THE DAMPENING OF DNA REPLICATION CYCLES AFTER X-IRRADIATION OR ULTRAVIOLET LIGHT IRRADIATION

DANIEL BILLEN *and* LAURA H. BRUNS

From the Radiation Biology Laboratory, Division of Nuclear Sciences, and Departments of Microbiology and Radiology, College of Medicine, University of Florida, Gainesville, Florida 32601

ABSTRACT *Escherichia coli* strains 15T⁻ (555–7) and B/r were grown in the presence of thymine-^{14}C to label all DNA. The ability of these parental DNA's to undergo cycles of replication subsequent to cellular irradiation with either X-ray or ultraviolet light (UV) was followed with density labels. Exposed cells were shifted into the density medium at times which were approximately multiples of normal rounds of DNA replication. A portion of the parental DNA, replicated semiconservatively once during an initial cycle following UV or X-irradiation in *E. coli*, failed to replicate again within the time studied. The time course of semiconservative parental DNA replication is altered.

INTRODUCTION

The characteristic response in DNA replication subsequent to UV irradiation in the D_{37} range in resistant bacterial cells is an inhibition followed by an apparent restoration to a normal synthesizing rate. Doudney and Young (1) have shown that, following UV exposure to doses allowing at least 10–20% survivors, most of the parental DNA undergoes a round of semiconservative synthesis as determined by density label. This was confirmed by Hewitt, Billen, and Jorgensen (2) and extended to X-irradiated cells (2). Similar density label experiments have been used to support the conclusion that DNA subjected to dark repair mechanisms behave in a "normal" way. For example, it has been reported that DNA subjected to repair replication will undergo a subsequent cycle of semiconservative replication (3), the implication being that the repaired segment is normal.

We have observed a dampening of DNA replication that extends over several postirradiation cycles. Dampening is here defined as a reduction in the final amount of parental DNA replicated. Thus, ability to undergo one postirradiation round of replication is not sufficient proof of the "normality" of the template strand since nonfunctional daughter strands may be produced.

MATERIALS AND METHODS

Bacteria and Bacterial Growth

The following multiauxotrophic strains of *E. coli* were used: *E. coli* 15T$^-$ (555–7) requiring thymine (2 μg/ml), arginine (38 μg/ml), methionine (30 μg/ml), and tryptophan (14 μg/ml) for growth; *E. coli* B/r (hcr+) requiring thymine (5 μg/ml) and tryptophan (14 μg/ml) for growth. For the 5-bromouracil (Bu) density-labeling experiments, the cells were grown with forced aeration at 37°C in a minimal-salts medium identified as MS-7 (4) and containing 10 μg/ml of Bu with the appropriate supplements. For the ^{15}N, ^{13}C density-labeling studies the cells were grown in a modified medium-C–salts medium (2) plus the required supplements.

Harvesting of Cells

Cells were harvested and washed with unsupplemented minimal-salts medium devoid of glucose on prewashed membrane filters of 0.45 μ pore size (Millipore Filter Corp., Bedford, Mass.) at ambient temperature.

Radioisotopes

For thymine-^{14}C labeling, 0.1 or 0.2 μCi/ml of thymine-2-^{14}C (specific activity [S.A.] = 51.6 mCi/mmole) was added to each ml of media. The final S.A. with strain 555–7 was either 5.7 or 10.3 μCi/μmole and for B/r was 2.4 or 4.7 μCi/μmole of thymine respectively.

In certain experiments with B/r, 0.5 μCi/ml of thymine-^3H (S.A. = 11.3 Ci/mmole) was added during postirradiation incubation (final S.A. was 12.5 μCi/μmole).

Irradiation of Bacteria

All irradiations were carried out at ambient temperature in minimal salts without glucose. The details for X-ray and UV exposure were as described elsewhere (2).

