A STUDY OF RADIATION SENSITIVITY IN *STREPTOMYCES COELICOLOR*,

by

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TEXT.

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ABBREVIATIONS

UV - ultraviolet light: NTG - N-methyl-N'-nitro-N-nitrosoguanidine: Hcr - host cell reactivation: LHR - liquid holding recovery: MMS - methyl methane sulphonate: TCA - trichloroacetic acid: Hfr - high frequency of recombination: p.f.u. - plaque forming units: MM - minimal medium: CM - complete medium: Arg - Arginine: Cys - Cystine: His - Histidine: Hom - Homoserine: Phe - Phenylalanine: Pro - Proline: Ura - Uracil: D37 - the increment in the UV dose resulting in 37% survival of the viable units at any point on the exponential part of a semi-log plot of survival. This is the dose causing an average of one lethal hit per viable nucleus at the starting point.

I. GENERAL INTRODUCTION

A. An introduction to *Streptomyces coelicolor*

1. *Streptomyces coelicolor*

The protokaryote nature of *Streptomyces coelicolor* was revealed by its cell wall composition (Cummins and Harris, 1958) and its fine structure shown by electron microscopy (Glauert and Hopwood, 1959, 1960, 1961; Hopwood and Glauert, 1960). Thus it had a gram-positive cell wall containing sugars, amino acids and amino sugars, including di-amino pimelic acid, and its fine structure showed membranous mesosomes, fibrillar nuclear material, protoplasm dense with ribosomes, but no nuclear membrane or mitochondria and no mitotic apparatus. Later, genetic studies showed it to have merozygotes and a circular genetic map which were both features possessed by other protokaryotes, such as *E. coli*.

The following considerations illustrate the suitability of *S. coelicolor* for genetic studies. Its vegetative and sexual life cycles are short, three to four days, in which time a single haploid spore can germinate, produce substrate mycelium, aerial hyphae and then haploid spores, as was shown by phase-contrast observations, (Hopwood, 1960). The spores are long-lived since sporulating slant cultures remain viable for more than a year. Up to 10^8 spores are obtained from a fresh slant culture. These spores are readily suspended in sterile water, and can be treated with a variety of mutagens. The wild-type strain, A3(2), grows on a simple defined medium, (see Section II), enabling many different auxotrophic mutations to be obtained. A sporulating culture of *S. coelicolor*

as discrete colonies or confluent growth on agar plates is readily replicated by a velvet pad to fresh agar plates, enabling screening of large numbers of spores, once they have grown to individual colonies, and therefore detection of rare mutants or recombinants.

2. The Genetic System of *S. coelicolor*.

Recombination was first demonstrated in *S. coelicolor* strain I.S.S. (Sermonti and Spada-Sermonti, 1955, 1956) and later in *S. coelicolor* strain A3(2) (Hopwood, 1957), the strain with which nearly all subsequent genetic studies have been made. Recombination occurred with variable frequency between any pair of the derivatives of A3(2); however, recombinants were always infrequent and were obtained for analysis by selecting them from amongst the bulk of asexual progeny by growing the progeny on media lacking nutrients required by the parent strains. This analysis at first revealed three linkage groups (Hopwood, 1959) which constituted a genome probably more than 200 recombination units long; later these became two linkage groups (Hopwood and Sermonti, 1962; Hopwood, 1965a).

When recombinants for closely linked markers were selected amongst the progeny of a cross, two types of colony appeared on the selective plating medium. The first type were larger regular shaped colonies, which yielded haploid spores all of the same genotype, indicating that the original cell was haploid. The second type, detected by their inability to replicate to the same selective medium as that on which they were grown, were smaller irregular shaped colonies, which produced a large majority of haploid spores with a variety of genotypes, while the minority retained the property of producing spore progeny with variable genotypes. This second type of colony was called a

heteroclone (Sermonti, Mancinelli and Spada-Sermonti, 1960).

Analysis of the genotypes of the haploid progeny of individual heteroclones revealed that complementary genotypes were not equal in frequency as they had been amongst haploid recombinant progeny of a cross as a whole. It was characteristic of heteroclones that, for each linkage group, recombinants containing at least one allele were deficient, or even absent, and the effect was not allele specific; commonly all of one parental set of alleles for one linkage group was missing. These findings were later interpreted (Hopwood, Mancinelli, Sermonti and Spada-Sermonti, 1961), to mean that the heteroclones arose from heterogenotes, that is *partially* diploid cells, in which one or more segments of either parental genome were missing and consequently alleles coupled to the missing regions entered haploid recombinants with reduced frequencies.

The variety of genotypes found in the progeny of heteroclones that were heterozygous for several markers indicated that the heterozygous condition was replicated many times before haploid segregation took place. Second order heteroclones were obtained on selective medium, especially if hyphae of first order heteroclones, rather than spores, were used as the inoculum; these heteroclones frequently showed more extensive deletions than their progenitor. (Hopwood, Sermonti and Spada-Sermonti, 1963).

Individual heteroclones provided a means of *non-selective* genetic analysis of their heterozygous region, and collectively of the whole genome. The effects on segregation of deleted regions could be taken into account, so that estimations of linkage could still be made (see Hopwood and Sermonti, 1962, and Sermonti and

Hopwood, 1964, for a review of the genetics of *S. coelicolor* at this stage). These analyses confirmed and extended the findings of the earlier selective analysis. Hopwood (1965a) later summarised the genetic techniques and their results for 28 loci, which still fell in two linkage groups of about 60 and 70 recombination units each.

The next step was taken when the two linkage groups were shown to be parts of a circular linkage map (Hopwood, 1965b). It was found that the segregation pattern of non-selected markers at both ends of one linkage group amongst recombinants selected for markers in the other linkage group was strongly dependent on the selection applied, and thus that the two linkage groups were connected at each end. The intervening regions must have been long, since all the markers in different linkage groups showed 50% recombination in heteroclones. This finding did not distinguish between a circular chromosome, or a linear chromosome that either was circularly permuted or else had constant ends, with viable recombinants arising only by even numbers of crossovers during the sexual process. That the chromosome did not have constant ends was later shown by 'phenotypic' analysis of heteroclones (Hopwood, 1966b). A large number of individual heteroclones obtained from one cross, selected to be heterozygous for a pair of complementing closely linked histidine *(his)* mutations, were analysed as a whole for heterozygosity or hemizygosity of a number of markers distributed throughout the genetically marked regions of the map. Practically all the heteroclones were heterozygous for a *continuous segment* of the genome, of variable length but always, of course, including the *his* region. The heterozygous regions of different heteroclones overlapped to cover the whole of the genome

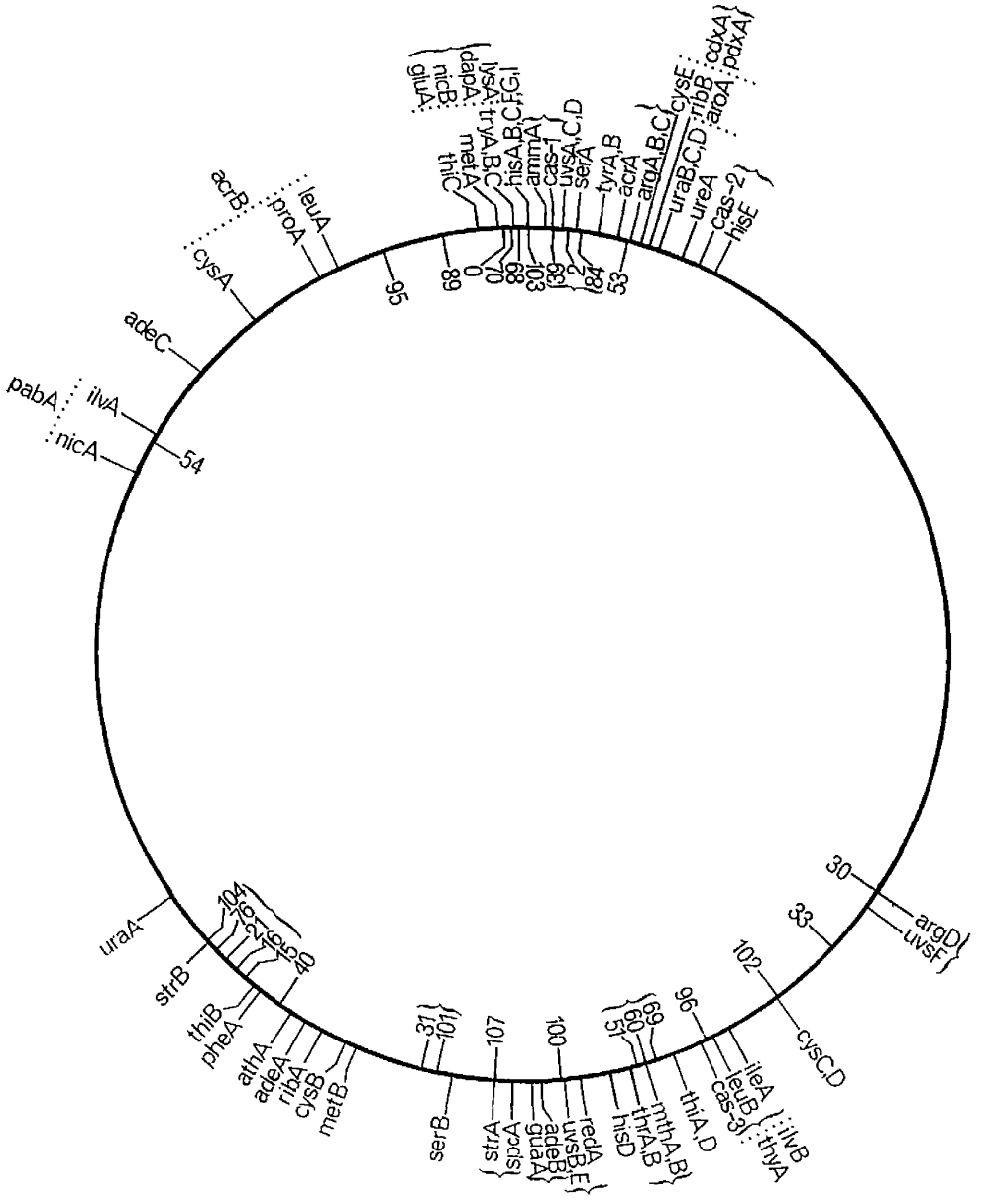
and thus the genome did not have constant ends. The data did not distinguish between closed genomes or genomes that were linear but circularly permuted. This distinction has still not been possible although as a working hypothesis the genome has been assumed to be a closed circle (Hopwood, 1967a).

There were certain features of the distribution of genes on the recombination map of *S. coelicolor* (see Figure 1). Firstly the known genes were concentrated in two regions separated by large 'silent' regions, each about $\frac{1}{4}$ of the total map; this uneven distribution included many temperature-sensitive mutations which might have been expected to occur in genes concerning a greater variety of processes than the bulk of the existing mutations which were concerned with biosynthetic processes (Hopwood, 1966a). Thus the silent regions contained genes controlling as yet undetected functions, or they were possibly regions that were not long in physical terms but in which recombination was abnormally frequent.

Secondly, there was some clustering of functionally related genes, suggesting the existence of operons (Hopwood, 1965a, 1965c); other functionally related genes were found to be arranged approximately diametrically in the two well marked regions of the map. Thus there were two sequences of genes (or clusters), one in each arc, containing functionally related genes at corresponding positions in the sequence, for which Hopwood (1967b) suggested an evolutionary origin.

With the knowledge of genome circularity, Hopwood (1967a) analysed the progeny of a cross containing markers widely distributed over the genetically marked segments of the map. He made six possible selections for recombinants between adjacent markers and found that,

FIGURE 1. The linkage map of *Streptomyces coelicolor*.
From Hopwood (1967a) with later additions.

