Photochem. Photobiol. Vol. 36, pp. 307 to 311, 1982
Printed in Great Britain.

0031-8655/82/090307-05$03.00/0

POSTREPLICATION REPAIR IN *uvrA* AND *uvrB* STRAINS OF *ESCHERICHIA COLI* K-12 IS INHIBITED BY RICH GROWTH MEDIUM

RAKESH C. SHARMA, THOMAS R. BARFKNECHT* and KENDRIC C. SMITH†
Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305, USA

(*Received* 24 *February* 1982; *accepted* 22 *April* 1982)

Abstract—*Escherichia coli* K-12 *uvrA* or *uvrB* strains grown to logarithmic phase in minimal medium showed higher survival after ultraviolet (UV) irradiation (254 nm) if plated on minimal medium (MM) instead of rich medium. This 'minimal medium recovery' (MMR) was largely blocked by additional *recA56* (92% inhibition) or *lexA101* (77%) mutations, was partially blocked by additional *recB21* (54%), *uvrD3* (31%) or *recF143* (22%) mutations, but additional *polA1* or *polA5* mutations had no effect on MMR. When incubated in MM after UV irradiation, the *uvrB5* and *uvrB5 uvrD3* strains showed essentially complete repair of DNA daughter-strand gaps (DSG) produced after UV radiation fluences up to $\sim 6\ J/m^2$ and $\sim 1\ J/m^2$, respectively, and then they accumulated unrepaired DSG as a linear function of UV radiation fluence. However, when they were incubated in rich growth medium *after* UV irradiation, they did not show the complete repair of DSG and unrepaired DSG accumulated as a linear function of UV radiation fluence. The fluence-dependent correlation observed for the *uvrB* and *uvrB uvrD* cells between UV radiation-induced killing and the accumulation of unrepaired DSG, indicates that the molecular basis of MMR is the partial inhibition of postreplication repair by rich growth medium. Rich growth medium can be just MM plus Casamino Acids or the 13 pure amino acids therein in order to have an adverse effect on survival, regardless of whether the cells were grown in rich medium or not before UV irradiation.

INTRODUCTION

Ultraviolet (UV)-irradiated, minimal medium-grown *uvrA*, *uvrB* and *uvrC* cells of *Escherichia coli* K-12 show a greater survival when plated on minimal medium than when plated on rich medium (Ganesan and Smith, 1968a, b). This higher survival on minimal medium plates has been referred to as 'minimal medium recovery' (MMR)‡ (Ganesan and Smith, 1968b, 1970; Smith, 1971). Since *uvrA*, *uvrB* and *uvrC* strains of *E. coli* are unable to excise pyrimidine dimers from their DNA (Howard-Flanders *et al.*, 1966), in the absence of photoreactivation they must rely on postreplication repair for recovery from UV radiation-produced DNA damage (Rupp and Howard-Flanders, 1968). This suggests that MMR is a postreplication repair phenomenon, and that rich growth medium may inhibit some step(s) in postreplication repair in excision repair-deficient strains of *E. coli*.

Postreplication repair [i.e. the repair of DNA daughter-strand gaps (DSG) produced after UV irradiation] is inhibited by a *recA* mutation in uvr^+ or uvr^- cells (Smith and Meun, 1970; Sedgwick, 1975a). In the *uvrA* and/or *uvrB* backgrounds, *recB* and *uvrD* (Youngs and Smith, 1976a), *lexA* (Sedgwick, 1975b; Youngs and Smith, 1976a), *polA* (Barfknecht and Smith, 1978), and *recF* (Ganesan and Seawell, 1975; Rothman and Clark, 1977b; Kato, 1977) mutations partially inhibit postreplication repair. Postreplication repair has been divided into two major independent pathways (both require a functional *recA* gene); one is dependent on the *recF* gene and the other is dependent on the *recB*, *lexA* and *uvrD* genes (Wang and Smith, 1981).

The intent of this study was to determine the genetic control of MMR in excision repair-deficient strains of *E. coli* K-12 in order to know whether MMR depends upon one or both major pathways of postreplication repair, and to determine if the molecular basis of MMR is the partial inhibition of postreplication repair by rich growth medium.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* K-12 strains used are listed in Table 1. The technique used for bacteriophage P1 transduction was essentially that of Miller (1972).

