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Repair of Radiation-induced Damage in *Escherichia coli*

I.† Effect of *rec* Mutations on Post-replication Repair of Damage due to Ultraviolet Radiation

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The *rec* mutants of *Escherichia coli* K12 are more sensitive to ultraviolet radiation than corresponding *rec*$^+$ derivatives. We have tested several of these *rec* mutants for post-replication repair after irradiation with ultraviolet light. The mutants carried *recA13*, *recA56*, *recB21* and *recC22*. Cultures growing exponentially were exposed to ultraviolet light (63 ergs/mm^2) and then labeled for ten minutes with [^{3}H]thymidine. The radioactive medium was removed and the culture divided into two portions, one of which was chilled while the other was reincubated in non-radioactive medium for various times. The DNA from the cells was then analysed on alkaline sucrose gradients. Labeled DNA from irradiated cells which had not been reincubated sedimented as short molecules. After reincubation, DNA from the *recA* mutants still behaved as short molecules, while DNA from *rec*$^+$, *recB* and *recC* cells assumed a position similar to that of DNA from unirradiated control cells. These results indicate that the two *recA* mutants tested were defective in their capacity for post-replication of damage induced by ultraviolet radiation. However, no deficiency in the *recB* and *recC* mutants was detected under the conditions used.

Even in *uvr*$^+$ cells, which are capable of excision-dependent repair, the formation of short molecules could be demonstrated immediately after irradiation. However, after a 40-minute post-irradiation incubation before labeling with [^{3}H]thymidine, only "normal-sized" DNA was formed. In contrast, in a *uvr*$^-$ strain, short molecules were still formed after a 90 minute post-irradiation incubation. These results indicate that excision-dependent repair can remove the lesions that lead to the synthesis of short pieces of DNA after ultraviolet-irradiation. Similarly, the exposure of ultraviolet cells to conditions of photoreactivation (which repairs pyrimidine dimers *in situ*) before labeling greatly diminished but did not completely eliminate the synthesis of short pieces of DNA.

1. Introduction

In *Escherichia coli* K12 there appear to be at least two systems for the dark repair of damage caused by ultraviolet radiation. The first to be described has been called excision-dependent repair. This system recognizes pyrimidine dimers (and other lesions) formed in DNA. Dimers opposite an intact region of a complementary strand are removed together with adjoining nucleotides. The excised material is then replaced with normal DNA by localised resynthesis using the undamaged region of the complementary strand as template (see Hanawalt *et al.*, 1968; Strauss, 1968 for reviews). Three genes, *uvrA*‡, *uvrB* and *uvrC* appear to control excision in K12. Cells carrying

† Paper II in this series is Kapp & Smith, 1970.

‡ Abbreviations used: genetic symbols are given in the legend to Table 1.

a mutation in any one of these genes fail to remove pyrimidine dimers from their DNA, and thus cannot undergo repair by this process (Howard-Flanders, Boyce & Theriot, 1966).

Even though *uvr* mutants are deficient in their capacity for excision-dependent repair, and consequently more sensitive to u.v. than corresponding *uvr*$^+$ strains, they still appear to be capable of repairing some u.v.-induced lesions. If cells of *uvr* mutants are plated on minimal medium after u.v. irradiation, a larger proportion survives than if they are plated on a complex medium such as nutrient agar. This effect has been attributed to recovery which can occur on minimal medium, but which is inhibited by complex medium (Ganesan & Smith, 1968*b*). Since recovery is believed to reflect the repair of DNA, this response indicates that *uvr* mutants retain some capacity for repair in minimal medium. In addition, the sensitivity of *uvr* mutants to u.v. can be increased by introducing a *rec* mutation into their genome (Howard-Flanders & Boyce, 1966; Howard-Flanders, Theriot & Stedeford, 1969). The *rec* mutations of *E. coli* K12 have several phenotypic consequences, including decreased capacity for genetic recombination, and increased sensitivity to u.v. and X-irradiation (Clark & Margulies, 1965; Howard-Flanders & Theriot, 1966). Derivatives of K12 containing a *uvr* mutation together with a *rec* mutation are more sensitive to u.v. than those carrying either mutation alone. From this it appears that the *rec* mutations inactivate some system which repairs damage caused by u.v. and that this system is different from the one controlled by the *uvr* genes (Howard-Flanders & Boyce, 1966).

It has been found that excision-deficient mutants can synthesize DNA after u.v. irradiation (Rupp & Howard-Flanders, 1968; Smith, 1969). This DNA appears to contain defects, however, such that when it is analyzed in alkaline sucrose gradients, it assumes a position characteristic of molecules shorter than those made by unirradiated cells (Rupp & Howard-Flanders, 1968; Howard-Flanders, Rupp, Wilkins & Cole, 1968). If the cells are incubated long enough after irradiation these defects in the newly synthesized DNA disappear, and the size of the DNA approximates that from unirradiated controls. The disappearance of defects has been attributed to a post-replication repair process which reconstructs at least one undamaged copy of the genome of each cell by exchanges between sister DNA duplexes. The exchanges envisioned for this type of repair resemble those involved in genetic recombination, and may, therefore, be mediated by some of the same enzymes (Rupp & Howard-Flanders, 1968).

If this were true, one would expect recombination-deficient (*rec*) mutants to be unable to repair the defects formed in DNA after u.v. irradiation (Ganesan & Smith, 1968*b*; Rupp & Howard-Flanders, 1968). In this paper we present the results of experiments designed to test this prediction by examining post-replication repair in several *rec* mutants of *E. coli* K12.

2. Materials and Methods

(a) *Bacterial strains*

Table 1 lists the bacterial strains used. We are grateful to R. P. Boyce for AB2487, AB2497, AB2499 and AB2500. The thymine-requiring derivatives SR74, SR78 and SR88 were selected from JC5088 (kindly provided by John Foulds), JC5743 and JC5489 (obtained through the courtesy of A. J. Clark). We used trimethoprim (a gift of G. H. Hitchings, Burrows Wellcome Co., Tuckahoe, N.Y.) as described by Stacey & Simson (1965) for this purpose.

