MUTANTS OF *ESCHERICHIA COLI* K-12 DEFECTIVE IN DNA REPAIR AND IN GENETIC RECOMBINATION

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BACTERIA are readily killed by exposure to ultraviolet light (UV), presumably as the result of the formation of UV photoproducts in the bacterial DNA. It is also known that pyrimidine dimers are among the photoproducts formed with a high yield, and that bacteria normally contain an efficient mechanism for the repair of DNA containing these products. Pyrimidine dimers are excised from DNA during incubation and can be recovered, still contained within a short oligonucleotide. This excision appears to involve the interruption of single DNA strands, and to be followed by local DNA breakdown and repair synthesis (SETLOW and CARRIER 1964; BOYCE and HOWARD-FLANDERS 1964; PETTIJOHN and HANAWALT 1964). These observations are of interest not only because they reveal the mechanism of an effective and possibly widespread process for the repair of injured DNA, but also because they suggest that local repair can occur in one strand of a double helix.

This mechanism for DNA repair may be related to the mechanism of genetic exchange. An insight into the mechanisms of genetic recombination was gained through the discovery that λ bacteriophage recombinants can be formed by joining fragments of preexisting phage DNA molecules (MESELSON and WEIGLE 1961). As continuity in the base sequence of the phage genome must be preserved, base pairing between overlapping single strands from each parental molecule is presumably required as a prelude to the formation of a recombinant (LEVINTHAL 1959). It has been suggested that the process of recombination may be completed by local DNA repair synthesis on either side of the overlap (MESELSON 1964) and that certain of the enzymes involved may serve in both genetic recombination and in repair after irradiation (HOWARD-FLANDERS and BOYCE 1964).

Further evidence in support of these concepts has been obtained through the isolation of mutants of *E. coli* K-12 that have lost the ability to form recombinants when mated with suitable donor strains. As these mutants are able to accept genetic material normally, it appears that they may be defective in the process of integrating the donor DNA into the recipient chromosome (phenotype symbol Rec^-). Because of the suggested relationship between repair and recombination, the strains were tested for ability to survive exposure to UV, and were found to be highly radiosensitive (CLARK and MARGULIES 1965). After exposure to UV, these mutants degrade their DNA excessively and fail to incorporate labeled thymidine (CLARK, CHAMBERLIN, BOYCE, and HOWARD-FLANDERS 1966).

TABLE 1

Characteristics of E. coli *K-12 strains*

Strain number	Origin or synonym	*uvr*	*rec*	Auxotrophic characters Thr	Leu	Pro	His	Met	Thi	Arg	Thy	Ile	Energy source utilization Lac	Gal	Ara	Xyl	Mtl	Phage growth T1	T4	T6	λ	Drug resistance Str	MC	Sex	Injection sequence	Obtained from
AB 259	Hfr Hayes	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	S	.	S	S	S	.	Hfr H	*pyr, thr, leu*	ADELBERG
AB2383	From P4 × 6	+	+	+	+	+	+	B	..	+	—	+	+	+	+	+	..	S	.	S	.	S	.	Hfr J2	*pro, leu, ara*	ADELBERG
AB2528	From AB313	+	+	..	—	+	+	+	—	+	+	—	—	+	+	+	+	S	.	S	.	R	.	Hfr 313	*mtl, xyl, str*	EGGERTSSON
	W 4520	+	+	+	+	+	+	+	..	+	+	+	..+/4	..	..	..	..	.	.	.	.	S	.	F-*gal*+	.....	ADELBERG
AB1886	NA mutation From AB1157	A	+	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	S	..	.....	.....
AB2429	Recombinant	A,C	+	..	..	+	—	+	—	+	+	+	—	—	+	—	..	S	.	R	.	R	S	..	.....	.....
AB1157	.....	+	+	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	R	F⁻	.....	ADELBERG
AB2462	NG mutation From AB1157	+	12	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	S	F⁻	.....	This paper
AB2463	NG mutation From AB1157	+	13	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	S	F⁻	.....	This paper
AB2464	NG mutation From AB1157	+	14	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	S	F⁻	.....	This paper
AB2465	NG mutation From AB1157	+	15	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	S	F⁻	.....	This paper
AB2472	NG mutation From AB1157	+	16	—	—	—	—	+	—	—	+	+	—	—	—	—	—	S	S	R	S	R	S	F⁻	.....	This paper
JC1569	.....	+	1	..	—	..	—	—	..	G	..	..	—	—	..	—	—	R	.	.	S	R	.	F⁻	.....	CLARK
AB2492	Recombinant*	+	13	—	—	+	+	+	—	—	—	+	.....					S	S	.	S	.	S	F⁻ Pm⁻	.....	This paper

The abbreviations for the auxotrophic characters signify requirements for threonine, leucine, proline, histidine, methionine, arginine, thymine and isoleucine; for energy source utilization signify lactose, galactose, arabinose, xylose and manitol; and for drug resistance signify streptomycin and mitomycin C.

* AB2492 is a non-permissive host for phage carrying amber mutations, and was obtained from AB259 Hfr H Pm⁻ × AB2487 *rec-13* Thy⁻. AB2487 is a Thy⁻ derivative obtained from AB2463 *rec-13*, and is able to grow with 2μg/ml of thymine.