Media and growth conditions. The minimal medium (MM) is a salts buffer (DTM) (Ganesan and Smith, 1968a) supplemented to a final concentration of 0.4% glucose,

*Present address: Research Triangle Institute, Chemistry and Life Sciences Group, P.O. Box 12194, Research Triangle Park, NC 27709, USA.
†To whom correspondence should be addressed.
‡*Abbreviations*: AA, amino acids; CAA, casamino acids; DSG, daughter-strand gaps; DTM, salt buffer; MM, minimal medium; MMR, minimal medium recovery; M_n, number average molecular weight; YENB, yeast extract-nutrient broth.

Table 1. List of strains of *E. coli**

Stanford Radiology No.	Relevant genotype	Other genotype	Source, derivation or reference
SR144	*polA1*	*rha-5 lacZ53 thyA36 deo(C2?) rpsL151*	J. D. Gross (JG137)
SR256	*uvrB5 recA56*	*rha-5 lacZ53 metE70 leuB19 deo(C2?) rpsL151*	Youngs and Smith (1976a)
SR257	*uvrB5 recB21*	Same as SR256	Youngs and Smith (1976a)
SR281	*uvrB5*	*rha-5 lacZ53 leuB19 thyA36 deo(C2?) rpsL151*	Youngs and Smith (1976a)
SR282	*uvrB5 uvrD3*	Same as SR281	Youngs and Smith (1976a)
SR283	*uvrB5 lexA101*	Same as SR281	Youngs and Smith (1976a)
SR349	*uvrA6*	*rha-5 lacZ53 leuB19 bioA2 metE70 thyA36 deo(C2?) rpsL151*	Youngs and Smith (1978)
SR552	*uvrB5*	Same as SR256	Exconjugant with SR256
SR553	*uvrB5*	Same as SR256	Cotransductant of SR257
SR788	*polA5*	*lacZ53 thyA36 deo(C2?) rpsL151*	CM4050, *E. coli* Genetic Stock Center
SR988†	*uvrA6*	*rha-5 lacZ53 leuB19 bioA2 thyA36 deo(C2?) rpsL151*	P1::Tn*9*cts·SR144 × SR349 (Select Met$^+$)
SR989†	*uvrA6 polA1*	Same as SR988	Same as SR988
SR1019	*uvrA6*	Same as SR988	P1::Tn*9*cts·SR788 × SR349 (select Met$^+$)
SR1020	*uvrA6 polA5*	Same as SR988	Same as SR1019
SR1040	*uvrB5*	Same as SR281	T. V. Wang
SR1041	*uvrB5 recF143*	Same as SR281	T. V. Wang

*Genotype symbols are those used by Bachmann and Low (1980). All strains are F$^-$ and λ^-. Met$^+$ means that transductants were selected for methionine prototrophy.

†Strain constructed by Dr. N. J. Sargentini.

10 μg/mℓ thymine (if required), 0.5 μg/mℓ thiamine·HCl, and each required amino acid at 1 m*M*. Minimal plating medium was MM solidified with 1.6% (wt/vol) Difco Noble agar. The rich plating medium (YENB plates) was Difco yeast extract (0.75%) plus Difco nutrient agar (2.3%). YENB liquid medium was Difco yeast extract (0.75%) and Difco nutrient broth (0.8%). When desired, Difco Casamino Acids (CAA, vitamin assay grade) were added to the minimal plating medium at a final concentration of 2 mg/mℓ (CAA plates). CAA are about 50% amino acid by weight and the relative ratios of the weights of the amino acids in CAA are arginine (1.00), aspartic acid (2.11), glutamic acid (6.63), glycine (0.68), histidine (0.74), isoleucine (1.53), leucine (2.11), lysine (2.32), methionine (0.57), phenylalanine (1.05), threonine (1.16), tyrosine (0.27) and valine (2.0) (Difco Laboratories, personal communication). Pure amino acids were mixed in these proportions and added to MM plates at a final concentration of 1 mg/mℓ (13AA plates).

