SENSITIVITY OF EXPONENTIALLY GROWING POPULATIONS OF *ESCHERICHIA COLI* TO PHOTO-INDUCED PSORALEN-DNA INTERSTRAND CROSSLINKS

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ABSTRACT Experimental survival curves for *Escherichia coli* K 12 (CR 34) were determined after exposure to 4,5',8-trimethylpsoralen and near ultraviolet light. The lethal action was shown to arise exclusively from interstrand crosslinks, cell vulnerability increasing markedly with the doubling time of the culture. To account for these results, two quite different models are considered. The first assumes that a cell survives as long as at least one copy of its genome remains undamaged; a variant of this permits repair by DNA strand exchange. The second model allows for a limited period of time during which DNA repair can take place. A crosslink in a stretch of DNA due to be replicated within this interval constitutes a fatal lesion. Theoretical survival curves are computed for bacterial populations with defined age distributions and chromosome configurations. While the first model completely fails to provide a satisfactory description of the experimental results, the second model does predict the presence of a shoulder in the survival curves and, in one of its forms, it seems to agree rather well with the measured data over a wide range of crosslink concentrations and doubling times.

INTRODUCTION

Exposure of bacteria or of mammalian cells to 4,5',8-trimethylpsoralen (TMP) and near ultraviolet light (NUV) produces both psoralen-DNA monoadducts and DNA interstrand crosslinks (1, 7, 8, 16). In *Escherichia coli,* the monoadducts are rapidly removed by excision repair and the crosslinks are the main cause of cell death (1, 8, 16). Psoralen-DNA interstrand crosslinks can be repaired, however, and extensive experimental data have led to a model describing the steps involved (9, 10). In the model an indispensable role is played by strand exchange with a homologous chromosome to produce one intact chromosome in the surviving cell, the ultimate lethal effect of any given crosslink being determined solely by the multiplicity of the DNA stretch containing the lesion. This model has gained some support from studies with ionizing radiation. Thus repair of double-strand breaks requires both the *rec*A gene product and a duplicate genome, suggesting that such breaks are repaired exclusively by strand exchange (13). In addition, we consider a kinetic model in which a crosslink is fatal only if it is reached by a replication fork before it is removed by some

unspecified repair mechanism. The proximity of a crosslink to an approaching replication fork thus determines its lethality.

We have performed experiments with TMP and NUV on cultures of *E. coli* growing under various defined conditions in which the distribution of chromosome configurations is known (11, 23), and compared the resulting survival curves with those predicted by each of the two models.

THEORY

Model 1 in its most general form (1*a*) states that any cell with crosslinks in all copies of multiply represented DNA (or a single crosslink in a unique DNA stretch), will die. A simplified version (1*b*) considers only crosslinks in unique DNA stretches to be lethal, any damage occurring in multiply represented stretches being subject to repair by strand exchange with a homologous sister chromosome (9). The two versions represent the extremes where the minimum distance needed for recombination between two homologous stretches has been set either at infinity (model 1*a*) or at zero (model 1*b*).

We also propose two versions of a kinetic model: all cells in which at least one DNA replication fork reaches a crosslink, will die (2*a*); only those cells die in which every fork of a synchronous replication wave (22) reaches a crosslink (2*b*).

Model 1

Let $u(a)$ be the stretch of unique, single-copy DNA in a cell of age a, and $2v(a)$, $4w(a)$, and $8x(a)$ the stretches of DNA present in two, four, and eight copies, respectively. It is convenient to express these lengths in units of C, the time it takes to replicate a chromosome, for then

$$u(a) + v(a) + w(a) + x(a) = 1, \tag{1}$$

provided we restrict ourselves to interdivision times $\tau > \frac{1}{2}C$ and $> D$, the time between termination of chromosome replication and the subsequent cell division.

According to model 1*a*, a cell will survive so long as at least one copy of its DNA remains devoid of crosslinks. The probability/unit time of incurring a fatal crosslink in the unique stretch of the chromosome is

$$\lambda u(a),$$

where λ is the probability of a crosslink/unit time per unit DNA. Similarly, the probability/unit time of crosslinks in both copies of the double-copy section of the chromosome resulting in fatal damage, is $[\lambda v(a)]^2$; for the other sections, it is $[\lambda w(a)]^4$ and $[\lambda x(a)]^8$.

We are interested in the probability of at least one such event. Since, in general, the probability of at least one of two events is the sum of the probabilities of the individual events less their joint probability, it follows that the probability/unit time of at least one fatal event $\beta(a)$ is given by

$$\beta(a) = \lambda_1 + \lambda_2 + \lambda_4 + \lambda_8 - \lambda_1\lambda_2 - \lambda_1\lambda_4 - \lambda_1\lambda_8 - \lambda_2\lambda_4 - \lambda_2\lambda_8 - \lambda_4\lambda_8 + \lambda_2\lambda_4\lambda_8 + \lambda_1\lambda_4\lambda_8 + \lambda_1\lambda_2\lambda_8 + \lambda_1\lambda_2\lambda_4 - \lambda_1\lambda_2\lambda_4\lambda_8, \tag{2}$$

where, for conciseness, we have introduced

$$\lambda_1 \equiv \lambda u(a),$$
$$\lambda_2 \equiv [\lambda v(a)]^2,$$
$$\lambda_4 \equiv [\lambda w(a)]^4,$$
$$\lambda_8 \equiv [\lambda x(a)]^8. \tag{3}$$

If we now define $S(a, t)$ as the number of cells of age a surviving at time t, then $dS(a, t)/dt = -\beta(a)S(a, t)$, or $S(a, t) = S(a, 0)e^{-\beta(a)t}$, because $\beta(a)$ is independent of time.

