THREE LOCI IN *ESCHERICHIA COLI* K-12 THAT CONTROL THE EXCISION OF PYRIMIDINE DIMERS AND CERTAIN OTHER MUTAGEN PRODUCTS FROM DNA

PAUL HOWARD-FLANDERS, RICHARD P. BOYCE AND LEE THERIOT

Radiobiology Laboratories, Yale University School of Medicine, New Haven, Connecticut

Received December 30, 1965

ULTRAVIOLET irradiation produces dimers of thymine, thymine-cytosine and cytosine in deoxyribonucleic acid (DNA) (BEUKERS, IJLSTRA and BERENDS 1960; WACKER, DELLWEG and WEINBLUM 1960; SETLOW, CARRIER and BOLLUM 1965). UV light inactivates transforming DNA and induces thymine dimers in a parallel manner, as if the dimers block transforming activity (SETLOW and SETLOW 1962). Thymine dimers and thymine-cytosine dimers are removed and probably monomerized by photoreactivation enzyme (WULF and RUPERT 1962; SETLOW, CARRIER and BOLLUM 1965). They are excised from DNA during incubation in the dark in wild-type strains of *E. coli*. However, this excision does not occur in the radiosensitive mutants B_{S-1} and K-12 *uvrA* (SETLOW and CARRIER 1964; BOYCE and HOWARD-FLANDERS 1964a).

T1 or λ bacteriophage irradiated with ultraviolet light (UV) form more plaques when plated on wild-type cells than when plated on these radiosensitive mutants (ELLISON, FEINER and HILL 1960; HOWARD-FLANDERS, BOYCE, SIMSON and THERIOT 1962; RORSCH, EDELMAN and COHEN 1963; HARM 1963). These results may be explained if the irradiated phage DNA is reactivated (host cell reactivation) in wild-type hosts, but not in these UV-sensitive mutants.

The reactivation of UV-irradiated T1 bacteriophage is controlled by three genetic loci, designated *uvrA*, *uvrB* and *uvrC*, the approximate map positions of which have been reported (HOWARD-FLANDERS 1964; VAN DE PUTTE, VAN SLUIS, VAN DILLEWIJN and RORSCH 1965). In the present paper, we report the genetic analysis of 23 such mutants, more accurate mapping by cotransduction of the *uvrA*, *uvrB* and the *uvrC* loci with other markers, some properties of the double mutants that carry two *uvr* mutations, and evidence that all three loci control the excision from DNA of UV-induced thymine dimers and thymine-cytosine dimers.

MATERIALS AND METHODS

Bacterial strains and media: The characteristics of the strains are listed in Table 1, which also acknowledges the origin of the strains obtained from other laboratories. The complete and the selective media used follow those described by ADELBERG and BURNS (1960). Yeast extract tryptone (YET) agar contains: 1% tryptone, 0.5% yeast extract, 1% NaCl and 2% agar.

The following abbreviations will be used for growth requirements: arginine, Arg; biotin, Bio; histidine, His; isoleucine, Ile; isoleucine or valine, Ilv; leucine, Leu; methionine, Met;

TABLE 1

Characteristics of E. coli *K-12 strains*

Strain Number	Origin or Synonym	uvr	Auxotrophic Characters												Energy Source Utilization					Phage Growth			Drug Resist		Suppressors	Sex	Injection Sequence	Obtained From
			Purine	Threonine	Leucine	Proline	Histidine	Methionine	Thiamine	Arginine	Thymine	Biotin	Isoleucine	Pyrimidine	Lactose	Galactose	Arabinase	Xylose	Mannitol	T1	T6	λ	Streptomycin	Mitomycin C				
AB 259	Hfr Hayes	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	S	S	S	S		Pm⁻	HfrH	pyr,thr,leu	Adelberg
AB 312	Hfr 312	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	S	S	S	R			Hfr312	str,mtl,met	Adelberg
AB 313	Hfr 313	+	+	(-)		+	+	+	-	+	+	+	+	+	-	+	+	+	+	S	S	R	R			Hfr313	mtl,str, his	Adelberg
AB 451	From Hfr J2	+	+	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	S	S	S	R			Hfr J2	pro,leu,ara	Adelberg
AB 492	From Hfr 311	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	R	S		R			Hfr311	his,try,gal	Eggertsson
AB 673	From PX 10	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	R	S					Hfr J4	met,ile,val	Adelberg
AB 2285	From AB312	+	-	+	+	+	+	+		-	+	+	88	+	+	+	+	-	+		S	S	S		H	Hfr312	str,mtl,met	Eggertsson
AB 2383	From P4 x 6	+	+	+	+	+	+	B		+	-	+	+	+	+	+	+	+		S	S		S			Hfr J2	pro,leu,ara	Eggertsson
AB 2433	AB259 x AB1884	C-34	+	+	+	+	-	+	-	+	+	+	+	+	-	+	+	-		S	S		R			Hfr H	pyr,thr,uvrB⁺,uvrC⁻	This paper
AB 2434	AB259 x AB1885	B-5	+	+	+	+	-	+	-	+	+	+	+	+	-	+	+	-		S	S		R			Hfr H	pyr,thr,uvrB⁻,uvrC⁺	This paper
AB 2435	AB2383 x AB1884	C-34	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-		S	S		R			Hfr J2	pro,leu,uvrA⁺,uvrC⁻	This paper
AB 2436	AB2383 x AB1885	B-5	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-		S	S		R			Hfr J2	pro,leu,uvrA⁺,uvrC⁺,uvrB⁻	This paper
AB 2437	AB2383 x AB1886	A-6	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+			S	S		R			Hfr J2	pro,leu,uvrA⁻	This paper
AB 2440	AB313 x AB1886	A-6	+	(-)		-		+	-	+	+	+	+	+	-	-	-	-		S	R		R			Hfr313	mtl,str,uvrC⁺,uvrB⁺,uvrA⁻	This paper
AB 1446		+	+	+	+	-	-	+	-	+	+	+	+	+	-	-		+			S		R			F-14		Pittard
AB 2407	Mut.from AB451	A-37	+	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	S	S	S	R			F⁺		This paper
AB 2414	Mut.from AB259	B-45	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	S	S	S	S			F⁺		This paper
AB 1157		+*	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	S	R	S	S	R	R	Pm⁺	F⁻		Adelberg
AB 1884	Mut.from AB1157	C-34*	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	S	R	S	S	R	S		F⁻		This paper
AB 1885	Mut.from AB1157	B-5*	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	S	R	S	S	R	S		F⁻		This paper
AB 1886	Mut.from AB1157	A-6*	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	S	R	S	S	R	S		F⁻		This paper
AB 1932		+	+	+	+	+	+	-		-	+	+	+	+	-	-	+	-	+	S	R			S			F⁻		Eggertsson
AB 2415		+	+	-	-	+	+	+		+	-	+	+	+	+	+	+	+		S				S			F⁻		Rudner
AB 2421	AB2414 x AB1886	B-45 A-6	+			-	-	+	-	-	+	+	+	+	-	+	-			S	R			R			F⁻		This paper
AB 2429	AB2407 x AB1884	A-37 C-34	+	-	-	+	-	+	-	+	+	+	+	+	-	-	+	-		S	R			R			F⁻		This paper
AB 2430	AB2414 x AB1884	B-45 C-34	+			-	-	+	-	-	+	+	+	+		+	-			S	R			R			F⁻		This paper
AB 2432	AB2383 x AB1886	A-6	+			-		B		-	+	+	+	+			-	+									F⁻		This paper
AB 2477	AB2435 x AB2279	C-34	+	+	+		-			+	+	+	-	+						S							F⁻		This paper
AB 3035	AT 1380	+	D	+	+	-	-	+	-	-	+	+	+	+													F⁻		Taylor
AB 3036	AT 1385	+	+	+	+	A	-	+		-	+	+	+	B	-	-		-	-		R			S			F⁻		Taylor
AB 3037	A 437	+	+	-	-	+	+	+		+	+	-	+	+		-				S							F⁻		Luria
AB 3042	AB259 x AB2500	A-6	+	-	-	-	+	+	-	-	-	+	+	+			-	-	-	S		S			Pm⁻		F⁻		This paper

Other strains are listed in Table 2. Except for *uvr* character, they are similar to AB1157 unless listed above.