Logarithmic phase cultures were obtained by diluting an overnight culture either 50- or 100-fold into fresh minimal medium containing thymine at 2 μg/mℓ (if required), followed by incubation in a shaking waterbath at 37°C. Cultures were grown to an optical density at 650 nm (OD_{650}) (Zeiss PMQ II spectrophotometer) of 0.4 ($\sim 3 \times 10^8$ cells/mℓ), harvested by centrifugation (5000 *g* for 7 min), washed twice with 0.067 *M* NaK-phosphate buffer (PB), pH 7.0 (Wang and Smith, 1981), and resuspended in PB to an OD_{650} of 0.1 ($\sim 0.8 \times 10^8$ cells/mℓ).

Irradiation. The method, source, correction for sample absorption and dosimetry for UV irradiation were as described (Sargentini and Smith, 1979). The samples (3.2 mℓ) of cells were irradiated (254 nm) at room temperature in uncovered 60 mm Pyrex Petri dishes on a rotary shaker. All experiments were performed under General Electric 'gold' fluorescent lights to prevent photoreactivation.

Survival determination. Appropriate dilutions of control and UV-irradiated cells were prepared in PB, and 0.1 mℓ samples were spread on plates. YENB, CAA and 13AA plates were incubated at 37°C for 24 h, while MM plates were incubated for 48 h before the survivors were scored.

Determination of postreplication repair. The DNA of logarithmic phase MM-grown cells was pulse labeled by adding 1.0 mℓ samples of cells that had been UV irradiated in DTM ($\sim 2 \times 10^8$ cells/mℓ) to 1.0 mℓ of DTM containing twice the normal concentration of organic supplements (omitting thymine), and [methyl-^{3}H]-thymidine (Amersham, 50 Ci/mmol) at a final concentration of 0.6 μg/mℓ (125 μCi/mℓ). After labeling for 10 min in a shaking waterbath at 37°C, a 1 mℓ sample was collected by filtration (Millipore, 0.45 μm pore size), washed with 5 mℓ of MM and resuspended in 1 mℓ of MM. Another 1 mℓ sample was collected similarly and resuspended in 1 mℓ of YENB. Both cultures were then incubated in a shaking waterbath at 37°C for 80 min before being converted to spheroplasts. No additional repair was observed in either MM or YENB for incubation times longer than 80 min (i.e. up to 150 min) (data not shown).

The method for spheroplast formation and lysis on alkaline sucrose gradients was that of Smith and Meun (1970), except that the final concentration of lysozyme (Worthington Biochemical Corp.) in the spheroplasting mixture was 100 μg/mℓ, and the number of cells layered onto the gradients was $\sim 4 \times 10^6$. The layered gradients were held for ~50 min in the dark at room temperature before being centrifuged at 20°C at 10000 rpm for 16 h (21000 rpm for 220 min for strain SR282) in SW 50.1 rotors in either a Beckman Model L2 or L2-65B ultracentrifuge. Bacteriophage T2, with [2-^{14}C]-thymine (New England Nuclear) labeled DNA, was used as a molecular weight marker in each centrifuge run. The procedures for producing and processing the gradients, and the calculation of the number average molecular weights (M_n) of the DNA samples have been described (Hamelin *et al.*, 1976; Youngs and Smith, 1976a, b).

RESULTS

We investigated the effect of various plating media on the UV radiation survival of logarithmic phase,