Determination of Per Cent Parental DNA Replicated

Details and rationale of the procedure for determining per cent of parental DNA replicated under a given set of conditions were as described previously (2). Briefly, "parental" DNA was identified by labeling with thymine-^{14}C during several generations growth (exponential phase) in ^{14}N, ^{12}C medium (light). Thus, parental DNA at the time of irradiation consisted of ^{14}N, ^{12}C components and contained ^{14}C label (buoyant density in CsCl of 1.710 g/cc). Immediately following irradiation the cells (1.0–2.0 $\times$ 10^8 viable cells/ml) were incubated in light medium unless otherwise indicated. Replication of parental DNA was determined either by Bu or ^{15}N, ^{13}C density label. For the Bu label a portion of the cells were shifted to a medium containing Bu instead of thymine. For the ^{15}N, ^{13}C density label the cells were shifted to a medium in which $^{14}NH_4Cl$ was replaced by $^{15}NH_4Cl$ and glucose-^{12}C replaced by 40–51% substituted glucose-^{13}C (^{15}N, ^{13}C medium, hereafter referred to as heavy medium). Cell lysates were prepared as previously described and then subjected to isopycnic analysis in CsCl (2). All replicated parental DNA (^{14}C-labeled DNA) was shifted to a banding position of hybrid density in the CsCl gradient (i.e. parental strand *light* and daughter strand *heavy*). For the Bu system the hybrid DNA was found to be approximately 1.755 g/cc and for the ^{15}N, ^{13}C system, 1.722–1.724 g/cc. The per cent DNA replicated was determined from that portion of the total ^{14}C counts shifted to the higher density region in the gradients. To avoid the complications of reaching stationary phase during the experiment, all cultures were diluted when

indicated with appropriate media such that the cell population did not exceed 6×10^8 cells/ml as monitored by turbidity measurements.

RESULTS

Postirradiation DNA Cycling in E. coli *15T⁻ (555–7): Use of 5-Bromouracil as a Density Label*

The ability of parental DNA of *E. coli* 15T⁻ (555–7) to undergo one or more cycles of replication subsequent to X-ray exposure was tested as follows. Cellular DNA