* Thymine requiring derivatives of AB1157, AB1884 *uvrC*, AB1185 *uvrB* and AB1886 *uvrA* that grow with 2μg/ml thymine are available and are numbered AB2497, AB2498 *uvrC*, AB2499 *uvrB* and AB2500 *uvrA* respectively.

P— [illegible] permissive host for phage with similar mutations

proline, Pro; purine, Pur; pyrimidine, Pyr; thiamine, Thi; threonine, Thr; thymidine, Thy. The loss of ability to utilize carbon sources will be abbreviated as follows: arabinose, Ara; galactose, Gal; lactose, Lac; manitol, Mtl; and xylose, Xyl. Streptomycin and Mitomycin C will be abbreviated Str and MC.

Crosses: The sites of *uvr* mutations in the chromosome of *E. coli* K-12 were investigated by the interrupted mating method and by the analysis of unselected markers (JACOB and WOLLMAN 1961; ADELBERG and BURNS 1960). The methods used for selecting *uvr*+ recombinants and scoring patches for *uvr*+ after replica plating differ slightly from those previously described (HOWARD-FLANDERS, BOYCE, SIMSON and THERIOT 1962). To determine the time of entry of the *uvr*+ allele, or to make the *uvr*+ selection, the zygotes were incubated for 3 hours on YET agar, then respread and exposed to 250–350 ergs/mm^2 of UV. Surviving colonies were inoculated in patches on plates which, after incubation, were printed onto YET agar and then exposed to 1500 ergs/mm^2 UV. They were also printed onto YET agar plates that had been spread with 10^7 T1 phage exposed to 600 ergs/mm^2 UV. Only the *uvr*+ patches produced confluent growth on the UV-irradiated plates, while only the *uvr*− patches grew on the plates spread with irradiated phage. The master plates were also printed onto selective agar so that the *uvr*+ patches could be analysed for unselected markers.

Irradiations: The source of UV was a 15 watt low-pressure mercury germicidal lamp. The intensity was measured with a General Electric germicidal light meter. The source of ionizing radiation was a 6 Mev linear accelerator which provided a dose rate of fast electrons of about 10^5 rads/minute to 10 ml of liquid in an 16 mm diameter tube irradiated from the side. The dose rate was measured with acid ferrous sulphate dosemeter solution.

The cells to be irradiated were grown overnight on YET agar, harvested and washed in buffered saline (0.13 M NaCl, 0.02 M phosphate at pH = 6.8). The suspension was diluted to 2×10^6 cells/ml for irradiation and exposed in a layer not more than 3 mm deep to the UV so that adsorption was insignificant. Suspensions were exposed to X rays in 10 ml amounts and bubbled with oxygen during the irradiation.

Transduction: Transduction was performed essentially according to the method of LENNOX (1955). Lysates of P1kc phage were harvested from YET agar containing 2.5×10^{-3} M $CaCl_2$, seeded with 2×10^7 donor bacteria and the same number of phage in soft agar. 10^9 recipient bacteria were mixed with from 1 to 5×10^9 P1kc phage in YET broth containing 2.5×10^{-3} M $CaCl_2$, incubated at 37°C for 20 minutes, washed and plated on selective media.

Measurement of thymine dimers and their excision from DNA: The methods for labeling the DNA of the bacteria with H^3-thymidine and for measuring the UV-induced thymine dimers in the cold acid precipitable and soluble fractions are similar to those already reported (BOYCE and HOWARD-FLANDERS 1964a). Each mutant was grown overnight at 37°C in EM9 medium containing 250 μg/ml deoxyadenosine and 50 μc/ml H^3-thymidine of specific activity 10 to 12.5 c/mmole. To this was added an equal volume of the same radioactive medium, and the culture was grown to early stationary phase with aeration. It was washed three times by centrifugation and diluted in M9 medium to give about 50% transparency to UV in a 2 mm deep layer. The suspension was exposed to the 2537 A light so that the average does to the cells in this layer was 2250 ergs/mm^2. The 5 ml cell suspension was supplemented with 0.04% Casamino acids and incubated at 37°C for 2 hours. It was centrifuged and the pellet was suspended in 5% trichloroacetic acid at 0°C. After 45 minutes, it was centrifuged, the pellet and supernatant were dried, and hydrolysed separately in 1 ml of trifluoroacetic acid in evacuated sealed tubes at 175°C for 90 minutes. The cold acid precipitable and soluble fractions were then subjected to descending paper chromatography in *n*-butanol acetic acid water (200–30–75 by volume) and passed through a strip scanner to measure the distribution of the radioactivity.

RESULTS

Isolation and sensitivity of mutants: Cultures of strains AB1157, AB259, AB451 and AB2415 were treated with nitrous acid and plated in soft agar containing

UV-irradiated T1 phage to eliminate unmutated cells as previously described (HOWARD-FLANDERS and THERIOT 1962). Single-colony isolates were made of the survivors. The majority proved to be T1 phage resistant and were rejected, but a number were T1 phage sensitive. Certain of these proved to be mutants with a greatly reduced plating efficiency for UV-irradiated T1 phage and were designated *uvr-1* etc. The number of plaques formed with T1 phage exposed to 500 ergs/mm^2 on various mutants are listed in Table 2. It is seen that the majority of mutants show approximately the same number of plaques as the strain AB1886 *uvrA6*, previously described, but some mutants show higher numbers, suggesting that these cells have residual ability to reactivate the UV-irradiated phage.

Position of uvrA *locus:* Certain F$^-$ *uvr* mutants were crossed with the Hfr strains AB259, AB451 or AB492 and the times of entry of the *uvr* and other mark-

TABLE 2

Properties of uvr *mutants.* (1) *Ability to form UV-resistant zygotes when mated with Hfr test strains.* (2) *Ability to propagate UV-irradiated T1 phage.* (3) *Levels of soluble and acid insoluble pyrimidine dimers in cells incubated for 2 hours following exposure to 2250 ergs/mm^2 UV*

Strain Number	Mutation Number	UV-resistant zygotes formed when mated with AB2435	AB2437	AB2433	AB2434	Fraction of T1 phage to form plaques on strain, UV dose to phage 500 ergs/mm^2	Acid Precipitable Thymine mean counts per minute	Acid Precipitable Dimer counts as % of thymine	Acid Soluble Thymine mean counts per minute	Acid Soluble Dimer counts as % of thymine	Number of Tests
Hfr AB259	+					7.34×10^{-1}			-	-	0
Hfr AB451	+					6.85×10^{-1}			-	-	0
F$^-$ AB1157	+					6.23×10^{-1}	60,000	0.6	5,000	4.6	8
F$^+$ AB2409	40					8.70×10^{-4}	46,000	0.6	3,200	< 1.0*	1
F$^+$ AB2414	(B) 45					1.95×10^{-4}	69,000	0.6	3,200	3.5	3
F$^-$ AB1881	A 14	+	-	-		1.70×10^{-4}	80,000	0.8	3,600	< 1.0	1
F$^-$ AB2404	(C) 31				(a)	1.35×10^{-4}	82,000	0.7	3,200	< 1.0	1
F$^-$ AB2401	B 27	-		+	- (b)	8.82×10^{-5}	48,000	0.7	3,000	< 1.0	1
F$^-$ AB2405	(C) 33				(a)	6.04×10^{-5}	58,000	0.7	3,500	< 1.0	2
F$^-$ AB1883	B 16	-		+	- (b)	5.83×10^{-5}	62,000	0.8	5,500	1.6	3
F$^-$ AB1890	(A) 20				(c)	5.57×10^{-5}	54,000	0.8	7,400	< 1.0	1
F$^-$ AB2419	B 49	-		+	-	4.25×10^{-5}	60,000	0.8	7,900	2.3	3
F$^-$ AB1893	A 24	+	-	-		4.10×10^{-5}	76,000	0.9	3,200	< 1.0	1
F$^-$ AB1885	B 5	-		+	-	3.90×10^{-5}	90,000	1.0	7,600	< 1.0	1
F$^-$ AB1894	A 25	+	-	-		3.84×10^{-5}	86,000	0.8	5,000	< 1.0	2
F$^+$ AB2413	44					3.83×10^{-5}	50,000	0.8	2,500	< 1.0	1
F$^-$ AB1892	A 23	+	-	-		3.83×10^{-5}	72,000	0.9	4,500	< 1.0	1
F$^-$ AB2403	B 30	-		+	-	3.79×10^{-5}	57,000	0.8	4,000	< 1.0	1
F$^-$ AB2402	B 29	-		+	-	3.68×10^{-5}	82,000	0.7	4,300	< 1.0	2
F$^-$ AB2417	A 48	+	-	-		3.61×10^{-5}	57,000	0.9	7,400	1.3	2
F$^-$ AB1891	A 22	+	-	-		3.61×10^{-5}	75,000	0.8	5,000	< 1.0	1
F$^-$ AB1895	A 26	+	-	-		3.58×10^{-5}	-	-	4,400	< 1.0	1
F$^-$ AB1889	A 19	+	-	-		3.54×10^{-5}	63,000	0.8	7,000	< 1.0	1
F$^+$ AB2407	(A) 37					3.53×10^{-5}	58,000	0.8	2,800	< 1.0	1
F$^-$ AB1882	15					3.52×10^{-5}	72,000	1.0	2,800	< 1.0	1
F$^-$ AB1888	A 18	+	-	-		3.52×10^{-5}	72,000	0.9	4,600	< 1.0	1
F$^-$ AB1886	A 6	+	-	-		3.49×10^{-5}	80,000	0.4*	4,900	< 1.0	2
F$^-$ AB1884	C 34	-	-	-	-	3.38×10^{-5}	48,000	0.3*	2,800	< 1.0	1
F$^+$ AB 2406	36					3.28×10^{-5}	66,000	0.7	3,500	< 1.0	1
F$^+$ AB2408	38					3.18×10^{-5}	60,000	0.8	2,800	< 1.0	1
F$^-$ AB2421	A6 (B) 45					3.62×10^{-5}					
F$^-$ AB2430	(B) 45 C34					3.12×10^{-5}		*UV dose was		*< 1.0% means	
F$^-$ AB2429	(A) 37 C34					3.06×10^{-5}		800 ergs/mm^2		undetectable	

