SENSITIVITY TO ULTRAVIOLET RADIATION AS A FUNCTION OF DNA CONTENT IN *ESCHERICHIA COLI* B/r

BURT V. BRONK AND DANA G. WALBRIDGE, *Departments of Physics and Microbiology, Clemson University, Clemson, South Carolina 29631 U.S.A.*

ABSTRACT Populations of *Escherichia coli* B/r A were grown to log phase at various growth rates determined by the richness of the medium. The genome content, G, was calculated from log phase doubling times by means of the Cooper-Helmstetter formula. Cell volumes were measured and found to vary linearly with this genome content. Cells with various DNA contents were prepared for ultraviolet irradiation and plated for dark repair under similar conditions. The resulting logarithmic survival curves were all similar in shape: convex up, with straight line portions having approximately the same slope (D_0 = 11.4 ± 0.2 J/m^2). The shoulders however increase in width with calculated DNA content giving an extrapolation number which varies roughly as $\exp(\overline{G})$ or $\exp(0.6\, G_{max})$.

INTRODUCTION

Chromosomal DNA is generally regarded as the primary target for damages leading to lethality after UV irradiation of living cells. A number of cellular repair systems function to eliminate deleterious chemical changes to the DNA of the cell. UV irradiation is particularly useful as a probe for studying quantitative aspects of repair since doses are reproducible and lead to measurable amounts of damage to the DNA.

A question of general interest with respect to DNA damage is: What is the relationship between the amount of DNA present per cell, and the degree of lethality induced by a given treatment? On the one hand, if lethality is simply due to the blocking of DNA replication by a damaged site, then increasing DNA content means a larger target and hence should lead to more lethality for a given level of treatment. On the other hand, more DNA may mean more "spare parts" and hence lead to more survival at a given level of damage. Both types of effect have been observed in different systems.

Higher plants show decreasing sensitivity to gamma irradiation with increasing chromosome number (1). The yeast *S. cerevisiae* similarly showed increased resistance to x-rays when diploid cells were substituted for haploid cells. However, on further increase of chromosome number, sensitivity increased (2). Increasing sensitivity to x-rays occurred for the sperm of the wasp, *Mormoniella,* when the chromosome number of the sperm was doubled, with dominant lethality the endpoint measured (3). No discernable difference in sensitivity to gamma irradiation was observed when sublines of certain mouse cultures with chromosome number varying from 53 to 109 were studied (4).

In the present paper we will study the variation in sensitivity to UV irradiation as a function of DNA content for the bacterial species, *Escherichia coli* B/r A, when dark repair takes

place under standard conditions. A similar study was made on the filamentous state of *E. coli* B. obtained after very low doses of UV. Here the DNA content increases with filament length (5). The UV and x-ray sensitivity as measured by the magnitude of the slope of the logarithmic survival curves increased with filament length but the curves remained exponential with no shoulder. Kubitschek et al. (19) found a different situation with regard to repair proficient *E. coli* cells whose DNA content is varied by selecting different stages of the cell cycle. Here the slopes of the exponential portion of the UV survival curves remained constant but the shoulder increased with increasing DNA content. A similar study (22) which concentrated on x-ray sensitivity of *E. coli* B/r as a function of position in the cell cycle indicated that the extrapolation number increased abruptly late in the cycle corresponding to an increase in the shoulder with increasing DNA content. Some change in slope was also suggested during the cycle, but in our opinion the data were not sufficient to establish this.

We varied DNA content by varying richness of the growth medium. The chromosomal content of both *Salmonella typhimurium* (6, 7) and *E. coli* (8, 9) have been found to increase as doubling time decreases when the growth rate varies as a function of the richness of the medium. Extensive studies by Helmstetter and collaborators have led to the formula:

$$\overline{G} = \{T/[C \ln(2)]\}\{\exp[(C + D) \ln(2)/T] - \exp[D \ln(2)/T]\}, \quad (1)$$

for $\overline{G}$, the average number of genome equivalents in a log-phase population of *E. coli* B/r *A* (ATCC 12407). Here C and D (assumed constant) are the times required for one complete round of chromosome replication and the time lapse between the end of the round and cell division, respectively, while T is the doubling time. Eq. 1 has been verified by direct measurements of DNA content per cell for substrains *A* and *F* of *E. coli* B/r (10). These experiments gave results in excellent agreement with the predictions of Eq. 1 with values for C (42 min) and D (22 min) for substrain *A*. These values are very close to those obtained earlier by labeling studies with synchronous cells (8, 9). We therefore used this equation and these parameters to obtain the average genome content as a function of our measured doubling time for a given medium. A value for G_{max}, the chromosome content for cells about to double, is obtained here using similar assumptions to those which led to Eq. 1 (see Appendix).