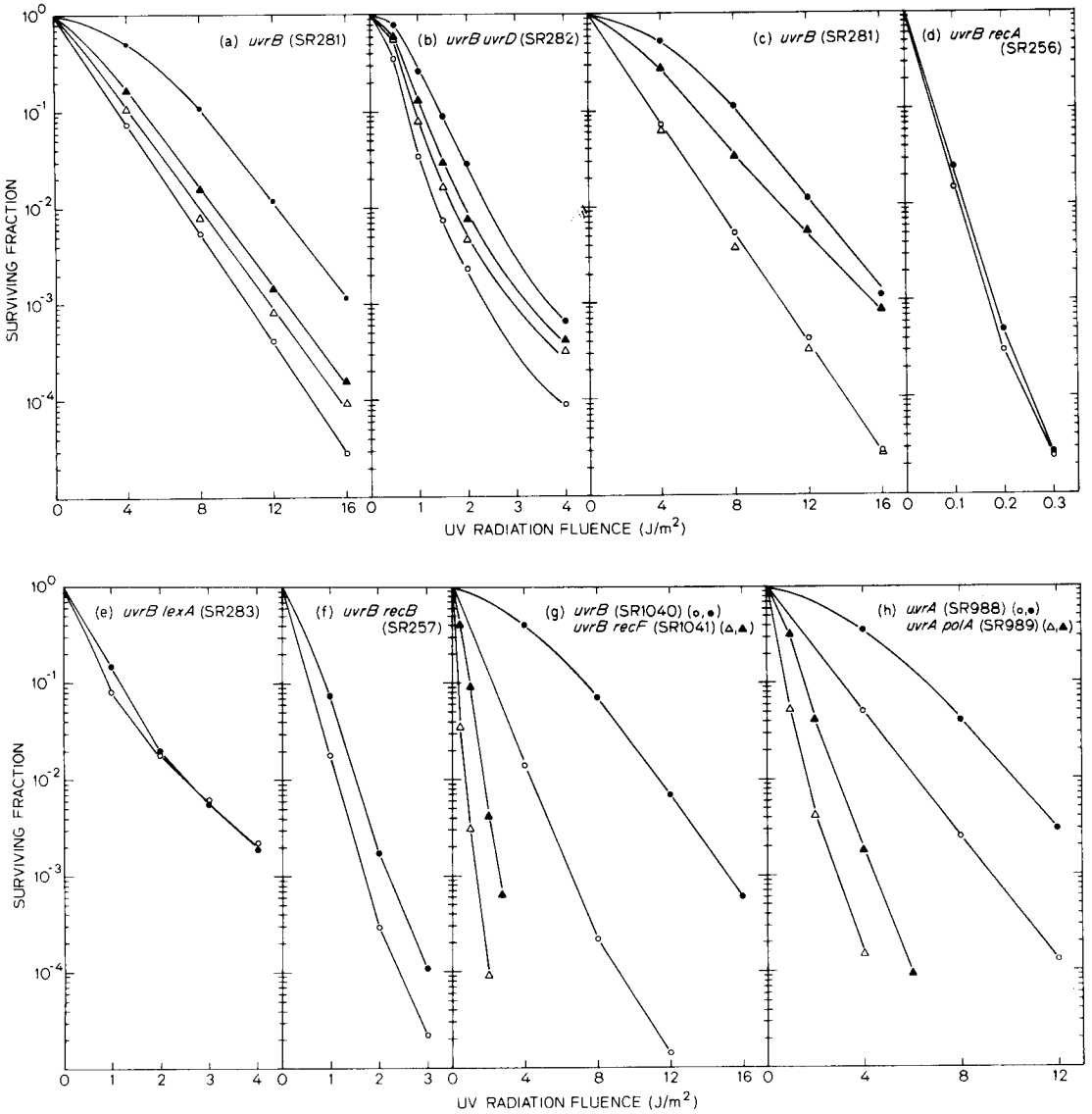

Figure 1. UV radiation survival curves of DNA repair-deficient strains of *E. coli* that were grown to logarithmic phase in MM, irradiated in PB, and plated on MM (●) and on YENB (○). The difference in survival on the two types of plates was used to calculate the amount of minimal medium recovery exhibited by the several strains (Table 2). (a) *uvrB5* (SR281)—cells were also plated on MM supplemented with Casamino Acids (△) and on MM supplemented with 13 amino acids at the concentrations reported for Casamino Acids (▲), (b) *uvrB5 uvrD3* (SR282)—MM + Casamino Acids (△) and MM + 13 amino acids (▲), (c) *uvrB5* (SR281)—cells were grown in MM, UV irradiated in PB and plated on MM (●) and YENB (○), or cells were grown in YENB and plated on MM (▲) and YENB (△), (d) *uvrB5 recA56* (SR256), (e) *uvrB5 lexA101* (SR283), (f) *uvrB5 recB21* (SR257), (g) *uvrB5* (SR1040) and *uvrB5 recF143* (SR1041), (h) *uvrA6* (SR988) and *uvrA6 polA1* (SR989). Each data point represents the average of at least two experiments.