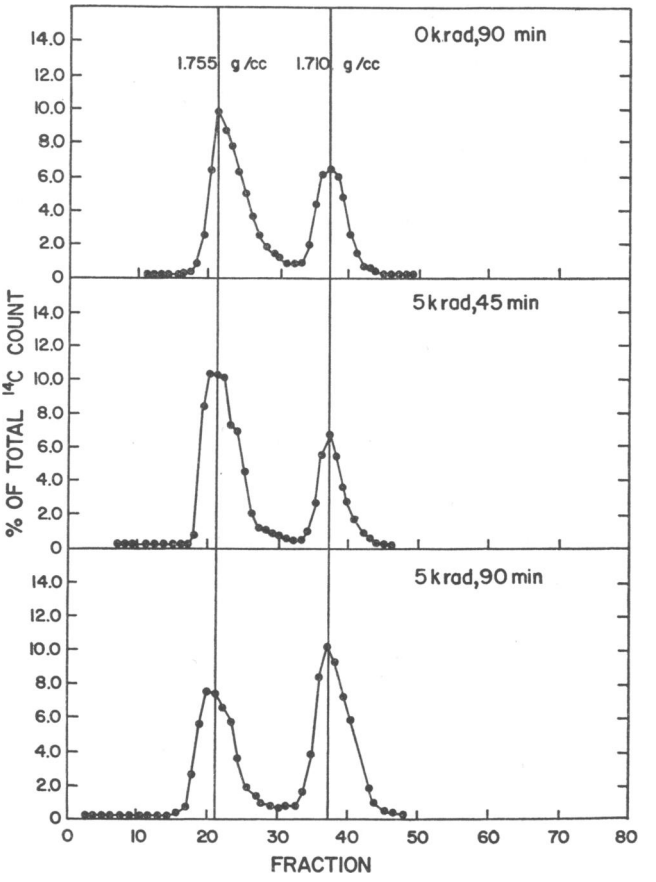

FIGURE 1 CsCl banding patterns of DNA from X-irradiated *E. coli* 15T⁻ (555–7) shifted during postirradiation incubation into a Bu medium. Cells were labeled as described in the text. At the time of X-irradiation all cellular DNA was ^{14}C-labeled. After exposure to 5 krad of X-rays (~40% survival) incubation was continued in fully supplemented thymine-containing medium (no radioisotopic label). At the times indicated the cells were harvested, washed, and shifted to a fully supplemented medium in which Bu (10 μg/ml) had been substituted for thymine. Shown is the DNA banding pattern, observed in CsCl gradients, with lysates obtained from cells incubated for *30 min* in the Bu medium. The dose and the time (min) of postirradiation transfer to the Bu medium are given in the upper right-hand corner of each frame.

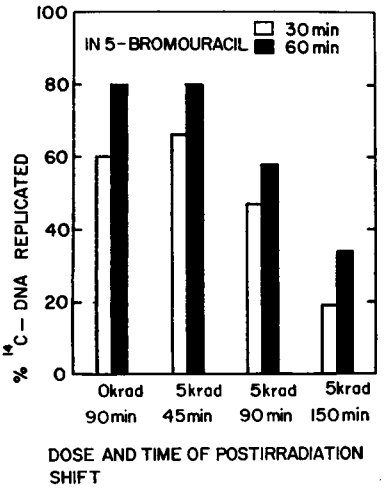

FIGURE 2 Ability of parental DNA to undergo additional cycles of replication with increasing postirradiation incubation times. At the times shown on the abscissa the irradiated cells were shifted to the Bu medium and sampled at 30 or 60 min. The per cent parental DNA replicated was determined from that portion of the ^{14}C-DNA appearing in the hybrid density position, as illustrated in Fig. 1. These data and those of Fig. 1 are from the same experiment.

was labeled by growth for several generations in thymine-^{14}C prior to exposure. This strain of *E. coli* has a division cycle and a DNA doubling time of about 45 min in MS-7 medium containing glucose.

Immediately after irradiation the cells were further incubated in MS-7 medium. At intervals (45, 90, and 150 min or approximately 1, 2, and 3.3 DNA replication cycle equivalents), a portion of the culture was shifted to a Bu medium. After an additional 30 or 60 min incubation in the Bu medium, 5 ml of the cell culture was harvested, the cells lysed, and subjected to isopycnic analysis in CsCl.

Typical CsCl density-gradient profiles from one such experiment are illustrated in Fig. 1. As seen in Fig. 2, unirradiated cells have replicated only 80% of their parental DNA in 60 min. This slower rate of DNA replication is expected as Bu substitution for thymine reduces the rate of DNA replication (2). Following exposure to 5 krad of X-rays, parental DNA is replicated at least once in that it behaves like the unirradiated control when shifted after 45 min to the Bu medium (Figs. 1 and 2).

However, as the time between irradiation and the density shift is increased (during which time one or more postirradiation cycles of replication are completed), less of the parental DNA is able to undergo additional replication during the density shift as illustrated in Figs. 1 and 2. By comparing the irradiated with the control data, it would appear that the parental DNA undergoes one postirradiation cycle of replication before dampening of replication capacity is observed.

The unirradiated cells (0 dose) incubated for 90 min before the shift into Bu medium, were still able to replicate the bulk of their DNA at the expected rate (Figs. 1 and 2, 0 krad, 90 min). Thus, the dampening of replication cycles observed in the irradiated population was not the result of attaining stationary phase conditions.