A related observation by Kubitschek (11) indicates that the ratio of DNA content to cell volume is a constant for *E. coli* cells. We therefore measured cell volume for the log-phase cells. We found that a value of G obtained from our measured cell volumes using Kubitschek's ratio (11) results in a number very close to the value of $\overline{G}$ obtained from Eq. 1 using our measured doubling times. This provides further support for the use of this equation.

MATERIALS AND METHODS

We attempted to reduce variation in conditions of irradiation and plating to a minimum. After growth to log-phase, cells were rinsed and later irradiated in ice-cold buffer. It has been observed that free amino acids in *E. coli* are released within a few seconds upon such treatment (12, 13) so that unincorporated nutrients should not be a factor contributing to differential sensitivity of cells grown in different media. The cells were irradiated at densities such that dose reduction by scattering and absorption was negligible. In every case, cells were plated into the same rich nutrient medium to eliminate variation in this step. Irradiation and plating was done under dim yellow lights to eliminate photorepair.

Organism

E. coli B/r *A* (ATCC 12407), which was kindly furnished by Dr. C. E. Helmstetter (Roswell Park Memorial Institute, Buffalo, N.Y.), was used throughout this project.

Media

The minimal salts solution (HC-salts) contained 2 g NH_4Cl, 6 g Na_2HPO_4 (anhydrous), 3 g KH_2PO_4, 3 g NaCl, and 0.25 g $MgSO_4 \cdot 7\ H_2O$ in 1 liter of glass distilled water (8) and was brought to pH 7.0 by adding ~0.2 ml of 50% wt/vol KOH solution. The HC-salts solution was used for dilutions and irradiation. For growth medium, sodium succinate (2 g/liter) or glucose (1 g/liter) with various combinations of amino acids (0.05 g/liter each) and/or Casamino acids (2 g/liter) was added to the HC-salts (see Table I). All plating was done on Difco Bacto "Nutrient Agar 1.5%" (31 g containing 8 g/liter NaCl added per liter distilled water) with Difco yeast extract (7.5 g/liter) added. Plating was done by spreading 0.1 ml of cells at an approximate dilution to obtain 100–400 colonies/plate. Spreading was done with five 6-mm diam sterile glass beads per plate.

Growth Conditions

Growth rates for each of the six media were determined during log-phase growth. Stock cultures of cells in stationary phase in the given medium were used to inoculate a flask at a sufficiently low concentration so that at least 8 h growth at 37°C with shaking was required for the cells to reach a concentration of 5–8 × 10^7 cells/ml. At this time cells were either rediluted to 2–4 × 10^6 cells/ml in the same medium for growth rate determination or prepared for irradiation. To insure maximum aeration ~50–80 ml medium

TABLE I
SUMMARY OF NUTRIENT CONTENTS AND AVERAGE DOUBLING TIMES FOR EACH MEDIUM

Medium No.	No. of Experiments	Doubling times, T		$\bar{G}$‡	G_{max}§	Nutrient Contents
		Present ± SEM	Published*			
		(min)	*(min)*			
1	4	47.8 ± 1.5	46	1.89	2.77	HC-salts (see section on media) plus glucose
2	5	40.2 ± 1.6	38	2.14	3.13	Medium 1 + Methionine, histidine, and arginine
3	3	30.4 ± 1.1	32	2.77	3.90	Medium 2 + Proline, leucine, threonine, serine phenylalanine, tryptophan, cysteine, glycine, and isoleucine
4	3	29.0 ± 1.2	28	2.91	4.24	Medium 3 + Aspartic acid, glutamic acid, and alanine
5	5	25.3 ± 0.4	25	3.43	5.12	Medium 1 + Casamino acids and tryptophan
6	5	124.5 ± 12.3‖	75	1.60 (1.33)	2.00	HC-salts + sodium succinate

*The previously published doubling times are from reference 8 except that the doubling time for medium 6 is from reference 17.

‡For all media except 6, $\bar{G}$ was calculated according to Eq. 1. For medium 6, we set $C = (2/3)T$, $D = (1/3)T$ according to the Cooper-Helmstetter model (9). The smaller value in parenthesis is obtained from Fig. 8 of Kubitschek and Newman (15).

§See Appendix.

‖A sixth growth experiment gave a doubling time of 256.4 min and was not averaged into the final result.

plus cells was placed in a 500 ml cotton stoppered flask and shaken at ~105 cpm with a 3.5-cm stroke length.

Preparation for Irradiation

When the cells reached a concentration of 5–8 × 10^7/ml the growth flask was removed from the 37°C bath and chilled on ice. The cell suspension was centrifuged at 10,000 rpm at 4°C using a Beckman J-21B centrifuge with a JA-20 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The pellet was resuspended and vigorously vortexed in chilled ~4°–8°C HC-salt solution. Centrifugation, resuspension, and vortexing were repeated, all at 0°–4°C and the cells were diluted to appropriate concentrations for irradiation (see Table II). 40 ml of the final suspension was placed in a 100-mm diam crystallizing dish kept on ice on a magnetic stirrer and mixed with a 4-cm stirring bar at ~200 rpm. The depth of the rotating suspension averaged ~0.5 cm. The suspension was exposed to 253.7-nm UV light at a distance of 53.3 cm from the center of an Atlantic Ultraviolet Germicidal lamp, No. 782130 (Atlantic Ultraviolet Corp., Bay Shore, N.Y.), at timed intervals at a dose rate of (2.4 ± 0.2) J/m^2s as measured by a Blak-ray meter, model J-225 (Ultraviolet Products Inc., San Gabriel, Calif.).