Letters in parentheses indicate the assignment to a regional group by crude mapping only. (a) The (C) mutants were crossed with AB313. *his*$^+$ recombinants isolated after 80 minutes contained 70 to 80% *uvr*$^+$ compared with 65% *uvr*$^+$ in the same cross and selection with AB1884 *uvrC34*. (b) Incubation for 48 hours gave rise to colonies. This was not due to mixed culture as shown by testing single colony isolates. (c) AB1890 maps in the *A* region. It fails to propagate UV-irradiated T1, but is unusual in being moderately UV resistant in colony forming ability. Thus, the complementation test could not be done on this mutant.

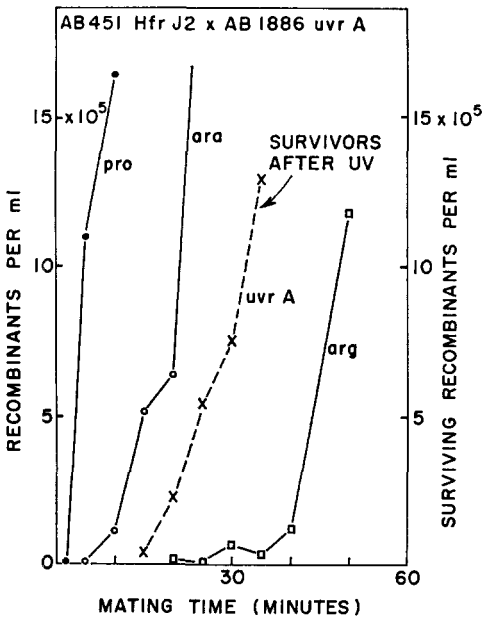

FIGURE 1.—The time sequence of transfer of genetic markers in *E. coli* K-12 AB451 Hfr J2 $T6^s$ × AB1886 F^- *uvrA6 pro⁻ ara⁻ arg⁻*. The numbers of recombinants per ml that formed colonies on selective agar and the numbers that survived UV-irradiation after incubation for 3 hours on YET agar, is plotted against the time at which mating was interrupted by the addition of T6 phage. The colonies were scored after incubation for one day on YET agar and after incubation for two days on selective media.

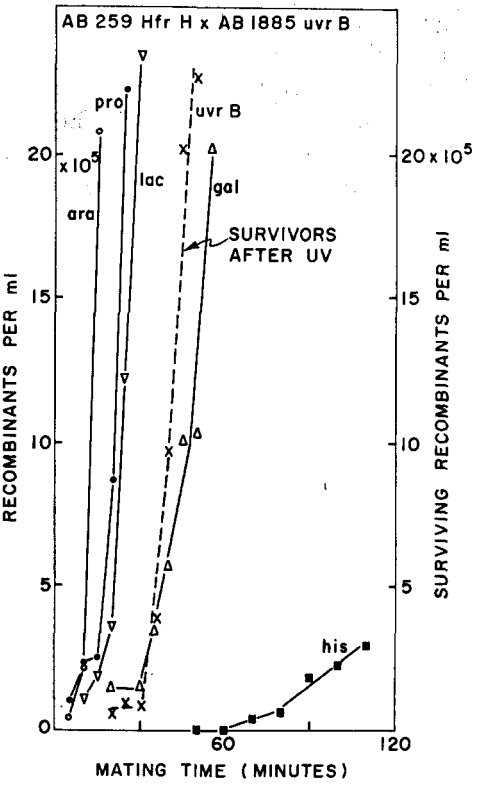

FIGURE 2.—The time sequence of transfer of genetic markers in *E. coli* K-12 AB259 Hfr H str^s × AB1885 F^- *uvrB5 str^r ara⁻ pro⁻ lac⁻ gal⁻ his⁻*. The number of recombinants per ml that formed colonies on selective agar and the numbers that survived UV-irradiation after incubation for 3 hours on YET agar is plotted against the time before mating was interrupted by agitation in a Vortex shaker and plating on media containing streptomycin.

ers were determined. It is seen in Figure 1 that in AB451 × AB1886, the *uvr*$^+$ gene required to make the zygote UV-resistant enters at about 15 minutes, between *ara* and *argA*. The previously reported analysis of unselected markers in AB451 Hfr J2 × AB1886 *uvr* supports this conclusion (HOWARD-FLANDERS, BOYCE, SIMSON and THERIOT 1962). Thus, the site of the mutation in strain AB1886 is at this locus between *arg* and *ara* which is designated *uvrA*. In crosses with AB1886, it was found that *uvrA* was not an early marker in the strains AB259 Hfr H or on AB673 Hfr J4. *uvrA* is cotransducible by phage P1kc with the methionine locus of AB 1932 *met-28*, which is an early marker, preceding arginine by about one minute, on AB673 Hfr J4 (see Figure 4) and is not on the

F-genote F′ 14 of AB1446 (PITTARD and ADELBERG 1964). Thus, *uvrA* must be close to the sex factor of Hfr J4. There were no *uvrA*$^+$ cotransductants among 492 *Met*$^+$transductants of AB2432 *uvrA6* that carried *metB1* from P4X-6 (PITTARD, LOUTIT and ADELBERG 1963). There were also no *uvrA* cotransductants among 287 Pyr$^+$ transductants of AT1385 *pyrB*, or among 82 Pur$^+$ transductants of AT1380 *purD*. *uvrA* was cotransduced in 7 out of 287 Met$^+$ transductants of AB1932 when AB2437 *uvrA6* was used as the donor, and in 18 out of 287 Met$^+$ transductants of AB1932 when AB2437 *uvrA6* was used as the donor. Thus, *uvrA* is cotransduced at a frequency of about 4% with this methionine locus, which is close to or the same as *met-A* described by TAYLOR and THOMAN (1964).

Position of uvrB *locus:* The time of entry of various markers in AB259 Hfr H × AB1885 are shown in Figure 2. It is seen that in this cross the *uvr* marker enters at about 32 minutes, shortly after *gal*, but well before *his*. This locus is designated *uvrB*. The results from the analysis of unselected markers in AB259 Hfr H × AB1885 *uvrB* are given in Table 3 and support the conclusion that *uvrB* is close to *gal*.