It will be shown in this paper that five mutants isolated because they were highly sensitive to X rays, were also found to be defective in their ability to form recombinants in suitable crosses. This finding further supports the idea that certain enzymes may serve both in genetic recombination and in repair after irradiation. The defect in the repair mechanism in these mutants is only partial, as it appears from the level of their sensitivity to UV, that about 95% of the pyrimidine dimers are still effectively repaired. It will be shown that, when labeled in their DNA by growth with H^3-thymidine, these recombinationless mutants also release an abnormally large amount of radioactivity following UV-irradiation. When log-phase mutants are exposed to UV, they show a prolonged inhibition of the capacity to incorporate exogenous thymidine. It appears that they are defective in their ability to terminate DNA breakdown and to initiate repair synthesis.

MATERIALS AND METHODS

Bacterial strains: The list of the strains of *E. coli* K-12, their characteristics and origins are given in Table 1.

Media: YET broth contained 5 g yeast extract, 10 g tryptone, 10 g NaCl, 120 mg NaOH per liter of water, and was supplemented with 1 g glucose for YET glucose medium. The YET agar contained YET broth with 2% agar. The selective media employed in the genetic crosses follow those described by ADELBERG and BURNS (1960). M9 medium contained 1g NH_4Cl, 11 g $Na_2HPO_4 \cdot 7H_2O$, 3 g KH_2PO_4, 5 g NaCl, 4 g glucose, 120 mg $MgSO_4$, 10 mg $CaCl_2$ and water to 1 liter. Enriched M9 (EM9) consisted of M9 with 2.5 g Casamino acids and 0.1 mg thiamine added per liter.

Isolation of radiation-sensitive, recombination-defective mutants: The original strain AB1157 carrying multiple genetic markers was grown overnight in 30 ml of YET broth, centrifuged, and resuspended in 1 ml of 50 mM sodium acetate buffer at pH 5.0. To this was added an equal volume of a freshly prepared solution of N-methyl-N'-nitro-N-nitrosoguanidine (NG) at 2 mg/ml in the same buffer. The culture was incubated at 37°C for 3½ hours, at which time about 1% of the cells survived. They were then washed, resuspended in 50 ml YET broth, divided into ten growth tubes and incubated overnight at 37°C. Each tube was sampled, diluted and plated onto YET plates, which were then incubated overnight. Before describing the procedure used to test for mutants among the colonies so formed, it must be recorded that a variant procedure was later employed and was used in the isolation of AB2472, one of the mutants to be described. This strain was derived from AB1886 which also came from AB1157, but carried an additional mutation at the *uvrA* locus. AB1886 was grown to log phase in YET broth with glucose. To 2 ml of this culture was added 0.1 ml of a freshly prepared solution of NG in phosphate buffer at pH 6.8, giving a final concentration of 50 μg/ml NG. The culture was aerated at 37°C, sampled at intervals for half an hour, diluted, plated on YET agar and incubated overnight. Only those plates for which the fraction of cells surviving was between 1 to 10% were kept for use. With both methods for applying the mutagen, colonies were picked and inoculated in patches on master plates of YET agar. After overnight incubation, the master plates were printed with sterile velvet onto YET plates in quadruplicate, and also onto media selective for Pro+ Str^R recombinants. These latter plates had been spread with strain AB2383 Hfr J2. One YET replica plate was exposed to 1500 ergs/mm² UV. Two of the YET replica plates were exposed to 15 and 25 kilorads of high-energy electrons from a 6 Mev linear electron accelerator. After incubation sufficient for good growth, the plates were scored. Mutants were picked from the master replica plates from any patches that appeared to be exceptionally radiation-sensitive. These were streaked twice and kept for further tests.

Crosses: The procedure for mating the F⁻ mutants with suitable Hfr or F+ strains followed those described by ADELBERG and BURNS (1960). Mating was continued for 2 hours unless otherwise stated.

Irradiations: The cells to be tested for their ability to survive irradiation were harvested from YET broth after overnight growth and were diluted and spread on plates immediately before UV-irradiation. For X-irradiation, the cells were diluted to about 2×10^6 cells/ml in EM9 and were bubbled with oxygen during the exposure. The suspension was sampled after various doses and diluted for planting on YET agar. The fractions of cells surviving were determined by colony count after 18 to 28 hours incubation at 37°C.

The UV irradiations were carried out at about 50 cm from a 15 watt low-pressure mercury germicidal lamp. The dose rate was 10 ergs/mm^2 per second, as measured with a General Electric Germicidal Light Meter. The printed replica plates were exposed to high energy electrons from a 6 Mev linear accelerator and irradiated through the bottom of the plate to permit a build-up of scattered electrons to occur before they passed through the bacteria. This high intensity source permitted the simultaneous irradiation of ten plates at a dose rate of about 5×10^4 rads per minute at about 2 meters from the electron window. The cell suspensions were exposed to 6 Mev X rays in a Pyrex glass tube and stirred by bubbling with oxygen. The dose rates of the X rays and the fast electrons were determined from the yield of ferric ions in aerated acid ferrous sulphate dosemeter solution. This contained 2.8 ml H_2SO_4, 280 mg $FeSO_4 \cdot 7H_2O$, 60 mg NaCl and three times distilled water to one liter, and was equilibrated with air before use. The optical density per cm was measured at 304 mμ and multiplied by 28 to give the dose in kilorads.

Release of radioactivity from cells labeled with H^3-thymidine after exposure to UV: To follow the effects of UV-irradiation on the stability of the DNA of the original and mutant strains of bacteria, they were grown overnight in EM9 and diluted tenfold into EM9 containing 0.5 to 2 μc/ml H^3-thymidine of specific activity 10 c/mM and 250 μg/ml deoxyadenosine. The culture was incubated at 37°C with aeration for about 3 hours until there were about 10^8 cells/ml.