The coefficient $S(a, 0)$ is the frequency function of age and, for an ideal distribution, is simply (17): $(2\ln 2/\tau)2^{-a/\tau}$. Thus,

$$S(t) \equiv \int_0^\tau S(a, t)da = \frac{2\ln 2}{\tau} \int_0^\tau 2^{-a/\tau} e^{-\beta(a)t} da. \tag{4}$$

There are two events in the life cycle of the cell that cause a discontinuous change in the chromosome configuration: initiation and termination (11). Together, they define three age intervals within each of which the lengths of the different DNA sections vary continuously with cell age (or remain constant). These intervals and the corresponding expressions for $u(a)$, $v(a)$, and $w(a)$ are listed in Table I for all values of $\tau \geq \frac{1}{2}C$, the expression for $x(a)$ following at once from Eq. 1. For any given λ, the λ_i can then be computed from Eq. 3 and substituted into Eq. 2 to get $\beta(a)$.

Model 1*b* states that a crosslink is fatal if and only if it occurs within a unique stretch of DNA. It can thus be treated mathematically as a special case of model 1*a:* all that is necessary is to set $\lambda_2 = \lambda_4 = \lambda_8 = 0$. We then have that $\beta(a) = \lambda u(a) = \lambda$ or $\lambda(\tau - D - a)/C$ or 0, depending on the range of a and τ, and Eq. 4 can be integrated directly.

Model 2

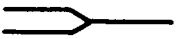
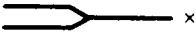
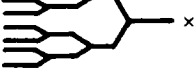
This model states that a finite time is required by a cell to remove a crosslink; if a replication fork should reach the site before repair is complete, the damage is permanent. In model 2*a,* such damage is invariably fatal whereas in model 2*b,* nonviable cells result only when all copies of a multicopy section of the genome have been damaged irreversibly.

Let θ be the time required to repair the damage caused by a crosslink. If $\xi(a)$ is the distance in advance of a fork (or origin) within which the presence of a crosslink would result in permanent damage—that is, the stretch of DNA to be replicated within θ—and p is the probability of such an event, then

$$p = \frac{\xi(a)}{G(a)} = \frac{t(a)/C}{G(a)/G_0}, \tag{5}$$

where $G(a)$ is the amount of DNA in a cell of age a, G_0 is the amount in a nonreplicating chromosome, and $t(a)$ is the time it takes the fork to travel the distance $\xi(a)$. The ratio $G(a)/G_0$ has been computed before (18) and is repeated here for convenience. We again use the age at initiation a_n and the age at termination a_m to divide τ into three intervals.

TABLE I

Range of τ*	Range of a	Chromosome configuration	$u(a)$	$v(a)$	$w(a)$
c'‡ $\leq \tau$	$0 \leq a \leq \tau + c'$		1	0	0
	$\tau - c' \leq a \leq \tau - D$		$(\tau - D - a)/C$	$1 - u(a)$	0
	$\tau - D \leq a \leq \tau$	×2	0	1	0
$C \leq \tau \leq c'$	$0 \leq a \leq \tau - D$		$(\tau - D - a)/C$	$1 - u(a)$	0
	$\tau - D \leq a \leq 2\tau - c'$	×2	0	1	0
	$2\tau - c' \leq a \leq \tau$	 ×2	0	$(2\tau - D - a)/C$	$1 - v(a)$
$\frac{1}{2}c' \leq \tau \leq C$	$0 \leq a \leq 2\tau - c'$		$(\tau - D - a)/C$	$1 - u(a)$	0
	$2\tau - c' \leq a \leq \tau - D$		$(\tau - D - a)/C$	τ/C	$1 - u(a) - v(a)$
	$\tau - D \leq a \leq \tau$	 ×2	0	$(2\tau - D - a)/C$	$1 - v(a)$
$\frac{1}{2}C \leq \tau \leq \frac{1}{2}c'$	$0 \leq a \leq \tau - D$		$(\tau - D - a)/C$	τ/C	$1 - u(a) - v(a)$
	$\tau - D \leq a \leq 3\tau - c'$	 ×2	0	$(2\tau - D - a)/C$	$1 - v(a)$
	$3\tau - c' \leq a \leq \tau$	 ×2	0	$(2\tau - D - a)/C$	τ/C

*Restricted to $\tau \geq D$.
‡$c' \equiv C + D$.