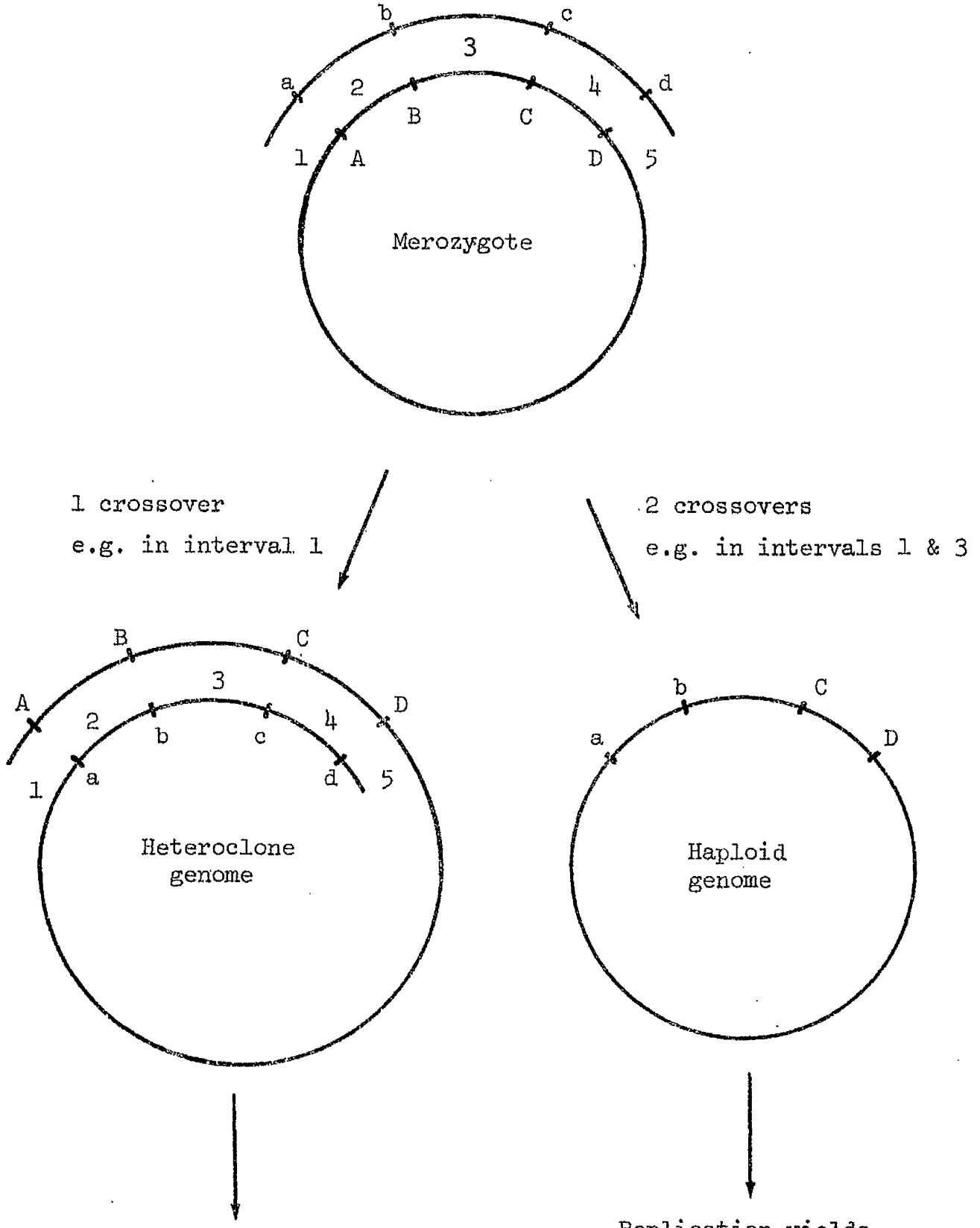


The circumference of the circle equals approximately 260 centiMorgans. The gene symbols indicated outside the circle and referred to in the thesis are explained in Table 9. Numbers inside the circle indicate the positions of indispensable temperature sensitive mutations. Brackets indicate unordered genes. Genes separated from a group of other ordered genes by dotted lines are unordered relative to the members of the group.

amongst each of the six samples of recombinants, the highest unselected recombination frequencies occurred in the regions immediately next to the region between the selected markers in which recombination was obligatory. The simplest explanation for this was zygote incompleteness; the region between the selected markers had to be heterozygous in order for recombination to yield a viable recombinant and the flanking regions would be more likely than distant regions to be also heterozygous and so contain unselected crossovers. There was no evidence for negative interference in the heterozygous regions of heteroclones so that negative interference as an alternative to zygote incompleteness in explaining these findings seemed unlikely. There was a negative correlation between non-selected crossovers in the two flanking intervals, favouring a model in which each merozygote contained a whole genome from one parent and a fragment from the other rather than one in which the contributions from both parents were incomplete. Each parent appeared to contribute the whole genome with about the same frequency, and the fragments had ends at random positions throughout the genome.

On the basis of this model for zygotes, Hopwood (1967a) proposed a model for the origin, by recombination, of heteroclones on the one hand and haploid recombinants on the other (see Figure 2). The segregation observed within the progeny of many individual heteroclones was compatible with this model, according to which a plating unit which could yield a heteroclone was derived from a merozygote when an odd number of crossovers (usually one) occurred in the disomic region, with the crossover usually near one end of this region. The terminally repeated genome thus produced could then replicate many

FIGURE 2. Model for the origin of heteroclone and haploid genomes from merozygotes (Hopwood, 1967a).



The circle and arc represent the genome contributions of the parents to the merozygote. Letters indicate marker alleles. Numbers between the circle and arc indicate intervals referred to in the text.

times during the growth of the heteroclone. The haploid progeny of a heteroclone would arise by further single or higher odd numbered crossovers which could occur in different places in different genomes within the same heteroclone colony, and this would explain the variable haploid progeny. Thus in the heteroclone genome in Figure 2, *a* will enter the haploid genome whenever the second crossover is in the intervals 2, 3, 4 or 5, and *A* only when it is in the interval 1; therefore *a* should be much more frequent in the haploid progeny than *A*. Similarly the frequency of *D* should exceed the frequency of *d*, with intermediate ratios for the intervening pairs of alleles. The allele frequencies observed amongst a random sample of the haploid progeny of a single heteroclone will form, therefore, two opposing gradients, ascending from a minimum for the alleles in coupling with genome ends to a maximum for the alleles in opposition to the ends. This situation was observed in most heteroclones. Most of the remainder had allele gradients compatible with the first crossover in the formation of the heteroclone occurring, not at the ends, but somewhere else in the disomic region (in intervals 2, 3 or 4).

Haploids would arise by an even number of crossovers within the heterozygous region of a merozygote (see Figure 2).

One major feature of this model was that heteroclone genomes were recombinant structures, and one way to test this would be to attempt heteroclone selections between strains, one or both of which were defective in recombination. Certain predictions could be made about the consequences if heteroclone genomes were recombinant structures.

3. The possible consequences of heteroclone selection in crosses with *rec* parents.

Firstly, consider the effect of selection for heteroclones amongst the progeny of a cross in which one parent was recombination deficient *(rec)*. If this mutation were recessive and located close to the points of selection, then it would be expected to have little effect since this region would be disomic in almost all zygotes. If, however, it was distant from the points of selection, then all zygotes in which the *rec* allele was hemizygous (those zygotes which contained a complete genome from the *rec* parent) would not be expected to produce heteroclones. This situation would be revealed by using the 'phenotypic' analysis of Hopwood (1966b) which determined whether haploid progeny of a particular heteroclone were all of one allele type or of both allele types for any unselected heterozygous markers in the cross. The heteroclone population would be expected to be polarised, with heteroclones arising almost entirely from zygotes containing a whole genome from the rec^+ parent and a partial genome from the *rec* parent. The progeny of heteroclones showing the opposite polarity should be heterozygous for a continuous sector from the points of selection to the *rec* location.

Secondly, consider the effect of selection from the progeny of a cross in which both parents were *rec*. If there were an absolute block to recombination then certainly haploids, and in addition heteroclones if they were recombinant structures, should be absent from the progeny. If the *rec* mutation resulted in a partial block in recombination, reducing the frequency of crossing-over, then amongst the reduced recombinant progeny of the cross, a higher fraction might be

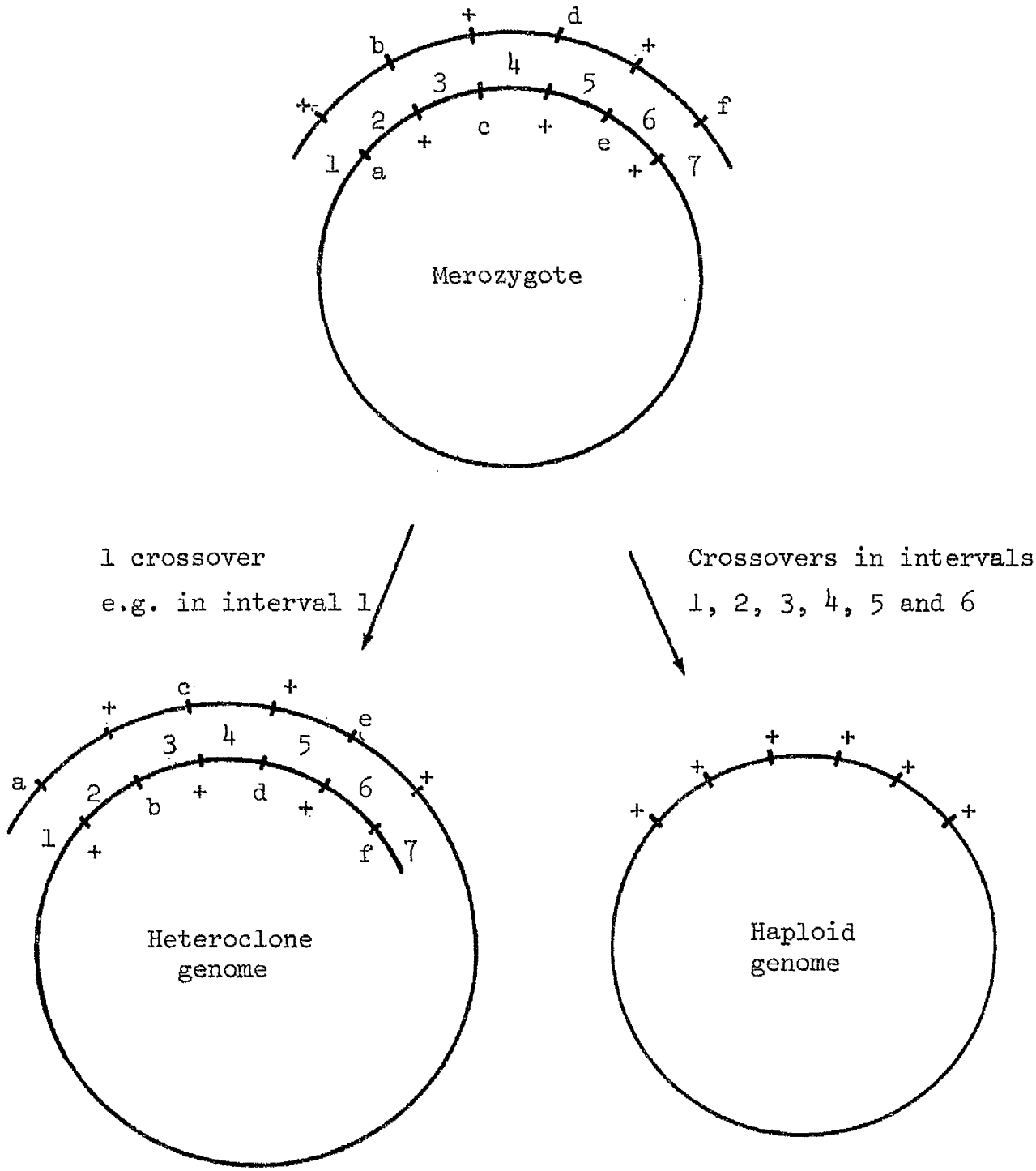
heteroclones (arising by *single* crossovers) compared with the equivalent heteroclone selection for *rec*$^+$ parents. These heteroclones should also be much more stable. This would present the problem of satisfactorily distinguishing stable heteroclones from true haploids. The following criteria might serve this purpose. Firstly, stable heteroclones due to hemi- or homozygosity for a *rec* mutation would still segregate haploids with variable genotypes at a low frequency, provided the block to recombination was not absolute. Secondly, if heteroclones could be selected from a combination of *rec* parent strains with the marker arrangements of Figure 3, then stable heteroclones with a long heterozygous region would have a phenotype which would be a rare multiple crossover class for a haploid, especially in a cross presumably having a reduced recombination frequency.

It thus appeared that the availability of *rec* mutations in *S. coelicolor* should allow a test of the hypothesis of heteroclone origin and they were therefore sought.

4. Rationale for the selection of *rec* mutants.

Rec mutants were first isolated in *E. coli* K12 by Clark and Margulies (1965) from a mutagen-treated F$^-$ *leu ade*$^+$ strain, detected as *rec* in crosses with an Hfr strain. When F$^-$ cells are mated with Hfr cells, the latter donate their DNA in a polarised manner in that all Hfr cells of a given strain begin donating at the same point on the genome and the donation proceeds with the same order of genes at the same rate. Thus all conjugating cells behave genetically in the same way. The mutagen treated F$^-$ cells were spread on agar plates and incubated to yield colonies. These were replicated to

FIGURE 3. Parental marker arrangements for a cross between leaky *rec* parents enabling selection of probable heteroclones from haploid recombinants.



Both types of recombinant have the same wild-type phenotype but the heteroclone genome requires only one crossover (that can occur anywhere in the heterozygous region) whereas the haploid genome requires six non-randomly distributed crossovers and is therefore likely to be the less frequent type.

+ represents wild-type alleles.

Lower case letters represent mutant alleles.

a lawn of Hfr *leu*$^+$ *ade* cells obtained by spreading a drop of a dense suspension of cells on an agar plate selecting for *leu*$^+$ *ade*$^+$ recombinants. In this way each individual F$^-$ colony was crossed with the Hfr strain. Any zygotes formed on the plates and yielding a suitable recombinant would give rise to a colony, and this occurred with a characteristic density of recombinant colonies for the majority of *rec*$^+$ F$^-$ colonies. A number of F$^-$ colonies containing a mutation affecting recombinational ability were identified by the much lower density of recombinants that they gave. Clark and Margulies were able to confirm that the reduced ability of two of these F$^-$ strains to yield recombinants with Hfr strains was at the level of recombination, rather than at the conjugation level, or due to intracellular breakdown of donated DNA. These *rec* mutations were also shown to render the strains UV sensitive.