TABLE 1

Escherichia coli *K12 derivatives used*

Designation	Mating type	Relevant genotype	Other markers
AB2487	F⁻	*recA13*	*thr leu arg his thi pro thy ara lac gal mtl xyl str*^{r} *T6*^{r} λ^s
AB2497	F⁻		*thr leu arg his thi pro thy ara lac gal mtl xyl str*^{r} *T6*^{r} λ^s
AB2499	F⁻	*uvrB5*	*thr leu arg his thi pro thy ara lac gal mtl xyl str*^{r} *T6*^{r} λ^s
AB2500	F⁻	*uvrA6*	*thr leu arg his thi pro thy ara lac gal mtl xyl str*^{r} *T6*^{r} λ^s
SR72	F⁻	*recA56 uvrB5*	*thr leu arg his thi pro thy* (*ara*) *lac* (*gal*) (*mtl*) (*xyl*) *str*^{r} *T6*^{r} λ^s
SR74	(Hfr)	*recA56*	*thr ilv thi thy* (*spm*^{r})
SR78	F⁻	*recB21*	*thr leu arg his thi pro thy* (*ara*) (*lac*) (*gal*) (*mtl*) (*xyl*) *str*^{r} *T6*^{r} λ^s
SR88	F⁻	*recC22*	*thr leu arg his thi pro thy* (*ara*) (*lac*) (*gal*) (*mtl*) (*xyl*) *str*^{r} *T6*^{r} λ^s

Abbreviations: (Demerec, Adelberg, Clark & Hartman, 1966; Taylor & Trotter, 1967; Curtiss, 1968). The symbols *arg, his, ilv, leu, pro, thi, thr, thy,* denote requirements for arginine, histidine, isoleucine and valine, leucine, proline, thiamin, threonine, thymine respectively; *ara, gal, lac, mtl* and *xyl*, the inability to utilize arabinose, galactose, mannitol and xylose, respectively; *T6, λ, spm* and *str*, response to the phages T6 and λ, and to the antibiotics, spectinomycin and streptomycin, (^{r} indicates resistance, ^{s}, sensitivity); *rec* denotes genes affecting genetic recombination and u.v. sensitivity; *uvr* designates genes affecting excision, host-cell reactivation and u.v. sensitivity. Markers in parenthesis have not been tested, but are inferred from the characteristics of the parent strains.

Cultures were incubated at 37°C. The phosphate-buffered minimal media used, both liquid and solid, have been described previously (Ganesan & Smith, 1968*a*). These were supplemented with amino acids to a final concentration of 10^{-4} M, 0·5 μg thiamin/ml. and 2 μg thymine/ml. when required for growth.

(b) *Irradiations*

An unfiltered General Electric G8T5 lamp emitting primarily at 254 nm was used at a distance of 43 cm from the surface of the cell suspension in a Petri dish on a rotary shaker (Eberbach, Ann Arbor). The dose rate was approximately 630 ergs/mm^2/min as measured by the photodecomposition of uranyl oxalate (Bowen, 1946). Until the cells were in the centrifuge all operations were performed under illumination from G.E. Gold fluorescent lamps. The procedures for irradiation have been given in more detail elsewhere (Ganesan & Smith, 1968*a*).

(c) *Sedimentation in alkaline sucrose gradients*

The design of the experiments and the conditions used for centrifugation are essentially those described by Rupp & Howard-Flanders (1968).

Exponentially-growing cultures (O.D.$_{650nm}$ (Zeis) = 0·2; ~0·5 to $1·0 \times 10^8$ cells/ml.) were divided into two portions, one of which was exposed to u.v. for 6 sec at room temperature (23°C) while the other served as the unirradiated control. [^{3}H]Thymidine (to a final concentration of 100 μc/ml.) was added and incubation at 37°C was continued for 10 min. The radioactive medium was removed by filtration. The unirradiated sample and one portion of the irradiated culture were washed and resuspended in chilled 0·05 M-Tris (pH 8·1) at a density of approximately 7×10^8 cells/min. The remaining sample of irradiated cells was washed in non-radioactive medium and resuspended in 2·5 to 3 times its original volume of non-radioactive medium and reincubated for 70 min. It was then chilled, washed and resuspended in Tris as described for the other samples.

In iced tubes, 0·3 ml. of cell suspension was mixed with 0·06 ml. of 30% sucrose in 0·6 M-Tris (pH 8·1), 0·4 ml. of 10% lysozyme (Worthington) solution and 0·1 ml. of 32 mM-EDTA. After 5 min on ice, 20 μl. of the spheroplast suspension (equivalent to approxmately 8×10^6 cells) thus formed were layered on 0·2 ml. of 0·5 N-NaOH on top of a 4·8 ml. gradient containing 5 to 20% (w/w) sucrose in 0·1 N-NaOH. The gradients were centrifuged at 20°C for 105 min at 30,000 rev./min in an SW50·1 rotor of a Spinco model L2-65B centrifuge.

After centrifugation, the tubes were punctured and about 40 5-drop fractions collected on Whatman 3 MM paper discs. The discs were dried, washed twice in 5% trichloroacetic acid, once in 95% ethanol, and once in acetone. They were then dried and counted in a liquid scintillation counter using counting solution containing 4 g 2,5-diphenyloxazole and 0·1 g 1,4-bis-2-(5-phenyloxazolyl) benzene per liter of toluene. The efficiency of counting was 3% under these conditions. The amount of radioactivity in each fraction is expressed as per cent of the total cold acid-precipitable counts recovered from the gradient.

Modifications of the general procedure are described in connection with individual experiments.

(d) *Chemicals*

[methyl-^{3}H]Thymidine (6·7 c/m-mole) was purchased from New England Nuclear.

