation of DNA becomes significant. After 788 erg/mm² (surviving fraction: $2.6 \cdot 10^{-3}$) about 10% of the DNA becomes acid-soluble before synthesis resumes.

Growth kinetics. The data for the growth kinetics of UV-irradiated *E. coli* B/r, *thy* are given in Fig. 9. During the first 30–60 min after irradiation there is a large increase in the number of CFU. It seems unlikely that this is due to growth since the doubling time for this increase after 472 erg/mm² is 21 min compared to 36 min for the growth of the control. Furthermore, a survival curve generated from the number of CFU present during the subsequent lag in growth is a linear function of dose (on a semi-log plot). The ratio of the D_{37} value for this curve to that for the

usual survival curve is 1.69, or a dose-reduction factor of 69%. This response would seem most likely to reflect some type of rapid-recovery phenomenon.

After this "recovery" phase, all the cells enter a period of division delay which persists for about 120 min or a little over 3 generation times. Cell division then resumes at a rate only slightly faster than the control. Before cell division resumes, the irradiated cultures have increased their DNA content by 3.4, 3.1, 2.8 and 2.3-fold for 158, 315, 472 and 630 erg/mm², resp.

Although the cell-division kinetics appear normal beyond 120 min after irradiation, the DNA-synthesis rates for the irradiated population are much less than the normal rate. We have previously discussed that irradiated cells cannot synthesize DNA at less than the normal rate and still divide at the normal rate. As pointed out above for *E. coli* B_{s-1}, *thy*, this apparent difference in rate is deceiving because the growth rate is due only to the viable cells while the radioactivity determinations also include the DNA synthesized by the dead cells. At some time beyond 300 min after UV irradiation, the DNA-synthesis kinetics of *E. coli* B/r, *thy* must resume at the normal rate. The time at which this occurs should be UV-dose dependent and will occur when the synthesis due to the viable cells dilutes out the contribution to synthesis by the non-colony forming cells (as shown in Fig. 4 for *E. coli* B_{s-1}, *thy*).

DISCUSSION

(a) *E. coli* B_{s-1}, *thy*

After UV irradiation *E. coli* B_{s-1}, *thy* continues to synthesize DNA at nearly the normal rate for about 15 min and then the rate is markedly reduced (Fig. 2). For short-term experiments, this could be interpreted as a permanent block to DNA synthesis. However, at about 90 min an increased rate of DNA synthesis is established which is inversely proportional to the dose of UV. DNA synthesis has been shown to continue in the non-colony forming cells for many generation times (Fig. 4). After only 1.5 erg/mm² (55% survival) it takes 460 min before the synthesis of the surviving cells dilutes out the contribution to DNA synthesis by the non-colony forming cells (Fig. 4). In support of the concept that DNA synthesis continues for long times in non-viable cells, we have observed that after UV irradiation some of the *E. coli* B_{s-1}, *thy* cells become elongated and form filaments. These filaments are roughly estimated to be about 10 times the length of the control cells, many times shorter than the filaments seen even in unirradiated cultures of *E. coli* B, *thy*.

During the first 15–30 min after irradiation, cells of *E. coli* B_{s-1}, *thy* show a rapid increase in viability equivalent to a dose-reduction factor of 33%. Strains B, *thy* and B/r, *thy* also show a qualitatively similar response and we suggest this may represent a unique repair mode. After this initial increase in viability, cells of *E. coli* B_{s-1}, *thy* divide at the control rate. There is no evidence of any division delay in the survivors of *E. coli* B_{s-1}, *thy* in contrast to the results for *E. coli* B, *thy* and B/r, *thy*.

Is a pyrimidine dimer lethal? Using a dose–response curve of 8 determinations for the formation of thymine dimers it has been determined that one cyclobutane-type thymine dimer is produced per erg at 2537 Å per cell of *E. coli* B/r, *thy* (ref. 17). The data cited by RUPP AND HOWARD-FLANDERS[10] suggest that 3 thymine dimers

are produced per erg at 2537 Å; however, this value is based on only one or two dose points by different authors. The results of SETLOW AND CARRIER[13] indicate that the total yield of pyrimidine dimers ($\widehat{TT}$, $\widehat{CC}$, $\widehat{TC}$) is about twice that of thymine dimers alone in *E. coli*. Therefore, our data[17] suggest that *two pyrimidine dimers are formed per erg at 2537 Å*.