Optical Density Measurements

Cells were prepared in the same manner as for irradiation but at several different concentrations ranging from 2.5 × 10^7 to 3 × 10^8. Absorbance measurements were made on a GCA/McPherson dual beam Spectrophotometer with sample chamber model Eu-707-12 and photomultiplier module model Eu-701-30. According to the manufacturer, the half angle for which light is received from the sample is ~1.1°. Each concentration of cells was shaken vigorously in a quartz cuvette of 0.9-cm optical path length before measurement against HC-salts as a reference.

TABLE II
MULTIPLICITIES

Medium	Single	Double	Triple	Quadruple	Others‡
	(%)	(%)	(%)	(%)	(%)
6*	84.45	13.21	1.65	0.70	—
1	79.83	15.76	2.16	2.16	0.09 (6)
2*	72.25	18.75	5.09	2.73	0.52 (5)
					0.52 (6)
					0.07 (8)
					0.07 (9)
2	79.94	19.65	—	0.31	0.10 (8)
2	80.62	19.14	—	0.24	—
3	78.74	20.06	0.70	0.50	—
4	81.67	18.33	—	—	—
5	81.85	18.07	0.09	—	—

Average§

Single	Double	Triple	Quadruple	Other
(%)	(%)	(%)	(%)	(%)
80.44 ± 1.19	18.50 ± 1.54	0.63 ± 0.85	0.54 ± 0.82	0.03 ± 0.05

The numbers in parentheses indicate the number of cells in the group.
*These experiments were not used in calculating overall average.
‡The percentage given is percent of groups.
§Errors are experimental standard deviations.

Cell Size Determination

Cell volumes for *E. coli* grown in each of the six media were determined with an Electrozone/Celloscope particle counter (Particle Data Inc., Elmhurst, Ill.) fitted with a 30-μm orifice tube. Calibration of the trigger levels determining the volume of the smallest particles counted, was performed by using latex spheres (Earnest F. Fullum Inc., Schenectady, N.Y.) of 1.099 and 2.02μm diam. The trigger level was linear in this range to >5%. Cells grown to log-phase ($\sim 5 \times 10^7$/ml) as described above were diluted into filtered counting solution (0.9% NaCl; 0.0925% Formaldehyde) to a concentration of 5×10^5 to 10^6 cells/ml and counted immediately at various settings of trigger level.

Multiplicity Counts

Survival curves must be corrected for any tendency of the cells to cluster. We therefore prepared cells as for irradiation and used a Petroff-Hausser counting chamber under a phase contrast microscope at a magnification of $\sim\times 400$ for counting. ~1,000 clusters of individual cells, pairs, triples, etc., were counted. When a constriction was noted, that was counted as a separation into two cells. The percentage of the total number of clusters was obtained for each type of cluster.

RESULTS

Growth curves were graphed on semilogarithmic plots of cell count against time in minutes. Each graph was checked by eye to eliminate the possibility of including lag-phase or stationary phase points. Generally at least seven points fell within the log-phase region, which was taken conservatively as the region with concentration from $\sim 5 \times 10^6$ to 1×10^8 cells/ml. The mean and standard deviation for each point was estimated from plate counts of at least 5 plates/point. To obtain doubling times for each medium, the points within the log-phase region were fit to a straight line using the method of least squares with points weighted by the inverse of their variance. Occasionally one, or at most two, points more than a standard deviation away from the straight line fit were ignored. Doubling times and standard deviations of the mean are presented in Table I. With the exception of the results for the succinate medium,[1] these results are in good agreement with those previously published. Hence the growth properties of our cells may be presumed identical to those determined when the genome content was measured in each of the media (8, 10).

Cell volume distributions as measured with the particle counter for each medium are presented in Fig. 1. The points represent the relative number of counts between two trigger level settings. Each point is an average over two or more experiments with cells grown on separate days. Smoothed curves were hand drawn through the (approximately) normalized experimental points. From these curves peak volumes, V_p, and average volumes, $\langle V_i \rangle$, were obtained and plotted vs. $\overline{G}$, the average genome content in Fig. 2. Here,

$$\langle V_p \rangle \equiv \frac{\int_0^\infty V f_i(V)\, dV}{\int_0^\infty f_i(V)\, dV},$$

[1]In succinate, an extremely long lag phase (1–3 d) occurred after cells were first introduced to the nutrient. The average doubling time, 124.5 min was quite different from the published value, 75 min. In one experiment (not included in the average) the doubling time was 256.4 min. These results suggest th a mutation may be required before *E. coli* B/r grows "rapidly" in this medium.