Specifically, $a_n \equiv (n + 1)\tau - c'$ and $a_m \equiv (m + 1)\tau - D$, where $c' \equiv C + D$, $n \equiv [c'/\tau]$, $m \equiv [D/\tau]$. Then for

$$
\begin{aligned}
a_n \leq a_m \qquad G(a)/G_0 &= N(n, m)/C, && 0 \leq a \leq a_n \\
&= N(n + 1, m)/C, && a_n \leq a \leq a_m \\
&= N(n + 1, m + 1)/C, && a_m \leq a \leq \tau
\end{aligned}
$$

and for

$$
\begin{aligned}
a_n \geq a_m \qquad G(a)/G_0 &= N(n, m)/C, && 0 \leq a \leq a_m \\
&= N(n, m + 1)/C, && a_m \leq a \leq a_n \\
&= N(n + 1, m + 1)/C, && a_n \leq a \leq \tau,
\end{aligned}
$$

where

$$N(n, m) \equiv (2\tau - a_n + a)2^n - (2\tau - a_m + a)2^m. \tag{6}$$

The function $t(a)$ depends on the value of τ and on the section of DNA involved. It also depends on whether sister chromosomes are considered individual entities, capable of independent survival, upon termination of chromosome replication (at age $\tau - D$) or only after cell separation (at age τ). These two possibilities, the genome version and the cell version, must be treated separately, as can be seen by comparing Table II and Table III, the construction of which follows directly if rather laboriously from the definition of $t(a)$.

The Tables cover the range $\theta \leq \tau$, but are easily extended by means of the identities:

$$\begin{aligned} t_2(a) &= C\upsilon(a) - t_2(a), \\ t_4(a) &= Cw(a) - t_4(a), \\ t_8(a) &= Cx(a), \end{aligned} \tag{7}$$

where the $t_i(a)$ on the left refer to the $t(a)$ in an i-fold section of DNA after separation of the genome (or of the cell, depending on the version) and those on the right are the corresponding quantities before separation; for the latter, one merely enters the Tables with $\theta = \tau$.

We now turn to the question of combining the probabilities within and between the different sections of the chromosome to obtain the total probability of fatal damage P_t. Let p_i be the probability of permanent damage in one copy of an i-fold section of DNA before separation and q_i the probability after separation. Then

$$p_i = 1 - (1 - p)^j$$

and

$$q_i = 1 - (1 - q)^j, \tag{8}$$

where p and q are the corresponding probabilities of a single crosslink causing permanent damage and j is the number of crosslinks in the cell. If P_i and Q_i are the combined probabilities in the i-fold sections before and after separation, respectively, it follows from the properties of the models that $P_i = 1 - (1 - p_i)^i$ for model 2*a*, $P_i = p_i^i$ for model 2*b*, and $Q_i = [1 - (1 - q_i)^{i/2}]^2$ for model 2*a*, $Q_i = q_i^i$ for model 2*b*. The overall probabilities before separation P and after separation Q are then $P = P_1 + P_2 + P_4 - P_1P_2 - P_1P_4 - P_2P_4 + P_1P_2P_4$, and $Q = Q_2 + Q_4 + Q_8 - Q_2Q_4 - Q_2Q_8 - Q_4Q_8 + Q_2Q_4Q_8$. Finally, the total probability of fatal damage P_t is just $P_t = P + Q - PQ$.

The proportion of cells of age a with a particular number of crosslinks j follows the Poisson distribution with mean l: $(l^j)/(j!e^l)$. Here, l is the average number of crosslinks in these cells and, if h is the average per nonreplicating chromosome (a measurable quantity, independent of cell age), then

$$l = h\frac{G(a)}{G_0}. \tag{9}$$

We then sum over all values of j and integrate over all values of a to get the fraction of

TABLE II

Genome	DNA section	Range* of τ	Range‡,§ of a	t (a)
Unseparated	Single	$c' \le \tau$	$M(\tau - c' - \theta) \le a \le M(\tau - D - \theta) - M(C - \theta)$	$\theta + a - (\tau - c')$
		$\frac{1}{2}C \le \tau \le c'$	$0 \le a \le M(\tau - D - \theta)$	θ
	Double	$C \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}c' \le \tau \le C$	$M(2\tau - c' - \theta) \le a \le M(\tau - D - \theta) - M(c' - D - \tau - \theta)$	$\theta + a - (2\tau - c')$
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$0 \le a \le M(\tau - D - \theta)$	θ
	Quadruple	$\frac{1}{2}C \le \tau$	$0 \le a \le \tau$	0
Separated	Double	$c \le \tau$	$\tau - M(c' + \theta - \tau) \le a \le \tau - M(\theta + D - \tau)$	$\theta + a - (2\tau - c')$
		$C \le \tau \le c'$	$M(2\tau - c' - \theta) \le a \le 2\tau - D - \theta - M(C - \theta)$	$\theta + a - (2\tau - c')$
		$\frac{1}{2}C \le \tau \le C$	$M(\tau - D - \theta) \le a \le \tau - D$	$\theta + a - (\tau - D)$
	Quadruple	$c' \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}c' \le \tau \le c'$	$\tau - M(c' + \theta - 2\tau) \le a \le \tau$	$\theta + a - (3\tau - c')$
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$M(3\tau - c' - \theta) \le a \le 3\tau - c'$	$\theta + a - (3\tau - c')$
	Eightfold	$\frac{1}{2}c' \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$\tau - M(c' + \theta - 3\tau) \le a \le \tau$	$\theta + a - (4\tau - c')$

*Restricted to $D \le \tau$. ‡For values of a outside the ranges indicated, $t(a) = 0$. §$M(y) = y$ if $y > 0$; otherwise, $M(y) = 0$.