3. Results

(a) *Preliminary experiments*

We examined three *rec*$^+$ strains to ascertain whether post-replication repair could be detected in all of them. Figure 1 presents the results obtained with *rec*$^+$ *uvr*$^+$ (AB2497) cells†. The *rec*$^+$ *uvr*B5 (AB2499) and *rec*$^+$ *uvr*A6 (AB2500) strains tested

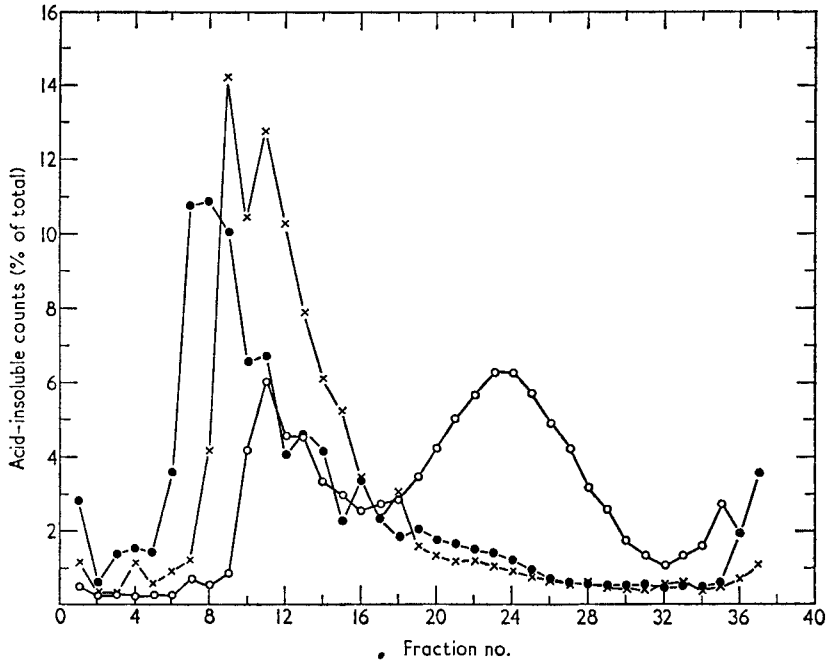

FIG. 1. Sedimentation in alkaline sucrose of DNA synthesized by irradiated cells of AB2497 (*rec*$^+$ *uvr*$^+$). Cells were irradiated with u.v. (63 ergs/mm^2 at 254 nm), then labeled with [^{3}H]thymidine as described in Materials and Methods. One sample was chilled immediately while the other was reincubated in non-radioactive medium for 70 min. When plated on minimal agar, 98% of the irradiated cells formed colonies.

—●—●—, Unirradiated control (100% = 37260 cts/min); —○—○—, irradiated but not reincubated (100% = 11780 cts/min); —×—×—, irradiated and reincubated (100% = 30380 cts/min).

† Some variation is observed in the positions of the peaks in the gradients of unirradiated and irradiated-reincubated cells relative to the position of the faster sedimenting peak of the irradiated-not-reincubated samples. These three peaks usually sediment identically, but occasionally the unirradiated or the reincubated samples sediment somewhat faster than "normal". The precise cause for this variability is not known. *Added in proof:* 3×10^6 cells (or less) per gradient give reproducible results.

behaved similarly. The DNA labeled with [^{3}H]thymidine for ten minutes after u.v. irradiation sedimented more slowly in alkaline sucrose density gradients than control DNA from unirradiated cells. If the irradiated cells were incubated for an additional 70 minutes after labeling before being lysed on the gradient, their DNA assumed a position similar to the one occupied by the control DNA.

These results were essentially the same as those reported by Rupp & Howard-Flanders (1968) and Howard-Flanders *et al.* (1968) for the *rec*$^+$ *uvr*A6 mutant, AB2500. It appeared, therefore, that under our conditions, post-replication repair could be detected in all of the *rec*$^+$ strains tested, even the one capable of excising u.v.-induced lesions from its DNA.

Consistent with the proposal that post-replication repair entails joining smaller molecules of DNA into larger ones (Howard-Flanders *et al.*, 1968), we found that DNA labeled immediately after u.v. irradiation began to increase in size in less than ten minutes after its synthesis, and appeared in progressively lower fractions of the gradients as repair proceeded (Fig. 2). The fact that it did not move directly from the region of the slowly sedimenting peak into that of the rapidly sedimenting peak indicated that the labeled material was not degraded into acid-soluble fragments and re-utilized for the synthesis of new DNA.

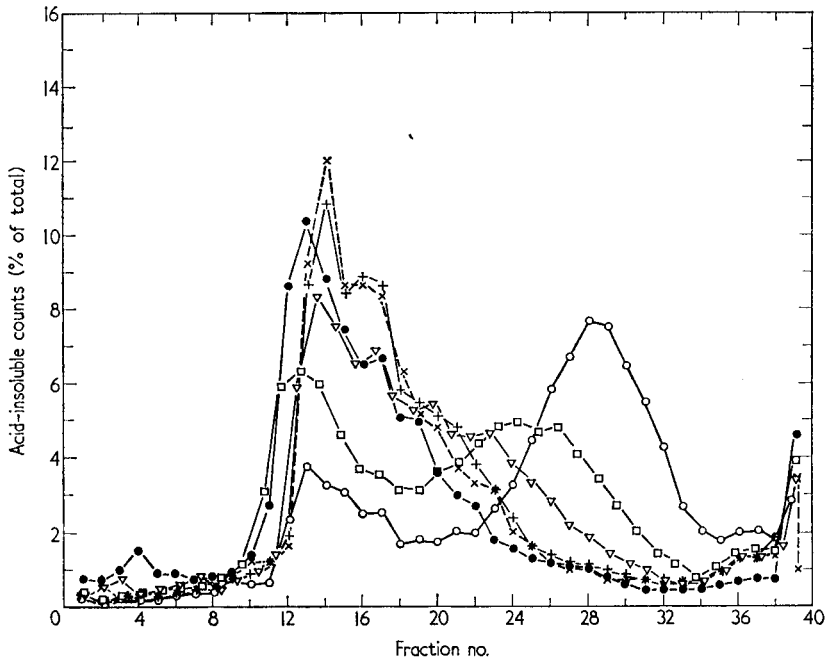

FIG. 2. The effect of different reincubation times on the sedimentation of DNA synthesized by irradiated AB2499 (*rec*$^+$ *uvrB5*). The procedure followed was the same as that for the experiment of Fig. 1, except that samples of the irradiated cells were reincubated for various times after labeling. Survival was not determined in this experiment, but in other experiments under the same conditions the survival of this strain on minimal medium was 80%.