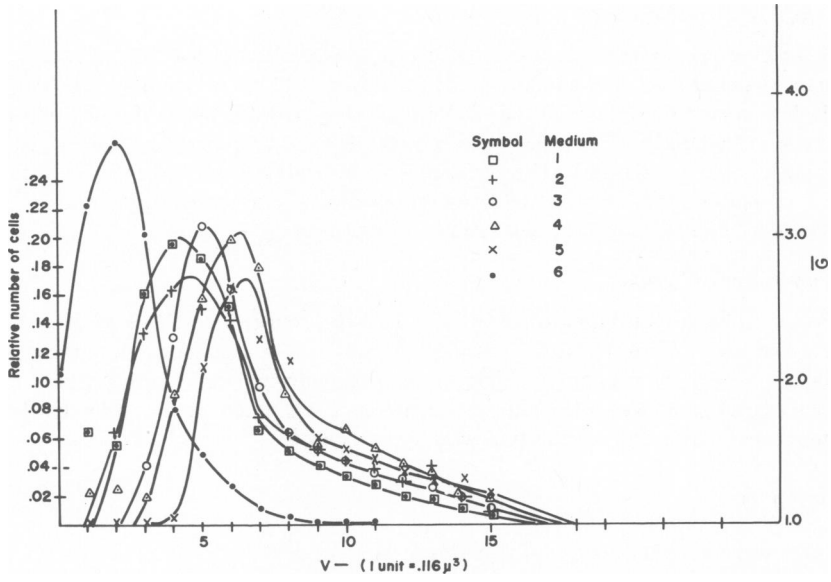

FIGURE 1 Relative number of cells vs. log-phase cell volume. Each point is average of values from two histograms taken after growth to log-phase on different days. One volume unit = 0.116 μm^2. Media: 1, ; 2, +; 3, ○; 4, Δ; 5, x; 6, ·;

where $f_i(V)$ is the smoothed density function from Fig. 1 for the ith medium. The volumes clearly increase with richness of the media and with the exception of the succinate grown cells, volumes increase linearly with $\overline{G}$ at a rate of 0.2 μm^3/genome. (This suggests a generalized gene dosage hypothesis: during balanced growth, constitutive molecules in a bacterial cell with certain notable exceptions [e.g., division septa] are produced in proportion to the number of genes present which pertain to the molecule.) A least squares straight-line fit (excluding succinate grown cells) of the volumes vs. $\overline{G}$ gives:[2]

$$V = a + b\overline{G}, \tag{2}$$

where the parameters giving V in cubic microns are: for average volumes, $a_a = 0.285 \pm 0.033$, $b_a = 0.20 \pm 0.01$; for peak volumes, $a_p = 0.18 \pm 0.13$, $b_p = 0.18 \pm 0.05$.

The value of $\langle V \rangle$ obtained from Eq. 2 for media 1 and 5 are 0.66 and 0.98 μm^3, respectively. These compare satisfactorily with the corresponding values of 0.72 and 1.27 μm^3 obtained from Kubitschek's formulation (11) using the present doubling times for the two media. It should be noted that in neither the present paper nor reference 11 are average volumes corrected for presence of multiple cells. Such a correction would involve multiplying both terms of Eq. 2 by the same factor, which would be considerably less than 1. This follows from the assumption, suggested by Kubitschek (16), that the volume-probability density curves (his Fig. 3) to a good approximation keep the same shape from one medium to another,

[2]The errors for a_a and b_a are estimated by the usual method of using the deviation of the fitted from the experimental point. Those for a_p and b_p are determined from a weighted least square fit (see reference 18). The volume of the succinate grown cells are apparently below the range of the instrument for the orfice used.