surviving cells $S(h)$:

$$S(h) = 1 - \int_0^{\tau} S(a, 0) \left[\sum_{j=1}^{\infty} \frac{l^j}{j!\, e^l} P_t \right] da. \tag{10}$$

MATERIALS AND METHODS

Bacterial Growth Conditions

E. coli K12 strain CR 34 (*thy* A$^-$, *drm*$^-$, *leu*$^-$, *thr*$^-$, *lac* Y$^-$) (14) was grown at 37°C in $A + B$ minimal medium (6) supplemented with leucine and threonine (50 μg/ml each), thymine (20 μg/ml) and deoxyguanosine (100 μg/ml). The carbon source was proline and alanine (0.04% each), glycerol (0.5%), glucose (0.2%) or glucose supplemented with casein hydrolysate (1%). Growth was followed turbidometrically, and all experiments were performed on cells that had been growing exponentially for at least four generations.

TABLE III

Cell	DNA section	Range* of τ	Range‡,§ of a	t (a)
Unseparated	Single	$c' \le \tau$	$M(\tau - c' - \theta) \le a \le M(\tau - D - \theta) - M(C - \theta)$	$\theta + a - (\tau - c')$
		$\frac{1}{2}C \le \tau \le c'$	$0 \le a \le M(\tau - D - \theta)$	θ
	Double	$c' \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}c' \le \tau \le c'$	$M(2\tau - c' - \theta) \le a \le \tau - \theta - M(c' - \tau - \theta)$	$\theta + a - (2\tau - c')$
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$0 \le a \le \tau - \theta$	θ
	Quadruple	$\frac{1}{2}c' \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$M(3\tau - c' - \theta) \le a \le \tau - \theta - M(c' - 2\tau - \theta)$	$\theta + a - (3\tau - c')$
Separated	Double	$c' \le \tau$	$\tau - M(c' + \theta - \tau) \le a \le \tau - M(\theta + D - \tau)$	$\theta + a - (2\tau - c')$
		$\frac{1}{2}C \le \tau \le c'$	$\tau - \theta \le a \le \tau - M(\theta + D - \tau)$	$\theta + a - \tau$
	Quadruple	$c' \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}c' \le \tau \le c'$	$\tau - M(c' + \theta - 2\tau) \le a \le \tau$	$\theta + a - (3\tau - c')$
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$\tau - \theta \le a \le \tau$	$\theta + a - \tau$
	Eightfold	$\frac{1}{2}c' \le \tau$	$0 \le a \le \tau$	0
		$\frac{1}{2}C \le \tau \le \frac{1}{2}c'$	$\tau - M(c' + \theta - 3\tau) \le a \le \tau$	$\theta + a - (4\tau - c')$

*Restricted to $D \le \tau$. ‡For values of a outside the ranges indicated, t(a) = 0. §M(y) = y if y > 0; otherwise, M(y) = 0.

Range of a	$t(a)$	Range of a	$t(a)$
$M(\tau - D - \theta) - M(C - \theta) \le a \le \tau - c' + M(C - \theta)$ $M(\tau - D - \theta) \le a \le \tau - D$	$C - M(C - \theta)$ $\tau - D - a$	$\tau - c' + M(C - \theta) \le a \le \tau - D$	$\tau - D - a$
$M(\tau - D - \theta) - M(c' - D - \tau - \theta) \le a \le 2\tau - c' + M(c' - \tau - D - \theta)$ $M(\tau - D - \theta) \le a \le \tau - D$	$\theta - M(\tau + D + \theta - c')$ $\tau - D - a$	$2\tau - c' + M(c' - \tau - D - \theta) \le a \le \tau - D$	$\tau - D - a$
$\tau - M(\theta + D - \tau) \le a \le \tau$	C		
$2\tau - D - \theta - M(C - \theta) \le a \le \tau - M(\theta + D - \tau) + M(\theta - C)$	$\theta - M(\theta - C)$	$\tau - M(\theta + D - \tau) + M(\theta - C) \le a \le \tau$	$2\tau - D - a$
$\tau - D \le a \le \tau - M(\theta + D - \tau)$	θ	$\tau - M(\theta + D - \tau) \le a \le \tau$	$2\tau - D - a$
$3\tau - c' \le a \le \tau$	θ		

Treatment with TMP and NUV

TMP (a gift of the Paul B. Elder Co., Bryan, Ohio) was added to the growing culture to a concentration of 2.28 μg/ml. The suspension was first incubated for 5 min at 37°C, then cooled rapidly and kept on ice for an additional 25 min (7), and finally transferred to a petri dish and irradiated with NUV. The incident flux, as measured by a calibrated black light meter (2), was in all experiments between 11 and 20 $Jm^{-2} \cdot sec^{-1}$ with a maximum at 365 nm (Sylvania tubes F15T8-BLB). The culture was maintained at ice temperature and stirred throughout the irradiation. Transmission through the 1-mm layer of growth medium containing casein hydrolysate was 90% at 320 nm and 95% at 360 nm; no correction for this absorption was attempted because the precise wavelength responsible for the photoreaction of psoralens is not known (5, 12, 20).

To determine viable counts, samples were removed, suitably diluted, and immediately spread on agar plates of the same composition as the original growth medium. The TMP monoadducts were measured using ^{3}H-TMP (kindly provided by Dr. G. Rodighiero); the assay for the DNA interstrand crosslinks was based on single-strand specific S_1 nuclease and has been described previously (2).