If the survival of a cell were directly dependent upon the production of pyrimidine dimers, then, from considerations of Poisson statistics, the surviving fraction after a given dose of UV should equal e^{-n} where n is the number of dimers produced by the dose of radiation. From the data for *E. coli* B_{s-1}, *thy* in Fig. 3, one can calculate that the surviving fraction after 2.5 erg/mm^2 is 0.2 (the extrapolated surviving fraction is 0.33). At this dose 5 pyrimidine dimers should be formed. The value for e^{-5} is 0.007. The actual surviving fraction is thus about 30 times higher than this ($e^{-1.61}$ equals 0.2 and $e^{-1.11}$ equals 0.33). After 5 erg/mm^2 the surviving fraction is 0.04 (the extrapolated surviving fraction is 0.1). The value for e^{-10} is 0.00004 or 10^3 less than the actual surviving fraction ($e^{-3.22}$ equals 0.04 and $e^{-2.3}$ equals 0.1). The D_{37} value for *E. coli* B_{s-1}, *thy* taken from data in Fig. 5 is 1.55 erg/mm^2. This dose would yield 3.1 pyrimidine dimers per cell.

This lack of agreement between the observed and calculated survival of *E. coli* B_{s-1}, *thy* could be explained either by the fact that: *(1)* the number of pyrimidine dimers formed per erg has been overestimated by us by a factor of 3; or *(2)* some pyrimidine dimers are not lethal. We prefer the latter conclusion and substantiate it by the fact that *E. coli* B_{s-1}, *thy*, which is deficient in its ability to excise pyrimidine dimers[12], can survive with as many as 3 dimers per cell (D_{37} dose) and after 10 erg/mm^2 0.26% of the cells survive with an average of 20 dimers per cell.

The delay in DNA synthesis per dimer. By determining the doubling time for DNA synthesis in *E. coli* B_{s-1}, *thy* from the final slopes of the curves in Fig. 2 (48, 56, 72, 93, and 114 min for 0, 2.5, 5, 7.5, and 10 erg/mm^2, resp.) and knowing the yield of dimers per erg, one can calculate that each pyrimidine dimer delays synthesis for 2–3 min. RUPP AND HOWARD-FLANDERS[10] have calculated a delay of 10 sec/dimer in *E. coli* K-12 AB2500 *uvrA 6* (an excision-deficient strain).

(b) E. coli B, thy

E. coli B, *thy* does not show an abrupt cessation of DNA synthesis after UV irradiation as does *E. coli* B/r, *thy*; nor does it continue at nearly the normal rate at early times as does *E. coli* B_{s-1}, *thy*, but rather shows a response intermediate between these two extremes. Because the viability of *E. coli* B, *thy* changed both up and down during the experiments, no unique conclusion can be made about its growth characteristics in minimal medium following UV irradiation. Similarly, the DNA-synthesis kinetics of *E. coli* B, *thy* are difficult to interpret because of ever-changing slopes.

E. coli B, *thy* differs from strain B/r, *thy* by one mutation[24]. *E. coli* B forms filaments and is FIL$^+$ while strain B/r does not form filaments and is FIL$^-$. It is expected that the UV sensitivity conferred by the *fil* genotype is associated with the inhibition of cell division after UV and not with any deficiency in ability to repair pyrimidine dimers or to replicate DNA after UV irradiation (WITKIN[24]). Nevertheless, the present results indicate a marked difference in the DNA-synthesis kinetics between strains B, *thy* and B/r, *thy* (compare data for 315 erg/mm^2 in Figs.

6 and 8) and they reflect the differences observed in UV survival between the two strains. SWENSON AND SETLOW[23] reported little difference in the DNA-synthesis kinetics of strains B and B/r in response to the same dose of UV radiation.