—●—●—, Unirradiated control (100% = 18400 cts/min); —○—○—, irradiated, but not reincubated (100% = 4430 cts/min); —□—□—, irradiated, reincubated for 10 min (100% = 4260 cts/min); —▽—▽—, irradiated, reincubated for 20 min (100% = 4510 cts/min); —+—+—, irradiated, reincubated for 40 min (100% = 4680 cts/min); -- × -- × --, irradiated, reincubated for 60 min (100% = 4850 cts/min).

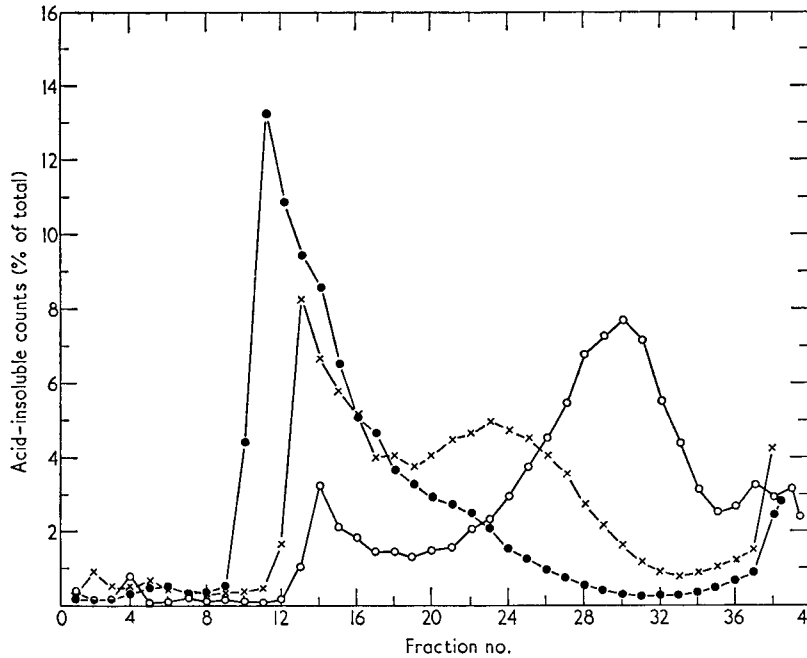

FIG. 3. The effect of photoreactivation upon the sedimentation of DNA subsequently synthesized by irradiated cells of AB2499 (*rec*$^+$ *uvrB5*). The procedure was the same as for the experiment presented in Fig. 1 except that immediately after irradiation, one sample of irradiated cells and one sample of unirradiated cells were exposed to the light from two Westinghouse black light bulbs (F15T8) for 20 min under conditions previously described (Smith & O'Leary, 1967). The irradiated sample which was not photoreactivated was shaken on a rotating platform for 20 min in the dark. The samples were then labeled for 10 min with [^{3}H]thymidine and analysed as indicated in Materials and Methods. The amount of photoreactivation was estimated by plating the cells on a complex medium to minimize dark recovery (Ganesan & Smith, 1968*b*). In the photoreactivated sample 81% of the cells formed colonies on complex medium, while only 2% did so in the unphotoreactivated sample.

—●—●—, Unirradiated control (100% = 16510 cts/min); —○—○—, irradiated but not photoreactivated (100% = 2400 cts/min); —×—×—, irradiated and photoreactivated (100% = 10950 cts/min).

If the length of the DNA molecules synthesized after u.v. irradiation depends upon the distance between pyrimidine present in the template at the time of replication (Rupp & Howard-Flanders, 1968), repair of dimers before synthesis begins should result in the formation of larger molecules of daughter DNA. Since both photoreactivation and excision remove pyrimidine dimers (Setlow, J. K., 1966; Setlow, R. B. & Carrier, 1964; Boyce & Howard-Flanders, 1964) we examined their effect upon the size of DNA molecules made by u.v.-irradiated cells. Cultures of AB2499 (*uvrB5*) were exposed to u.v., then to photoreactivating light before being labeled with [^{3}H]thymidine. As Figure 3 shows, the labeled DNA from the photoreactivated cells sedimented at a position intermediate between DNA from unirradiated control cells and DNA from irradiated cells which had not been photoreactivated. When *uvr*$^+$ cells (AB2497) were irradiated, and then incubated in the dark to permit excision to occur before [^{3}H]thymidine was added to them, the size of the DNA synthesized after irradiation increased with the time of incubation before labeling (Fig. 4(a)).