minimal medium-grown *uvrB* cells (SR281). The F_{10} (fluence to yield 10% survival) for cells on YENB plates was reduced 2.3-fold compared to that on MM plates (Fig. 1a and Table 2). The F_{10} was reduced 1.9-fold when assayed on CAA plates, and 1.6-fold when assayed on 13AA plates. Similar results (1.9-, 1.5- and 1.3-fold, respectively) were obtained for the *uvrB uvrD* strain (Fig. 1b).

To determine whether the medium in which cells are grown *before* irradiation has an effect on MMR, *uvrB* cells (SR281) were grown in YENB and MM and tested for their ability to demonstrate MMR. The cells grown in YENB showed less MMR than the cells grown in MM (Fig. 1c). This decrease in MMR for YENB-grown cells was the result of a decreased survival on MM plates, as both the MM-grown and YENB-grown cells exhibited the same survival on YENB plates (Fig. 1c). Similar results were obtained for the *uvrB uvrD* strain (data not shown).

As a further test of this pregrowth phenomenon, *uvrB* cells (SR281) were grown to logarithmic phase in MM plus 13AA, UV irradiated and then plated on

Table 2. Genetic control of MMR

Stanford Radiology No.	Relevant genotype	F_{10}* (J/m²) MM	F_{10}* (J/m²) YENB	Amount of MMR†	% Inhibition of MMR‡
SR988	*uvrA6*	6.50	3.06	2.1	0
SR989	*uvrA6 polA1*	1.59	0.76	2.1	0
SR1019§	*uvrA6*	5.80	2.60	2.2	0
SR1020§	*uvrA6 polA5*	2.60	1.20	2.2	0
SR281	*uvrB5*	8.13	3.52	2.3	0
SR282	*uvrB5 uvrD3*	1.44	0.77	1.9	31
SR283	*uvrB5 lexA101*	1.18	0.92	1.3	77
SR553§	*uvrB5*	6.93	3.01	2.3	0
SR257	*uvrB5 recB21*	0.90	0.57	1.6	54
SR552§	*uvrB5*	8.45	3.76	2.3	0
SR256	*uvrB5 recA56*	0.062	0.057	1.1	92
SR1040	*uvrB5*	7.33	2.24	3.3	0
SR1041	*uvrB5 recF143*	0.97	0.34	2.8	22

*The F_{10} (determined from the data in Fig. 1) is the UV radiation fluence that is required to inactivate 90% of the cell population.

†MMR was calculated by dividing the F_{10} for cells plated on MM by the F_{10} for cells plated on YENB. A value of 1.0 indicates the absence of MMR. Because of the variability (as yet unexplained) in the absolute amount of MMR between the various *uvr* strains, we have always compared the MMR of a double mutant with a closely related *uvr* strain.

‡Calculated by dividing the MMR value for the double mutant by the MMR value for the appropriate single mutant after subtracting 1.0 from each value, and subtracting this value from 1.0 and multiplying by 100.

§Data are from the average of two survival curves (not shown).

the various types of plates (data not shown). The survival results on YENB plates were identical to those shown in Fig. 1a. The relative survival on CAA, 13AA and MM plates was the same, although the absolute survival was increased slightly above that shown in Fig. 1a. Therefore, the pure 13AA added to MM plates had an adverse effect on survival whether or not the cells were grown in the presence of the 13AA *before* UV irradiation.

To determine the genetic control of MMR, *uvrA* or *uvrB* strains carrying an additional mutation in genes that affect postreplication repair were tested for their ability to demonstrate MMR (Table 2). An additional *recA* mutation resulted in a 92% inhibition of MMR (Fig. 1d), *lexA* a 77% inhibition (Fig. 1e), *recB* a 54% inhibition (Fig. 1f), *uvrD* a 31% inhibition (Fig. 1b), *recF* a 22% inhibition (Fig. 1g) and *polA* no inhibition of MMR (Fig. 1h).