The cells were washed twice and resuspended in five times the original volume of unsupplemented M9 to permit UV-irradiation in a layer about 3 mm deep without undue absorption of UV. After irradiation, the cell suspensions were supplemented with 20 μg/ml of nonradioactive thymidine to reduce the reutilization of any radioactive thymine released from the cell. Control and UV-irradiated cell suspensions were incubated at 37°C with aeration in dim light to avoid photoreactivation, and 1 ml samples were taken at intervals and mixed with an equal volume of cold 10% trichloracetic acid. After holding for at least one hour at 0°C, .05 ml of 1% serum albumin was added to increase the bulk of the precipitate. The mixture was centrifuged at 10,000 rpm for 15 minutes and duplicate 0.1 ml samples were taken from the supernatant for assay of the radioactivity of the total acid soluble material in both cells and medium. The pellet was then broken up in the same 5% TCA supernatant and the mixture was heated to 90°C for 30 minutes to extract the hot acid soluble material. After allowing to stand, 0.1 ml samples were again taken from the supernatant. The cold acid soluble and hot acid soluble material were counted by pipetting 0.1 ml into 10 ml of liquid scintillator, which was then counted in a scintillation counter. Each liter of the scintillator contains 670 ml toluene, 330 ml ethanol, 2.7 g 2,5-diphenyloxazole and 33 mg 1,4-bis 2-(5-phenyloxazolyl)-benzene.

Incorporation of radioactive thymidine into acid precipitable material following irradiation: Cultures of the various strains of *E. coli* K-12 were grown in EM9 to about 10^8 cells/ml, washed and resuspended in five times the volume of EM9. The suspension was divided into three fractions and exposed to various doses of UV. Cell suspensions were then supplemented with 250 μg deoxyadenosine and 2 μc H^3-thymidine or 0.2 μc, C^{14}-thymidine per ml. The supplemented cultures were then incubated at 37°C with aeration and duplicate 0.1 samples were taken at time 0 and at intervals. The samples were pipetted onto filter discs and washed in cold 5% trichloroacetic acid for one hour. When dry, the discs were counted in 10 ml of liquid scintillator fluid.

RESULTS

Among the mutants isolated, five (designated AB2462 through AB2465 and AB2472) were found to have the highest sensitivities to X-irradiation. The fraction of cells, retaining the ability to form colonies on YET agar after exposure to

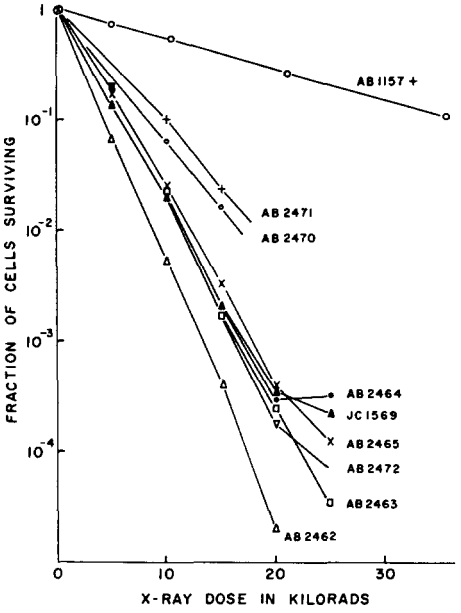

FIGURE 1.—The fraction of the cells forming colonies on complete medium is plotted against the X-ray dose in kilorads.

Cells were harvested from overnight growth in YET broth, and resuspended in EM9 before irradiation in a Pyrex vessel in which they were bubbled with oxygen. After irradiation, the suspensions were diluted, plated on YET agar, incubated 18 to 24 hr at 37°C and scored for the numbers of visible colonies formed. (We are indebted to DR. J. B. STEDEFORD for these data.)

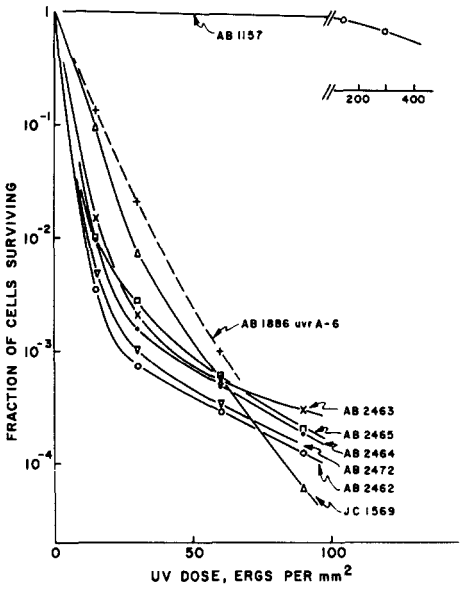

FIGURE 2.—The fraction of cells forming colonies on complete medium is plotted against the UV dose.

The cells were harvested after overnight growth in YET broth and diluted and plated onto YET agar. They were exposed to various doses of UV without further delay and incubated 18 to 28 hours. The fraction of survivors was then determined from the numbers of visible colonies formed.

various doses of X-rays, is shown in Figure 1, both for the original strain AB1157 and the various mutants. Also included for comparison are results obtained with the mutant JC1569 (CLARK and MARGULIES 1965), which exhibits a similar radiosensitivity. It is not certain that two of the mutants, AB2464 and AB2465 were of independent origin, as they came from the same growth tube. The estimated probability of their being derived from the same mutation is, however, less than 5%. Strains AB2470 and AB2471 are slightly less radiosensitive and appear to be recombination-defective mutants of another class, which will be described in a subsequent publication.