(c) E. coli B/r, thy

DNA synthesis stops abruptly in *E. coli* B/r, *thy* after UV irradiation. At doses up to about 315 erg/mm² (12% survival) the time of cessation is a function of the dose of radiation. Even at higher doses, however, the cessation of DNA synthesis does not persist for longer than about 30 min (0.75 generation time). This is in agreement with the data of DOUDNEY AND YOUNG[2] who used a direct chemical analysis for DNA. These results, however, are inconsistent with the isotope-incorporation data of SWENSON AND SETLOW[23] which suggest that DNA synthesis is inhibited for much longer than a generation time. The possible reasons for this discrepancy have been discussed[21].

By determining the doubling time for DNA synthesis in *E. coli* B/r from the final slopes of the curves in Fig. 8 and assuming a 2.5-min delay per dimer (as found for *E. coli* B_{s-1}, *thy*) we can estimate the number of unrepaired dimers in *E. coli* B/r, *thy* after synthesis resumes. The doubling times are 40, 63, 75, and 83 min for 0, 158, 315 and 472 erg/mm², resp. This suggests that 90 min after 158 erg/mm², *E. coli* B/r, *thy* has 9 dimers unrepaired (307 dimers repaired); after 315 erg/mm², there are 14 dimers unrepaired (616 dimers repaired); and after 472 erg/mm², there are 17 dimers unrepaired (927 dimers repaired).

The data for the growth kinetics of *E. coli* B/r, *thy* reveal a rapid increase in viability during the first 30–60 min after irradiation, equivalent to a dose-reduction factor of 69%. This response would seem to reflect some type of rapid-recovery phenomenon. This recovery phase is followed by a division delay that persists up to about 120 min after irradiation (about 3 generation times). Cell division then resumes at a rate only slightly faster than the control. Before division resumes, the cultures have increased their DNA content by about 3-fold.

The D_{37} for *E. coli* B/r, *thy* is 117 erg/mm² or 234 pyrimidine dimers per cell. The difference in D_{37} between *E. coli* B/r, *thy* and B_{s-1}, *thy* (D_{37} = 1.55 erg/mm²) is about 115 erg/mm² or about 230 cyclobutane-type pyrimidine dimers.

Correlation of survival kinetics with DNA-synthesis kinetics. The final slopes for the DNA-synthesis curves for *E. coli* B_{s-1}, *thy* in Fig. 3 are 1, 1.19, 1.54, 1.96 and 2.44 (ratios of values of x (control = 1) at a fixed value of y) for 0, 2.5, 5, 7.5 and 10 erg/mm², resp. The final slopes for the DNA-synthesis curves for *E. coli* B/r, *thy* in Fig. 10 are 1, 1.28, and 1.54 for 0, 158, and 315 erg/mm², resp. A plot of these values allows one to calculate the relative slopes of the dose–effect curves for slowing down DNA synthesis in *E. coli* B_{s-1}, *thy* and B/r, *thy*. The ratio is 63 which is in reasonable agreement with the value of 75 for the slope ratios for the survival curves for *E. coli* B_{s-1}, *thy* and B/r, *thy* (Fig. 5). Similar data for *E. coli* B, *thy* relative to *E. coli* B_{s-1}, *thy* are 29 for the ratio of DNA-synthesis kinetics (using the data for the final slope after 210 erg/mm²) while the ratio of D_{37} values is 37. There would thus appear to be a close correlation between survival and DNA-synthesis kinetics after UV irradiation.