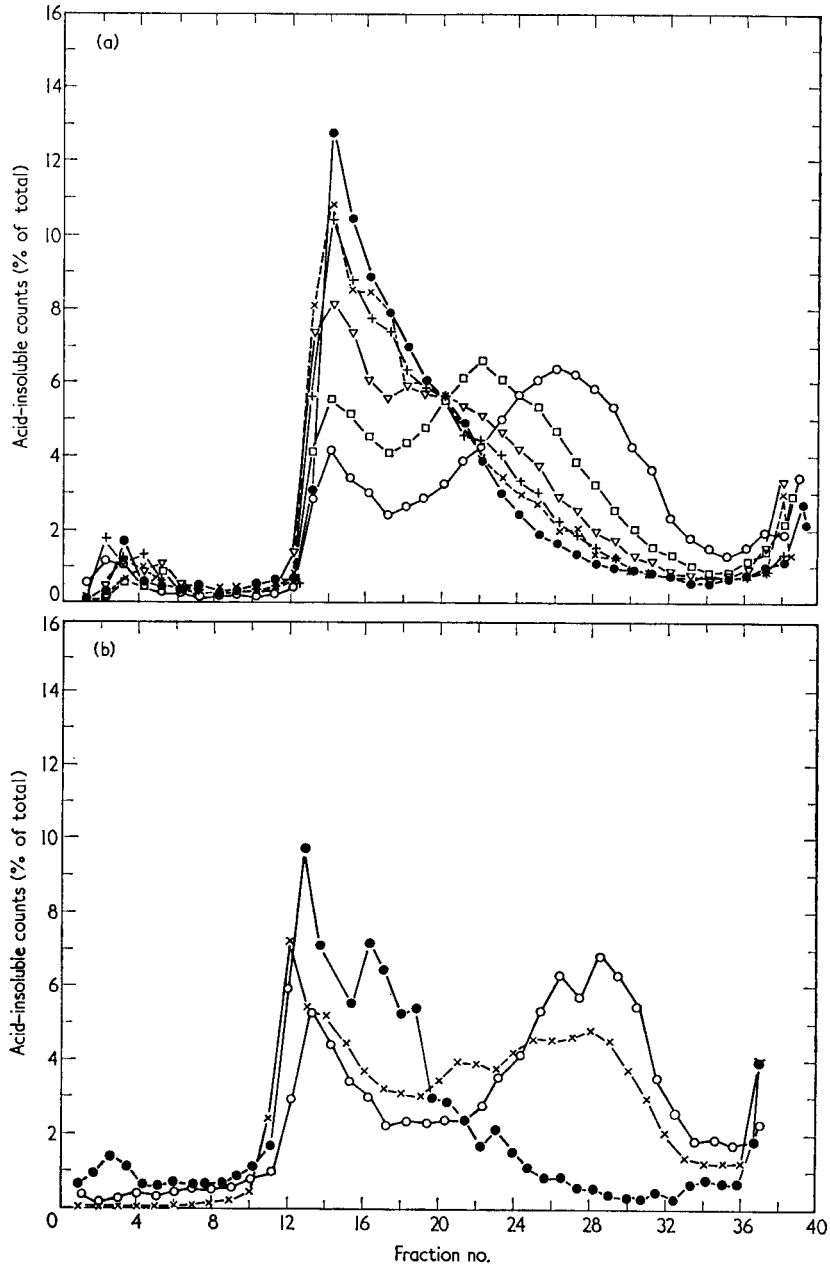

FIG. 4. The effect of excision on the sedimentation of DNA synthesized by irradiated cells. Conditions were the same as for Fig. 1 except that samples of the irradiated cells were incubated in the dark for various periods of time before being labeled with [^{3}H]thymidine.

(a) Cells of an excision proficient strain (AB2497 *rec*$^+$ *uvr*$^+$). —●—●—, Unirradiated control (100% = 17560 cts/min); —○—○—, irradiated but not incubated before labeling (100% = 6510 cts/min); —□—□—, irradiated, incubated for 10 min before labeling (100% = 10430 cts/min); —▽—▽—irradiated, incubated for 20 min before labeling (100% = 12970 cts/min); —+—+—, irradiated, incubated for 40 min before labeling (100% = 14750 cts/min); — × — × —, irradiated, incubated for 60 min before labeling (100% = 12150 cts/min).

(b) Cells of an excision-deficient mutant (AB2499 *rec*$^+$ *uvrB5*) —●—●—, Unirradiated control (100% = 29560 cts/min); —○—○—, irradiated, but not incubated before labeling (100% = 4420 cts/min); — × — × —, irradiated, incubated 90 min before labeling (100% = 9320 cts/min).

After 40 minutes incubation it was nearly indistinguishable from DNA from unirradiated cells. In contrast, DNA synthesized by an excision-deficient mutant, AB2499 (*uvrB5*), under the same conditions did not increase appreciably in size even after 90 minutes incubation before labeling (Fig. 4(b)). These data support the hypothesis that under these conditions pyrimidine dimers in template DNA are the major determinants of the length of the daughter molecules synthesized from it.

(b) *Tests of* rec *mutants*

Three *recA* derivatives, AB2487 (*recA13 uvr*$^+$), SR74 (*recA56 uvr*$^+$) and SR72 (*recA56 uvrB5*), were tested for post-replication repair. All three behaved in essentially the same way. Figure 5 shows the results obtained with SR72. Labeled DNA synthesized in irradiated cells sedimented more slowly than that formed in unirradiated controls. However, reincubation of the irradiated cells after labeling did not result in any detectable increase in the rate of sedimentation of the labeled DNA, in contrast with the results for the *rec*$^+$ strains. Under these conditions the *recA* mutants did not appear to undergo post-replication repair.

However, much of the labeled DNA was degraded into acid-soluble material during the reincubation period, reducing the sensitivity with which repair could be detected. This occurred in both *uvr*$^+$ and *uvr*$^-$ derivatives of *recA* mutants. As indicated in

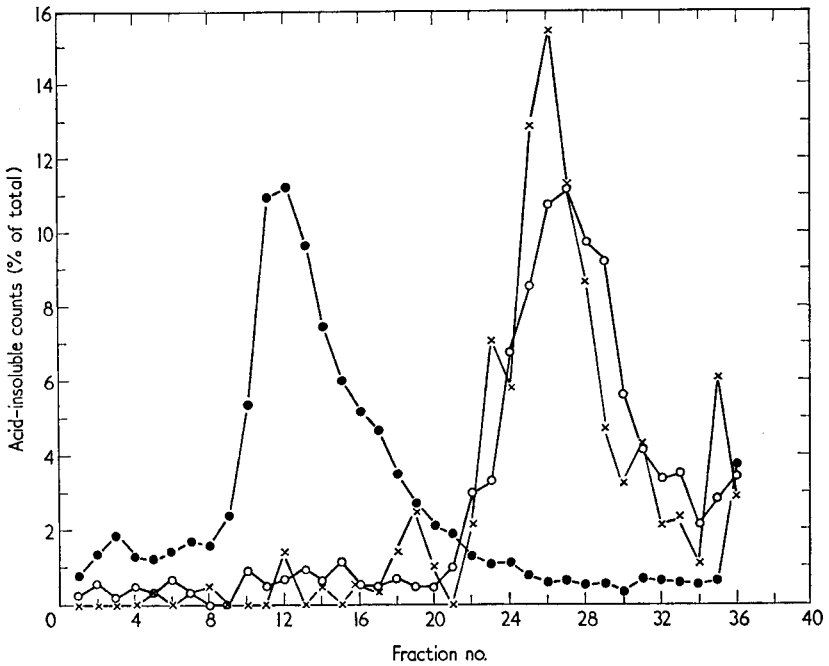

FIG. 5. Sedimentation in alkaline sucrose of DNA from irradiated cells of SR72 (*recA56 uvrB5*). The procedure was the same as described in Fig. 1 except that only 10 μl. of spheroplasts from the unirradiated culture were put on the gradient. Of the irradiated cells <0·1% formed colonies on minimal agar.