Figure 2 shows the results of similar experiments on the fraction of cells surviving exposure to UV-irradiation. Also included in this figure are the results obtained with strain AB1886 *uvrA6* which was previously shown to be sensitive to UV by virtue of having lost the ability to excise thymine dimers (BOYCE and HOWARD-FLANDERS 1964).

Figure 3 shows the fraction of UV-irradiated T1 phage able to form plaques when plated on the various mutants and the original strain, as a function of the UV dose to the bacteriophage. It can be seen from the number of plaques formed, that the UV-irradiated bacteriophage is reactivated almost as effectively in the

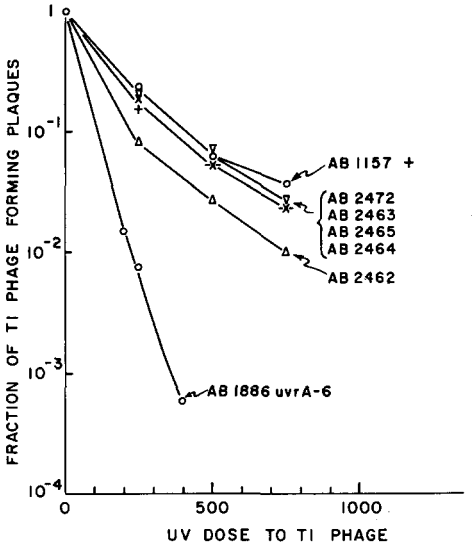

FIGURE 3.—The numbers of UV-irradiated T1 bacteriophage forming plaques are expressed as a fraction of the numbers formed by unirradiated phage, and are plotted against the UV dose in ergs/mm² given to the phage.

The phage were irradiated in phosphate buffer at pH 7.0 and plated in soft agar seeded with the various strains of bacteria poured over YET agar plates. The plaques were scored after incubation at 37°C overnight.

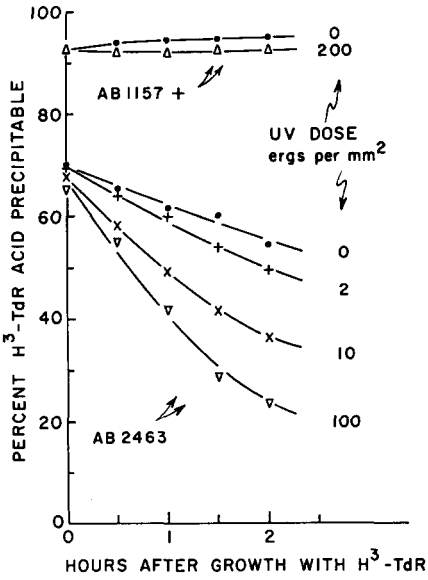

FIGURE 4.—The fraction of the total radioactivity of the cells and suspending medium that is precipitable in cold 5% TCA is plotted as a function of the time of incubation at 37°C in M9 medium after labeling with radioactive thymidine and exposure to UV.

Cells of the original strains AB1157 and AB2463 Rec⁻ were labeled by growth in EM9 medium supplemented with 2 μc/ml H^3-thymidine and 250 μg/ml deoxyadenosine. The cells were washed, resuspended in M9 medium, and exposed to various doses of UV. The culture was supplemented with 20 μg/ml thymine and incubated in dim light at 37° with aeration. One-ml samples were taken at intervals. These contained over 10^4 count/min per ml. The fraction of the total radioactivity that was cold acid precipitable was determined and is shown plotted against the time of incubation. Other details of these procedures are given in METHODS.

Rec⁻ mutants, as it is in the original strain. Although AB2472 was isolated from AB1886, which is mutant at the *uvrA* locus, it appears to have reverted and recovered the ability to reactivate the UV-irradiated T1 phage.

As the repair of DNA damaged by irradiation is thought to involve local DNA breakdown and repair synthesis, the next experiments were designed to test for any abnormality in the postirradiation DNA degradation and synthesis in the mutants. Figure 4 shows the results of experiments in which strains AB1157 and AB2463 were labeled in their DNA by growth in the presence of H^3-thymidine as described in METHODS. The cells were harvested, washed three times and resuspended in M9 medium. The suspension was divided into aliquots which were exposed to various doses of ultraviolet light or kept as control. After irradiation, the suspensions were supplemented with 20 μg/ml nonradioactive thymidine to minimize reincorporation of any radioactive nucleotides released, and incubated with aeration at 37° in dim light. It is seen in Figure 4 that exposure of the wild-

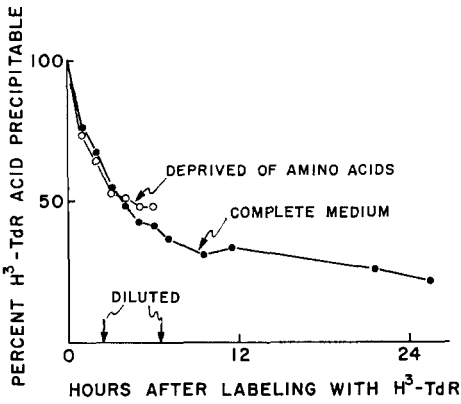

FIGURE 5.—The H^3 radioactivity remaining acid precipitable, is expressed as a percent of the initial acid precipitable radioactivity in a culture of AB2463 Rec⁻ during incubation with aeration, after labeling by growth with H^3-thymidine.