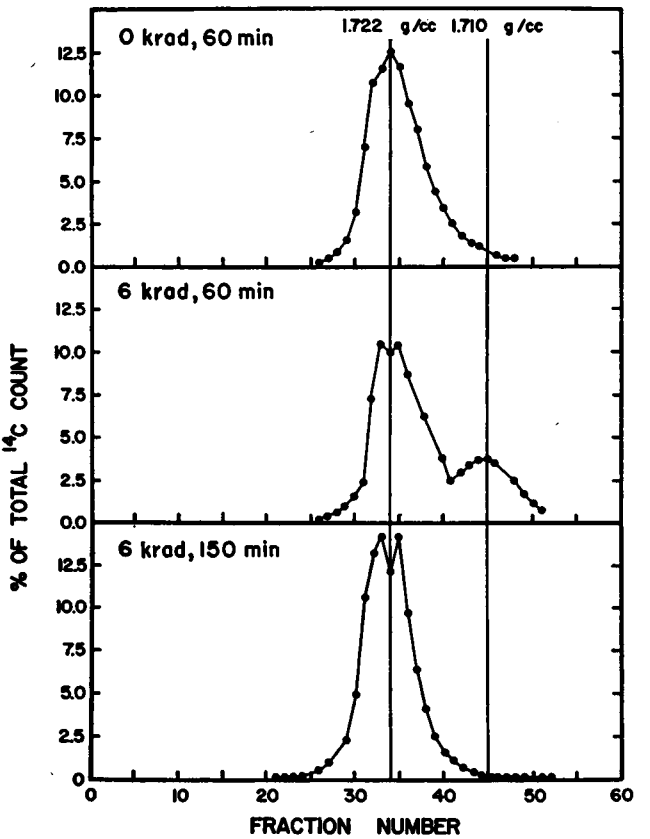

FIGURE 3 CsCl banding patterns of DNA from X-irradiated *E. coli* 15T⁻ (555–7) shifted during postirradiation incubation into a ^{15}N, ^{13}C medium. Cells were grown in ^{14}N, ^{13}C medium in the presence of thymine-^{14}C as described in the text. After exposure to 6 krad of X-rays the cells were added to a fully supplemented ^{15}N, ^{13}C medium. Shown is the DNA banding pattern observed in CsCl gradients with lysates obtained from cells growing in the ^{15}N, ^{13}C medium for the indicated times. The dose and then the length of incubation time in the heavy medium are given in the upper left-hand corner of each frame.

Postirradiation DNA Cycling Patterns in E. coli *15T⁻ (555–7) and* E. coli *B/r (hcr+); Use of ^{15}N, ^{13}C as Density Label*

X-Irradiation: E. coli *15T⁻ (555–7).* To determine which round of DNA replication first exhibited inhibition, the following experiments were carried out using the ^{15}N, ^{13}C density system (Bu toxicity is usually strongly expressed in second rounds [5] and, therefore, is not an appropriate density label in these experiments). *E. coli* were grown for several generations in light medium containing thymine-^{14}C. When the population reached $\sim 2 \times 10^8$ cells/ml the cells were harvested, washed, and a portion exposed to 6 krad of X-rays. Another portion was identically treated

except for X-ray exposure. The cells were then shifted to heavy medium immediately after exposure. The per cent of parental ^{14}C-labeled DNA replicated as ascertained by determining that portion of the ^{14}C-labeled DNA shifted to the hybrid density position in a CsCl gradient. As expected, all the parental DNA in the unirradiated cells was replicated at least once in 60 min (Fig. 3, 0 krad, 60 min). About 75% of the parental DNA in the irradiated population was replicated in a similar time interval (Fig. 3, 6 krad, 60 min). However, upon further incubation the remaining unreplicated parental DNA was seen to replicate (Fig. 3, 6 krad, 150 min).

To determine whether all the parental DNA that replicated once would complete a second cycle, irradiated ^{14}C-labeled cells were incubated for 45 min in light medium and then shifted into heavy medium (no ^{14}C-thymine during postirradiation period). The results are summarized in Table I. During the initial 45 min in heavy medium, about ⅔ of the parental DNA was replicated. In the next 90 min another 16% was replicated. However, at least 20% remained unreplicated during an additional generation time. Thus, dampening of DNA replication in this strain, at the X-ray dose used, starts during a second postirradiation round.