When mating was interrupted at 35 minutes, the 121 *uvrB*$^+$ recombinants which were also Lac$^+$, had 97% *gal*$^+$. Among the 149 Gal$^+$ recombinants which were Lac$^+$, 77% were *uvr*$^+$. These results suggest that the order of the markers is *lac*, *gal*, *uvrB*. Advantage was taken of the zygotic induction that occurs when λ prophage enters a nonlysogenic zygote (JACOB and WOLLMAN 1956) to determine if λ prophage enters before *uvrB*. AB1885 *uvrB* was mated with AB259 Hfr H and AB259 Hfr H (λ). It was found that in the Gal$^+$ selection from AB259 Ffr H × AB1885 *uvrB*, 75% were *uvr*$^+$, while in the Gal$^+$ selection from AB259 Hfr H (λ) × AB1885 *uvrB*, only two out of 160 were *uvr*$^+$. These results suggest that λ prophage enters before *uvr*$^+$.

Further support for the order of markers *gal*, λ, *uvrB* was obtained from P1 transduction of A437 (*gal*$^-$ *bio*$^-$ *uvr*$^+$) to Gal$^+$ or Bio$^+$, using the donor AB2434 *uvrB*. *bio* and *gal* are on opposite sides of λ (ROTHMAN 1965). An analysis of unselected markers among 257 Gal$^+$ transductants between strains that did not carry the λ prophage showed that 19.5% were *uvrB*, and 22.6% were Bio$^+$. Of 169 Bio$^+$ transductants, 40.2% were Gal$^+$, while 80.5% were *uvrB*. *uvrB* was not cotransduced in four Gal$^+$ transductants of strain AB1885 by λdg. This locates *uvrB* near to *bio*, with λ between these markers and *gal*.

TABLE 3

Analysis of recombinants from AB259 Hfr H × AB1885 uvrB *for unselected markers*

Selected marker	Mating time (Minutes)	Number tested	Unselected markers				
			pro$^+$	*lac*$^+$	*gal*$^+$	*uvrB*$^+$	*his*$^+$
pro+	20	96	100%	0	0	0	0
lac+	25	64	98%	100%	2%	0	0
gal+	35	204	68%	72%	100%	75%	0
uvr+	35	222	51%	54%	87%	100%	0
his+	50	198	46%	47%	40%	41%	100%

Position of uvrC *locus:* The mutant AB1884 was of interest because the site of the *uvr* mutation differed from that of either of the two strains AB1886 *uvrA* or AB1885 *uvrB*. This was shown by the facts, first that in AB451 Hfr J2 × AB1884 *uvr*, there were no uvr^+ recombinants in the 45-minute Arg^+ selection, while there were about 80% uvr^+ in the same selection from AB451 Hfr J2 × AB1886 *uvrA*. Secondly, in AB259 Hfr H × AB1884 *uvr*, there was only 1% uvr^+ in the 45-minute Gal^+ selection, while there were 74% uvr^+ in the same selection from AB259 Hfr H × AB1885 *uvrB*. As the site of the mutation in AB1884 differed from either *uvrA* or *uvrB*, the locus was designated *uvrC* and its position was determined from further crosses. Figure 3A shows the results of a time of entry experiment with AB259 Hfr H × AB1884 *uvrC*. It is seen that *uvrC* enters at about 80 minutes and is linked to *his*. Figure 3B shows results from AB492 Hfr 311 × AB1884 *uvrC* and that both *his* and *uvrC* enter at about 15 minutes. The results obtained in the analysis of unselected markers from these two crosses are shown in Table 4. The fact that 77% of zygotes tested were uvr^+ in the His^+ selection from the cross with AB259 Hfr H, while 54% of the zygotes tested were uvr^+ in the His^+ selection from the cross with AB492 Hfr 311 suggests that the order of markers is *his*, *uvrC* and *try*. This order was confirmed by P1 transduction analysis. AB2477 (*uvrC*, *his*$^-$, *ilv-88*) was transduced to His^+

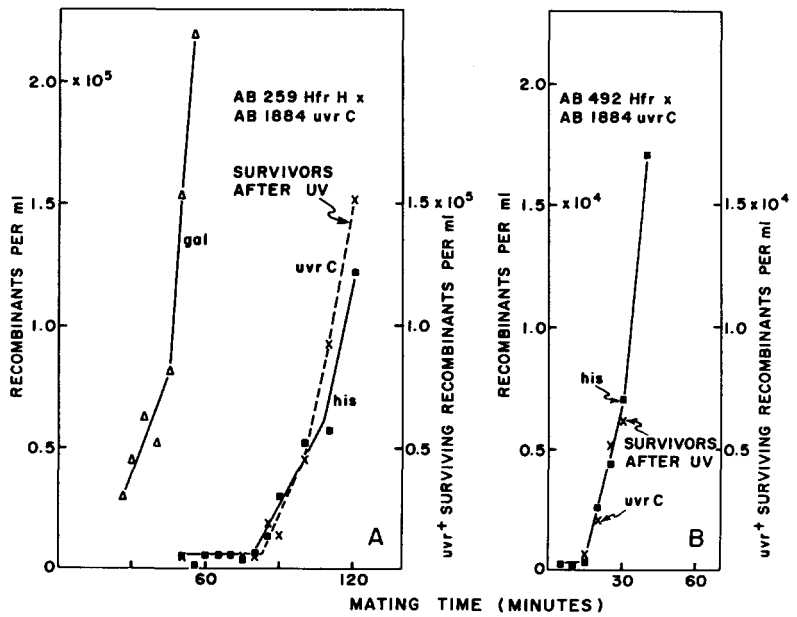

FIGURE 3.—The numbers of recombinants per ml that formed colonies on selective agar and the numbers that survived UV-irradiation after incubation for 3 hours on YET agar is plotted against the time before mating was interrupted. A.—The time sequence of transfer of genetic markers in *E. coli* K-12 AB259 Hfr *str*^{s} × AB1884 F$^-$ *uvrC34 gal*$^-$ *his*$^-$. Mating was interrupted by agitation in a Vortex shaker and plating on media containing streptomycin. B.—The sequence of transfer of genetic markers in *E. coli* K-12 AB492 Hfr 311 T6^s × AB1884 F$^-$ *uvrC34 gal*$^-$ *his*$^-$. Mating was interrupted by the addition of T6 phage.

TABLE 4

Analysis of recombinants from crosses with AB1884 uvrC *for unselected markers*

Cross	Selected marker	Mating time (minutes)	Number tested	Unselected marker		
				gal+	*uvr*+	*his*+
AB1884 × AB259	*uvr*+	110–120	123	6.5%	100%	46.3%
AB1884 × AB259	*his*+	110–120	161	13.7%	77%	100%
AB1884 × AB492	*uvr*+	40–50	134	...	100%	74%
AB1884 × AB492	*his*+	40–50	238	...	54.1%	100%

or *supH12* (phenotype Ilv+) using P1 phage from the donor AB2285 (*uvr*+, *his*+, *ilv-88*, and *supH12* which suppresses *ilv-88*) (EGGERTSSON and ADELBERG 1965). An analysis of unselected markers among 300 His+ transductants showed 15.7% *supH12* and 0.33% *uvr*+. Thus, the order of markers appears to be *his*, *supH12*, *uvrC*, *try*.

Of the 23 *uvr*- mutants investigated, 13 were in or near *uvrA*, seven were in or near *uvrB* and only 3 proved to be in or near *uvrC*. The approximate positions deduced for the markers *uvrA*, *uvrB* and *uvrC* in relation to other markers (TAYLOR and THOMAS 1964) is shown in Figure 4.

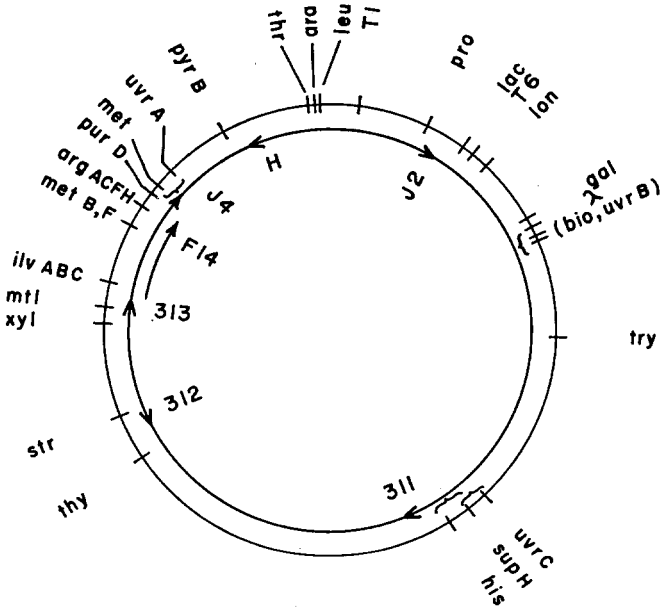

FIGURE 4.—Genetic map of *E. coli* from TAYLOR and THOMAN (1964) and HAYES (1964) with positions of various genetic markers. The origins and direction of transfer of various Hfr strains is shown by the arrowheads and the approximate position and length of F14 is shown by the arrow (PITTARD and ADELBERG 1963). The positions of the *uvrA*, *uvrB* and *uvrC* deduced from the present experiments are shown. Cotransduction is indicated by the curved brackets and the cotransduction frequencies in λ sensitive strains are as follows: *uvrA* with *met* 4%; *uvrB* with *gal* 20%; *uvrB* with *bio* 80%; *uvrC* with *sup* H-12 17%; *uvrC* with *his* 0.3%.