The property of UV sensitivity conferred by the *rec* mutations in *E. coli* was used as the criterion for attempting to isolate *rec* mutations in *S. coelicolor* since at the time a test of recombination similar to that devised by Clark and Margulies could not be applied, depending as it does on the different roles (donor and recipient of chromosome fragments) played by the two strains in a cross in *E. coli*. However, by no means all UV sensitive mutations in *E. coli* were associated with recombination deficiency and thus many UV sensitive mutations in *S. coelicolor* that did not affect its recombinational ability were expected. Nevertheless it was a reasonable expectation that UV sensitive mutations should include at least some of the desired *rec* type.

In view of the fact that none of the UV sensitive mutations

discussed in this thesis has in fact been shown to affect recombination, a summary of UV sensitive mutations as a whole in *E. coli* and other micro-organisms is appropriate here.

B. Radiation sensitivity in *Escherichia coli*.

1. UV and X-ray Sensitivity in *Escherichia coli* B.

The first UV sensitive mutant in any micro-organism was isolated and described in *Escherichia coli* B (Hill, 1958) as one of 22 survivors of 2.2×10^6 cells exposed to a high dose of UV. Subsequently eleven other UV sensitive strains were isolated in the same way and described by Hill and Simson (1961) and Hill and Feiner (1964). Table 1 summarises their findings. Two of the UV sensitive mutants (Bs1 and Bs2) were also X-ray sensitive (Hill and Simson, 1961); Bs4, Bs5, Bs6, Bs7, Bs9, Bs10 and Bs11 were also stated to be X-ray sensitive by Chung and Greenberg (1969) but no data on these mutants appear to have been published. The mutants differed in the extent of cell elongation after UV, a property typical of strain B, and also in their sensitivities to crystal violet and furacin. Four strains, Bs1, Bs3, Bs8 and Bs12, were unable to support the development of plaques by UV irradiated T1 bacteriophage, a phenomenon called *H*ost *c*ell *r*eactivation (Hcr) and first described for B and Bs1 (Ellison, Feiner and Hill, 1960); these strains were therefore called Hcr^-.

Several workers have located the mutant genes in these strains and also that in a UV and X-ray resistant strain of *E. coli* B isolated and described by Witkin (1947) and designated B/r. These genetic results are summarised in Table 2.

TABLE 1. Phenotypes of *Escherichia coli* B strains.

(Adapted from Hill and Feiner, 1964)

Strain	UV sensitivity	Elongation after UV (a)	Inhibitory concentration of crystal violet	Inhibitory concentration of furacin	HCR ability	X-ray sensitivity (b)
B	wild-type	4+	1-2 ug/ml	0.25	+	wild-type
B/r	resistant	2+	more than 8 ug/ml	3.3	+	resistant
Bs1	sensitive	1+	more than 8 ug/ml	0.23	-	sensitive
Bs2	sensitive	1+	more than 8 ug/ml	0.53	+	sensitive
Bs3	sensitive	4+	2-4 ug/ml	0.25	-	not tested
Bs4	sensitive	2+	more than 8 ug/ml	0.75	+	not tested
Bs5	sensitive	2+	more than 8 ug/ml	0.67	+	not tested
Bs6	sensitive	2+	more than 8 ug/ml	0.53	+	not tested
Bs7	sensitive	2+	more than 8 ug/ml	0.59	+	not tested
Bs8	sensitive	2+	more than 8 ug/ml	0.87	-	not tested
Bs9	sensitive	2+	more than 8 ug/ml	0.59	+	not tested
Bs10	sensitive	2+	more than 8 ug/ml	0.73	+	not tested
Bs11	sensitive	1+	1-2 ug/ml	0.20	+	not tested
Bs12	sensitive	4+	2-4 ug/ml	0.20	-	not tested

(a) 1+ to 4+: relative elongation at 1% survival after UV irradiation.

(b) From Hill and Simson (1961).

TABLE 2. Genes affecting radiation sensitivity in *Escherichia coli* B.

Strain	Mutation	Location	Technique	Reference	Comments
B	wild-type				
B/r	*sul*	between *lac* and *ara*	Phenotypic analysis of selected recombinants from Hfr x F⁻ crosses	(a)	
Bs1	*hcr(uvr-1)*	near *gal*	P1 cotransduction	(b)	Double mutant (see also Greenberg, 1967).
	exr-1	near *met-3(malB)*	P1 cotransduction		
Bs2	UV3	near *malB*	Phenotypic analysis of selected recombinants from K12 Hfr x B F⁻ cross	(c)	K12 and Bs2 differ by two genes UV2 *(lon)* and UV3 *(exr-2)*.
Bs2	*exr-2*	near *malB*	P1 cotransduction	(d)	
		near *metA* and *malB*	P1 cotransduction	(e)	
Bs3	*uvr-3*	near *his*	P1 cotransduction	(d)	
Bs4	*exr-4*)				
Bs5	*exr-5*)	near *malB*	P1 cotransduction	(d)	
Bs6	*exr-6*)				
Bs7	*exr-7*)				
Bs8	*uvr-8*	near *gal*	P1 cotransduction	(d)	See reference (f)
Bs9	*exr-9*	near *malB*	P1 cotransduction	(d)	
Bs10	*exr-10*	near *malB*	P1 cotransduction	(d)	
Bs11	*exr-11*	not located		(d)	
Bs12	*uvr-12*	near *malB*	P1 cotransduction	(d)	Closely linked to *uvrA* of K12.
-	*phr*	near *gal*	Time of entry in K12B (Hybrid) Hfr x B F⁻ cross	(g)	

(continued overleaf)

TABLE 2. Genes affecting radiation sensitivity in *Escherichia coli* B (continued).

References:

(a) Donch, Chung and Greenberg (1969).

(b) Mattern Zwenk and Rorsch (1966).

(c) Greenberg (1964b).

(d) Donch and Greenberg (1968a).
Exr-11 was possibly a *rec* mutation since no recombinants were obtained from P1 cotransduction or normal K12 Hfr x B F^- matings.
These authors concluded that the close linkage of *uvr-2*, *uvr-4*, *uvr-5*, *uvr-6*, *uvr-7* and *uvr-10*, and their phenotypic similarity for UV sensitivity, Hcr^+ and lack of elongation after UV exposure, was sufficient evidence that they are mutant in the same cistron which they proposed designating *exr* although no published evidence had shown that they were X-ray sensitive, nor had complementation tests been carried out.
Later work (Chung and Greenberg, 1969), showed that when P1 transduction was performed from strains Bs2 *exr-2*, Bs4 *exr-4*, Bs7 *exr-7* or Bs12 *uvr-12* to further strains which were *exr* or *uvr* derivatives of these strains, there was a tenfold to eighteenfold greater survival after exposure to UV for *exr* x *uvr* transductions, than *exr* x *exr* or *uvr* x *uvr* transductions. The surviving transductants were almost entirely unstable heterogenotes, and demonstrated complementation between *uvr* and *exr* mutations, but not between *exr* mutations.

(e) Chung and Greenberg (1968). *Uvr-2(exr)* ordered with respect to *uvrA6* as *metAmalBuvr-2uvrA6*.

(f) Donch and Greenberg (1968b). Bs8 contained a *lon* suppressor which was not located. Its presence was inferred since Bs8 was less sensitive than K12 *lon uvr-8*, yet Bs8 still contained the *lon* mutation of its progenitor, strain B.

(g) Van de Putte, van Sluis, van Dillewijn and Rorsch (1965).

These mutations can be divided by phenotype and genetic location into six groups:

Group	Mutation	Phenotype	Location
a	*uvr-1,-8*	UV sensitive, Hcr^-	near *gal* and *uvrB* of K12
b	*uvr-3*	UV sensitive, Hcr^-	near *his* and *uvrC* of K12
c	*uvr-12*	UV sensitive, Hcr^-	near *malB* and *uvrA* of K12
d	*exr-1,-2,-4, -5,-6,-7,-9,-10*	X-ray sensitive UV sensitive, Hcr^+	near *malB* and *lex* of K12
e	*exr-11*	X-ray sensitive UV sensitive, Hcr^+ possibly *rec*.	Not located.
f	*sul*	suppressor of *lon* phenotype	between *lac* and *ara*

A *phr* strain lacking photoreactivating enzymes was obtained by Dr. W. Harm, and the *phr* gene located near *gal* by van de Putte, et al. (1965). It is included in this section because it was an *E. coli* B strain.

Wild-type *E. coli* B was more radiosensitive than wild-type *E. coli* K12, being similar in sensitivity and phenotype (except for mucoid production), to K12 *lon* mutants which are described later. K12 has radiosensitivity similar to B/r. Greenberg (1964a), analysing recombinants from K12 Hfr x BF^- crosses, located a gene of K12 designated UV2 conferring UV resistance, closely linked to *tsx* ($T6^r$), a gene for resistance to bacteriophage T6. All UV resistant recombinants tested were NTG resistant like K12. Donch and Greenberg (1968d) cotransduced with $proC^+$ a gene for UV sensitivity from *E. coli* B to a lon^+ K12 derivative. The UV sensitive transductants were filament forming and mucoid, and since *lon* of K12 is linked to *proC* this was evidence that *E. coli* B carried a *lon* type mutation. It presumably also contained a suppressor of mucoidy. This *lon*-type

gene was also linked to *tsx* and was presumably the allele of UV2 described above.

Genetic and phenotypic studies of two further derivatives of *E. coli* B, *syn* (UV sensitive) and *fil* (UV resistant) are summarised in Table 3. *Syn* has a similar phenotype and location to *uvr-3* of *E. coli* B and *uvrC* of *E. coli* K12. The *fil* gene resembles *lon* of K12 in its phenotype but not in its location, with *fil* equivalent to *lon*$^+$ and *fil*$^+$ equivalent to *lon*. Genotypically *fil* may not be equivalent to *lon* of K12, but may be like the *sul* gene in which a mutation serves to suppress the *lon* phenotype of *E. coli* B.

Kato and Kondo (1967) isolated a number of radiation sensitive strains from a B/r type *E. coli* strain by selecting for Hcr$^-$ mutants using the method of Howard-Flanders and Theriot (1962). All had greater UV sensitivity than the wild-type and none were filament forming. They were characterised to varying degrees, defining four phenotypes for UV and X-ray sensitivity, and Hcr of UV and X-ray inactivated bacteriophage. Mutants of the first phenotypic group were UV sensitive and Hcr$^-$ for UV inactivation; they showed slight X-ray sensitivity and an intermediate level of Hcr for X-ray inactivation. In these respects they were similar in phenotype to the *uvrA*, *B* and *C* mutants of *E. coli* K12. They also showed enhanced UV mutability for a given dose, which was not further enhanced by the presence of acriflavine after the irradiation, and were cross-sensitive to NTG and MMS. A single mutant representing the second group was UV and X-ray sensitive but Hcr$^+$ for UV irradiated bacteriophage, and had abnormal DNA metabolism after UV irradiation. In these respects it was similar to the *exr* or *lex* mutants of *E. coli* B

TABLE 3. Phenotypes and genetics of mutations in the *syn* or *fil* genes of *Escherichia coli* B.

Genotype	Phenotype				
	UV Sensitivity (a)	Hcr (c)	UV Inhibition of Growth (a)	Susceptibility to Filamentation (a)	Nitrous Acid Sensitivity (a)
syn+fil+	wild-type	Hcr^+	wild-type	wild-type susceptible	wild-type
syn+fil	resistant	not tested	wild-type	not susceptible	resistant
syn fil+	sensitive	Hcr^-	increased	wild-type susceptible	sensitive
syn fil	varied with culture conditions	not tested	increased	not susceptible	moderately resistant

Gene	Genotype: Location	Technique	Reference
fil	between *gal* and *trp*	Phenotypic analysis of selected recombinants from $B\ F^+ \times B\ F^-$ cross	(d)
syn	near *his*	Phenotypic analysis of selected recombinants from K12 Hfr x $B\ F^-$ cross	(b)

References:

(a) Rorsch, Edelman, Van de Kamp and Cohen (1962). Filamentation was induced by UV, novobiocin, penicillin, and crystal violet.

(b) Greenberg (1965).

(c) Rorsch, Edelman and Cohen (1963). They compared the growth of UV irradiated bacteriophage on *E. coli* C and on an *E. coli* C (B *syn* transductant).

(d) Van de Putte, Westenbroek and Rorsch (1963).

and *E. coli* K12 respectively. In contrast to *exr* mutations it showed normal UV mutability, which was further increased by post-irradiation treatment with acriflavine, and it was Hcr^- for X-irradiated bacteriophage. A single representative mutant of the third group was UV and X-ray sensitive, but Hcr^+ for both UV and X-ray irradiated bacteriophage. In these respects it resembled *rec* mutants. The fourth group contained mutants that were UV sensitive and had intermediate Hcr ability for UV inactivated bacteriophage, and were of intermediate X-ray sensitivity. These mutants were not further characterised and none of the mutations involved was located.