The bacteria were grown overnight in EM9 and diluted 1/10 in EM9 supplemented with H^3-thymidine at 25 μc/ml and 250 μg/ml deoxyadenosine and incubated with aeration at 37°C for about 3 hours until the optical density at 650 mμ reached 0.8. The cells were washed twice and resuspended in five times the original volume of M9 or EM9 medium. The suspension was supplemented with 20 μg/ml nonradioactive thymine and incubated at 37°C with aeration. One-ml samples were taken at intervals and assayed for acid precipitable radioactivity, as described in the METHODS. The total initial radioactivity of the labled cells was 80,000 count/min per ml. This is expressed as a percentage of the initial acid precipitable radioactivity. The culture in EM9 was diluted 1/9 in the same medium but without added radioactivity after 2 1/2 hours, and then diluted 1/9 again after 6 1/2 hours, at which time there were 800 count/min per ml of cell suspension. This kept the turbidity reading below an optical density of 1.0 in the culture and the cells in almost continuous logarithmic growth for 9 hours. Dilution of the cells in M9 was not required as the cells were deprived of amino acids required for growth, and the optical density remained below 0.4.

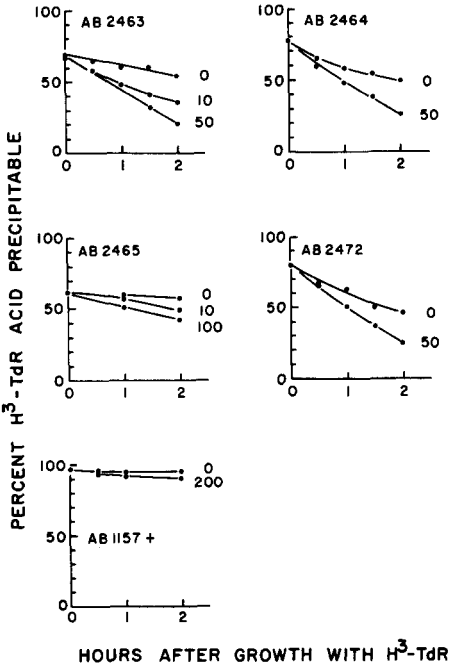

FIGURE 6.—The percentage of H^3 radioactivity in the cells, plus medium that is acid precipitable is plotted against the time of incubation at 37° with aeration after labeling and exposure to various doses of UV. The experiment was carried out as described in the legend of Figure 4 and in METHODS. (We are indebted to DR. J. B. STEDEFORD for these results.)

type strain to 200 ergs/mm² causes about 3% release of radioactivity. In contrast, the Rec⁻ mutant AB2463 exhibited an abnormally high acid soluble pool of radioactivity after labeling with thymidine. There was a spontaneous release of radioactivity from the acid precipitable form in the control culture and a greatly increased rate of release following exposure to doses of 10 or 100 ergs/mm².

The results of a further investigation into the spontaneous release of radioactivity is shown in Figure 5. After labeling by growth in the presence of H^3-thymidine, cells of strain AB2463 were washed three times. One half was resuspended in complete medium EM9, while the second half was suspended in the minimal medium M9, in which this auxotrophic strain was deprived of several amino acids required for growth. The cultures were incubated with aeration at 37°C and sampled at intervals. The amount of cold-acid soluble and acid

precipitable radioactivity of the sample containing both cells and suspending medium, was measured. The culture in EM9 medium was diluted after 2½ and after 6½ hours into fresh nonradioactive medium so that the culture was kept in logarithmic growth for a total period of 8 or 9 hours. The culture in M9 medium could not grow through lack of the required amino acids, and was not diluted. It is seen in Figure 5 that there is a progressive release of acid soluble radioactivity, and that this release occurs to almost the same extent if the cells are deprived of the four amino acids required for growth.

The spontaneous and UV-induced release of acid precipitable radioactivity from several other mutants is shown in Figure 6. Although there are reproducible differences between the strains in the initial level of acid soluble radioactivity and the spontaneous and UV-induced release of radioactivity, it is seen that the release is altogether greater in these mutants than in the original strain.

The effect of UV irradiation on the incorporation of radioactive thymidine into an acid precipitable form was investigated in the original strain AB1157, the UV-sensitive mutant AB2429 *uvrA uvrC*, that is unable to excise pyrimidine dimers (BOYCE and HOWARD-FLANDERS 1964), and the mutant AB2463 *rec-13*. Log phase cultures of the strains were divided into two halves. One was kept as control and the other was exposed to 200 ergs/mm² UV. The cultures were then supplemented with deoxyadenosine and H^3-thymidine and then incubated in subdued light. The results shown in Figure 7 indicate that UV-irradiation of the original strain causes a delay of about 30 minutes in the incorporation of radioactivity, following which the incorporation increases rapidly. The *uvr* mutant AB2429 shows an almost complete inhibition of DNA synthesis over the first hour to be followed by synthesis at a rate of no more than 10% the rate in the control. The mutant

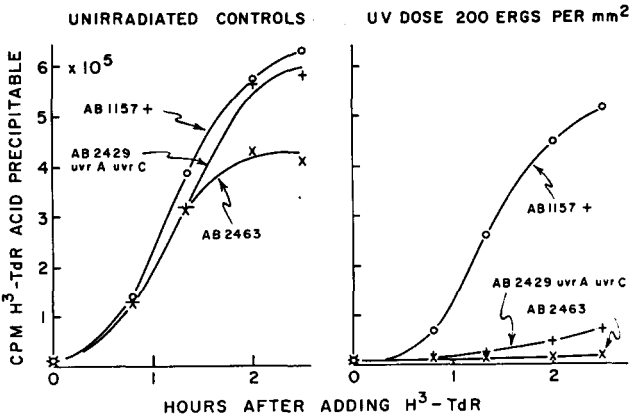

FIGURE 7.—The counts per minute of acid precipitable H^3 radioactivity in the bacterial suspension is plotted against the time after adding H^3-thymidine and incubating at 37°C with aeration.