Preparation of double uvr *mutants:* If the three *uvr* loci affect a single function or single biochemical pathway, then a strain carrying a mutation at two of the *uvr* loci might be no more sensitive than the most sensitive single *uvr* mutants, while if two mutations affect the repair processes in different ways, the double mutants might prove to be more radiosensitive. Strains containing two *uvr* markers were prepared by mating F^+ *uvr*$^-$ strains from AB259 Hfr H and AB451 Hfr J2 with F^- cells that were mutant at a different *uvr* locus. An F^+ *uvr*$^-$ mutant, AB2414, which was isolated from AB259 Hfr H and was found to have changed from Hfr to F^+ has a level of colony survival after UV-irradiation intermediate between those of AB259 Hfr H and AB1886 *uvrA6*, as seen in Figure 5. As seen in Figure 6 and Table 2, the number of plaques formed by UV-irradiated T1 phage is also intermediate. In a cross between AB2414 F^+ *uvr*$^-$ and AB1885 *uvrB*, the 45-minute Gal^+ selection contained 58 *uvr*$^-$ and no *uvr*$^+$ recombinants. As the same selection from a cross using AB259 Hfr H yields 70 to 80% *uvr*$^+$ among the Gal^+ recombinants, it was inferred that the mutation of AB2414 is in or near

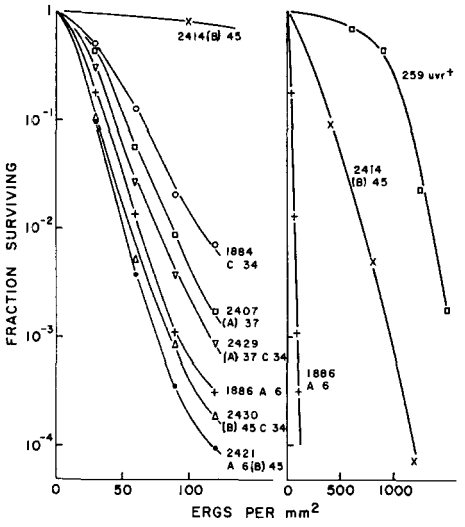

FIGURE 5.—The fraction of cells surviving to form visible colonies is plotted against the dose of UV on two different dose scales. Overnight cultures of the various mutant strains were grown in YET broth without aeration, spun and resuspended in buffered saline so as to be 80% transparent to the UV. They were exposed, diluted and plated on YET agar. The fraction surviving was determined from the number of visible colonies after incubation at 37°C for 24 to 28 hours.

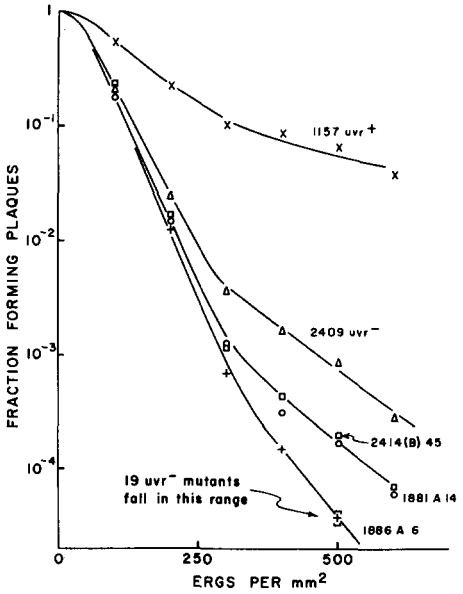

FIGURE 6.—The fraction of T1 phage to form plaques after exposure to various doses of UV. They were plated in soft agar seeded with the various mutant strains of *E. coli* K-12, spread over YET agar and incubated at 37°C. The strains used include: AB1157 *uvr*+, AB2409 *uvr*−, AB2414 *uvrB45*, AB1881 *uvrA14* and AB1886 *uvrA6*. The fraction forming plaques on various mutants after exposure to 500 ergs/mm² UV is also given in Table 2, which lists the 19 mutants that permit from 3.2 to 4.2 $\times$ 10^{-5} surviving fraction.

the *uvrB* locus. This mutant will be called AB2414 *uvr(B)45*. In a similar fashion, it was determined that the site of mutation in AB2407 *uvr*, a mutant of AB451 Hfr J2 also changed to F^+, must be in or near the *uvrA* locus, and this mutant will be called AB2407 *uvr(A)37*. As seen in Figures 5 and 6 and Table 2, its colony survival after UV-irradiation and plaque number with UV-irradiated T1 phage are similar to those of AB1886.

To prepare an *uvrA uvrB* mutant and an *uvrB uvrC* mutant, F^+ AB2414 *uvr(B)45* was crossed with AB1886 *uvrA6* or with AB1884 *uvrC34*. Many of the gal^+ recombinants should have received the nearby *uvrB* marker. A recombinant from each cross was tested by backcrossing with AB451 Hfr J2, which introduces about 80% $uvrA^+$ among Arg^+ recombinants, or with AB492 Hfr 311, which yields about 50% $uvrC^+$ among His^+ recombinants. No uvr^+ were recovered when the prospective double mutants were thus backcrossed. Recombinants from these backcrosses showed either of two levels of plating efficiency for UV-irradiated T1 phage or of survival after UV-irradiation, corresponding to the low level observed with the F^+ strain AB2414 *uvr(B)45*. These recombinants appeared to be the required mutants and were called AB2421 *uvrA6 uvr(B)45* and AB2430 *uvr(B)45 uvrC34*.

To prepare an *uvrA uvrC* mutant, AB2407 F^+ *uvr(A)37* was mated with AB1886 *uvrC34*, and an Arg^+ recombinant was isolated. To test if AB2429 carried *uvr(A)37*, it was backcrossed with AB492 Hfr 311. As there were no uvr^+ among 69 His^+ recombinants, AB2429 must carry *uvr(A)37* and *uvrC34*.

The levels of survival of the double mutants after exposure to UV are shown in Figure 5 and correspond approximately to those of a maximally sensitive single *uvr* mutant, and afford no definite evidence that they affect survival in different ways. In contrast, double mutants of the type uvr^- lon^- are about 15 times, and uvr^- rec^- are over 50 times more UV sensitive as judged by the dose that kills 90% of cells (HOWARD-FLANDERS, SIMSON and THERIOT 1964; HOWARD-FLANDERS and THERIOT, unpublished).

Test of the dominance of $uvrA^+$ *in zygotic partial diploids:* To see if a $uvrA^+$ allele can be expressed in a zygote containing the $uvrA^-$ allele, AB2383 Hfr J2 uvr^+ Str^S $T6^S$ was mated with AB1886 *uvrA* Str^R $T6^R$ for 50 minutes, sufficient for the entry of $uvrA^+$. Mating was interrupted by the addition of T6 phage and 200 μg streptomycin/ml. The mixture was incubated at 37°C, sampled at intervals, and plated on media selective for Arg^+ Ara^+ Str^R Thy^+ recombinants in duplicate. One of each duplicate pair was exposed to 300 ergs/mm^2 UV and all plates were then incubated. Figure 7 shows that immediately after the 50 minute mating period, more than 10% of the Arg^+ Ara^+ zygotes from the cross with AB1886 *uvrA* survived UV-irradiation. This compares with 30% survival in a similar cross with AB1157 uvr^+ and none in the cross with AB1885 *uvrB*. This indicates that $uvrA^+$ transferred in the Hfr chromosome is able to increase the UV resistance of the zygote almost to the wild-type level, and that $uvrA^+$ is dominant in this zygote.