2. UV sensitivity in *Escherichia coli* K12.

A similar series of mutants has been characterised in *E. coli* K12 and a derivative of K12, strain CR34. Genetic analysis was much more easily performed in these strains than B, because of the inter-fertility of K12 strains. The genes *uvrA*, *uvrB*, *uvrC* (Howard-Flanders, Boyce and Theriot, 1966), *uvrD* (Ogawa, Shimada and Tomizawa, 1968) and *lon* (Howard-Flanders, Simson and Theriot, 1964a; Adler and Hardigree, 1964) have been identified. The *uvrA*, *B*, *C* and *D* mutants analysed were selected to be Hcr^- by the method of Howard-Flanders and Theriot, (1962) and the *lon* mutants were selected either for their increased UV sensitivity or for excess mucoid secretion. Donch and Greenberg (1968c) subdivided *lon* mutants of *E. coli* K12 into two groups on the basis of cotransduction with *proC*; *lonA* 24-29% cotransduction and *lonB* 12-17%. However, *lonA* and *lonB* mutants did not appear to complement each other. Tables 4 and 5 summarise the phenotypic and genetic results.

TABLE 4 Phenotypes of UV sensitive mutants of *Escherichia coli* K12

Mutation	UV Sensitivity	Hcr	UV induced DNA degradation	X-ray Sensitivity	Susceptibility to filamentation	Mucoidy	Mitomycin C sensitivity
uvrA) *uvrB*) *uvrC*)	Sensitive (c) (d)	Hcr^- (c)(d)	less than normal (d)	slight (c)(d)	not susceptible (wild-type) (g)	non-mucoid (wild-type) (g)	sensitive (h)
uvrD	intermediate (f)	Hcr^- (f)	excessive (f)	moderate (f)	not tested	not tested	not tested
lon	sensitive (b)	Hcr^+ (b)	not tested	sensitive (a) (b)	susceptible (a) (b)	mucoid (g)	not tested
darA	sensitive (e)	Hcr^+ (e)	not tested	not tested	not susceptible (wild-type) (e)	non-mucoid (wild-type) (e)	not tested

References:

(a) Adler and Hardigree (1964): *lon* strains were more X-ray sensitive than wild-type only in the presence of glucose.

(b) Howard-Flanders, Simson and Theriot (1964a): *lon* strains were UV sensitive only on complex medium.

(c) Howard-Flanders, Boyce, Simson and Theriot (1962).

(d) Howard-Flanders, Boyce and Theriot (1966).

(e) Van de Putte, Van Sluis, Van Dillewijn and Rorsch (1965).

(f) Ogawa, Shimada and Tomizawa (1968).

(g) Howard-Flanders, Simson and Theriot (1964b).

(h) Howard-Flanders and Boyce (1966).

TABLE 5 Genes affecting Radiation Sensitivity in *Escherichia coli* K12 and its derivative CR34.

Gene	Location	Technique	Reference
uvrA	near *metA*	Time of entry in Hfr x F^- cross P1 cotransduction	(a) (b)
uvrB	near *bio*	Time of entry in Hfr x F^- cross P1 cotransduction	(b)
uvrC	near *supH*	Time of entry in Hfr x F^- cross P1 cotransduction	(b)
uvrD	near *metE*	P1 cotransduction	(e)
lon	between *lac* and *gal*	Time of entry in Hfr x F^- cross Genotypic analysis of selected recombinants	(c) (d)
darA	near *ilv*	Time of entry in Hfr x F^- cross	(f)

References:

(a) Howard-Flanders, Boyce, Simson and Theriot (1962).

(b) Howard-Flanders, Boyce, and Theriot (1966).

(c) Howard-Flanders, Simson and Theriot (1964a).

(d) Adler and Hardigree (1964).

(e) Ogawa, Shimada and Tomizawa (1968).

(f) Van de Putte, van Sluis, van Dillewijn and Rorsch (1965).

Note: Markovitz (1964) has shown that *lon-1* was mutant in a regulator gene for capsular polysaccharide biosynthesis, now designated *capR*.

A further series of mutants that were UV sensitive, but not filament forming (eliminating any *lon* mutants) were isolated by van de Putte et al, (1965) in a strain CR34. Their results indicated that most of these *dar* (*d*ark *r*epair) mutations were alleles of the *uvrA*, *B* or *C* loci already defined, but *dar-2* which was Hcr$^+$ mapped at a different position to known UV sensitivity genes of *E. coli* and presumably defined another gene, (called *darA* by Taylor and Trotter, 1967), see Tables 4 and 5 for its phenotype and genetic location.

3. Recombination deficient mutants of *Escherichia coli* K12.

Three loci controlling recombination ability have so far been defined, *recA* (Willetts, Clark and Low, 1969), *recB* and *recC* (Emmerson, 1968), on the basis of genetic and physiological findings. The phenotypes and locations of these mutants are summarised in Tables 6 and 7.

Clark (1967) divided 25 *rec* mutations into three phenotypic groups in an attempt to get an indication of the number of genes involved. They were designated Rec1, Rec2 and Rec3, on the basis of their fertility level with strain HfrKL16, (dependent upon the relative dominance relationship of the *rec* mutation with *rec*$^+$ and the location of the *rec*$^+$ gene with regard to the Hfr origin), their degree of UV sensitivity and their spontaneous induction levels of λ lysogens.

Rec1 mutations had lower UV sensitivity and relatively high fertility with KL16 and showed normal spontaneous induction of λ lysogens. Rec2 mutations had higher UV sensitivity and high fertility with KL16 and abnormal spontaneous induction of λ lysogens.

TABLE 6 Phenotypes of *rec* mutants of *Escherichia coli* K12.

Mutation	UV sensitivity	Hcr	Pyrimidine dimer excision	DNA degradation: Spontaneous	DNA degradation: UV Induced	UV effect on DNA synthesis	X-ray sensitivity	Mitomycin C sensitivity
recA	sensitive (c)	Hcr$^+$ (c)	Excision proficient (b)	high (b)(c)	high (reckless) (b) (c)	complete inhibition (b) (c)	sensitive (c)	sensitive (d)
recB	sensitive (e)	Hcr$^+$ (d)	not tested	normal (e)	low (cautious) (e)	not tested	sensitive (e)	sensitive (d)
recC	sensitive (e)	not tested	not tested	normal (e)	low (cautious) (e)	not tested	sensitive (a) (e)	not tested
lex	sensitive (d)	Hcr$^+$ (d)	not tested	normal (d)	high (d)	longer inhibition than wild-type (d)	sensitive (d)	sensitive (d)

References:

(a) Emmerson and Howard-Flanders (1967).

(b) Clark, Chamberlin, Boyce and Howard-Flanders (1966).

(c) Howard-Flanders and Theriot (1966).

(d) Howard-Flanders and Boyce (1966).

(e) Emmerson (1968).

TABLE 7 Genetics of *rec* mutations of *Escherichia coli* K12.

Mutation	Location	Technique	Phenotypic classification group (Clark, 1967)	Reference
recA	between *cysC* and *pheA*	Delayed time of entry of *thyA* until *recA*$^+$ enters zygote. P1 cotransduction with *cysC* and *pheA*	Rec2 and Rec3	(a)
recB	between *lysA* and *argA*	P1 cotransduction with *lysA*, *argA* and *thyA*	Rec1	(c)
recC	between *lysA* and *argA*	P1 cotransduction with *lysA*, *argA* and *thyA* *recC* defined by complementation of *rec-22* with *recB21*	Rec1	(c)(d)
lex	linked to *metA* and *uvrA*	Cotransduction with *metA* and *uvrA*		(b)

References:

(a) Willetts, Clark and Low (1969).

(b) Howard-Flanders and Boyce (1966).

(c) Emmerson (1968).

(d) Emmerson and Howard-Flanders (1967).

Rec3 mutations had higher UV sensitivity and low fertility with HfrKL16, and abnormal spontaneous induction of λ lysogens. Clark demonstrated that two of the *rec* mutants were recessive to rec^+ and that the UV sensitivity and recombinational ability were properties of the same gene or of very closely linked genes.

A Rec1 mutation (*rec-21*) was shown to complement the Rec2 (*rec-13* and *rec-56*) and Rec3 (*rec-67* and *rec-12*) mutations, but these Rec2 and Rec3 mutations did not complement one another. All three groups were nearly normal in their formation of lac^+ merodiploids, indicating a defect in recombination rather than merely in chromosome transfer.

Low (1968) examined the types of recombinants and the linkage of unselected markers amongst those recombinants that were obtained from crosses between a rec^+ donor and *recA13*, *recB21* or *recC22* recipients in which the rec^+ allele had not had time to enter any zygotes. The absolute yields of recombinants were in all cases much less than in rec^+ x rec^+ crosses. *RecA13* was shown to yield no real recombinants; all the progeny were either F' strains, being apparently recombinants because of their merodiploid nature, or they were non-donor merodiploids which carried only part of an F factor. *RecB21* and *recC22* gave similar results to one another; both yielded recombinants with normal linkage relationships which were F^- and apparently normal recombinants. Thus although the absolute level of recombinants was depressed by *recB* and *recC* mutations, when recombination did occur within a zygote, it occurred with normal frequency relative to map length.

Witkin (1969) reported that UV induced mutability was greatly reduced by the *recA1* (no mutations were detected) and *recB* mutations,

and reduced to a lesser extent by *recC22*.

A fourth mutation, *lex*, is included in this section and Tables 6 and 7 because of its closer similarity to *rec* than to *uvr* or *lon* mutations. It confers a phenotype similar in many respects to the *exr* mutations of *E. coli* B (Howard-Flanders and Boyce, 1966).

Further mutations having an effect on UV sensitivity and recombination were described by Holland (1967) and Holland & Threlfall (1969). Four, RefII mutants of *E. coli* K12 which had been selected for refractivity to colicin E2, were also found to be UV sensitive. These RefII (uvs) mutants shared some properties with those of known *rec* mutants (see Table 8 which summarises their results). The refractivity and UV sensitivity were properties of a single mutation or very closely linked mutations, with a location near to *thr*, and to mutations with a RefII (uvs^+) phenotype. The authors suggested that, since colicin E2 acted on sensitive cells by causing DNA degradation, the RefII (uvs) mutants, which in the two cases tested appeared to be defective also in recombination, may have been deficient in a nuclease or some aspect of control of the action of a nuclease. A *rec* mutant and genes controlling restriction and modification were also located in this region, suggesting a region of the chromosome concerned with DNA metabolism.

4. Studies with double mutants.

Double mutants constructed to contain two mutations in different genes sharing some phenotypic properties have been studied for the interaction of the two component mutations. The rationale involved was that if the two components expressed themselves independently in the double mutant and their common properties were approximately

TABLE 8 Ref II (uvs) mutants of *Escherichia coli* K12.

Strain	Genotype	Location	Technique	Phenotype	Reference
ASH110	Ref II (uvs)	not located	-	Hcr^+	(a)
ASH111	Ref II (uvs)	not located	-	Hcr^+. UV and colicin E2 induced (reckless) release of H^3-thymidine from DNA. Normal spontaneous release.	(a)
ASH112	Ref II (uvs)	close to *thr*	Time of entry in Hfr by F^- cross. (b)	Hcr not tested. All E2 sensitive recombinants were UV resistant.	(a)
				Rec^-. Low recombination deficiency index.	(b)
ASH113	Ref II (uvs)	not located	-	Rec^-. High recombination deficiency index.	(b)
ASH 54 ASH 55	Ref II (uvs^+)	close to *thr*	Time of entry in Hfr by F^- cross. (b)	Wild-type for these characteristics except E2 sensitivity	

References:

(a) Holland (1967).

(b) Holland and Threlfall (1969).

Note: *rec-35* showed greater spontaneous, UV and colicin E2 induced release of H^3-thymidine from its DNA than any of the above mutants.

additive, then independent pathways of action of the genes were indicated. If this was not so, then a common pathway of action was indicated.

For example, Howard-Flanders, Boyce and Theriot (1966) showed that *uvrAuvrB*, *uvrAuvrC* and *uvrBuvrC* double mutants were only a little more UV sensitive than the most sensitive allele of their components, and *uvrA*, *B* and *C* were therefore presumed to act in a common pathway of repair.

Similarly Ogawa et al. (1968) found a *uvrBuvrD* double mutant to be only three times as sensitive to UV as its least sensitive component, slightly more sensitive to gamma rays and to have slightly less Hcr of UV irradiated bacteriophage. It showed much less UV induced DNA degradation than the *uvrD* mutant. They concluded that the genes *uvrB* (and therefore *uvrA* and *uvrC*) and *uvrD* were functionally related.

Uvr lon double mutants (Howard-Flanders, Simson and Theriot 1964b), however, were 15 times more sensitive than their most sensitive component at the 10% survival level, and the radiation resistance of recombinants in an Hfr uvr^+ lon^+ x F^- *uvr lon* cross increased in two steps. The different phenotypes of *uvr* and *lon* were both expressed in the double mutant and these were presumed to act by different mechanisms.