X-Irradiation of E. coli *B/r.* To determine if the altered behavior of DNA was unique to *E. coli* 15T$^-$ (555–7), these studies were extended to E. coli B/r (hcr+). The general plan was similar to that described for the 555–7 study. Parental DNA was ^{14}C-labeled during growth of the bacteria in light medium containing thymine-^{14}C. At various times *postirradiation* incubation in light medium (thymine-^{14}C omitted), portions of the culture were shifted to an ^{15}N, ^{13}C medium and incubated for an additional 30 or 60 min for analysis of DNA replicative capacity.

In this strain, after 6 krad of X-rays and immediate postirradiation shift to heavy medium, only about 60% of parental DNA replicated during a 60 min incubation (Fig. 4, 6 krad, 0 min) as compared to 90% in the unirradiated controls (Fig. 4, 0

TABLE I

REPLICATION OF PARENTAL DNA FOLLOWING X-IRRADIATION OF *Escherichia coli* 15T$^-$ (555-7)

Time in ^{15}N, ^{13}C medium	Per cent parental DNA replicated in ^{15}N, ^{13}C medium when density shift delayed 45 min postirradiation*
(*min*)	
45	61.4
90	77.4
135	81.4
180	80.7

* 100% of control parental DNA replicated in 45 min in the ^{15}N, ^{13}C medium.

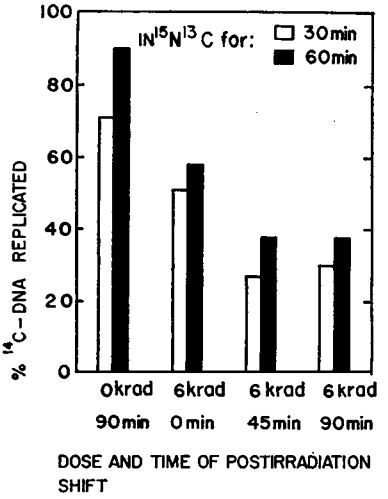

FIGURE 4 Ability of parental DNA to undergo additional cycles of replication during postirradiation incubation of X-irradiated *E. coli* B/r (hcr+). Parental DNA was ^{14}C, ^{14}N, ^{12}C-labeled by several generations growth of the bacteria in an ^{14}N, ^{12}C medium supplemented with thymine-^{14}C. Following harvesting and washing, a portion of the cells were then exposed to 6 krad of X-rays. Incubation was then continued in ^{14}N, ^{12}C medium. At the indicated times portions of the population were shifted to ^{15}N, ^{13}C medium. After 30 or 60 min in the ^{15}N, ^{13}C medium, samples were removed, the cells lysed, and the lysate analyzed by CsCl gradients. The bars represent the per cent parental DNA replicated as determined by that portion of the ^{14}C label appearing in the hybrid DNA. In this experiment 18% of the irradiated cells survived.

krad, 90 min). With increasing delays between irradiation and the density shift, decreasing amounts of parental DNA were capable of further rounds of replication.

UV Irradiation of E. coli *B/r (hcr+).* A basically similar but slightly modified experiment on UV effects was conducted in which replication of postirradiation progeny DNA would be detected. The radioisotopic and density labels were as described for the X-ray study. However, during the postirradiation density shift, thymine-^{3}H was also present. Whereas in the previously described experiments there was no radioisotopic label during the density shift, and, therefore, no identifying radioisotope in the heavy strand of the hybrid DNA, in this experiment all DNA synthesized during ^{15}N^{13}C incubation was ^{3}H-labeled. Unreplicated parental DNA (light) should contain only ^{14}C label. (^{3}H label due to repair synthesis would not be detectable here because of the low specific activity used.) The immediate progeny native DNA (hybrid) should contain ^{14}C and ^{3}H label in the parental (light) and daughter (heavy) strands respectively. One-half of the progeny of a second round of postirradiation DNA replication in the ^{3}H, ^{15}N, ^{13}C medium should contain DNA heavy and ^{3}H-labeled in both strands.