Range of a	$t(a)$	Range of a	$t(a)$
$M(\tau - D - \theta) - M(C - \theta) \le a \le \tau - c' + M(C - \theta)$ $M(\tau - D - \theta) \le a \le \tau - D$	$C - M(C - \theta)$ $\tau - D - a$	$\tau - c' + M(C - \theta) \le a \le \tau - D$	$\tau - D - a$
$\tau - \theta - M(c' - \tau - \theta) \le a \le \tau - \theta + M(\tau + \theta - c')$ $\tau - \theta \le a \le \tau$	$\theta - M(\tau + \theta - c')$ $\tau - a$	$\tau - \theta + M(\tau + \theta - c') \le a \le \tau$	$\tau - a$
$\tau - \theta - M(c' - 2\tau - \theta) \le a \le \tau - \theta + M(2\tau + \theta - c')$	$\theta - M(2\tau + \theta - c')$	$\tau - \theta + M(2\tau + \theta - c') \le a \le \tau$	$\tau - a$
$\tau - M(\theta + D - \tau) \le a \le \tau$ $\pi - M(\theta + D - \tau) \le a \le \tau$	C $\tau - D$		

Computing Procedure

It is apparent from Table I that for model 1*a*, $\beta(a)$ will turn out to be a polynomial in a of degree ≤ 10; analytical integration of Eq. 4 is thus not possible in general, and we have resorted to standard numerical methods (15). In the case of model 1*b*, Eq. 4 can be integrated directly.

The procedure for model 2 is far more complex and differs slightly, according to the kind of process envisaged: constant repair time per cell or constant repair capacity per cell mass. (In the latter, $\theta = kj/2^{a/\tau}$, where k is a proportionality factor independent of cell age and dose level, in the former, θ itself is constant.) First we assign numerical values to C, D, τ (restricted to $> \frac{1}{2}C$ and $> D$), θ (or k, when θ is not constant), and h. For any given age a, Eq. 6 is then used to calculate $G(a)/G_0$ and Eq. 9 to calculate l. If necessary, θ is then computed for $j = 1$. Next, Table II or Table III is entered, depending on the version, and the $t(a)$ for each section before and after separation is obtained (six values in all); for $\theta > \tau$, recourse must first be had to Eq. 7. Eq. 5 is now substituted in Eq. 8 to get the corresponding p_i and q_i, and these are combined via the P_i and Q_i to give P and Q and finally P_t. This is repeated for all $j > 1$ and the weighted summation carried out as indicated in Eq. 10. The resulting expression is of course a function of a and must be integrated numerically over all ages between 0 and τ to get the fraction of surviving cells for the particular h chosen. The entire procedure can then be repeated for other combinations of h, θ (or k), and τ to cover the range of experimental interest.

LIST OF SYMBOLS

Symbol	*Description*	*Eq. or reference*
a	cell age	
C	replication time	ref. 11
c'	$c' = C + D$	
D	interval from termination of replication to cell division	ref. 11
G_0	amount of DNA in a nonreplicating chromosome	Eq. 5
$G(a)$	amount of DNA in a cell of age a	Eq. 5
h	average number of crosslinks per nonreplicating chromosome	Eq. 9
j	actual number of crosslinks in a particular cell	Eq. 10
k	proportionality factor between repair capacity and cell mass	
l	average number of crosslinks per cell	Eq. 9
$S(a, t)$	cells of age a surviving at time t	Eq. 4
$t(a)$	time needed for replication fork to travel distance $\xi(a)$	Eq. 5
$u(a)$	unique DNA	Eq. 1
$v(a)$	two-copy DNA	Eq. 1
$w(a)$	four-copy DNA	Eq. 1
$x(a)$	eight-copy DNA	Eq. 1
$\beta(a)$	probability per unit time of at least one fatal event	Eq. 2
θ	time required to repair a crosslink	
λ	probability of a crosslink/unit time per unit DNA	
$\xi(a)$	distance to be replicated within time θ	Eq. 5
τ	doubling time of culture	

RESULTS

Lethal Action of TMP and NUV

The average number of psoralen-DNA interstrand crosslinks and of monoadducts photoinduced per unit DNA are independent of the growth rate of the cells, as can be seen in Fig. 1. Crosslinks are considered to be the dominant cause of cell death following exposure to TMP

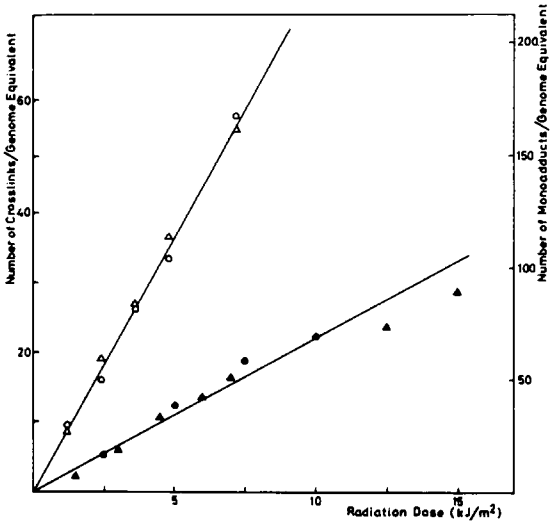


FIGURE 1 Formation of TMP-DNA adducts by NUV. Total number of monoadducts (open symbols) and of interstrand crosslinks (closed symbols) per genome equivalent of DNA (2.5×10^9 daltons) as functions of radiation dose. (●, ○) glucose plus casein hydrolysate medium (τ = 28 min), (▲, △) proline-alanine minimal medium (τ = 144 min).