Strain Bs1, which was a double mutant from the outset, being *uvr-1 exr-1* suggests that *exr* and *uvr* act by different mechanisms since strains obtained when each was transduced out of Bs1 into B had only part of the mutant phenotype of Bs1. (Mattern, Zwenk and Rorsch, 1966).

Donch, Green and Greenberg (1968) studied *exr lon* strains

constructed with *exr-2* and *exr-7* from strains Bs2 and Bs7 and *lon-1* from *E. coli* K12. In these strains filamentation induced by a variety of agents including UV was suppressed and the UV sensitivity was less than additive. However, since pantoyl lactone was shown to prevent filamentation and decrease UV sensitivity simultaneously, filamentation itself would appear to have been the lethal factor. Then the less than additive UV sensitivity of *exr lon* would be expected even though *exr* and *lon* otherwise operate through different mechanisms. *Exr lon* strains were still mucoid, another phenotypic property of *lon* strains (except those obtained from strain B where mucoidy was suppressed). The suppression of *lon* filamentation by *exr* was supported by the reduced recovery and therefore suppression of filamentation brought about in *exr lon* compared with exr^+ *lon* by pantoyl lactone, minimal medium recovery or liquid holding recovery. The implication of these results is that *exr* and *lon* operate by different routes; that *exr* involves functions not totally affected by *lon* was confirmed by the detection of *exr* mutants in strain B which contained a *lon* type mutation.

Howard-Flanders, Theriot and Stedeford (1969) constructed a *uvr rec* strain and compared it with uvr^+ rec^+, *uvr* rec^+ and uvr^+ *rec* strains. The different phenotypes of the two types of mutation were expressed independently in the doubly mutant strain. Thus *uvr* and *rec* appeared to act by independent pathways. A further observation was that the *uvr rec* strain had a survival of 37% at a UV dose which induced about 1.3 pyrimidine dimers per genome.

The values for uvr^+ *rec*, *uvr* rec^+ and uvr^+ rec^+ strains were

22, 60 and 3,700 dimers per genome respectively (Howard-Flanders and Boyce, 1966).

Witkin (1967) examined the phenotypes of all possible combinations of genotypes of *fil*,*fil*$^+$; *hcr*,*hcr*$^+$; *exr* and *exr*$^+$ obtained by successive mutation for increased UV resistance starting from Bs1 *hcr-1 exr-1 fil*$^+$ and Bs2 *hcr*$^+$ *exr-2 fil*$^+$. Apart from the suppression of filament formation of *fil*$^+$ in the presence of *exr*, the three pairs of phenotypes were expressed independently in all the strains. Thus from these studies, *uvr*, *lon (fil)* and *exr(lex)* genes appeared to operate by different mechanisms, *rec* operated independently of *uvr*, and although it was not tested in double mutants, was independent of *lon* on the basis of their phenotypes. *Rec* and *lex* were not tested for interaction, but have similar mutant phenotypes and possibly therefore closely related functions.

5. Mechanisms of UV repair.

Various biochemical studies have been made on the state of the DNA synthesised before and after UV irradiation, in wild-type cells and in *uvr*, *rec* or *uvrrec* mutants.

Before the study of the early radiation sensitive mutants thymine dimers between adjacent thymine residues in DNA were already known to be produced by UV and were correlated with UV killing of bacteria *in vivo*. A photoreactivating enzyme of yeast could eliminate the dimers in the presence of visible light (Wacker 1963 for a review). Subsequently it was shown that the dimers were induced by UV between adjacent pyrimidines in DNA, whether cytosine or thymine residues, that any of these were removed by photoreactivating enzymes in the presence of light and that uracil dimers, which were produced by

heat treatment of cytosine dimers, were monomerised by the photo-reactivating enzyme; presumably all dimers were in fact monomerised *in situ.* (see Setlow 1966 for a review).

The finding of Howard-Flanders, Boyce, Simson and Theriot (1962) that *uvr*$^+$ cells could no longer reactivate UV irradiated T1 bacteriophage if thymidine was substituted by 5-bromodeoxyuridine had implicated thymine as the target for UV induced damage which could be reactivated in the dark in wild-type cells.

Boyce and Howard-Flanders (1964) and Howard-Flanders, Boyce and Theriot (1966) showed that the *uvr*$^+$ *E. coli* K12 could excise from its DNA thymine dimers (they became TCA soluble during incubation) and thymine-cytosine dimers induced by UV. Strains mutant at *uvr*A, *uvr*B or *uvr*C were deficient in this function (the dimers remained TCA insoluble) and in the partial UV induced DNA degradation associated with excision which was typical of the wild-type.

Setlow and Carrier (1964) showed that similarly wild-type *E. coli* B and the B/r mutant were able to excise UV induced thymine dimers, whereas strain Bs11 performed this function at half the rate of B and strain Bs1 excised no dimers. In addition the dimers in Bs1 remained intact in the DNA whereas those of B/r did not.

Pettijohn and Hanawalt (1964) demonstrated in *E. coli* TAU-bar, wild-type for radiation sensitivity, that some DNA degradation follows UV irradiation, as was shown already in *E. coli* K12 (Boyce and Howard-Flanders 1964), and at the same time H^3-5 bromouracil was incorporated into DNA. The DNA was extracted and banded on centrifuge density gradients; much of the radioactivity was associated with DNA of the same density as pre-existing DNA with some at positions intermediate

between this and that of semi-conservatively replicated hybrid DNA. This was not at all characteristic of bromouracil incorporation into replicating unirradiated DNA which shows semi-conservative replication, with DNA banding discretely at light, hybrid and heavy positions. Thus the newly synthesised DNA occurred in short segments randomly distributed throughout the pre-existing DNA. Photoreactivation before bromouracil incorporation restored normal semi-conservative replication, implying that the random synthesis of short segments of new DNA was dependent upon the presence in DNA of pyrimidine dimers, and presumably upon their excision. It was concluded that wild-type cells repair UV damage by single strand excision of the defect and repair of the single strand gap using the undamaged strand as a template.

Rupp and Howard-Flanders (1968) followed post irradiation DNA synthesis in a strain containing the mutation *uvrA6* and therefore unable to excise pyrimidine dimers. This strain shows 37% survival at a UV dose which produced an average of 50 pyrimidine dimers per genome. Thus the cells apparently have a mechanism for overcoming the potentially lethal effect of many pyrimidine dimers. This strain incorporated H^3 thymidine into its DNA at a reduced rate after a low dose of UV; the dimers inhibited but did not block DNA synthesis. Analysis of sedimentation rates on alkaline sucrose gradients of the DNA synthesised immediately after UV irradiation showed that it contained alkali labile bonds or gaps which with further incubation became alkali stable or repaired. The evidence for this was that the newly synthesised DNA sedimented more slowly than, and then with further incubation as fast as, the pre-existing DNA. An estimate

of the number of gaps per genome corresponded approximately with the number of dimers induced per genome by the UV dose used. The presence of gaps rather than alkali labile bonds was confirmed by sedimentation analysis of denatured newly synthesised DNA on neutral sucrose gradients when it still sedimented more slowly than pre-existing DNA. (Howard-Flanders et al., 1968).

Howard-Flanders et al. (1968) also made use of the conjugation system in the further analysis of the *uvr* and *rec* functions. They worked on the assumption that the chromosome donated during conjugation was one of a pair of daughter chromosomes produced by replication at the time of conjugation. A number of findings were reported.

1. The donation of both chromosomal DNA and episomal DNA from irradiated excision defective cells was not greatly affected although the donated DNA presumably contained single strand gaps in its newly synthesised strand.
2. Irradiated excision defective F *lac*$^+$ donors yielded an increasing proportion of *lac*$^+$ recombinants rather than secondary F *lac*$^+$ donors in crosses with *rec*$^+$ recipients, whether *uvr*$^+$ or *uvrA*, as the UV dose was increased.
3. When the recipients were *recA*, whether *uvr*$^+$ or *uvrA*, the number of Lac$^+$ progeny from crosses with irradiated excision defective F *lac*$^+$ donors fell sharply with increasing UV dose, and all the Lac$^+$ progeny were secondary F *lac*$^+$ donors. *RecA* recipients appeared to be able to form a Lac$^+$ colony only if they received an intact episome, since the UV dose for 37% inactivation induced an average of about one dimer per transferred episomal strand. Similarly *recB* recipients whether

uvr$^+$ or *uvrB* had the same kinetics of Lac$^+$ colony formation in crosses with UV irradiated excision defective F *lac*$^+$ donors but some of these (the proportion increasing with dose) were *lac*$^+$ recombinants.

An intact *uvr*$^+$ system in the above two experiments made no difference to the dose response curves indicating that excision repair was not effective on the irradiated, replicated and transferred DNA.

4. Photoreactivation of the recipients in 3 above after conjugation produced an increased yield of F *lac*$^+$ secondary donors, with a long half life for photoreactivation treatment (about 1.5 hours), that is the dimers were very stable in the recipients.

Thus the pyrimidine dimers in the *transferred* DNA were accessible to photoreactivation but not excision repair indicating that the single strand gaps were opposite the dimers and not displaced from them, since if this had been the case, both processes should be capable of repairing the dimers.

5. When chromosomal transfer from excision defective donors to *uvr*$^+$ *rec*$^+$ recipients was performed without irradiation, a high proportion, 70%, of recombinants selected for a particular donor allele also inherited a continuous sequence of three unselected donor alleles. After a dose of irradiation, although the yield of recombinants for a single male marker was 20% to 50% of control levels, a high proportion of the recombinants now possessed only the selected donor allele and only 1.5% possessed the continuous sequence of the selected and three

unselected donor alleles. Dimers induced after mating had little effect on the pattern of inheritance of alleles (Wilkins and Howard-Flanders, 1968).

Defects induced before donation, when transferred, perhaps induced and necessitated recombination events to incorporate only short segments of donor DNA into recombinants.

6. Other observations on the frequencies of *lac*$^+$ recombinants arising from irradiated F *lac*$^+$ donors crossed with *rec*$^+$ recipients suggested that the irradiated donor DNA increased the recombination frequency.

7. There was recovery of F *lac*$^+$ episomes transferred from irradiated excision defective *rec*$^+$ donors to *recA* recipients, if the donors were incubated between irradiation and mating. The incubation had no effect with *rec*$^+$ recipients, which showed a steady higher level of Lac$^+$ progeny, presumably largely *lac*$^+$ recombinants. In similar experiments with irradiated excision defective *recA* donors, essentially no Lac$^+$ progeny were formed with or without the incubation period, when *recA* recipients were used. With *rec*$^+$ recipients the incubation period caused a decline of Lac$^+$ progeny.

These results suggested that an intact recombination system was needed for recovery of episomes in the excision defective donor.

The findings described in this section, taken as a whole, have resulted in the following models for the mechanisms of dark repair of UV damage.

UV induces pyrimidine dimers in DNA.

Before replication.

1. An enzyme detects the pyrimidine dimer and cuts the DNA deoxyribose phosphate backbone.

2. A deoxyribonuclease releases the dimer and a number of other bases to form a single strand gap.

3. A DNA polymerase repairs the gap using the opposing single strand as template.

4. A DNA ligase restores the continuity of the DNA deoxyribose phosphate backbone.

(Boyce and Howard-Flanders,1964; Setlow and Carrier,1964; Pettijohn and Hanawalt,1964).

At replication.

1. The dimer delays the replication process.

2. Replication commences beyond the dimer leaving a short single strand gap opposite the dimer in one daughter chromosome and a normal DNA duplex in the other.

(Rupp and Howard-Flanders,1968; Howard-Flanders et al., 1968).

After replication.

1. A recombination mechanism uses the intact information of the normal DNA duplex of one of the daughter chromosomes to repair the single strand gap opposite the dimer of the other daughter chromosome, or recombination between sister chromosomes could create one perfect chromosome and another containing all the defects.

(Howard-Flanders et al., 1968; Howard-Flanders, Theriot and Stedeford, 1969).

6. Summary of the gene functions in *Escherichia coli*.

Uvr mutations affect the ability of cells to perform dark repair of the irradiated DNA before replication by excision of pyrimidine dimers. *Lon* or *fil*$^+$ mutations act by inhibiting cell wall formation after UV irradiation and other treatments, perhaps due to some defective control mechanism linking cell wall synthesis (mucopolysaccharide) to DNA replication, leading to lethal filament formation.

Rec mutations act by affecting steps in recombinational repair, acting on daughter chromosomes after replication of the damaged chromosome, possibly being unable to initiate DNA degradation ('cautious', *recB*, *recC*) or stop it once started ('reckless', *recA*). *RecA* virtually eliminates normal recombination whereas *recB* and *recC* depress the number of zygotes yielding recombinants but permit normal recombination frequency when it does occur.

Exr and *lex* mutations are also implicated in recombinational repair since *lex* at least has a similar phenotype to *rec* mutants and *exr* mutants have a pronounced effect on reducing UV induced mutation, in fact, eliminating it altogether (Witkin, 1967).