When unirradiated control cells (0 dose) were incubated for 90 min in light medium and then shifted to heavy medium, a round of replication was essentially completed in 45 min (Fig. 5, 0 ergs, 90 min). Exposed cells (200 ergs/mm^2) shifted immediately to the heavy medium after irradiation replicated about 2/3 of their DNA in 90 min (Fig. 5, 200 ergs, 0 min). In addition, a second cycle of replication had been initiated as evidenced by the presence of DNA in a heavy-band position (both strands heavy, banding at a density of 1.734 g/cc).

If, however, the exposed cells were held in light medium during the initial 90 min postirradiation period to allow at least one replication cycle (data of Fig. 5, 200 ergs, 0 min shows that a second round of replication is well underway for a portion of the

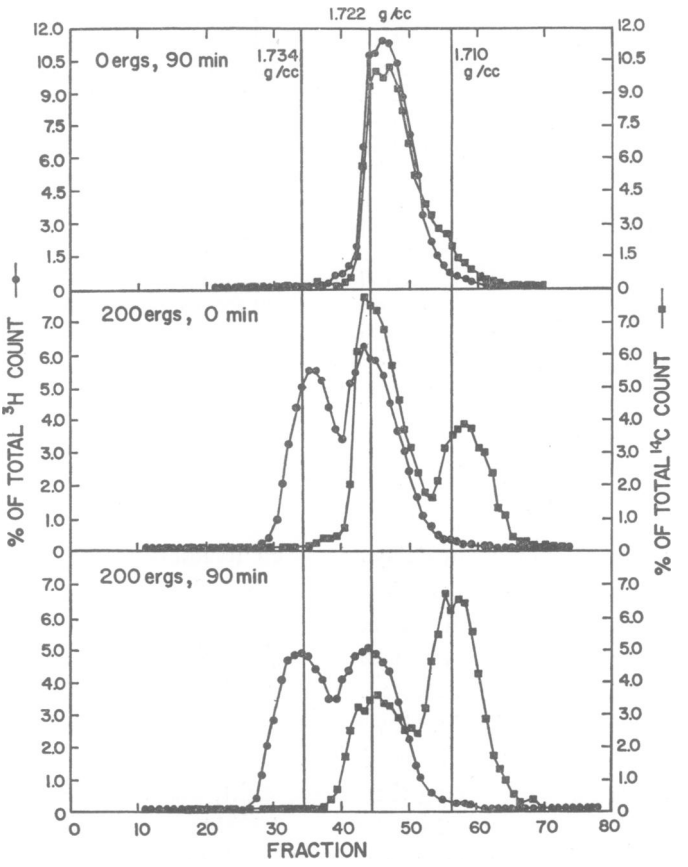

FIGURE 5 CsCl banding patterns of DNA from UV exposed *E. coli* B/r (hcr+) shifted after postirradiation incubation into a ^{3}H, ^{15}N, ^{12}C medium. At the time of irradiation, all parental DNA was ^{14}C, ^{14}N, ^{12}C-labeled. After exposure to 200 erg/mm^{2} (40% survival) incubation was continued in ^{14}N, ^{12}C medium. At the times indicated in the left-hand corner of each frame the cells were shifted to an ^{15}N, ^{12}C medium containing thymine-^{3}H. The unirradiated cells were incubated for 45 min and the irradiated cells for 90 min in the heavy medium before harvesting and lysis.