The use of Hfr strains to distinguish between uvrA, B *and* C *mutants:* Hfr strains that carry *uvr* loci were made by mating AB1886 *uvrA6* AB1885 *uvrB5*

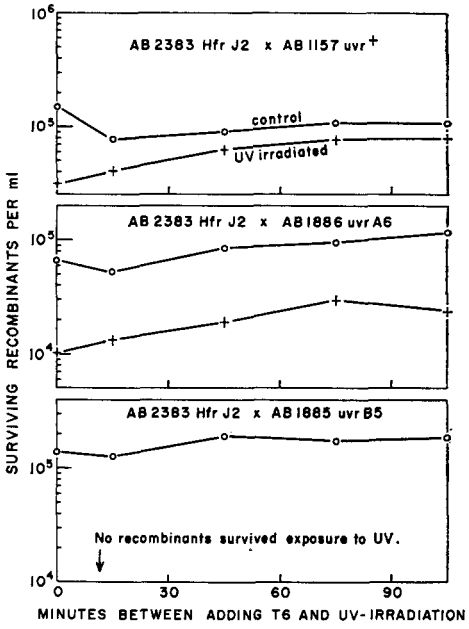

FIGURE 7.—The numbers of *arg*+ *ara*+ recombinants that survive exposure to 0 or 300 ergs/mm² UV is plotted as a function of the time after adding T6 phage to interrupt mating. The results are for:

AB2382 Hrf J2 *str*^{s} × AB1157 F⁻ *uvr*+ *arg*⁻ *ara*⁻

AB2383 Hfr J2 *str*^{s} × AB1885 F⁻ *uvrB5* *arg*⁻ *ara*⁻

AB2383 Hfr J2 *str*^{s} × AB1886 F⁻ *uvrA6* *arg*⁻ *ara*⁻

AB2383 introduced *uvrA*+ at about 20 minutes. Mating was permitted for 50 minutes before adding T6. The high survival after UV of the recombinants from AB2383 × AB1886, as compared with AB2383 × AB1885, indicates that the *uvrA*+ allele is able to express itself and is dominant in the zygote.

and AB1884 *uvrC34* with AB2383 Hfr J2, AB259 Hfr H or AB313 Hfr, and selecting for the distal marker. Hfr strains were isolated from among the recombinants and are listed in Table 1. These Hfr *uvr*⁻ strains were used as test stocks to determine whether the site of mutation in an F⁻ strain is at the *A*, *B* or *C* locus. 0.1 ml of a log phase culture containing about 2×10^8 cells per ml was mixed with 0.4 ml of a culture containing about 2×10^8 cells per ml of the F⁻ strain to be tested, and allowed to mate for 2 hours at 37°C. Aliquots of the mating mixture were spread on YET plates, exposed to 300 ergs/mm² of UV and incubated at 37°C. AB2433 Hfr H *uvrC34* injects *uvrB*+ early and forms from 10 to 50 times more UV resistant zygotes with *uvrB* than with *uvrA* or *uvrC* recipient strains. Similarly, AB2435 Hfr J2 *uvrC34* injects *uvrA*+ early, and makes UV resistant zygotes in large numbers only with *uvrA* recipients. AB2440 Hfr 313 *uvrA6*, which injects *uvrC*+ earlier than *uvrB*+, forms more survivors after UV-irradiation when mated with *uvrC* than with *uvrA* or *uvrB* strains. This strain is less fertile than the other males and is possibly no longer Hfr.

These tests can be used to distinguish between F⁻ strains that carry *uvrA*, *B* or *C* mutations. They can also be used for crude mapping to separate other *uvr* mutants into three regional groups A, B and C. Strain AB1888 *uvr18* for example, was placed in group A as, when mated with AB2435 Hfr J2 *uvrC34* there were many UV survivors (more than 2×10^5 per ml and so scored +). Presumably the wild-type allele for *uvr-18* is injected with high frequency by Hfr J2 and promotes the UV resistance of the zygote by complementation with or without recombination.

A further test is needed to determine whether a mutation in the A group such

as *uvr-18* is in the *uvrA* locus. Strain AB1888 was mated with AB2437 Hfr J2 *uvrA6*, but there were less than 10^3 UV survivors per ml (score −). The fact that UV resistance did not develop in any appreciable fraction of the zygotes from this cross, suggests that the *uvrA*$^+$ allele is required for UV resistance of the zygotes when mated with AB2435. Each F$^-$ mutant assigned to the A group was tested in this way, in the hope that one might be found that would form UV resistant zygotes with both AB2435 and AB2437, and so reveal the existence of another uvr locus in this region. However, as seen in Table 2, each mutant in the A group scored − by this test when mated with AB2437. No second locus injected with a high frequency by Hfr J2 was detected. While *uvr*$^+$ recombinants were recovered from crosses such as AB2437 Hfr J2 *uvrA6* × AB1888 *uvrA18*, they were formed in such small numbers that they did not interfere with the tests just described. Thus a score + for the UV resistance of zygotes when mated with AB2435, and a score − when mated with AB2437, was taken as evidence that a given mutation in the A group was in the *uvrA* locus. Similarly, mutants were allocated to the regional group B, and were accepted as *uvrB* if they scored + when mated with AB2433 Hfr H *uvrC34*, but − when mated with AB2434 Hfr H *uvrB5*. The results obtained with mutants in the C group were less clear cut and it was not possible to determine whether *uvr-31* and *uvr-33* were at the *uvrC* locus. The use of parenthesis in the locus designations in Table 2 indicates assignment to the regional group by crude mapping methods.

Measurement of excision of thymine dimers: The release of thymine dimers during incubation after UV-irradiation was measured in all the *uvr*$^-$ mutants and in the original *uvr*$^+$ strain. In these experiments the UV dose was higher than in previous work, in the expectation that this would give the maximum rate of release of dimers during the period of incubation, and therefore give maximum sensitivity in the detection of reduced rates of release. At this dose, only a fraction of dimers were released from the *uvr*$^+$ strain during the 2-hour incubation, and the majority remained in an acid precipitable form. The results are presented in Table 2 with the mutants listed in order of the numbers of plaques formed with UV-irradiated T1 bacteriophage. Data are given for the total radioactivity in the thymine peak at Rf about 0.6, and for the radioactivity in the two dimer peaks at Rf 0.19 and 0.27 added together and expressed as a percentage of the thymine radioactivity. These measurements were made on the cold acid precipitable and acid soluble material in the cells at the end of the 2-hour incubation. Neither the initial distribution of radioactivity nor the amount extruded into the medium was measured in these experiments, as interest was centered on whether dimers could be detected in the acid soluble material at the end of the incubation. It is seen that the levels of radioactivity in the two dimer peaks were undetectable (less than 20 count/min above background or less than 1.0% of the thymine count/min) in the majority of strains tested, irrespective of whether they were *uvrA*, *B* or *C* mutants. Several mutants exhibited an intermediate level of thymine dimer excision or formed an intermediate number of plaques with UV-irradiated T1 phage and will be referred to in the discussion.

A second photoproduct runs at Rf = 0.18 in a butanol acetic acid chromatog-

raphy system and is excised in wild-type cells along with thymine dimers from the DNA (BOYCE and HOWARD-FLANDERS 1964). This product has been characterized as a thymine-uracil dimer in three different chromatography systems (BOYCE, unpublished data) and is formed by the deamination during heating of a thymine-cytosine dimer (SETLOW, CARRIER and BOLLUM 1965). As this product did not appear on the chromatograms from the *uvr* mutants that did not release dimers, the excision of thymine-cytosine dimers must also be defective in these mutants. The results in Table 2 refer to the amount of radioactivity in both photoproduct peaks expressed as a fraction of that in the thymine peak at Rf = .63 on the same chromatogram. Where appropriate, it was shown that the excised photoproducts were thymine-containing dimers by eluting them, exposing the solution to UV and rechromatographing the product, which then ran as thymine with an Rf = .63.