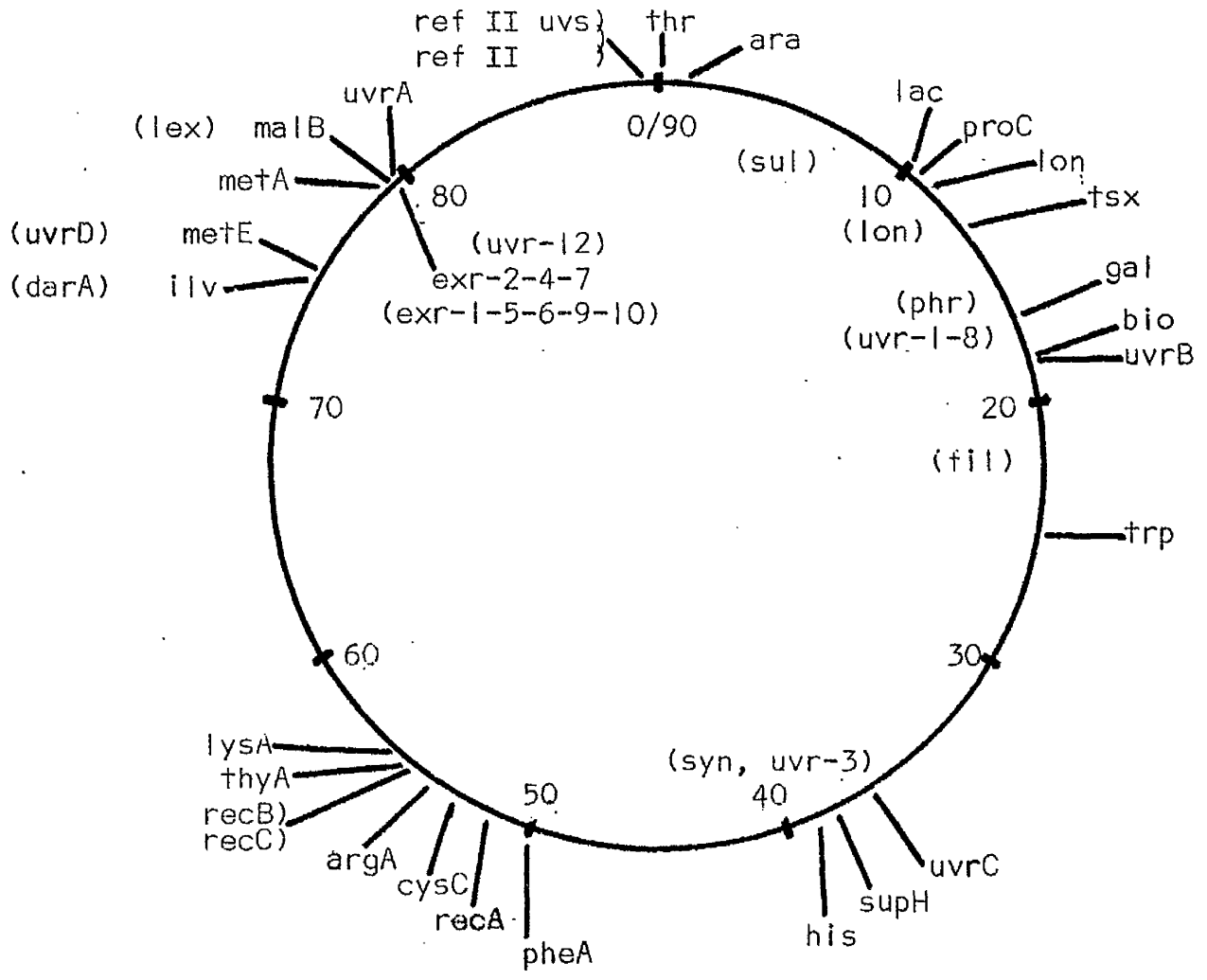
Uvr and *rec* mutants can still overcome the lethal effect of some UV damage, but *uvr recA* mutants can apparently overcome none. All the genes of *E. coli* mentioned in this section are shown on the genetic map in Figure 4.

C. Radiation Sensitivity in other Micro-organisms.

1. Protokaryotes.

Mutations affecting radiation sensitivity have been isolated in a number of other bacteria but none of these organisms has been

FIGURE 4 The linkage map of *Escherichia coli*.
Adapted from Taylor and Trotter (1967).



Only genes referred to in this thesis are included. Genes described in *E. coli* K12 are indicated outside the circle and those in *E. coli* B inside the circle.

( ) indicate imprecisely located genes.

} indicate unordered genes.

The numbers inside the circle are the map length in minutes.

analysed in as much detail as *E. coli*.

Hcr$^-$ strains have been found in *Bacillus subtilis* (Mahler, 1965; Reiter and Strauss, 1965; Munakata and Yonosuke, 1969); *Haemophilus influenzae* (Setlow et al., 1968); *Micrococcus lysodeikticus* (Feiner, 1967); *Pseudomonas aeruginosa* (Holloway, 1966a); *Salmonella typhimurium* (Skavronskaya et al., 1969); *Serratia marcescens* (Winkler, 1964) and *Streptococcus pyogenes* (Malke, 1967). These presumably correspond to the *uvr* mutants of *E. coli*.

Strains which were sensitive to both UV and X-rays have been obtained in *Haemophilus influenzae* (Barnhart and Cox, 1968; Setlow et al., 1968). The strain of Setlow et al. may be *rec*, whereas that of Barnhart and Cox, which showed small X-ray sensitivity and some Hcr deficiency, may be *uvr*.

Mutants affected in recombination have been obtained in *Bacillus subtilis* (Okubo and Romig, 1966; Hoch et al., 1967); *Proteus mirabilis* (Bohme, 1968); *Pseudomonas aeruginosa* (Holloway, 1966b) and *Salmonella typhimurium* (Wing et al., 1968).

Micrococcus radiodurans is an extremely radiation resistant organism, such that UV and X-ray killing probably depends as much on cytoplasmic damage as damage to DNA. Moseley (1967, 1969) isolated radiation sensitive mutants in this organism which were partially defective in pyrimidine dimer excision such that radiation damage to DNA was now the most important factor for killing cells.

UV sensitive strains which also have high spontaneous mutability have been isolated in *Neisseria meningitidis* (Jyssum, 1968) and *Proteus mirabilis* (Bohme, 1967); such strains are similar to that recently isolated in *E. coli* by Hill (1968), except that the

mutation conferring high mutability in this strain did not also confer UV sensitivity.

This summary of the results obtained in other protokaryotes indicates that repair mechanisms for UV damage, which have been best characterised in *E. coli*, are widespread amongst the protokaryotes.

2. Eukaryotes.

UV sensitive mutants have been isolated in *Aspergillus nidulans* (Lanier, Tuveson and Lennox, 1968); *Chlamydomonas reinhardi* (Davies, 1967); *Neurospora crassa* (Chang and Tuveson, 1967); *Saccharomyces cerevisiae* (Cox and Parry, 1968; Nakai and Matsumoto, 1967; Snow, 1967); *Schizosaccharomyces pombe* (Haefner and Howrey, 1967) and *Ustilago maydis* (Holliday, 1965).

The picture that has emerged from these studies in eukaryote micro-organisms is that UV sensitivity control and its relationship to recombination is more complex than in protokaryotes. For example, Cox and Parry (1968) attempted to detect most of the genes affecting UV sensitivity in *Saccharomyces* by isolating 96 mutants. However, these were located at 22 loci, ten of which were only represented once, indicating that several more were still undetected. The mutants isolated in other organisms have been too few to extend this result, but where they have been mapped, loci were frequently represented by only one mutation.

Some of these mutants were also X-ray or gamma ray sensitive. Thus mutants at five of the loci of Cox and Parry (1968) and one of the mutants of Makai and Matsumoto (1967) in *Saccharomyces* were sensitive to gamma or X-rays, two of the phenotypic groups of mutants of *Ustilago* were X-ray sensitive to different degrees

(Holliday, 1965), and the five mutants of *Chlamydomonas* were gamma ray sensitive (Davies, 1967).

By construction of double mutants, Nakai and Matsumoto (1967) demonstrated that their two UV sensitive mutations had closely related functions since the double mutant did not show greater sensitivity than the single mutants. In contrast, the X-ray sensitive mutant affected a different function since, in combination with either UV sensitive mutation, the double mutants were very UV sensitive. Similarly Davies (1967), Haefner and Howrey (1967) and Holliday (1967) all obtained double mutants of greater sensitivity to UV than either component mutation in *Chlamydomonas*, *Schizosaccharomyces* and *Ustilago* respectively, indicating the existence of at least two different mechanisms controlling UV sensitivity in every one of these organisms.

Kilbey and Smith (1969) compared one of the UV sensitive mutants of Nakai and Matsumoto (1967) with a wild-type *Saccharomyces* strain for photoreactivability, the effect of LHR on photoreactivability, and sensitivity to diepoxybutane and NTG. They concluded that the mutant was qualitatively similar to Hcr^- strains of *E. coli* and therefore that it may have been deficient in excision repair.

Some of these mutants affected recombination, or showed effects that may have resulted from defective recombination. Holliday (1967) showed that *uvs-1* and *uvs-2* of *Ustilago* had considerable effects in reducing mitotic gene conversion, and causing increased or decreased UV induced mitotic segregation respectively in diploids; and that *uvs-2* blocked meiosis.

Other observations were that UV sensitive mutants of

Saccharomyces affected UV induced intergenic mitotic recombination in a complex way (Snow, 1968); that five mutants of *Saccharomyces* reduced sporulation in diploids, a step occurring after recombination (Cox and Parry, 1968); that *uvs-1* of *Neurospora* when homozygous in crosses caused a high degree of ascus and ascospore abortion although crossover frequencies were normal (Chang and Tuveson, 1967); and that matings homozygous for UV sensitivity in *Aspergillus* yielded sterile, dwarf cleistotheca (Lanier, Tuveson and Lennox, 1968).

II. GENERAL MATERIALS AND METHODS

A. *Streptomyces coelicolor* strains

1. Origin of the strains.

These were derivatives of a single clone wild-type isolate designated A3(2) (Hopwood, 1959), obtained by successive steps of mutagenesis or recombination. All these strains were inter-fertile. They were grown by incubation at 30°C unless otherwise stated.

In the study concerned with photoreactivation another wild-type isolate, K673, was used. This strain was obtained from Dr H.J. Kutzner and was considered by Kutzner and Waksman, (1959) to be correctly named *S. violaceoruber*. A3(2) closely resembled K673 and differed from *S. coelicolor* as defined by these authors and therefore A3(2) should strictly be called *S. violaceoruber*. However, for reasons explained by Hopwood and Sermonti (1962), A3(2) has continued to be called *S. coelicolor* in papers concerned with genetic recombination. Recombinants have not so far been obtained from mixed cultures of K673 with A3(2).

2. Nomenclature.

All loci in *S. coelicolor* are described by three letter symbols following the recommendations of Demerec et al. (1966), for bacterial genetics, and to conform with this, loci which control sensitivity to ultraviolet light were designated *uvs* (*u*ltra*v*iolet *s*ensitivity). Table 9 lists the loci involved in this study, the particular alleles used, and their mutant phenotype. The genotypes of strains will be described in full in the text as appropriate.

TABLE 9. The loci used in this study, the mutant alleles and their characteristics. (Taken from Hopwood, 1967a).

Locus	Alleles	Phenotype
ammA	*amm-5*	Unable to utilise nitrate.
argA	*arg-1*	Requirement for arginine (citrilline) or ornithine.
adeA	*ade-3*	Requirement for purines.
cysA	*cys-15*	Requirement for cysteine.
cysC	*cys-3*	Requirement for cysteine or S_2O_3 or S_2O_4
cysD	*cys-18*	Requirement for cysteine or S_2O_3 or S_2O_4 or S_2O_5.
guaA	*gua-1*	Requirement for guanine.
hisA	*his-1*	Requirement for histidine.
hisC	*his-9*	Requirement for histidine or histidinol.
hisD	*his-3*	Requirement for histidine or histidinol.
mthB	*mth-2*	Requirement for methionine plus threonine or homoserine.
pheA	*phe-1*	Requirement for phenylalanine.
proA	*pro-1*	Requirement for proline.
serA	*ser-1*	Requirement for serine or glycine.
strA	*str-1*	Resistance to streptomycin.
tps	*tps-30*	Temperature sensitive. Unable to grow at 37°C.
uraA	*ura-1*	Requirement for uracil.
uvsA	*uvs-2, 4, 9, 15, 19, 20, 22, 23, 24*	Sensitive to ultraviolet light.
uvsB	*uvs-6*	
uvsC	*uvs-7, 8, 10, 14, 16, 17*	
uvsD	*uvs-1, 3, 5, 11, 18*	
uvsE	*uvs-13*	
uvsF	*uvs-25*	Enhancer of sensitivity to ultraviolet light of some *uvsC* and *uvsD* mutants.

B. Media (Hopwood, 1967a)

1. Minimal Medium (MM).

This had the following composition per litre:

asparagine, 0.5g; K_2HPO_4, 0.5g; KOH, 0.3g; $MgSO_4.7H_2O$, 0.2g; $FeSO_4.7H_2O$, 0.01g; agar, 15g; and glucose, 10g.