Cultures were grown in EM9 medium to about 10^8 cells/ml, washed and resuspended in five times the volume of EM9. The suspension was divided into three fractions and exposed to various doses of UV in a thin layer to avoid adsorption. The cell suspensions were then supplemented with 250 μg/ml deoxyadenosine and 2 μc/ml H^3-thymidine of specific activity 10 c/mm. The suspensions were incubated at 37° with aeration in dim light and duplicate 0.1 ml samples were taken at intervals. The samples were pipetted onto filter discs and washed 1 hr in cold 5% trichloroacetic acid. When dry, the discs were counted in 10 ml of liquid scintillator in a scintillation counter.

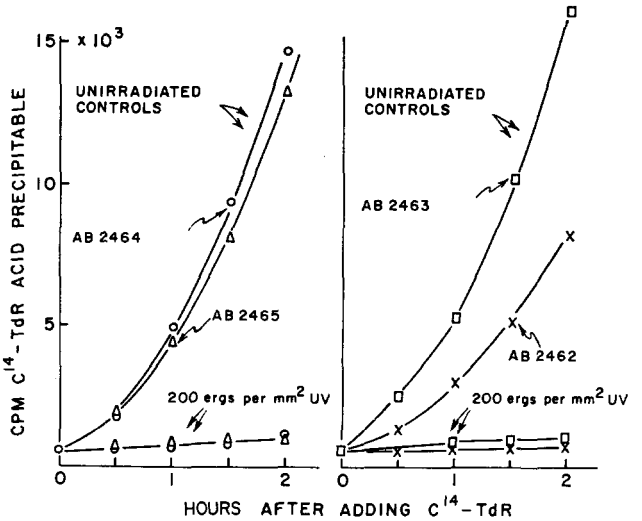

FIGURE 8.—The counts per minute of acid precipitable C^{14} radioactivity in the bacterial suspension is plotted against the time after adding H^3-thymidine and incubating at 37°C with aeration.

Cultures were grown in EM9 medium to about 10^8 cells/ml, washed and resuspended in five times the volume of EM9. The suspension was divided into two fractions and one was exposed to 200 ergs/mm² UV in a thin layer to avoid adsorption. The cell suspensions were then supplemented with 250 μg/ml deoxyadenosine and 0.5 μc/ml C^{14}-thymidine. The suspensions were incubated at 37° with aeration in dim light, and duplicate 0.1-ml samples were taken at intervals. The samples were pipetted onto filter discs and washed 1 hr in cold 5% trichloroacetic acid. When dry, the discs were counted in 10 ml of liquid scintillator in a scintillation counter.

AB2463, however, shows a virtually complete inhibition of DNA synthesis over the whole period of 2½ hours, the incorporation being less than 2% that in the control. Similar results with three of the other mutants are shown in Figure 8.

The ability of these F^- mutants to conjugate with, and accept, the sex factors from F′ strains, and to form recombinants with Hfr donor strains was next investigated. The original strain AB1157 and each one of the five mutants were mated 60 min with W4520 F-*gal*$^+$ and the number of Str^R Gal^+ recipients was determined by plating on selective agar. Similarly, the strains were mated 2 hr with AB259 Hfr H and AB2383 Hfr J2 and then plated on agar selective for Thr^+ Leu^+ Str^R recombinants. They were also mated 2 hr with AB2528 Hfr 313 and plated on agar selective for Ile^+ His^+ recombinants. The results are presented in Table 2, which shows the numbers of partial diploids and recombinants formed in crosses with the various donor strains. The results are expressed by giving the absolute yields as well as the percentage yields formed by the mutants as compared with the number formed by the original strain AB1157 in comparable crosses. It is seen that in crosses with the F-*gal*$^+$ strain W4520, the numbers of Str^R Gal^+ partial diploids formed with the mutants lies between 50 and 76% of the number formed with the original strain. The lower yield may reflect the smaller input numbers of viable cells rather than any lack of ability to conjugate. In contrast, the mutants form less than 1% of the normal numbers of recombinants in crosses in which the part of the Hfr chromosome near *thr* and *leu* is introduced. The yield of recombinants is low enough for the mutants to be considered defective in their mechanism for forming recombinants. The number

TABLE 2

The numbers of F-gal+ recipients and recombinants formed in crosses with the original strain and mutants of E. coli *K-12*