—●—●—, Unirradiated control (100% = 12020 cts/min); —○—○—, irradiated, but not reincubated after labeling (100% = 2420 cts/min); —×—×—, irradiated, reincubated for 70 min after labeling (100% = 550 cts/min).

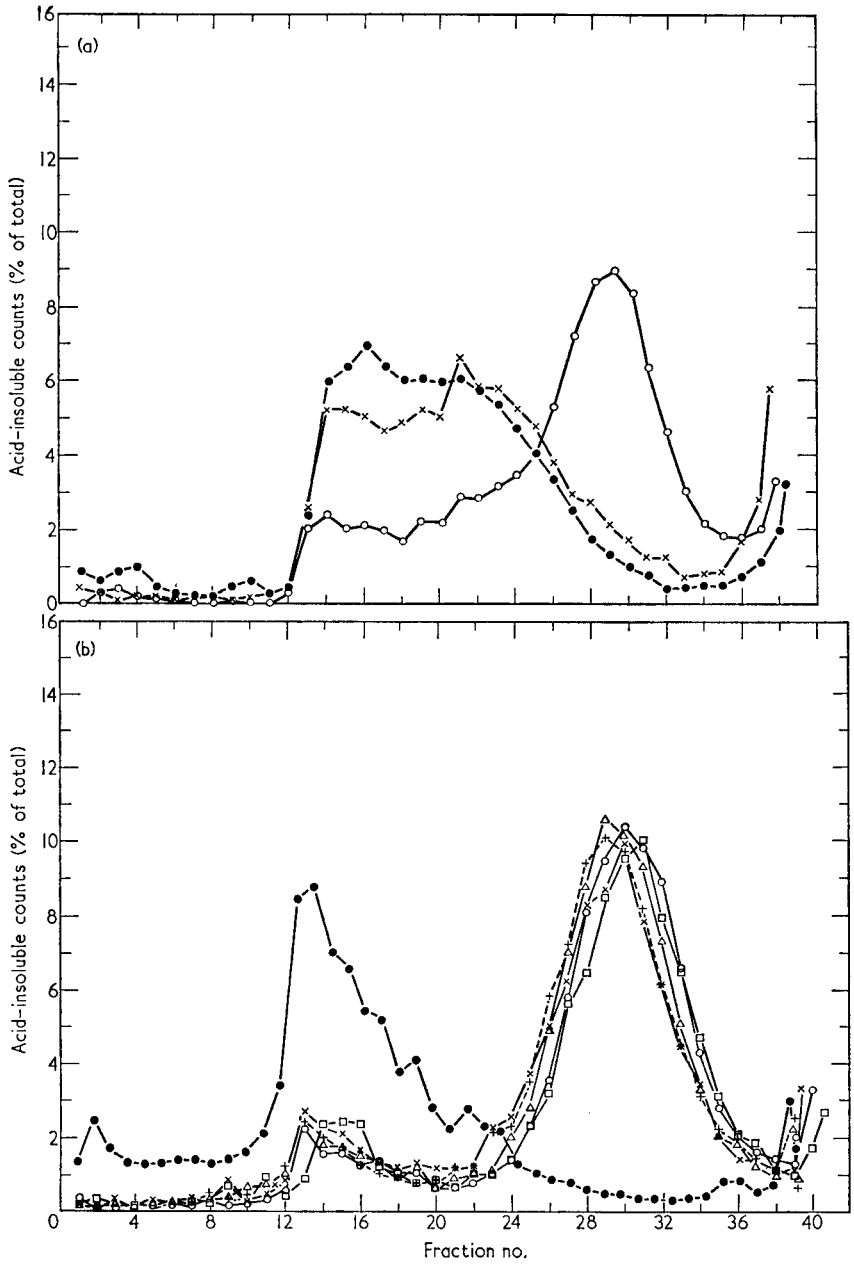

FIG. 6. Sedimentation in alkaline sucrose of DNA synthesized after u.v. irradiation by cells starved for glucose and amino acids for 90 min before irradiation. Glucose, but not amino acids, was added after irradiation. Otherwise, the samples were processed as described in Fig. 1.

(a) AB2499 (*rec*$^+$ *uvrB5*)

—●—●—, Unirradiated control (100% = 4630 cts/min); —○—○—, irradiated, but not reincubated after labeling (100% = 3000 cts/min). When plated on minimal agar, 63% of the irradiated cells survived; —×—×—, irradiated, reincubated for 60 min after labeling (100% = 2190 cts/min).

(b) AB2487 (*recA13 uvr*$^+$)

—●—●—, Unirradiated control (100% = 6300 cts/min); —○—○—, irradiated, but not reincubated after labeling (100% = 2680 cts/min). When plated on minimal agar, 0·4% of the irradiated cells formed colonies; —△—△—, irradiated, reincubated for 10 min after labeling (100% = 2510 cts/min); – – + – – + – –, irradiated, reincubated for 20 min after labeling (100% = 2360 cts/min); —□—□—, irradiated, reincubated for 30 min after labeling (100% = 2270 cts/min); —×—×—, irradiated, reincubated for 60 min after labeling (100% = 2160 cts/min).