The glucose was autoclaved separately as a 50% solution. The medium was supplemented when necessary as follows (per litre); amino acids except histidine, 50mg; histidine, 70mg; adenine and uracil, 10mg; vitamins, 1mg; dihydrostreptomycin sulphate 50mg.

2. Complete Medium (CM).

This consisted of (per litre): K_2HPO_4, 5g; NaCl, 0.5g; $MgSO_4.7H_2O$, 0.5g; peptone (Difco), 2g; yeast extract (Yeastrel), 1g; Casamino acids (Difco), 1.5g; yeast nucleic acid hydrolysate, 5ml, (boil 2g of nucleic acid in 15ml of 1N NaOH for 10 minutes; boil 2g of nucleic acid in 15ml of 1N HCl for 10 minutes, mix the two solutions, adjust to pH 6.0, filter hot, make up to 40ml with water); vitamin solution, 1ml, (riboflavine, 0.1%; nicotinamide, 0.1%; p-aminobenzoic acid, 0.01%; pyridoxine HCl, 0.05%; thiamine HCl, 0.05%; biotin, 0.02%); agar, 15g; glucose, 25g; histidine, proline, threonine, tryptophan and tyrosine (when dealing with the relevant auxotrophs), 50mg each. CM was adjusted to pH 7.2 with 1N HCl and could be autoclaved at 115°C for 10 minutes without excessive breakdown of the glucose.

C. Use and Maintenance of Cultures

1. Equipment.

a. Slants of 5ml CM in 125mm x 16mm test tubes plugged with

cotton wool were used for maintaining stock cultures which were incubated for about a week and then stored at 4°C. These needed to be subcultured once a year to fresh slants of CM.

b. Slants of 15ml CM or supplemented MM in 150mm x 25mm test tubes inoculated and then incubated at 30°C for 3 to 5 days were used for the preparation of spore/hyphal suspensions from single or mixed cultures and for the maintenance of stock cultures in current use.

c. Filter tubes were constructed from 125mm x 16mm test tubes in the bottom of which a hole approximately 5mm in diameter was blown. This hole was closed with 1" of packed cotton wool, and the mouth of the tube plugged with cotton wool. The tube was inserted through a cotton wool plug in the neck of a 150mm x 25mm test tube.

d. 100mm x 12.5mm thick walled tubes were used for centrifugation.

e. Agar plates were prepared by pouring 15ml of molten CM or supplemented MM into sterile 9cm glass or plastic Petri dishes. Since aeration improves sporulation on agar plates in *S. coelicolor* glass Petri dishes were covered with metal lids lined with 11cm filter papers, and the plastic Petri dishes had vented lids.

f. Subcultures to agar slants or plates were made with nichrome wire needles or loops.

g. Spore suspensions were spread over agar plates with glass rod spreaders constructed of 3mm glass rod. These were sterilised in a bunsen flame and allowed to cool immediately before use.

h. Sterile velvet pads held by a stainless steel ring over a

stainless steel block were used to make replicas to agar plates from a master plate by the technique of Lederberg and Lederberg (1952).

2. Inoculation of Cultures.

Inocula in general consisted of hyphae in all stages of development including substrate and aerial hyphae and spores. These were obtained either from confluent cultures on an agar slant from which an area about 5mm square was dug out, or from an agar plate containing mature colonies from which a whole colony was taken.

a. Inoculation of Slants.

The inocula were taken and transferred on a nichrome wire needle and placed on the new slants where they were broken up and streaked over the agar surface.

Slants were inoculated with only one or with two different strains. In the first case, all the spores grown on the surface of the confluent culture were of the same genotype except for rare spontaneous mutations. In the latter case, spores formed on the confluent culture consisted predominantly of the genotypes of the two parental strains, but a proportion varying between about 1% and less than 0.0001% depending upon the fertility of the two strains, had recombinant genotypes. These arose from zygotes formed between the hyphae of the two parent strains during growth of the mixed culture. Such mixed cultures are referred to as "crosses" between the two parents involved.

b. Inoculation of agar plates.

This was carried out either with an inoculum taken from a

confluent culture, or a single colony; or with a spore suspension.

i. A confluent culture or single colony inoculum was taken and streaked with a nichrome wire. In some cases, two or more strains were streaked to sectors of a plate, so that their phenotypes could be compared by replica plating or some other tests applicable to agar plates. In other cases, the inoculum of a single strain was streaked to yield single colonies.

When the inoculum consisted of a spore suspension, the intention was usually to obtain isolated colonies. Spore suspensions did in fact consist of a variable proportion of spores and aerial hyphal fragments, depending upon the strain and the time at which a culture was harvested. They contained a small proportion of substrate hyphal fragments.

There were three ways in which a spore suspension was treated:

ii. The first of these three methods was used when isolated colonies were required, but the number of colony-forming units in the suspension was not to be calculated. 0.1 ml of the suspension was pipetted on to the surface of an agar plate. 0.1 ml of sterile distilled water was pipetted on to one or more further plates of the same composition. A glass spreader was used to spread the undiluted suspension and then the sterile distilled water on the subsequent plates without intermediate sterilisation of the spreader. This accomplished very approximate ten-fold dilutions,

since about 10% of the plating units in the initial suspension were transferred on the spreader and distributed in the 0.1 ml of water on the next plate, and so on. One of these plates usually yielded 50 to 100 isolated colonies.

iii. The second method was used when both isolated colonies and an approximate estimate of the concentration of the plating units from which they had arisen was required. Ten-fold dilutions of the starting suspension were made by successive transfer of a sample at one dilution into an appropriate volume of sterile distilled water to make the next dilution. 0.1 ml of each of a range of these ten-fold dilutions was pipetted on to the surface of one of a series of agar plates and each 0.1 ml was spread over the surface of its plate with a newly sterilised glass spreader. This spreading technique, however, introduced an error into the dilutions, since about 10% of the spores were removed from each plate on the spreader. This yielded a series of plates, each successive plate in the series having $^{1}/_{10}$th as many colonies as the one before. Single colonies when required were taken with ease from any plate with less than about 300 colonies. Colony counts when required were made with accuracy on any plates with less than about 500 colonies.

iv. The third method was used when it was desired only to obtain accurate counts of viable plating units by counting isolated colonies; the colonies were not required for further

testing or subculturing. Ten-fold dilutions were made in sterile distilled water. 1.0 ml of each of the appropriate range of dilutions was pipetted into a separate Petri dish. 15ml of molten agar of the desired composition, held at 50°C, was poured into the Petri dish and spores evenly dispersed by agitating the dish before the agar solidified. Upon incubation this yielded isolated colonies growing within the agar. Up to 1500 colonies per plate could be counted with little difficulty. This method gave the most accurate estimate of the concentration of viable plating units, which was calculated from the colony counts of two or three replica platings of each sample.

3. Harvesting a confluent culture as a spore suspension.

Inocula on large (25mm x 150mm) slants yielded confluent substrate hyphal growth within two days. In general, aerial hyphae were produced after 1 to 2 days and spores after 2 to 3 days. Slants were harvested after three or more days incubation by the following method. 9ml of sterile distilled water from a "Universal" container was poured on to the slant. A heat-sterilised wire loop was used to scrape the surface of the slant, at first gently, and then with increasing pressure, but avoiding as far as possible tearing up the agar surface. This crude spore suspension was returned to the Universal container and vigorously agitated for about 30 seconds on a Whirlimixer (Fison's Scientific Apparatus, Ltd., Loughborough, Leicestershire) to break up spore chains and large hyphal fragments. The suspension was then filtered through the cotton wool plug of the filter described above

to remove agar fragments and large clumps of aerial hyphae and spores. The spore suspension was centrifuged for 10 minutes in an MSE Angle 50 Centrifuge, with the 15ml, 12 place head, at about 1550 x g. The supernatant which contained the bulk of any compounds dissolved from the slant was discarded and the spore pellet dispersed in a suitable medium, usually sterile distilled water. The technique yielded pellets containing a maximum of about 10^9 viable units when plated on suitably supplemented MM.

D. Ultraviolet Sources.

1. The different sources.

Five UV sources were used during the course of this study. UV lamps 1, 2, 3 and 4 were Hanovia 'germicidal' mercury lamps and UV lamp 5 was a Philips 30 watt TUV mercury vapour lamp, all emitting light primarily with a wavelength of 253.7 nm.

a. UV lamp 1 was used for the induction by UV of the *uvs* mutations, and for their recognition. It was also used in the early genetic studies when a qualitative differentiation of recombinants with wild-type from those with mutant sensitivity was required. The dose-rate of this lamp was not determined accurately, but was in the region of 10 ergs mm^{-2} sec^{-1}.

b. UV lamp 2 was used from August 1966 until August 1968 and replaced UV lamp 1 for the qualitative studies. Its dose-rate was determined as 13.2 ergs mm^{-2} sec^{-1}, at the sample surface of 47.5 cm.

c. UV lamp 3 was used for the quantitative studies performed up

to August 1968. Its dose-rate was determined as 7.5 ergs mm^{-2} sec^{-1} at the sample surface of 45.5 cm. The voltage of the electricity supply to this lamp was controlled by an Advance Voltstat, Type C.V. 75A, obtained from Advanced Components, Ltd., Hainault, Essex.

d. UV lamp 4 was used for qualitative studies from September 1968 onwards. Its dose-rate was determined as 23.6 ergs mm^{-2} sec^{-1} at the standard irradiating distance of 23 cms from the lamp.

e. UV lamp 5 was used for the quantitative studies performed since September 1968. Its dose-rate was determined as 11.8 ergs mm^{-2} sec^{-1} with the lamp masked down to leave $1\frac{1}{4}$ cms exposed, and 57.3 cms from the sample surface.

2. Determination of the dose-rate of UV lamps 3, 4 and 5 by T2 bacteriophage survival curves.

T2 bacteriophage in T2 buffer give a reproducible survival curve when exposed to UV light, and the $\log_{10}$ of the surviving fraction is plotted against some measure (time of irradiation) of the UV dose received by the bacteriophage. Standard survival curves calibrated in ergs mm^{-2} may be referred to in the literature (Jagger, 1967), which enables calibration of a new UV source.

$T2H^{+}$ bacteriophage and a host indicator strain *E. coli* BR2, which is sensitive to $T2H^{+}$ were kindly provided by Dr D.A. Ritchie, Institute of Virology, Glasgow University.

a. Media for $T2H^{+}$ bacteriophage survival curves were made with the following compositions:

i. Phage broth.

Difco Bacto peptone, 15g; Difco nutrient broth, 8g;

NaCl, 8g; glucose, 1g; water, 1000ml.

ii. Top agar.

0.6% agar in water.

iii. Base medium.

Difco tryptone, 10g; Difco agar, 10g; NaCl, 8g; glucose, 1g; water 1000ml.

iv. T2 bacteriophage buffer.

0.1M $MgSO_4$, 20ml; 0.01M $CaCl_2$, 20ml; 1% gelatin, 2ml; $Na_2HPO_4.12H_2O$, 15.2g; KH_2PO_4, 3g; NaCl, 8g; K_2SO_4, 10g; water, 2000ml.

b. Experimental procedure.

Viable $T2H^+$ bacteriophage were detected by mixing them at a suitable dilution with a dense suspension of *E. coli* BR2 in 2.5ml of 0.6% agar which was poured as a thin layer over plates of base medium. After overnight incubation at 37°C, each viable bacteriophage or plaque forming unit (p.f.u.) produced a clear plaque against the turbid background growth of uninfected *E. coli* BR2. These plaques were counted for suitable dilutions of samples of a bacteriophage suspension after various exposures to radiation from the UV lamps. 5ml of $T2H^+$ bacteriophage suspended in T2 bacteriophage buffer at a concentration of about 10^8 p.f.u. per ml was pipetted into a 9 cm glass Petri dish with a metal cover. The irradiation was carried out at the standard irradiation distances from the UV lamps with the Petri dish on a magnetic stirrer base, using a 1" magnetic stirrer at 700 r.p.m. (nominal). The UV irradiation and subsequent plating of

dilutions of the bacteriophage suspension were carried out in a room with a Philips 35W SOX sodium vapour lamp as the only source of light, which emits light almost entirely at 589 nm, to avoid photoreactivation. These were also the standard conditions for all the survival curve experiments performed with *S. coelicolor*. 0.1ml samples of the $T2H^{+}$ bacteriophage suspension were taken at zero and a series of predetermined doses. The suspension was exposed to UV by removing the metal cover from the dish for the appropriate period to the first sampling time, when it was replaced, and the dish removed from under the lamp and the sample taken. This procedure was repeated for the increment of time to the next sampling time and so on. The 0.1 ml samples were diluted immediately ten or 100 fold into T2 bacteriophage buffer, depending upon the first dilution to be plated. Further dilution of these samples was by ten fold dilution of 0.1 ml or 0.5 ml samples. A preliminary experiment had indicated which dilutions of the bacteriophage suspension would yield between 50 and 500 plaques per plate at a particular UV dose. For these predetermined dilutions between 0.1 ml and 0.5 ml samples were separately diluted into 2.5 ml of molten top agar held at $45^{\circ}C$ which was already mixed with 2 drops (approximately 0.1 ml) of a stationary culture of *E. coli* BR2 grown overnight at $37^{\circ}C$ in 10 ml of phage broth. This mixed bacteriophage and bacteria suspension was quickly poured on to an agar plate of approximately 15 ml of base medium which had been dried

at 37°C for a few hours before use. The drying ensured adequate gelling of the top layer. The plaques were counted after overnight incubation at 37°C.

c. Results.