To test the effect of postirradiation growth medium on postreplication repair, MM-grown cells were UV irradiated in DTM, pulse labeled in MM and then incubated in either MM or YENB. The *uvrB* (Fig. 2a) and *uvrB uvrD* (Fig. 2b) cells that were incubated in MM showed essentially complete repair of DSG up

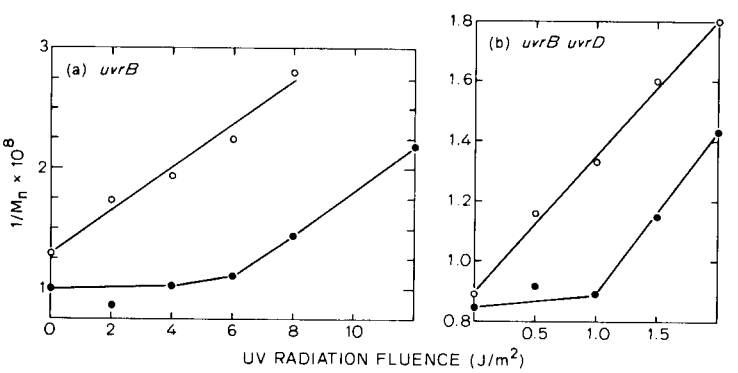

Figure 2. Repair of DNA daughter-strand gaps in UV irradiated *E. coli*. (a) *uvrB5* (SR281) cells were grown in MM, UV irradiated in DTM, and pulse labeled with [^{3}H]-thymidine in MM, but were allowed to repair in MM (●) or YENB (○) medium for 80 min at 37°C. The reciprocal of the number average molecular weight (M_n) (which is related to the number of unrepaired DNA daughter-strand gaps) is plotted as a function of UV radiation fluence. The data are from a representative experiment. (b) *uvrB5 uvrD3* (SR282) cells were treated as in (a). Each data point is the average of two experiments.

to UV radiation fluences of ~6 J/m^2 and ~1 J/m^2, respectively and then accumulated unrepaired DSG as a linear function of UV radiation fluence. The cells incubated in YENB accumulated unrepaired DSG in a linear manner over the whole fluence range.

DISCUSSION

As an explanation for the phenomenon of MMR, Ganesan and Smith (1968a, b; 1970) suggested that rich growth medium (YENB, as in the present experiments) may contain a component that is inhibitory to the recovery of UV-irradiated excision repair-deficient cells. A major component of YENB is hydrolyzed proteins, and much of the YENB effect could be duplicated by adding Casamino Acids or 13 pure amino acids to MM plates (Fig. 1a, b). These results suggested that the enhanced lethality seen on rich medium plates might be due to a 'shift-up' in growth medium. However, when the *uvrB* cells were grown in YENB and plated on YENB, they showed the same low survival after UV irradiation as did cells grown in MM and plated on YENB (Fig. 1c). Thus, the enhanced lethality observed on rich medium plates is not a shift-up phenomenon.

When *uvrB* cells were grown in YENB, UV irradiated and plated on MM vs. YENB (Fig. 1c) or were grown in MM plus 13AA, UV irradiated and plated on MM vs. 13AA plates (data not shown), an enhanced survival was observed on MM plates. Such an increase in survival has been called 'shift-down recovery' (Witkin, 1967; Rothman and Clark, 1977a). Perhaps this is a better description of what we have called MMR. In any event, our data indicate that the mere presence of a mixture of nonrequired amino acids in the growth medium *after* UV irradiation (whether or not these amino acids were present in the growth medium before UV irradiation) can reduce cell survival.

In addition, the presence of rich medium *after* UV irradiation inhibits postreplication repair. There is a good correlation between the fluence-dependency for survival and for the repair of DSG for the *uvrB* (cf. Figs. 1a and 2a) and *uvrB uvrD* (cf. Figs. 1b and 2b) strains when incubated in MM or YENB after UV irradiation. That is, the UV radiation fluence at the end of the shoulder region of the survival curve on MM approximates the fluence at the end of the threshold for the 'complete' repair of DSG in MM; the absence of a shoulder on the survival curves in YENB correlates with the absence of a threshold for 'complete' repair in YENB.