Search for evidence of a sequential action of uvr *genes:* It is possible that the three *uvr* genes required for the excision of defects may act in a particular sequence rather than simultaneously. For example, it might be necessary for the gene product of *uvrA* to act before that of *uvrB* can function. If so, it can be imagined that excision might take place if UV-irradiated DNA was exposed first to the cytoplasm of a *uvrB uvrC* mutant (containing presumably the active *uvrA* gene products) and then transferred to the cytoplasm of a *uvrA* mutant (containing presumably active *uvrB* and *uvrC* gene products). Excision should not occur, however, if the sequence of transfer is reversed.

To search for an effect of this kind, cultures of *uvr* mutants were infected with T1 phage and incubated for 5 minutes. Ten μg/ml chloramphenicol was added to inhibit lysis and incubation was continued for 10 minutes, at which time the complexes had become resistant to UV-irradiation. The infected cells were then exposed to 350 ergs/mm² UV and incubated for 45 minutes in the presence of 100 μg/ml 5-fluorodeoxyuridine to reduce DNA synthesis and supposedly to increase the time available for enzyme action on the UV-photoproducts in the phage DNA. The cells were diluted 30-fold into fresh broth, incubated for 5 minutes and lysed by shaking with chloroform. The lysates were plated on indicator strains of genotype identical to, or complementary to, that of the first host, as shown in Table 5. The numbers of plaques formed on the *uvr*⁻ indicator strains represent only about 20% of those on wild type, so that the 4/5 of the

TABLE 5

The numbers of plaques formed by lysates from UV-irradiated T1 phage-infected uvr *mutants when plated on various indicator strains*

First host AB2421 *uvrA uvr(B)*			First host AB2429 *uvr(A) uvrC*			First host AB2430 *uvr(B) uvrC*		
	Genotype of indicator	Infective centers per 0.1 ml		Genotype of indicator	Infective centers per 0.1 ml		Genotype of indicator	Infective centers per 0.1 ml
AB2421	*uvrA uvr(B)*	5	AB2429	*uvr(A) uvrC*	26	AB2430	*uvr(B) uvrC*	14
AB1884	*uvrC*	19	AB1885	*uvrB*	29	AB1886	*uvrA*	16
AB1157	+	80	AB1157	+	158	AB1157	+	106

phage must have contained UV-induced defects that could be repaired in the *uvr*$^+$ host. As there was no marked difference between the numbers of plaques formed upon *uvr* mutants of genotype similar to, or complementary to, that of the first host, there is no evidence that any one of the three gene products can act before the other two.

Sensitivity of uvr *mutants to ionizing radiation:* Overnight YET broth cultures of strains AB1157 *uvr*$^+$, AB1886 *uvrA*, AB1885 *uvrB* and AB1884 *uvrC* were spun and resuspended at about 2×10^6 cells/ml in 3 XD, a glycerol-salts minimal medium with Casamino acids. The suspensions were bubbled with oxygen and exposed to high energy electrons from a 6 Mev electron accelerator, a convenient high intensity source of ionizing radiation. After irradiation, the cultures were diluted and plated on YET agar. The fraction of cells surviving is plotted as a function of dose in Figure 8. It is seen that all three *uvr* mutants are about 30% more sensitive than the original strain AB1157 *uvr*$^+$ to this ionizing radiation which is similar to X rays as regards the nature of the radiation products formed in the cell.

DISCUSSION

The results show that these *E. coli* K-12 mutants with defective ability to reactivate UV-irradiated T1 bacteriophage (phenotype Hcr$^-$) carry a mutation at one of three loci designated *uvrA*, *uvrB* and *uvrC*. The approximate positions of these loci in the genetic map of *E. coli* K-12 were determined from crosses with Hfr strains and more accurate positions were found by cotransducing with other markers.

As in earlier work (HOWARD-FLANDERS and THERIOT 1962; HILL 1964), it has been found that there is a correlation among *uvr* mutants between (1) the levels of survival of colony-forming ability of UV-irradiated cells and (2) the

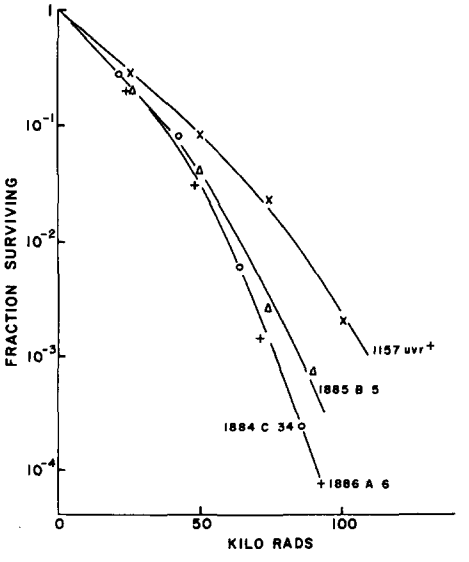

FIGURE 8.—The fractions of cells surviving to form colonies on YET agar after exposure to various doses of ionizing radiation. Overnight cultures of AB1157 *uvr*$^+$, AB1884 *uvrC34*, AB1885 *uvrB5* and AB1886 *uvrC6* grown without aeration in YET broth were bubbled with oxygen and exposed to the electron beam from a 6 Mev electron accelerator at a dose rate of about 10^5 rads/minute. They were diluted, plated on YET agar and incubated for 18 to 24 hours.

levels of plaque forming ability of the cells when used as host for UV-irradiated T1 bacteriophage. Thus, these mutants appear to be defective in the repair of both phage DNA and the DNA of their own genome.

No thymine dimer excision was detected in 17 out of 23 mutants, which included the ten *uvr* mutants that formed the smallest numbers of plaques with the UV-irradiated T1 bacteriophage. A loss of ability to excise thymine dimers can result from a mutation at any one of the three loci. Thus, it seems to be unlikely that different kinds of dimers are excised by different enzymes, controlled by the *A*, *B* and *C* loci. However, as seen in Table 2, six mutants, 2 *uvrA*, 3 *uvrB* and one *uvr(C)* were exceptional in showing an intermediate level of dimer excision while they formed a low to intermediate number of plaques. Three mutants, AB1881, AB2404 and AB2414, one in each group, showed a somewhat raised number of plaques with UV-irradiated phage, but no detectable thymine dimer excision. Another mutant, AB1890, showed little reactivation of phage DNA, but was relatively UV-resistant, as judged by the survival of colony-forming ability. The behavior of these intermediate mutants requires further investigation. The results on the majority of mutants, however, suggest that *E. coli* normally contains a defect excision endonuclease able to act on phage or host DNA alike, and that this enzyme (or enzymes) is determined by the three *uvr* loci. The functions of the products from the three genes is not known, and the attempt to detect a sequential action gave negative results.

The products excised include oligonucleotides containing both thymine dimers and thymine-cytosine dimers and the free dimers are recovered in the acid soluble fraction only after hot acid hydrolysis (Boyce and Howard-Flanders 1964a; Setlow, Carrier and Bollum 1965). There is indirect evidence that certain other defects are excised. Mitomycin C, which is considered to cross-link purines (Iyer and Szybalski 1964), causes extensive DNA breakdown in bacteria (Reich, Shatkin and Tatum 1961). However, it has been found that this occurs only in wild type and not in *uvr* mutants. Moreover, the *uvrA*, *B* and *C* mutants are all very much more sensitive to the lethal effects of mitomycin C than are uvr^+ strains (Boyce and Howard-Flanders 1964b). These results are readily explained if the mitomycin C-induced defects are excised by the same enzymes that release thymine dimers and if DNA breakdown is initiated at the site of excision. The *uvr* genes have a similar but much smaller effect upon survival after X-irradiation, as if about one quarter of the X-ray products are repaired by a mechanism requiring active excision enzymes. These three genes also affect survival after nitrogen mustard or nitrous acid treatment, but are without effect upon survival after exposure to methyl methanesulphonate (Howard-Flanders and Finesilver, to be published). These results suggest that the *uvr* genes may control excision enzymes that act on certain but not all defective bases in DNA.

If a mutant is unable to excise thymine dimers, it might be anticipated that each dimer would then be a lethal block. However, to judge from the sensitivity of colony-forming ability to UV, this is not the case. As the dose required to reduce the number of survivors by one natural logarithm is about 10 ergs/mm^2,

which will suffice to induce more than 50 pyrimidine dimers in the DNA of the genome, it is evident that these *uvr* mutants are able to by pass or repair a considerable number of dimers, in spite of carrying a mutation at one of the *uvr* loci. The mechanism by which these cells survive in spite of the dimers, is not clear from the present results.