(d) General comments

A precise evaluation of the effects of UV on bacterial cells has been hindered by the implication that pyrimidine dimers constitute permanent blocks to DNA synthesis in excision-*minus* strains and, until recently, by the fact that all repair has been considered to be equivalent to excision-repair. The present data clearly demonstrate that UV irradiation does not cause a permanent inhibition of DNA synthesis in the non-excising strain, *E. coli* B_{s-1}, *thy*. Furthermore, there is little or no delay in cellular division in the survivors. The fact that dimers do not constitute absolute blocks to DNA synthesis in excision-*minus* strains has also been demonstrated by a physical-chemical method. Using an excision-*minus* strain of *E. coli* K-12, RUPP AND HOWARD-FLANDERS[10] demonstrated with alkaline sucrose gradients that the DNA synthesized for the first 10 min after UV irradiation consists of single-strand pieces smaller than those seen under similar conditions in unirradiated cells. Upon further incubation of the cells, however, the low molecular weight DNA labeled during the 10-min pulse attains essentially the same size distribution as in the control. It has been proposed that a break is left in the daughter strand as replication proceeds past a dimer (in the parent strand) and that these gaps are subsequently repaired (by a mechanism presently unknown).

GANESAN AND SMITH[3] have demonstrated that certain excision-*minus* strains of *E. coli* K-12 show a large recovery in viability after UV irradiation if they are grown in minimal medium instead of complex medium. This recovery in minimal medium is not observed for excision-*minus* strains that are also recombination-deficient. This recovery in viability after UV irradiation depends upon the presence of genes required for genetic recombination. The molecular basis of this recombination mode of recovery is suggested by the observations of RUPP *et al.*[9,10], cited above, and by the fact that certain mutants which are both excision-*minus* and recombination-deficient (*rec*A) do not join together the small pieces of DNA synthesized after UV irradiation[22].

Attempted replication past an excised region (before the hole is patched) is expected to be lethal[4]. Therefore, the excision mode of repair would seem to be favored by a cessation in normal DNA replication. This would allow more time for the lesions to be cut out and the holes patched. Since pyrimidine dimers do not constitute permanent blocks to DNA synthesis, conditions permitting continued DNA replication would favor the recombination mode of recovery. In a strain that is both excision- and recombination-proficient, one might imagine that the excision mode of recovery is the major one operating during a period of UV-induced synthesis inhibition, but that upon resumption of synthesis the recombination mode of recovery would dominate.

Certain of the observations for which we have no ready explanation are listed here in the hope that future information on recovery mechanisms may allow a more meaningful evaluation of DNA-synthesis kinetics following UV irradiation.

(1) Why is there a delay in DNA synthesis and cell division in the resistant strain *E. coli* B/r, *thy* but no delay in the sensitive strain *E. coli* B_{s-1}, *thy*? Is this due to the large (75-fold) difference in doses used to achieve the same viability or is it another phenotypic expression of the genes controlling the excision process?

(2) What is the mechanism for stopping DNA synthesis in UV-irradiated *E.*

coli B/r, *thy* and why does this cessation in synthesis persist only up to a maximum of about one generation time?

(3) After a 57% survival dose of UV radiation, why does *E. coli* B/r, *thy* (after a 15-min cessation of synthesis) synthesize DNA at the control rate for about 60 min before changing to a new rate that appears inversely proportional to the dose of UV (Fig. 8)? It is difficult to imagine how the cells can synthesize at the normal rate in the presence of 316 pyrimidine dimers (if they are not all repaired during the 15-min lag in synthesis). If they can synthesize at the normal rate for 60 min, why do they then change to a slower rate inversely proportional to the UV dose?

(4) Is the rapid increase in viability shown by *E. coli* B_{s-1}, *thy*, B, *thy* and B/r, *thy* during the first 30–60 min after UV a new repair mechanism?

(5) Part of the problem in interpreting the differences in response of *E. coli* B/r, *thy* and B_{s-1}, *thy* to UV-induced modification of growth and DNA-synthesis kinetics is that strain B_{s-1}, *thy* differs from strain B/r, *thy* by 3 mutations[24]. Ideally the present experiments should be repeated on representative single mutants and on various double mutants. However, we have already discussed the problem of interpreting the results for *E. coli* B, *thy*, which differs by only one mutation from *E. coli* B/r, *thy*.

Since the currently observed modes of repair involve the genes controlling excision and recombination, it may be advantageous to use the present methodology to investigate growth and DNA-synthesis kinetics in strains having mutations only at the *uvr* and *rec* loci.

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