Figure 5, the amount of acid-precipitable radioactivity recovered from the gradient containing irradiated cells reincubated for 70 minutes after labeling was only about 23% of that from the gradient of irradiated cells which had not been reincubated. It had been observed that degradation of DNA synthesized by *recA* cells before exposure to u.v. could be inhibited by starving the cells for amino acids required for growth (Ganesan & Smith, 1968*b*; Horii & Suzuki, 1968). To minimize degradation, and permit labeling of DNA after irradiation, the following procedure was devised. Glucose, as well as amino acids, was removed for 90 minutes before irradiation of the cells. After irradiation, glucose was added together with [^{3}H]thymidine for the 10-minute labeling period†. The [^{3}H]thymidine was removed by filtering and the cells, resuspended in complete medium minus amino acids, were either chilled immediately or reincubated before analysis on alkaline sucrose gradients. The starvation procedure appeared to reduce degradation, and very little of the radioactivity incorporated into DNA was lost during reincubation in the absence of amino acids. Control experiments indicated that repair did occur in *rec*$^+$ cells under these conditions (Fig. 6(a)). Thus, even in the absence of degradation there was no evidence for repair after 63 ergs/mm^2 in the *recA* mutant examined (Fig. 6(b)).

One *recB* and one *recC* mutant were examined for post-replication repair. Figure 7 presents the results obtained with the *recB* mutant. The *recC* strain behaved simi-

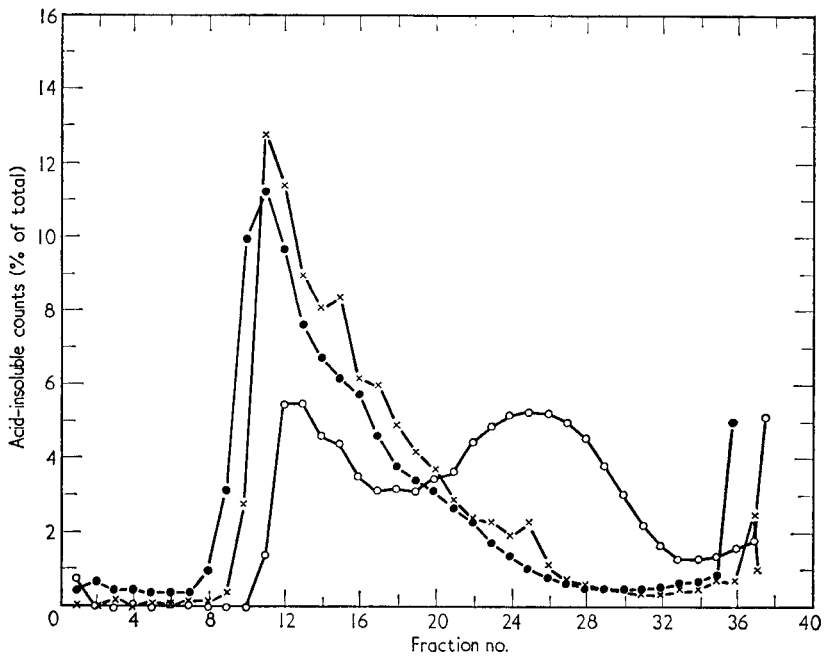

FIG. 7. Sedimentation in alkaline sucrose of DNA from irradiated cells of SR78 (*recB21 uvr*$^+$). The same procedure described in Fig. 1 was used. Survival was not determined in this experiment, but in other experiments under the same conditions, the survival of this strain on minimal medium was 28%.

—●—●—, Unirradiated control (100% = 9740 cts/min); —○—○—, irradiated, but not reincubated after labeling (100% = 2330 cts/min); —×—×—, irradiated, reincubated for 70 min after labeling (100% = 7300 cts/min).

† The results for *uvrB5* cells (non-starved) in Fig. 2 compared with the results in Fig. 6(a) (starved) indicate that the amount of DNA synthesized by the starved cells during the pulse was about 25% of the non-starved controls and 67% of the non-starved irradiated cells.

larly. In both, DNA synthesized by irradiated cells sedimented more slowly than that made under the same conditions by unirradiated cells, In both, reincubation after labeling led to an increase in sedimentation rate of the labeled DNA. It appeared from this that *recB* and *recC* cells, unlike *recA* cells, were able to repair the majority of the defects formed in DNA synthesized after exposure to 63 ergs/mm^2 of u.v. radiation. The same appeared to be true for the *recC* mutant when examined after exposure to 120 and 180 ergs/mm^2 of u.v. radiation.

As expected, no significant degradation was observed in the *recB* and *recC* mutants during reincubation (Emmerson, 1968). Instead, it appeared that incorporation of radioactivity into DNA continued during reincubation. For example, in the *recC* mutant, after filtering and washing to remove exogenous thymidine, 45% of the total radioactivity taken up by the cells after irradiation (63 ergs/mm^2) was soluble in cold trichloroacetic acid. After 60 minutes reincubation, however, the acid-soluble fraction had decreased to 10%, while the total radioactivity remained relatively constant. This effect appeared to be more pronounced after 120 and 180 ergs/mm^2, as judged by the amount of acid-precipitable radioactivity recovered from gradients of the *recC* mutant. Preliminary data indicate that there is a much larger acid-soluble pool of pyrimidines in pulse-labeled irradiated cells compared to unirradiated cells. This pool is not removed when the cells are washed with growth media on filters.