We have confirmed (Fig. 1d) that a *recA* mutation blocks MMR (Ganesan and Smith, 1970). Our data for the other DNA repair-deficient strains (Table 2) suggest that, of the two major pathways of postreplication repair (Wang and Smith, 1981), rich growth medium mainly inhibits the *recB* (*lexA*, *uvrD*) pathway, the *recF* pathway being much less affected.

Barfknecht and Smith (1977) have shown that the enrichment of minimal medium with only a small amount of nutrient broth (0.02%, wt/vol), as is frequently used in mutation assay plates when scoring for the reversion of an amino acid auxotroph, is sufficient to inhibit MMR and to enhance Leu$^+$ reversion of the *leuB* missense mutation in *uvrA* and *uvrB* strains of *E. coli* K-12. Taken together with the present results, this suggests that rich growth medium may selectively inhibit a type of error-free postreplication repair in excision repair-deficient strains.

Acknowledgements- We thank Drs. Neil J. Sargentini and Tzu-chien V. Wang for helpful discussions. This research was supported by US Public Health Service research grant CA-02896 and research program project grant CA-10372 from the National Cancer Institute, DHHS. T.R.B. acknowledges the support of a National Institutes of Health National Research Service Award 77-GM-0112 from the National Institute of General Medical Sciences, DHHS.

REFERENCES

Bachmann, B. J. and K. B. Low (1980) *Microbiol. Rev.* **44**, 1–56.
Barfknecht, T. R. and K. C. Smith (1977) *Photochem. Photobiol.* **26**, 643–645.
Barfknecht, T. R. and K. C. Smith (1978) *Mol. Gen. Genet.* **167**, 37–41.
Ganesan, A. K. and P. C. Seawell (1975) *Mol. Gen. Genet.* **141**, 189–205.
Ganesan, A. K. and K. C. Smith (1968a) *J. Bacteriol.* **96**, 365–373.
Ganesan, A. K. and K. C. Smith (1968b) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 235–242.
Ganesan, A. K. and K. C. Smith (1970) *J. Bacteriol.* **102**, 404–410.
Hamelin, C., D. A. Youngs and K. C. Smith (1976) *J. Bacteriol.* **127**, 1307–1314.
Howard-Flanders, P., R. P. Boyce and L. Theriot (1966) *Genetics* **53**, 1119–1136.
Kato, T. (1977) *Mol. Gen. Genet.*, **156**, 115–120.
Miller, J. H. (1972) In *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Rothman, R. H. and A. J. Clark (1977a) *Mol. Gen. Genet.* **155**, 267–277.
Rothman, R. H. and A. J. Clark (1977b) *Mol. Gen. Genet.* **155**, 279–286.
Rothman, R. H., T. Kato and A. J. Clark (1975) In *Molecular Mechanisms for Repair of DNA* (Edited by P. C. Hanawalt and R. B. Setlow), Part A, pp. 283–291. Plenum Press, New York.
Rupp, W. D. and P. Howard-Flanders (1968) *J. Mol. Biol.* **31**, 291–304.
Sargentini, N. J. and K. C. Smith (1979) *J. Bacteriol.* **140**, 436–444.
Sedgwick, S. G. (1975a) *J. Bacteriol.* **123**, 154–161.
Sedgwick, S. G. (1975b) *Proc. Natl. Acad. Sci. USA* **72**, 2753–2757.
Smith, K. C. (1971) *Photophysiology* **6**, 209–278.
Smith, K. C. and D. H. C. Meun (1970) *J. Mol. Biol.* **51**, 459–472.
Wang, T. V. and K. C. Smith (1981) *Mol. Gen. Genet.* **183**, 37–44.
Witkin, E. M. (1967) *Brookhaven Symp. Biol.* **20**, 17–55.
Youngs, D. A. and K. C. Smith (1976a) *J. Bacteriol.* **125**, 102–110.
Youngs, D. A. and K. C. Smith (1976b) *Photochem. Photobiol.* **24**, 533–541.
Youngs, D. A. and K. C. Smith (1978) *Mutat. Res.* **51**, 133–137.