	Recipient strain	Input viable recipient cells per ml	Donor strain, mating time, selection and input viable donor cells per ml			
			W4520 F-gal+ 60 minutes Str^R Gal^+ 9×10^7	AB259 Hfr H 120 minutes Str^R Thr^+ Leu^+ 7×10^7	AB2383 Hfr J2 120 minutes Str^R Thr^+ Leu^+ 1.2×10^8	AB2528 Hfr 313 120 minutes His^+ Ile^+ 1×10^8
Experiment I						
	AB1157 +	1.7×10^8	2.1×10^8 100%	2.1×10^7 100%	1.5×10^7 100%	1.4×10^6 100%
	AB2462	6.2×10^7	1.05×10^8 50%	7×10^2 .003%	1.0×10^2 .001%	6×10^2 .04%
	AB2463	7.7×10^7	1.5×10^8 71%	1.3×10^4 .06%	2.2×10^3 .01%	9.4×10^4 6.7%
	AB2464	6.7×10^7	1.3×10^8 62%	1.5×10^4 .07%	3.4×10^3 .02%	2.6×10^4 1.8%
	AB2465	6.3×10^7	1.05×10^8 50%	9.5×10^3 .04%	2.6×10^3 .02%	1.5×10^5 11%
Experiment II						
			7.4×10^7	4.9×10^7	5.1×10^7	6.3×10^7
	AB1157 +	1.5×10^8	2.5×10^8 100%	1.9×10^7 100%	1.5×10^7 100%	2.1×10^6 100%
	AB2472	1.0×10^8	1.9×10^8 76%	2.7×10^4 .14%	5.0×10^3 .03%	4.0×10^4 1.9%

of recombinants formed was much greater, however, in the crosses with AB2528 Hfr 313 in which the part of the Hfr chromosome between *mtl* and *his* was transferred. Presumably the Hfr AB313 transfers *rec*$^+$ early, thus promoting recombination in the partial diploid so formed.

DISCUSSION

The results of the genetic crosses suggest that the X-ray sensitive mutants whose isolation is described, are able to conjugate almost normally with F′ strains and to accept genetic material from the donor. However, they form remarkably few Thr$^+$ or Leu$^+$ recombinants in crosses with Hfr H or Hfr J2, even though these are early markers on the Hfr chromosomes and mating was continued for two hours. This suggests that these mutants are defective in their ability to integrate the donor DNA to form recombinants, and may be classed as recombination-defective. In crosses where the portion of the Hfr chromosome between *his* and *mtl* enters the zygote, however, a greater relative yield of recombinants is obtained. This may reflect the entry of the wild-type allele for the recombination locus and its expression in time to promote the formation of at least a fraction of the normal number of recombinants. Other potential recombinants are presumably lost during the time before expression is complete.

The position of the recombination locus in the genetic map of *E. coli* has been determined more closely in one of the mutants, AB2463 and found to lie between *his* and *thy* in experiments to be described elsewhere (CLARK, personal communication).

The yield of pyrimidine dimers in *E. coli* is about 1.8×10^{-6} T-T dimers and 1.4×10^{-6} T-C dimers per thymine per erg/mm^2 at 2537 Å wavelength (SETLOW, SWENSON and CARRIER 1963; BOYCE and HOWARD-FLANDERS 1964; SETLOW, CARRIER and BOLLUM 1965). The release of thymine radioactivity from the original strain in two hours following exposure to 200 ergs/mm^2 usually lies between 3% and 10% and corresponds to a yield of from 50 to 140 thymines per dimer. Assuming that the other three bases which are present in similar amounts are released to the same extent, the total amount of DNA degraded must correspond to between 200 and 500 nucleotides per pyrimidine dimer in the original strain. The UV-induced release of radioactivity in the various Rec$^-$ mutants lies between 0.5% and 2% per erg/mm^2 during a 2-hour incubation. This corresponds to from 5×10^3 to 2×10^4 nucleotides per pyrimidine dimer, and is 20 to 40 times greater than that in the original strain. It is not yet known whether the release of radioactivity results from a similar breakdown at each of many sites where the single strands are interrupted, or whether it is due to a very uneven breakdown that occurs mainly in certain parts of the DNA or in certain cells. As pointed out below, many UV-induced single-strand breaks in Rec$^-$ strains appear to be repaired. This suggests that breakdown at these sites is limited and that it must be every extensive elsewhere. The spontaneous breakdown of DNA, that is so marked in these Rec$^-$ strains when grown as described, may also reflect a very extensive breakdown at a small number of sites.

Recombination defective mutants of different types can be recognised. The yields of recombinants from the crosses listed in Table 2 show considerable varia-

tions, and are lowest with AB2462, which is also the most X-ray sensitive mutant isolated. Other mutants such as AB2470 do not show the abnormal spontaneous and radiation induced DNA breakdown observed with AB2463, and will be described elsewhere.

It is seen in Figure 7 that there is a marked inhibition of the ability to incorporate radioactive thymidine into an acid precipital form in bacteria immediately following exposure to UV-irradiation. This may, in part, be due to the inhibition of DNA replication as the polymerase reaches a pyrimidine dimer in the DNA (SETLOW, SWENSON and CARRIER 1963). This cannot be the only cause of the lack of uptake, for the incorporation is inhibited more completely in the Rec⁻ mutant AB2463 than in the *uvr*⁻ mutant AB2429 although the latter strain cannot excise thymine dimers. This inhibition of thymidine uptake was immediate even in Rec⁻ cells already labeled in their DNA (CLARK, CHAMBERLIN, BOYCE, and HOWARD-FLANDERS 1966). This result may reflect either (1) an inhibition of the formation of thymidine triphosphate from exogenous thymidine and a preferential utilization of DNA breakdown products for new synthesis. (2) an inhibition of the DNA polymerase responsible for chromosomal replication by single-strand cuts resulting from incomplete repair, or (3) the action of a DNA replication inhibitor, triggered by injury to the DNA or by the resulting breakdown products. The present results do not permit any one of these alternatives to be ruled out with certainty.

All these X-ray sensitive Rec⁻ mutants appear to repair single strand breaks with a reduced efficiency and frequently fail to control DNA breakdown. Thus the higher level of survival of the original strain AB1157 suggested that wild-type bacteria normally repair most of the X-ray induced products in their DNA by single strand breakdown and repair synthesis.