and NUV (1, 3, 8); that psoralen-DNA monoadducts play an insignificant role can be rigorously demonstrated by comparing survival at a given density of DNA crosslinking in the presence and in the absence of an excess of monoadducts. The degree of survival in the presence of surplus monoadducts was determined after periods of simultaneous treatment with both TMP and NUV. The same amount of crosslinking but with a minimum of monoadducts was achieved by removing the unbound psoralen and subsequently reirradiating. The results show (Fig. 2) that cell survival depends only on the number of crosslinks—that is, on damage that affects both DNA strands at a particular site.

Unlike the level of crosslinking, cell survival at a given dose of NUV varies markedly with the growth rate of the culture. To avoid possible complications caused by shifts in growth rate and to ensure a well-defined distribution of chromosome configurations over the cell population, survival was measured as colony-forming units on agar plates of the same composition as the original growth medium. Cells grown on proline and alanine as carbon source (τ = 144 min) have a D_{37} of only seven interstrand crosslinks per genome equivalent of DNA while those on glucose and casein hydrolysate (τ = 28 min) are much more resistant, with a D_{37} of 27 crosslinks per genome equivalent; cells grown on glycerol (τ = 88 min) or on glucose minimal medium (τ = 57 min) display intermediate sensitivities (Fig. 3). The response of *E. coli* K12 (CR 34) to the lethal action of pyrimidine dimers produced by shortwave UV-light (254 nm), is completely independent of the doubling time of the culture (data not shown).

Simulated Survival Curves

Survival curves were calculated from model 1*a* with C = 40 min and D = 20 min independent of the growth rate (4). The shape of the curves reflects the composition of the population at

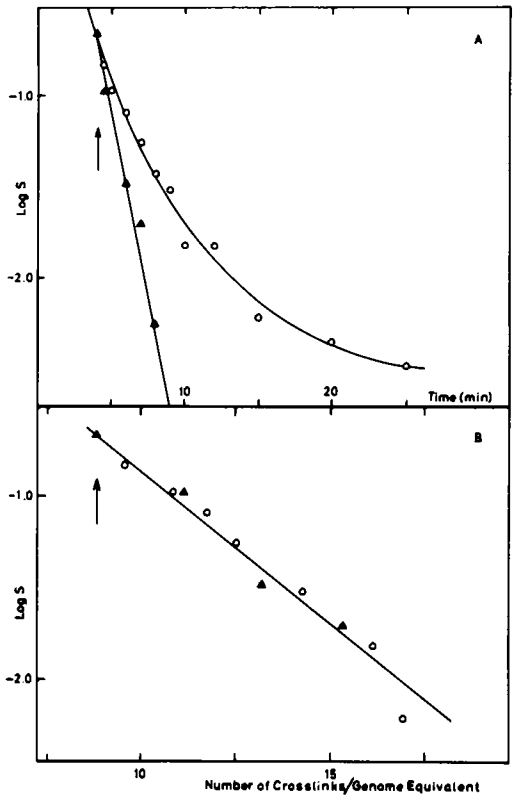


FIGURE 2 Fraction S of *E. coli* K-12 (τ = 144 min) surviving as a function of (*A*) the time exposed to near NUV and (*B*) the number of crosslinks formed per genome equivalent (note semilogarithmic scale). (▲) continuous treatment with TMP and NUV, (○) 4-min treatment with TMP and NUV, removal of unbound TMP (arrow), reirradiation with NUV.

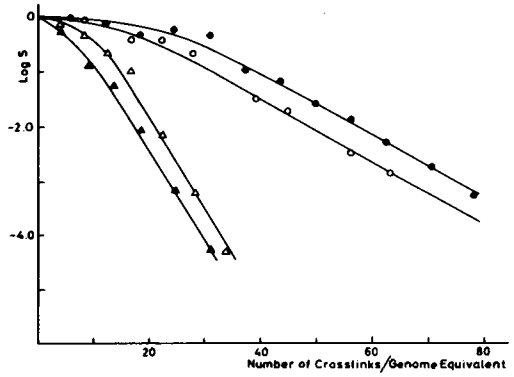


FIGURE 3 Fraction S of *E. coli* K-12 surviving as a function of psoralen-DNA crosslinks per genome equivalent. (●) glucose plus casein hydrolysate medium (τ = 28 min), (○) glucose minimal medium (τ = 57 min), (△) glycerol minimal medium (τ = 88 min), (▲) proline-alanine minimal medium (τ = 144 min).