One can compare the relative efficiency of repair in the several strains by estimating the proportion of their labeled DNA present after irradiation and reincubation which

TABLE 2

Proportion of labeled DNA present as rapidly sedimenting material from lysates of various mutants of Escherichia coli *K12*

Designation	Relevant genotype	Percentage labeled DNA as rapidly sedimenting† u.v.-irradiated		
		Control	Not reincubated	Reincubated
AB2497	*rec*$^+$ *uvr*$^+$‡	75	34	86
AB2500	*rec*$^+$ *uvrA6*§	67	25	63
AB2499	*rec*$^+$ *uvrB5*‖	77	29	75
SR78	*recB21 uvr*$^+$§	73	32	70
SR88	*recC22 uvr*$^+$§	62	25	54
AB2487	*recA13 uvr*$^+$¶	70	15	24
SR74	*recA56 uvr*$^+$§	62	6	13
SR72	*recA56 uvrB5*§	79	10	6

† See Fig. 1 and Materials and Methods section for experimental details and Table 1 for a description of the mutants used. The data shown in the Figures are combined with available data on the other strains. The point of separation between fast and slowly sedimenting DNA was taken as the minimum value between the two peaks of the non-reincubated u.v. irradiated samples (around fraction 18). This point was also used for calculating the DNA distribution in the control and u.v. irradiated sample that had been reincubated.

‡ Data are the average of 3 experiments for control, 4 for u.v. irradiated, not reincubated and 1 for u.v. irradiated, reincubated.

§ One experiment.

‖ Average of 3 experiments for control and 5 for u.v. irradiation ± reincubation.

¶ Average of 2 experiments.

has a sedimentation rate similar to that of unirradiated control DNA. Table 2 presents a summary of this information compiled from the experiments presented in the Figures and others which have not been shown. These data indicate that gradients prepared from irradiated *recA* cells before reincubation contained less DNA in the rapidly sedimenting fraction than gradients prepared from *rec*$^+$, *recB* or *recC* cells (irrespective of their *uvr* genotype). After reincubation, the *recA* cells showed almost no increase in the proportion of rapidly sedimenting DNA. In the *rec*$^+$, *recB* and *recC* cells, however, the proportion of rapidly sedimenting DNA increased to levels similar to those present in unirradiated controls.

4. Discussion

Following u.v.-irradiation, excision-deficient strains of *E. coli* K12 (*uvrA6* and *uvrB5*) synthesize DNA in short pieces compared to unirradiated control cells, but on continued incubation these short pieces are joined together to form "normal-sized" DNA by a mechanism believed to be responsible for the post-replication repair of radiation damage. An excision-proficient strain (AB2497) also behaves in the same manner. This result indicates that for at least the first ten minutes after u.v.-irradiation, excision-dependent repair neither removes a sufficient amount of the damage to obviate this response nor does it otherwise interfere with this type of repair.

When an irradiated excision-deficient mutant, AB2499 (*uvrB5*), was incubated for 90 minutes before pulse labeling the sedimentation property of the labeled DNA was comparable to that for DNA synthesized immediately after irradiation (i.e., small pieces of DNA were synthesized). However, when *rec*$^+$ *uvr*$^+$ cells (AB2497) were allowed to grow in the dark for 60 minutes after u.v.-irradiation before the ten-minute pulse of [^{3}H]thymidine, only large pieces of DNA were synthesized, comparable in size to those synthesized by the unirradiated controls. Therefore, the excision repair controlled by the *uvr* genes must remove the u.v.-induced damage that causes the synthesis of small pieces of DNA. The results of Achey & Billen (1969) indicate that the excision-mode of repair is complete by about 45 minutes after u.v.-irradiation. This result is consistent with our observation that after about 40 minutes of incubation of u.v.-irradiated cells prior to labeling with [^{3}H]thymidine normal-sized DNA is synthesized in an excision-proficient strain (but not in an excision-deficient strain, *uvrB5*).

The importance of pyrimidine dimers in causing the synthesis of the short pieces of DNA after u.v. is further indicated by our results showing that the repair of dimers by photoreactivation prior to the pulse greatly reduced but did not completely eliminate the synthesis of short pieces of DNA (see also Rupp, 1968 *5th Intern. Cong. Photobiol.* Abstr. Gh-3). This residual synthesis of intermediate sedimenting material could reflect the presence of unpaired pyrimidine dimers or indicate the importance of other types of photochemical lesions in causing the synthesis of short sections of DNA. We have preliminary evidence which indicates that u.v.-induced DNA–protein cross-links (Smith & O'Leary, 1967) can cause the synthesis of slowly sedimenting DNA.

Our results indicate that *recA* mutants of *E. coli* K12 are deficient in their capacity for post-replication repair of lesions caused by u.v. radiation. In all of the K12 derivatives examined, *rec*$^+$ and *rec*$^-$ (irrespective of *uvr* genotype), the DNA synthesized immediately after irradiation behaved as small molecules in alkaline sucrose gradients. The rejoining of these small molecules into larger ones, believed to repre-

sent repair, was observed after 63 ergs/mm^2 (254 nm) in *rec*$^+$, *recB* and *recC* strains, but not in the *recA* mutants tested.

The large amount of DNA degradation characteristic of *recA* mutants (Clark, Chamberlin, Boyce & Howard-Flanders, 1966; Horii & Suzuki, 1970) complicates the interpretation of the data. However, our results with amino acid-starved cells, where little or no degradation occurred, suggest that the lack of repair in *recA13* is due to a deficiency in repair function rather than to the inhibition of repair by DNA breakdown. The *recA* mutants clearly differ from *rec*$^+$ and the *recB* and *recC* mutants in their ability to reconstruct DNA synthesized after u.v. irradiation. Their behavior is thus consistent with the idea that the *recA* gene product is necessary for post-replication repair as well as for genetic recombination.

Our experiments do not provide evidence that the *recB* and *recC* genes participate in this type of repair. The *recB* and *recC* mutants are more resistant to u.v. than strains carrying *recA* mutations (Howard-Flanders & Theriot, 1966; Clark, 1967; Emmerson, 1968; Willetts & Clark, 1969; and Figs 6(b) and (7)), and they appear to retain some capacity for genetic recombination (Clark, 1967; Low, 1968; Wilkins, 1969). Perhaps their ability to perform post-replication repair is limited (as seen for their repair of X-ray damage; Kapp & Smith, 1970), but the doses of u.v. used in our experiments (up to 180 ergs/mm^2) may have been too low to reveal their deficiency in this function. Alternatively, if the *recB* and *recC* genes control steps involved in post-replication repair their behavior may indicate that these steps occur subsequent to the joining of the small molecules into larger ones.

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