The 37% survival dose for T1 phage plated on strain AB1886 *uvrA* is about 40 ergs/mm². Assuming that T1 phage contains 2.5×10^4 thymines and the yield of T-T + T-C dimers per thymine to be 3.2×10^{-6} for a dose of 1 erg/mm² at 2537A (SETLOW, SWENSON and CARRIER 1963; BOYCE and HOWARD-FLANDERS 1964; SETLOW, CARRIER and BOLLUM 1965), the number of pyrimidine dimers per lethal hit is 4. This low figure suggests that the majority of photoproducts can act as lethal hits and that there can be little effective repair of T1 phage DNA in the host that carries a mutation in one of the *uvr* loci.

The UV dose at which 37% of the Rec⁻ mutants survive is about 3 ergs/mm², which will induce about 25 dimers in the bacterial genome of 10^7 nucleotides. As the dimers will presumably be excised from the DNA of Rec⁻ strains that carry no *uvr* mutation, it appears that over 90% of the single-strand cuts may be successfully repaired. At higher doses, the rate of inactivation per unit dose is less and the fraction of single-strand breaks to be repaired must be even higher. Thus, there is a loss of efficiency rather than an absolute block in the DNA repair system. This suggests that all the enzymes required of repair must be present, even if they do not operate with normal efficiency. It has already been found that the levels of endonuclease I, exonucleases I, II and III as well as DNA polymerase activity in crude extracts are approximately normal in the Rec⁻ strain JC1569 (CLARK, CHAMBERLIN, and HOWARD-FLANDERS 1966).

Several hypotheses as to the nature of the defect in these Rec⁻ mutants may be considered. (1) *Modified DNA hypothesis.* The recombination gene may control a chemical modification of the DNA. Thus, the DNA may differ in its susceptibility to enzymatic degradation following single strand interruption, according to whether it comes from a Rec⁻ or normal strain. (2) *Uncontrolled exonuclease hypothesis.* The nuclease responsible for the degradation that follows single-strand interruption, may be defective in these Rec⁻ mutants, being abnormally slow in releasing the site of action in the DNA to the repair polymerase. To explain the apparent dominance of the wild-type recombination gene in crosses, it may be assumed that this gene determines an enzyme subunit. This subunit is required for a correctly phased changeover from nuclease to repair polymerase at the site of action in the DNA. Phage may induce their own regulatory substance to control exonuclease action. (3) *Exonuclease regulator hypothesis.* Normal cells may contain a substance that may be called an exonuclease regulator. The function of the exonuclease regulator is to control single-strand breakdown, by ensuring that the exonuclease is removed and replaced by the repair polymerase at the site of action in the DNA at the correct time. This substance may be missing or defective in these Rec⁻ mutants. As this mutation does not appear to influence the repair of phage DNA, it is possible that the postulated exonuclease regulators are normally attached to the host chromosome, and that certain phage may have their own.

The present results are not sufficient to rule out any one of these hypotheses. Whatever the primary cause of the undue breakdown may prove to be, it can be understood that a mutation causing uncontrolled and excessive DNA degradation at single-strand breaks is likely to affect processes that involve single-strand interruption, such as repair and recombination. Undue breakdown may cause the loss of DNA essential to stability or continuity in the process of forming a recombinant.

In conclusion, the finding that certain recombination-defective mutants are highly sensitive to radiation, and that certain mutants selected for high sensitivity to X rays are also defective in genetic recombination, strongly supports the idea that there are common enzyme-mediated steps involved in the two processes. These steps may involve the orderly release of nucleotides from single-strand gaps, DNA repair synthesis and rejoining of the phospho-diester backbones.

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SUMMARY

Five X-ray sensitive mutants were isolated from a genetically marked F⁻ strain of *E. coli* K-12 following treatment with nitrosoguanidine and testing survivors by replica plating for sensitivity to radiation. Each one of the five most X-ray sensitive mutants was found to be defective in ability to form recombinants in suitable crosses. These mutants are able to conjugate with F-*gal*⁺ donors to form Gal⁺ recipient strains with almost normal frequency. However, in crosses with

Hfr H or Hfr J2 in which transmission of the early markers *thr* and *leu* was required, the numbers of recombinants was less than 1% of that formed in comparable crosses with the original strain. In crosses with Hfr 313 that transmitted the chromosome from *mtl* to *his*, the number of recombinants formed was between 1 and 10% of those formed with the original strain. These results suggest that the wild-type allele for the recombination defect in these mutants lies between *mtl* and *his*, and that the entry of the wild-type allele is able to promote the formation of some of the recombinants which would otherwise be lost. These mutants are able to reactivate ultraviolet (UV)-irradiated T1 bacteriophage (Hcr^+) and carry out the repair of UV-induced pyrimidine dimers in their own DNA with a reduced efficiency. When labeled in their DNA by growth with H^3-thymidine and then irradiated, the mutants show both a spontaneous release of radioactivity during incubation and a greatly increased release if exposed to UV. Unlabeled log phase mutants exposed to UV show an immediate and prolonged inhibition of ability to incorporate radioactive thymidine. These Rec^- mutants appear to be defective in their ability to terminate DNA breakdown and initiate repair synthesis correctly, following single-strand interruption.—The finding that certain recombination-defective mutants are highly sensitive to radiation and that certain mutants selected for high sensitivity to X rays are also defective in genetic recombination, supports the idea that common enzyme mediated steps are involved in the two processes. These steps may include the widening of single-strand gaps, DNA repair synthesis, and joining phospho-diester bonds.

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