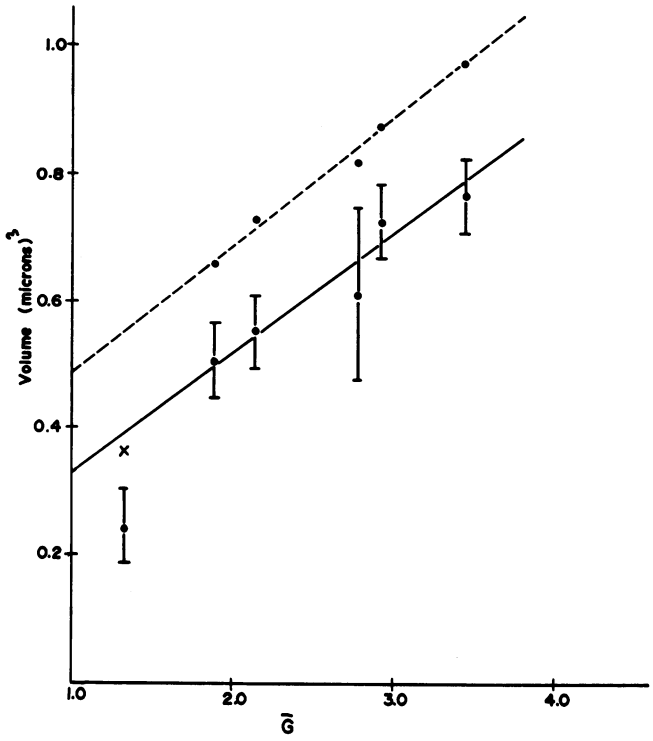

FIGURE 2 Peak volumes (—) and average volumes (---) vs. average calculated genome number per cell. Volume units are cubic microns. Errors on peak volumes are estimates based on fact that peaks from separate experiments fell within one unit (0.116 μm^3) of each other except in the case of medium 3, where they were 2½ units apart. The point marked x gives the average volume for medium 6 vs. $\bar{G}$ from reference 15.

TABLE III
PARAMETERS OF SURVIVAL CURVES

Medium	Zero dose	−Slope	ln intercept	N_0	χ_ν^2	o.d.	$\langle I/I_0 \rangle$	−Slope/$\langle I/I_0 \rangle$
	(*cells/ml*)	(m^2/J)						
6	7.8×10^7	0.090 ± 0.005	0.96 ± 0.26	2.62	3.17	0.34	0.81	0.11
1	6.2×10^7	0.084 ± 0.004	1.9 ± 0.4	6.96	0.63	0.27	0.84	0.10
2	4.8×10^7	0.099 ± 0.004	2.6 ± 0.4	13.30	0.26	0.22	0.87	0.11
2	8.9×10^7	0.088 ± 0.004	2.3 ± 0.5	9.60	0.54	0.41	0.78	0.11
3	2.7×10^7	0.093 ± 0.007	1.9 ± 0.8	6.80	0.31	0.16	0.90	0.10
3	1.26×10^8	0.088 ± 0.005	2.0 ± 0.5	7.33	1.60	0.77	0.64	0.14
4	4.8×10^7	0.086 ± 0.006	2.2 ± 0.7	8.92	0.026	0.21	0.88	0.097
4	1.12×10^8	0.087 ± 0.003	2.85 ± 0.38	17.32	0.45	0.49	0.74	0.12
5	3.7×10^6	0.089 ± 0.004	3.65 ± 0.41	38.47	0.93	0.025	0.98	0.091
5	6.8×10^6	0.083 ± 0.007	2.9 ± 0.7	18.75	0.95	0.045	0.97	0.086
5	6.3×10^7	0.086 ± 0.004	2.8 ± 0.5	16.28	0.20	0.41	0.78	0.11

Slopes and intercepts are from straight line fits of ln (surviving fraction) vs UV dose (J/m^2) to the data sets with the standard deviation obtained from the least squares analysis. $N_0 = \exp$ (ln intercept) is the extrapolation number. χ_ν^2 is the reduced value for chi-square of the fit (see Ch. 10 of reference [17]). o.d. Denotes optical density measured against buffer in a 0.9-cm cuvette. $\langle I/I_0 \rangle$ denotes the average fractional intensity assuming no multiple scattering. The slopes are given in units of natural log of viable fraction per unit dose in J/m^2.

and also because the proportion of multiple cells is the same for all media considered (see Table III).

The data for survival after irradiation were tabulated.[3] S_x, the observed surviving fraction, required correction for the fact that a fraction of the cells stay in clusters or chains of two or more cells. The percentages of total clusters having various numbers of cells are listed in Table II for each medium. A corrected surviving fraction, S, is obtained from the equation:

$$S_x = \Sigma c_i[1 - (1 - S^i)]/100, \tag{3}$$

where c_i is the percentage[4] of clusters with i cells. Eq. 3 follows from the assumption that each cluster will give rise to exactly one colony if one or more cells in it survive.

Examples of corrected logarithmic survival curves are shown in Fig. 3. Their behavior is typical of all the curves in that each is convex-up and has a shoulder followed by a clearly