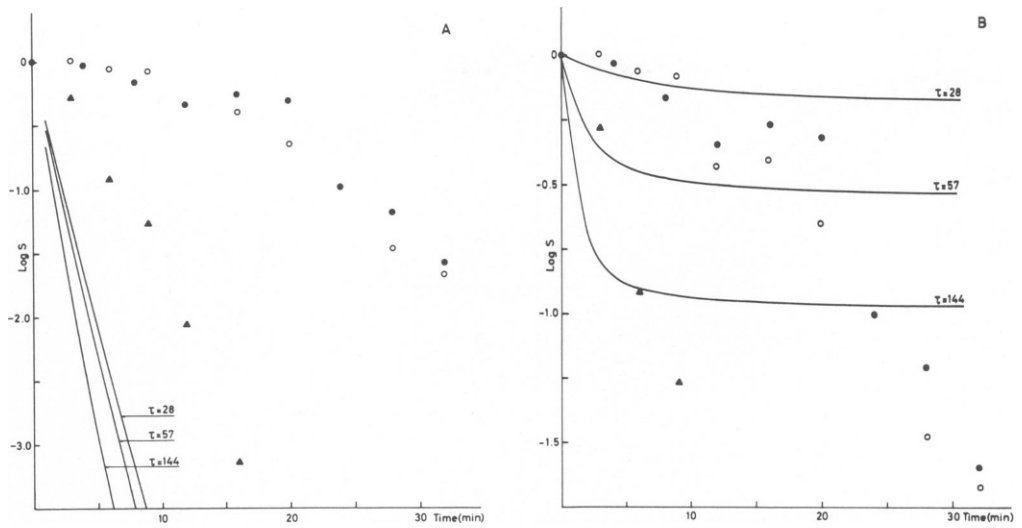


FIGURE 4 Fraction S of cells surviving as a function of the time of irradiation with NUV light. Point symbols: experimental data, solid curves: theoretical predictions according to (A) model 1a and (B) model 1b, with the doubling time τ indicated in minutes. (The chromosome replication time C and the interval between termination of replication and the subsequent cell division D were taken to be constant at 40 and 20 min, respectively.) (●) τ = 28 min, radiation intensity = 1.56 crosslinks/genome equivalent per min; (○) τ = 57 min, radiation intensity = 1.41 crosslinks/genome equivalent per min; (▲) τ = 144 min, radiation intensity = 1.56 crosslinks/genome equivalent per min.

the given growth rate: the older a cell is, the higher the multiplicities of its DNA stretches and the more resistant it is expected to be (Fig. 4 A). While the curves do predict an increase in the sensitivity of the cells with doubling time, they are grossly at variance with the experimental results. Model 1b (Fig. 4 B) exhibits the same general features and, in addition, predicts that a portion of the cells will be totally refractory to the lethal action of DNA-crosslinks; this fraction represents cells in D phase and increases with the growth rate, from 10% at τ = 144 min to 64% at τ = 28 min.

The survival curves based on model 2 are not affected much by the particular values chosen for C and D. In general, the predictions of model 2b seem to come slightly closer to the experimental data than do those of model 2a, and the version that considers the entity capable of survival to be the individual terminated chromosome (Table II) differs very little from the one that considers it to be the entire cell (Table III); only the latter is shown in Fig. 5, and only for model 2b. Finally θ, the time required to repair a crosslink, may not necessarily be constant—proportionality to the number of crosslinks j, for example, would occur if the repair system were saturated and acting at full capacity at all dose levels. We have chosen to treat the case of constant repair capacity per cell mass; that is,

$$\theta = kj2^{-a/\tau}, \tag{11}$$

where k is an adjustable parameter. The theoretical survival curves for τ = 28 min display an initial shoulder and fit the experimental data reasonably well both for θ constant at ~3 min and for k = 0.03 min/crosslink (Fig. 5 A). At the slower growth rates, the curves for constant