It may be asked whether pairing errors between otherwise normal bases in DNA are subject to repair by excision, and if so, whether this process is controlled by the *uvr* genes. Some indirect evidence that heterozygous regions in λ bacteriophage may be removed in wild-type but not *uvr* mutants has been obtained. The yield of $c/c^+\lambda$ phage heterozygotes is twofold higher from a cross in a *uvr* mutant as compared with a wild-type host, as if the heterozygous region was repaired in the latter strain only (WEIGLE and BODE, personal communication, 1964). However, as this result is open to other interpretations, there is a need for a more direct test for determining whether heterozygous regions or pairing errors are enzymatically removed.

We are greatly indebted to DR. E. A. ADELBERG and DR. G. EGGERTSSON for the provision of strains and guidance in the transduction of the *bio*, *met* and *supH* loci. This work was supported by Public Health Service Grants CA-06519 and AM K 6 9397.

SUMMARY

Twenty-three mutants sensitive to ultraviolet light (UV) were isolated, that plate T1 phage normally but form abnormally few plaques when used to plate UV-irradiated phage. These mutants are defective in their ability to repair either their own DNA or that of infecting phage. All the mutants map at one of three loci designated *uvrA*, *uvrB* and *uvrC*, as determined from the time of entry of markers in crosses with various Hfr donor strains. The loci are cotransducible as follows: *uvrA* with *met* 4%; *uvrB* with *bio* 80% and *gal* 20%; *uvrC* with *supH* 17% and *his* 0.3%. Double *uvr* mutants of types *A*, *B*; *B*, *C* and *C*, *A* are no more than about 20% more sensitive to UV than the most sensitive single mutants. *uvrA*, *uvrB* and *uvrC* mutants are phenotypically similar in all respects so far tested, and there is no evidence of sequential action, or specificity in acting on particular types of defects. A convenient test to distinguish between the *A*, *B* and *C* mutants depends upon the development of UV resistance in the zygotes in crosses with Hfr strains that carry a complementary *uvr* mutation. The development of such resistance indicates that in all three loci the wild-type allele is probably dominant in the zygote.—The mutants were also labeled with H^3-thymidine, exposed to UV and tested for ability to excise thymine dimers. None of the fully UV sensitive *uvrA*, *B* or *C* mutants were able to excise detectable amounts of thymine dimer during incubation. Certain mutants that were not so sensitive to UV showed an intermediate level of dimer excision, as if some residual activity was retained, but UV sensitivity did not show a strict relationship to the level of dimer release. *uvr* mutations, at all three loci, have little effect on the sensitivity to ionizing radiation, but they affect control of the sensitivity of *E. coli* to certain other mutagens, including bifunctional alkylating agents, nitrous acid and mitomycin C, as

well as the DNA degradation that occurs after treatment with UV or mitomycin C. *uvrA*, *uvrB* and *uvrC* may thus control an excision nuclease, specific for certain types of defect in DNA while the observed breakdown of DNA may be secondary to excision.

LITERATURE CITED

ADELBERG, E. A., and S. N. BURNS, 1960 Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**: 321–330.

BEUKERS, R., J. IJLSTRA, and W. BERENDS, 1960 The effect of ultraviolet light on some compounds of the nucleic acids, VI. The origin of the ultraviolet sensitivity of deoxyribonucleic acid. Rec. Trav. Chem. **79**: 101–104.

BOYCE, R. P., and P. HOWARD-FLANDERS, 1964a Release of ultraviolet-light induced thymine dimers from DNA in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S. **51**: 293–300. —— 1964b Genetic control of DNA breakdown and repair in *E. coli* K-12 treated with mitomycin C or ultraviolet light. Z. Vererb. **95**: 345–350.

EGGERTSSON, G., and E. ADELBERG, 1965 Map positions and specificities of suppressor mutations in *E. coli* K-12. Genetics **52**: 319–340.

ELLISON, S. A., R. FEINER, and R. HILL, 1960 A host effect on bacteriophage survival after ultraviolet irradiation. Virology **11**: 294–296.

HARM, W., 1963 Repair of lethal ultraviolet damage in phage DNA. pp. 107–118. *Repair from Genetic Radiation Damage*. Edited by F. H. SOBELS. Macmillan, New York.

HILL, R. F., 1964 Relationship between ultraviolet sensitivity and ability to propagate UV-irradiated bacteriophage. J. Bacteriol. **88**: 1283–1287.

HOWARD-FLANDERS, P., 1964 Discussion remark in SETLOW, R. B., 1964 Physical changes and mutagenesis. J. Cell. Comp. Physiol. **64**: (Suppl. 1): 51–68.

HOWARD-FLANDERS, P., R. P. BOYCE, E. SIMSON, and L. THERIOT, 1962 A genetic locus in *E. coli* K-12 that controls the reactivation of UV-photoproducts associated with thymine in DNA. Proc. Natl. Acad. Sci. U.S. **48**: 2109–2115.

HOWARD-FLANDERS, P., and L. THERIOT, 1962 A method for selecting radiation-sensitive mutants of *Escherichia coli*. Genetics **47**: 1219–1224.

HOWARD-FLANDERS, P., E. SIMSON, and L. THERIOT, 1964 The excision of thymine dimers from DNA, filament formation and sensitivity to ultraviolet light in *Escherichia coli* K-12. Mutation Res. **1**: 219–226.

IYER, V. N., and W. SZYBALSKI, 1964 Mitomycin and porfiromycin: chemical mechanisms of activation and cross-linking of DNA. Science **145**: 55–58.

JACOB, F., and E. WOLLMAN, 1961 *Sexuality and Genetics of Bacteria*. Academic Press, New York. —— 1956 Sur les processus de conjugaison et de recombinaison génétique chez *E. coli*. L'induction par conjugaison ou induction zygotique. Ann. Inst. Pasteur **91**: 486–510.

LENNOX, E., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**: 190–206.

PITTARD, J., and E. ADELBERG, 1964 Gene transfer by F′ strains of *Escherichia coli* K-12. III. An analysis of the recombination events occurring in the F′ male and in the zygotes. Genetics **49**: 995–1007.

PITTARD, J., J. S. LOUTIT, and E. ADELBERG, 1963 Gene transfer by F′ strains of *Escherichia coli* K-12. I. Delay in initiation of chromosome transfer. J. Bacteriol. **85**: 1394–1401.

REICH, E., A. SHATKIN, and E. TATUM, 1961 Bacteriocidal action of mitomycin C. Biochim. Biophys. Acta **53**: 132–149.

RORSCH, A., A. EDELMAN, and J. COHEN, 1963 The gene controlled radiation sensitivity of *Escherichia coli*. Biochim. Biophys. Acta **68**: 263–270.

ROTHMAN, J., 1965 Transduction studies on the relationship between prophage and host chromosome. J. Mol. Biol. **12**: 892–912.

SETLOW, R., and W. CARRIER, 1964 The disappearance of thymine dimers from DNA; an error-correcting mechanism. Proc. Natl. Acad. Sci. U.S. **51**: 226–231.

SETLOW, R., W. CARRIER, and F. BOLLUM, 1965 Pyrimidine dimers in UV-irradiated Poly dI:dC. Proc. Natl. Acad Sci. **53**: 1111–1118.

SETLOW, R., and J. SETLOW, 1962 Evidence that ultraviolet-induced thymine dimers in DNA caused biological damage. Proc. Natl. Acad. Sci. U.S. **48**: 1230–1257.

TAYLOR, A., and M. THOMAN, 1964 The genetic map of *Escherichia coli*. Genetics **50**: 659–667.

VAN DE PUTTE, P., C. A. VAN SLUIS, J. VAN DILLEWIJN, and A. RORSCH, 1965 The location of genes controlling radiation sensitivity in *Escherichia coli*. Mutation Res. **2**: 97–110.

WACKER, A., H. DELLWEG, and D. WEINBLUM, 1960 Strahlenchemische Veränderung der Bakterien Desoxyribonucleinsaure *in vivo*. Naturwissenschaften **47**: 477–480.

WULFF, D. L., and C. S. RUPERT, 1962 Disappearance of thymine photodimer in ultraviolet-irradiated DNA upon treatment with a photoreactivating enzyme from Baker's Yeast. Biochem. Biophys. Res. Commun. **7**: 237–240.