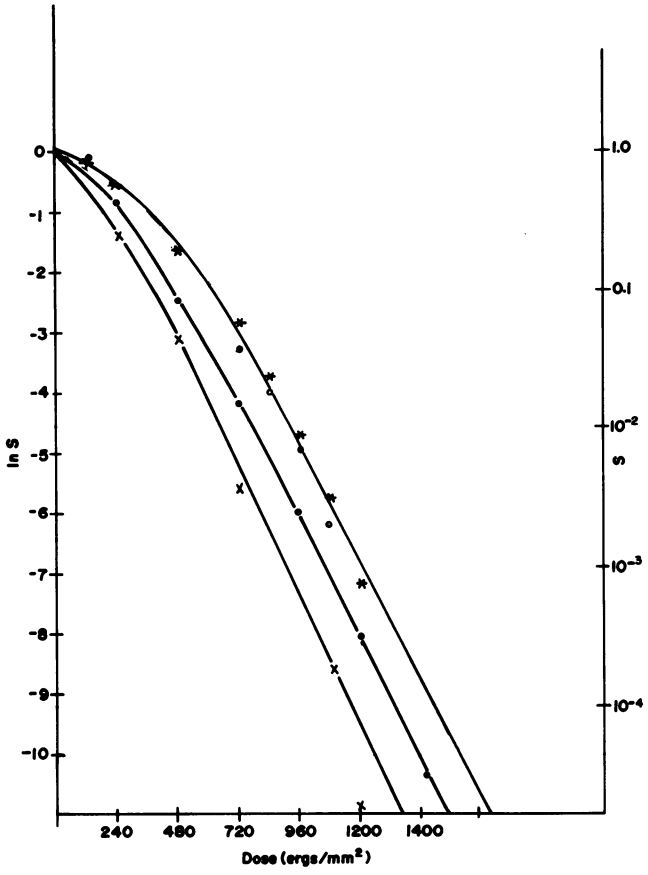

FIGURE 3 Examples of the logarithmic survival curves. Medium 6, x; Medium 1, ·; Medium 5, * and o. Curves are hand drawn.

[3]This table including estimated errors is available on request.

[4]For media 1–5 cluster size is apparently constant so tabulated averages are used. For $i > 2$, c_i are small, and the average size of these was taken as 4. For medium 6, c_i were-used as listed.

defined straight line portion at higher doses. Weighted least squares fits were made to the straight line portions (generally four or five points) which were taken as the region with doses ≥ 72.0 J/m^2 for media 1–5; ≥ 24.0 J/m^2 for medium 6. In most cases, survival for doses $\geq 1{,}400$ erg/mm^2 fell above the straight line portion and had large variance. These points[5] were not included in the fit. In Table III we have tabulated the results of this analysis. Most of the reduced χ^2 values are <1 which indicates adequate fits have been obtained. Optical densities (OD) of cell suspensions were measured to estimate an upper bound to the dose reduction due to scattering from other cells. Estimates of OD for each data set based on a straight line fit of measured OD vs. cell concentration are presented in Table III along with the average fractional intensity, $\langle I/I_0 \rangle$ calculated from the equation (reference 20):

$$\langle I/I_0 \rangle = \int_0^D e^{-Ec\,(2.303)x}\,dx/D, \tag{4}$$

where $Ec = \mathrm{OD}/0.9$ and D is the average depth of the cell suspension. In the last column of Table III, $(-\text{slope}/\langle I/I_0 \rangle)$ gives an upper bound on the magnitude of the slope of the survival curve for a suspension with negligible scattering. The dose reduction calculated from measured ODs and Eq. 4 is an overestimate since there is evidently no clear trend toward uncorrected slopes of higher magnitude for more transmission (less cells). Thus scattered light must often be absorbed by a second bacterium. This is not surprising since all light falling outside a very narrow angle ($\sim 1°$) is counted as scattered in our OD measurement. It is important to note that reduction in intensity of UV light due to scattering can only change the measured slope, but not the value of the intercept since the cells are thoroughly mixed, making any dose reduction uniform. Hence the only difference should be a scale change on the dose axis.

The results in Table III indicate that the final slopes of the log-survival curves are independent of the growth medium and hence calculated genome content. The average slope[6] from column 3 of the table is 0.0874 ± 0.0015 (J/m^2)$^{-1}$, which gives $D_0 = 11.43 \pm 0.19$ J/m^2 (Errors are standard deviations of the mean.)

The natural log of the intercept of the survival curve increases with increasing calculated genome content as indicated in Fig. 4. Although there is no *a priori* reason to expect a linear relationship, a least squares fit to a straight line is a convenient way of summarizing the data relating the log intercept (L.I.) and average ($\overline{G}$) or maximum ($G_{\max}$) genome content per log-phase cell. Hence we set L.I. $= a_{\mathrm{avg}} + b_{\mathrm{avg}}\overline{G}$ and L.I. $= a_{\max} + b_{\max}G_{\max}$ from which the following parameters were obtained: $a_{\mathrm{avg}} = -0.18 \pm 0.18$; $b_{\mathrm{avg}} = 0.95 \pm 0.17$; $a_{\max} = -0.21 \pm 0.43$; $b_{\max} = 0.65 \pm 0.11$. Thus the extrapolation number, n_0 is roughly equal to $\exp(\overline{G})$ or $\exp(0.6\,G_{\max})$.

DISCUSSION

The above results are in agreement with those of Kubitschek et al. (19) who found that the slope of the UV survival curve remained constant for *E. coli* strains B_{s-1} and 15 THU as the

[5]Additional survival at very high doses could be due to shielding of $\sim 1/10^4$ cells at the center of large clusters which were occassionally observed.