DNA by the end of the initial 90 min), and then were shifted to the heavy medium, then only ⅓ of the parental DNA strands replicated during the subsequent 90 min in the heavy medium (Fig. 5, 200 ergs, 90 min). Since ⅔ of the parental DNA replicated during the first 90 min, only half (or less, since some of the prelabeled DNA might be replicated for the first time during the final 90 min incubation) of the progeny of the initial cycle was capable of undergoing a second cycle. As before, some of the DNA replicated during the 90 min density shift had undergone a second replication cycle. The per cent of parental DNA replicated during the density shift is summarized in Table II.

TABLE II

REPLICATION OF PARENTAL DNA FOLLOWING UV EXPOSURE OF *Escherichia coli* B/r

Postirradiation shift	Per cent replicated in ^{15}N, ^{13}C*	
min	*45 min*	*90 min*
0	39	63
45	—	37
90	25	36

* 100% of control (0 erg) parental DNA replicated in 45 min.

DISCUSSION

The experiments described here show that DNA replicated once after irradiation exposure may fail to undergo additional rounds of synthesis. The extent of this dampening effect would appear to be strain dependent. Thus, in X-irradiated *E. coli* (555–7), DNA will undergo one cycle of replication before dampening is observed, whereas in *E. coli* B/r, dampening of DNA replication is seen during the initial postirradiation cycle and continued through a second cycle (however, with the latter strain it is possible that incubation times beyond 90 min would allow additional postirradiation replication of parental DNA but at a much reduced rate, see Fig. 4). In addition, at least a portion of the parental DNA destined to eventually replicate is more slowly replicated. Either the travel time for a length of DNA which the replication fork traverses is increased (reduced rate) in a given time, or there are several classes of DNA with variable travel times.

These observations on initial postirradiation DNA replication with UV irradiated *E. coli* B/r are similar to those reported for the same strain by Doudney and Young (1). Using the analytical centrifuge, these investigators observed that a portion of the parental DNA did not undergo any postirradiation replication.

The implications of these observations are severalfold. 1. They are especially germaine to interpretation of the biological significance of data from experiments on (*a*) semiconservative replication of "repaired" template DNA strands (3) and (*b*) replication of template between dimers and subsequent gap closing in the initial progeny strand (6). 2. Lethal sectoring observed with irradiated *E. coli* (7) may in part reflect failure to provide adequate genetic information to daughter cells.

The failure of DNA replicated once to undergo continued serial replication may help explain cell death in "resistant" cells, i.e., those cells containing repair systems. In this regard, the dampening of DNA replication postirradiation could involve template, initiators, membrane attachment, DNA conformation, etc. The possible role of alternate initiation sites (8) and abnormally methylated nascent DNA (9) in postirradiation dampening effect is currently being considered in our laboratory. Our approach is based on the belief that aberrant postirradiation DNA replication is in

part due to abnormalities in regulatory operations concerned with insuring the controlled production of a functionally sequenced genome.

This investigation was supported by Contract AT-(40-1)-3596 from the U. S. Atomic Energy Commission.

Received for publication 28 October 1969 and in revised form 12 January 1970.

REFERENCES

1. DOUDNEY, C. O., and C. S. YOUNG. 1962. *Genetics.* **47**:1125.
2. HEWITT, R., D. BILLEN, and G. JORGENSEN. 1967. *Radiat. Res.* **32**:214.
3. HANAWALT, P. 1967. *Nature (London).* **214**:209.
4. BILLEN, D. 1959. *Biochim. Biophys. Acta.* **34**:110.
5. HEWITT, ROGER, JOAN C. SUIT, and DANIEL BILLEN. 1967. *J. Bacteriol.* **93**:86–89.
6. RUPP, W. D., and P. HOWARD-FLANDERS. 1968. *J. Mol. Biol.* **31**:291.
7. HAEFNER, K., and U. STRIEBECK. 1967. *Mutat. Res.* **4**:399.
8. BILLEN, D. 1969. *J. Bacteriol.* **97**:1169.
9. WHITFIELD, B. L., and D. BILLEN. 1969. Proceedings of the Annual Meeting of the American Society for Microbiology. The American Society for Microbiology, Washington, D. C. 65.