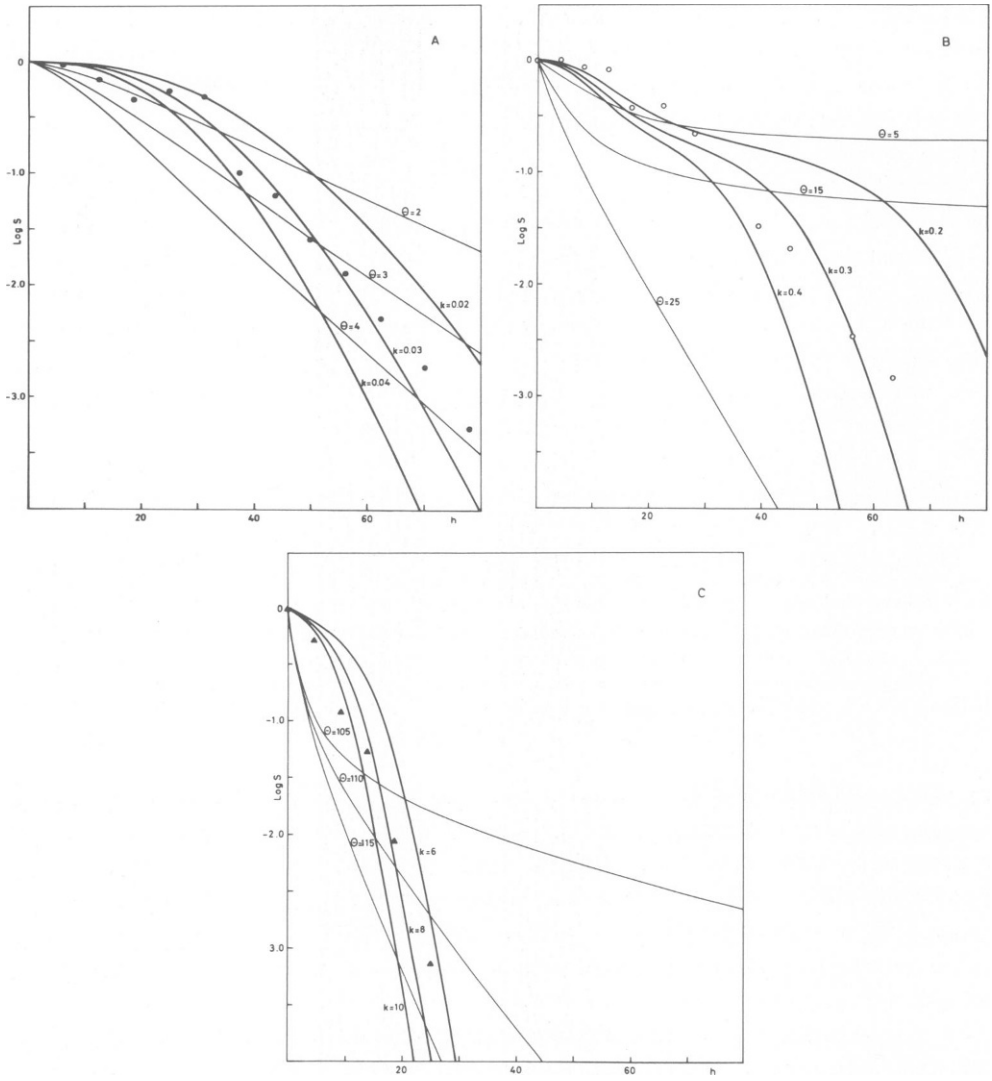


FIGURE 5 Fraction S of cells surviving as a function of h, the average number of crosslinks per genome equivalent. Point symbols: experimental data, solid curves: theoretical predictions according to model 2*b* with the individual cell as the entity of survival. (*C* and *D* as in Fig. 4.) Solid curves, light: constant θ version, value of θ indicated in minutes, solid curves, heavy: θ equal to $kj/2^{a/\tau}$, value of k indicated in min/crosslink. (*A*) τ = 28 min, (*B*) τ = 57 min, (*C*) τ = 144 min.

θ fail to exhibit a shoulder but a satisfactory fit is still provided by Eq. 11, with k = 0.3 min/crosslink for τ = 57 min (Fig. 5 *B*) and k = 8 min/crosslink for τ = 144 min (Fig. 5 *C*).

DISCUSSION

The lethal action of TMP and NUV is due solely to interstrand crosslinks (Fig. 2). Even at slow growth rates, however, all cells are capable of repairing a limited number of such lesions (Fig. 3). The repair mechanism proposed by Cole (9, 10) involves DNA strand exchange with

a homologous chromosome. This implies survival curves that differ markedly from the experimental data obtained with TMP and NUV at all growth rates (Fig. 4); specifically, in medium supporting only slow growth, repair of some seven crosslinks per genome equivalent occurs even when most cells contain only unique DNA and so cannot possibly make use of homologous chromosomes.

An alternative model, for which we propose no specific molecular mechanism, defines a time θ during which the repair can take place: if a crosslink is reached by a replication fork first, the damage becomes permanent. One version of this model (*2b*) describes the survival behavior of fast growing cells rather well (Fig. 5 *A*) with θ constant. For the slower growing cells, a shoulder on the survival curve is predicted only for θ proportional to the crosslink concentration in the individual cell (Fig. 5 *B* and *C*). The other versions of this model, in which varying amounts of permanent damage are needed to render the cells incapable of forming a colony, are either similar or inferior to that shown in Fig. 5.

There are two major potential sources of error that can influence the quality of the fit between the predicted and the measured survival. First, a small proportion of the cells in the growing culture could conceivably have failed to divide normally and so not be included in the age distribution used to compute the integrated survival curves—such cells occur in great abundance during various treatments that are known to interfere with macromolecular synthesis (21). We took great care to ensure steady-state growth conditions, and added deoxyguanosine to prevent thymine limitation (23); still, there is probably at least 1 abnormal cell/1,000 in any culture. Such low numbers can be expected to go undetected in cell size studies (25) but would greatly distort the experimental survival distributions obtained at higher dose levels.

The second source of uncertainty concerns the relationship between θ and j. We have taken θ proportional to $j/2^{a/\tau}$, which would be the case if the repair process were always operating at full capacity and that capacity were proportional to cell mass, regardless of the radiation dose. But it is also possible that θ could, with increasing j, attain values where enzymes induced and synthesized *de novo* after the damage, began participating in the repair; the dependence then of θ on j would be highly complex. As before, the greatest effect is at high doses, where cell death would be less than that predicted on the basis of simple linearity.

The value of the parameter k required to provide a satisfactory fit, varies considerably with the doubling time τ of the culture (Fig. 5). Although some variation with growth rate, such as proportionality between k and τ (and, of course, between k and the mean cell mass at that τ), would not be too surprising, in view of a similar relationship for ribosomal protein synthesis (19). The dependence found here is far stronger, and we can offer no simple explanation. Additional assumptions are necessary to make our description of the inactivation of *E. coli* by TMP and NUV completely quantitative.

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