[6]The data with the larger slope for medium 2 are left out of the averages.

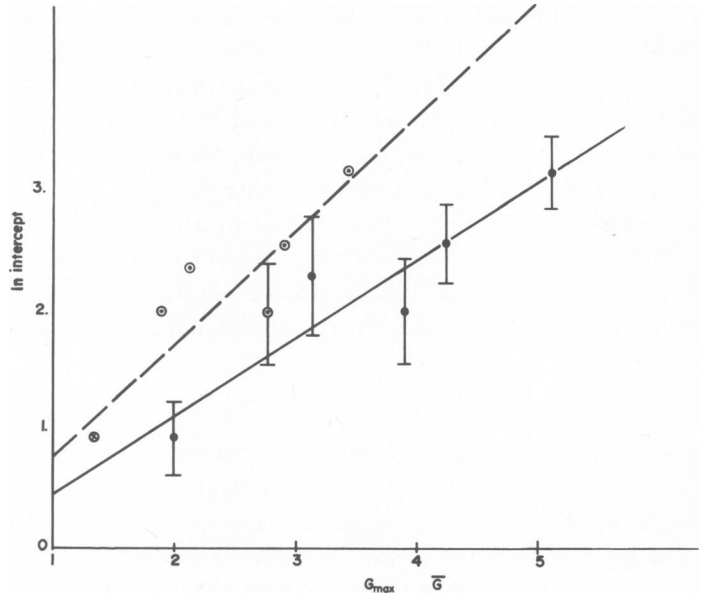

FIGURE 4 The log of the intercept of the straight line portion of the survival curve vs. calculated values for $\overline{G}$ (⊙) or G_{max} (·). The point marked ⊗ is with $\overline{G}$ calculated according to Kubitschek and Newman (15) (see Table I). Standard deviations of mean shown only for G_{max} curve but apply to both. Note that open circle point shown with error bar coincides with closed circle point.

DNA content varied as a result of progression through the cell cycle. However, while the survival curve remained a simple exponential for the repair deficient strain, B_{s-1}, the curve for the repair proficient strain had a shoulder for which the extrapolation number, n_0, increased with DNA content.

Our main conclusions are that the final slopes of the logarithmic curves for survival vs. UV dose do not vary significantly as a function of calculated genome content for log-phase cells irradiated and plated under the conditions described in this paper, whereas the extrapolation number or log intercept do increase with calculated genome content. The reader should be aware that these experiments could have an alternative interpretation in which either the words growth rate or cell volume could be substituted for calculated genome content in the statements of this paragraph.

The observed relationship: constant sensitivity plus a shoulder width which increases as a function of genome content is qualitatively similar to the predictions of a single-hit multi-target model. In the case of rapidly replicating *E. coli,* however, the DNA is continuously replicated and not all the units can be considered separate entities. Thus a quantitative model which correctly describes damage and repair should include rearrangements of DNA so that increasing the genome content has an effect as though the number of targets were increased.

APPENDIX

It is of interest to obtain the maximum number of chromosomes per cell in a log-phase population predicted by a model with the same assumptions (8, 9) which lead to Eq. 1 for average genome content. These assumptions may be stated as follows: The intervals C and D are set at fixed time lengths which do

not change with division rate. C is the time to replicate a single genome. The rate of replication is assumed constant. D is the time between the end of replication cycle and division. The interdivision time,[7] τ_c is assumed $>D$. To accommodate increased rates of division, an exactly adequate number of replication forks form such that: (*a*) The total genome is traversed exactly once by replication forks during a replication cycle. Various portions are traversed by 1, 2, . . . 2^n pairs of forks on as many branches, depending on whether the branch traversed is to be completed 0, 1 . . . n generations later. (*b*) Each initiation starts at a time such that at an interval $(C + D)$ following that initiation (or a time D following completion) division occurs.

We will consider three different regimes of growth rate: (*a*) $C + D \leq \tau_c$. In this case $G_{max} = 2$ because exactly two genomes are present before division. (*b*) $\tau_c < (C + D) \leq 2\tau_c$. In addition to the two genomes (one pair of forks) completed during the present (ith) division period, two more (two pairs of forks) are being synthesized for the $(i + 1)$th generation for an interval which equals $(C + D - \tau_c)$ by the end of the ith. Then a replication rate of 1/C gives:

$$G_{max} = 2 + 2\left(\frac{C + D - \tau_c}{C}\right)$$

(*c*) $2\tau_c < (C + D) \leq 3\tau_c$. Here two genomes are completed during the ith generation, with two additional to be completed during the $(i + 1)$th generation and four more (four pairs of forks) to be completed after the $(i + 1)$th division and before the $(i + 2)$th division. These four have been replicating a time $(C + D) - 2\tau_c$ by the end of the ith generation. For this case,

$$G_{max} = 2 + 2\frac{(C + D) - \tau_c}{C} + 4\frac{(C + D) - 2\tau_c}{C}.$$

We would like to thank C. E. Helmstetter for the cell strain and for an enlightening discussion.

Received for publication 23 January 1979 and in revised form 5 May 1980.

REFERENCES

1. SPARROW, A. H., and H. J. EVANS. 1961. Nuclear factors affecting radiosensitivity. I. The influence of nuclear size and structure, chromosome complement, and DNA content. *Brookhaven Symp. Biol.* **14**:76–100.
2. MORTIMER, R. K. 1958. Radiobiological and genetic studies on a polyploid series (haploid to hexaploid) of *Saccharomyces cerevisiae*. *Rad. Res.* **9**:312–326.
3. MORTIMER, R. K., and R. C. BORSTEL. 1963. Radiation-induced dominant lethality in haploid and diploid sperm of the wasp *Mormoniella*. *Genetics* **48**:1545–1549.
4. TILL, J. E. 1961. Radiosensitivity and chromosome numbers in strain L mouse cells in tissue culture. *Rad. Res.* **15**:400–409.
5. DEERING, R. A. 1959. Radiation sensitivity of filamentous *Escherichia coli*. *Biochim. Biophys. Acta.* **31**:11–19.
6. SHAECTER, M., O. MAALØE, and N. O. KJELDGAARD. 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. *J. Gen. Microbiol.* **19**: 592–606.
7. MAALØE, O., and N. O. KJELDGAARD. 1966. Control of Macromolecular Synthesis. W. A. Benjamin, New York. 158.
8. HELMSTETTER, C. E., and S. COOPER. 1968. DNA synthesis during the division cycle of rapidly growing *Escherichia coli* B/r. *J. Mol. Biol.* **31**:507–518.
9. COOPER, S., and C. E. HELMSTETTER. 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. *J. Mol. Biol.* **31**:519–540.

[7]The interdivision time is approximately, but not exactly, equal to the doubling time. For precise definitions and an experimental example see references 23 and 24.

10. HUCUL, J. A., and C. E. HELMSTETTER. 1978. A comparison of DNA content between two substrains of *Escherichia coli* B/r. *Biochem. Biophys. Res. Commun.* **80**:970–974.
11. KUBITSCHEK, H. E. 1974. Constancy of the ratio of DNA to cell volume in steady-state cultures of *Escherichia coli* B/r. *Biophys. J.* **14**:119–123.
12. BRITTEN, R. J., and F. T. MCCLURE. 1962. The amino acid pool in *Escherichia coli*. *Bacteriol. Rev.* **26**:292–335.
13. PIPERNO, J. R., and D. L. OXENDER. 1968. Amino acid transport systems in *Escherichia coli* K12. *J. Biol. Chem.* **243**:5914–5920.
14. PIPERNO, J. R., and D. L. OXENDER. 1966. Amino acid bound protein released from *Escherichia coli* by osmotic shock. *J. Biol. Chem.* **241**:5732–5743.
15. KUBITSCHEK, H. E., and C. N. NEWMAN. 1978. Chromosome replication during the division cycle in slowly growing steady-state cultures of three *Escherichia coli* B/r strains. *J. Bacteriol.* **136**:179–190.
16. KUBITSCHEK, H. E. 1969. Growth during the bacterial cell cycle: analysis of cell size distribution. *Biophys. J.* **9**:792–808.
17. HELMSTETTER, C. E. 1974. Initiation of chromosome replication in *Escherichia coli*. I. Requirements for RNA and protein synthesis at different growth rates. *J. Mol. Biol.* **84**:1–19.
18. BEVINGTON, P. R. 1969. Data Reduction and Error Analysis for the Physical Sciences. Chs. 5 and 6. McGraw-Hill, New York.
19. KUBITSCHEK, H. E., A. C. FINNEY, and R. E. KRISCH. 1973. Constancy of UV sensitivity of *E. Coli* during the cell cycle. *Photochem. Photobiol.* **18**:365–370.
20. MOROWITZ, H. 1950. Absorption effects in volume irradiation of microorganisms. *Science (Wash. D.C.)*. **3**:229–230.
21. NORMAN, A. 1954. The nuclear role in the ultraviolet inactivation of *Neurospora conidia*. *J. Cell Comp. Physiol.* **44**:1–10.
22. CLARK, D. J. 1968. Effects of ionizing radiation on synchronous cultures of *Escherichia coli* B/r *J. Bacteriol.* **96**:1150–1158.
23. BRONK, B. V., G. J. DIENES, R. SCHINDLER, and J. R. GAUTSCHI. 1974. Kinetic cell-cycle analysis of a cultured mammalian cell population. *Biophys. J.* **14**:601–624.
24. BRONK, B. V., G. J. DIENES, and A. PASKIN. 1968. The stochastic theory of cell proliferation. *Biophys. J.* **8**:1353–1398.