The results of the experiments for UV lamps 3, 4 and 5 are presented in Table 10 and are plotted as survival curves in Figure 5. Jagger (1967) recommended that, in using T2 bacteriophage survival curves to calibrate a new UV source, the published data obtained with strains and conditions as similar as possible to your own, should be used. The data of Zelle and Hollaender (1954) which gave a D37 of 33 ergs mm^{-2}, were obtained using T2 bacteriophage plated on *E. coli* B which had been irradiated in phosphate buffer. This was the system closest to my own amongst those quoted by Jagger (1967). The D37 values in seconds taken from Figure 5 for UV lamps 3, 4 and 5 were 4.6 secs, 1.4 secs and 2.8 secs respectively corresponding to the respective dose-rates of 7.2 ergs mm^{-2} sec^{-1}, 23.6 ergs mm^{-2} sec^{-1} and 11.8 ergs mm^{-2} sec^{-1}.

3. Determination of the dose-rates of UV lamps 2 and 3 using a UV sensitive meter.

a. Another method of calibration which was used for UV lamps 2 and 3 was provided by apparatus loaned by Dr P. Gormley of the Institute of Virology, Glasgow University. This consisted of a UV sensitive light meter, connected to a counter which accumulated one count for every 32.26 ergs mm^{-2} received. This value had been calculated by comparison of

TABLE 10 The survival of T2H$^+$ bacteriophage as a function of time of irradiation with UV lamps 3, 4 or 5, when grown on *E. coli* BR2 (plotted as survival curves in Figure 5).

UV lamp No. and symbol in Figure 5		UV dose (secs)	Sample size (ml)	Dilution factor	Number of plaques	p.f.u./ml	Surviving fraction
3	Closed circles	0	0.1	1×10^{-5}	124	134×10^6	1.0
			0.3	1×10^{-5}	413		
		10	0.2	1×10^{-5}	67	34×10^6	2.5×10^{-1}
		30	0.2	1×10^{-5}	154	77×10^4	5.8×10^{-3}
		60	0.2	1×10^{-5}	33	17×10^2	1.3×10^{-5}
3	Open circles	0	0.1	1×10^{-5}	111	122×10^6	1.0
			0.3		378		
		10	0.1	1×10^{-5}	29	32.5×10^6	2.7×10^{-1}
			0.3		101		
		20	0.1	1×10^{-4}	39	51.5×10^5	4.2×10^{-2}
			0.3		167		
		30	0.1	1×10^{-4}	3	4×10^5	3.1×10^{-3}
			0.3		12		
		50	0.1	1×10^{-2}	9	8×10^3	6.8×10^{-5}
			0.3		24		
		60	0.1	1×10^{-1}	23	27×10^2	2.2×10^{-5}
			0.3		85		
4	Closed triangles	0	0.1	1×10^{-5}	112	113×10^6	1.0
			0.5		564		
		5	0.1	1×10^{-4}	31	29×10^5	2.6×10^{-2}
			0.5		145		
		10	0.1	1×10^{-2}	76	96×10^3	8.5×10^{-4}
			0.5		497		
		15	0.1	1×10^{-1}	202	202×10^2	1.8×10^{-4}
			0.5		-		
		20	0.1	1×10^{-1}	42	40×10^2	3.5×10^{-5}
			0.5		200		

(continued overleaf)

TABLE 10 (continued)

UV lamp No. and symbol in Figure 5		UV dose (secs)	Sample size (ml)	Dilution factor	Number of plaques	p.f.u./ml	Surviving fraction
5	Closed squares	0	0.1	1×10^{-5}	59	59×10^{6}	1.0
		10	0.1	1×10^{-4}	16	16×10^{5}	2.7×10^{-2}
		20	0.1	1×10^{-2}	61	61×10^{3}	1.0×10^{-3}
		40	0.1	1×10^{-1}	44	44×10^{2}	7.5×10^{-5}
5	Open squares	0	0.1	1×10^{-5}	85	100×10^{6}	1.0
			0.3		314		
		5	0.1	1×10^{-4}	100	100×10^{5}	1×10^{-1}
		5	0.1	1×10^{-5}	17	14×10^{6}	1.4×10^{-1}
			0.3		37		
		10	0.1	1×10^{-3}	271	271×10^{4}	2.7×10^{-2}
		10	0.1	1×10^{-4}	32	25×10^{5}	2.5×10^{-2}
			0.3		68		
		15	0.1	1×10^{-2}	428	428×10^{3}	4.3×10^{-3}
		15	0.1	1×10^{-3}	51	38×10^{4}	3.8×10^{-3}
			0.3		132		
		20	0.1	1×10^{-2}	107	110×10^{3}	1.1×10^{-3}
			0.3		332		
		30	0.1	1×10^{-1}	258	258×10^{2}	2.6×10^{-4}
		40	0.1	1×10^{-1}	55	160×10^{2}	1.6×10^{-4}
			0.3		640		

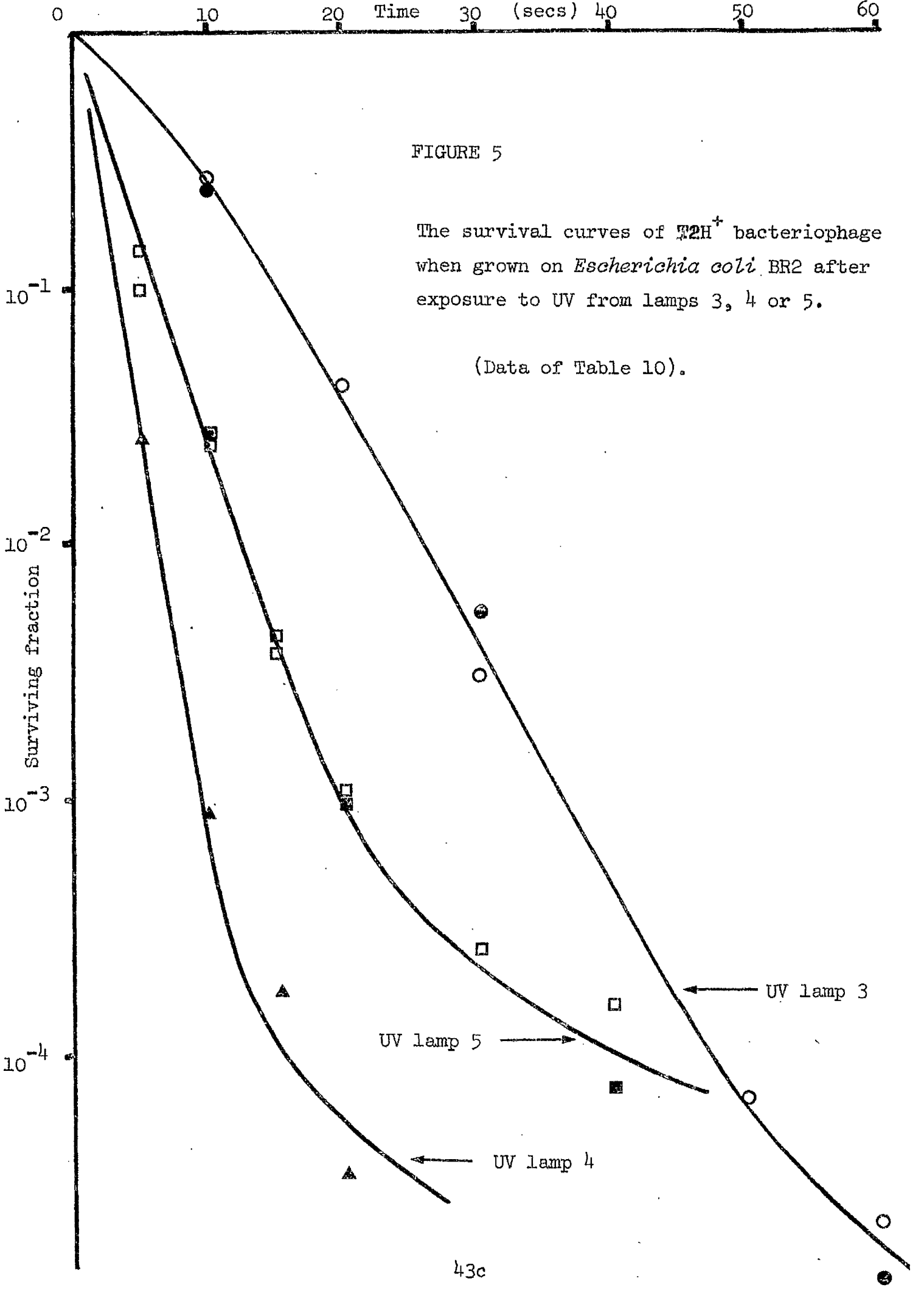


FIGURE 5

The survival curves of T2H^{+} bacteriophage when grown on *Escherichia coli* BR2 after exposure to UV from lamps 3, 4 or 5.

(Data of Table 10).

the light meter's response with a T2 bacteriophage survival curve of the type described above.

b. The results are tabulated in Table 11. These measurements were taken at the distances from the lamps, and under the light conditions, which were normally used during irradiation experiments with these lamps on *S. coelicolor* strains.

i. UV lamp 2, which had been switched on barely half an hour before measurements were commenced, did not appear to have reached stable emission until the latest measurements were taken. At 47.5 cms from the meter surface, the distance at which irradiation was normally carried out, 2.44 secs were equivalent to 1 count.
This gives a dose rate of 13.2 ergs mm^{-2} sec^{-1}. So that a direct comparison of the dose rates of UV lamps 2 and 3 could be made, measurements were also taken at 45.5 cms from the meter surface, the distance at which measurements had been taken for UV lamp 3. 2.18 secs were equivalent to 1 count. This gives a dose rate of 14.8 ergs mm^{-2} sec^{-1} which was slightly less than twice the dose rate of UV lamp 3 at this distance. This reduction in output of UV lamp 3 was presumed to be due mainly to the voltage controlled supply of this lamp.

ii. UV lamp 3, which had been switched on for more than 45 minutes before measurements were taken, appeared to have reached stable emission. Then 4.2 secs were equivalent to 1 count, and therefore to 32.26 ergs mm^{-2}. This gave a dose rate of 7.7 ergs mm^{-2} sec^{-1} which was in good

TABLE 11 Dose-rate determinations for UV lamps 2 and 3 by means of a UV sensitive meter.

UV Lamp No.	Distance from lamp to meter (cm)	Counts	Seconds	Increment (secs) per 100 counts
2	47.5	100	267	267
		200	523	256
		300	776	253
		400	1020	250
		500	1273	247
		600	1518	245
		700	1762	244
2	45.5	100	224	224
		200	444	220
		300	663	219
		400	881	218
3	45.5	50	210	420
		100	419	419
		200	842	423

1 count is equivalent to 32.26 ergs mm^{-2}.

UV dose-rates are calculated from these data in the text.

agreement with the value obtained directly from the T2 bacteriophage survival curve, which was 7.2 ergs mm^{-2} sec^{-1}. The average value of 7.5 ergs mm^{-2} sec^{-1} was taken as the dose rate of the lamp.

4. Estimation of the dose rate of UV lamp 1.

An indication of the dose rate of this lamp was obtained by the following comparison with UV lamp 2. Both of these lamps were used for qualitative studies of the UV sensitivities of *S. coelicolor* strains. Replica plates were irradiated with doses of UV from these lamps which barely visibly affected the growth of *uvs*$^{+}$ strains on replicas, but which killed practically all of the replica plated spores of most *uvs* strains. Two minute exposures were used with UV lamp 1 and 1.5 minute exposures with UV lamp 2. Since UV lamp 2 had a dose rate of 13.2 ergs mm^{-2} sec^{-1} at the distance normally used, the dose rate of UV lamp 1 was calculated to be $^{1.5}/_{2}$ x 13.2 or approximately 10 ergs mm^{-2} sec^{-1}.

III. ISOLATION AND CHARACTERISATION OF *UVS* MUTANTS

A. Method of isolation.

Mutants were isolated amongst the survivors of spores treated with UV or NTG. The spores were prepared as a pellet by the method described in Section II C 3 from confluent slant cultures, each of which was inoculated with a different single colony of one of the following strains:-

A3(2)

916 *hisA1 mthB2 pheA1 strA1*

749 *proA1 argA1 cysD18 uraA1*

The single colony inoculum ensured that any mutant isolated from one spore suspension was different in origin from those isolated from other spore suspensions.

1. Mutagenesis by UV.

A spore pellet was suspended in 10ml of sterile distilled water to a spore density of up to 10^8 per ml. This was placed in a 9cm glass Petri dish and exposed to UV from lamp 1 for eight or ten minutes (approximately 5000 or 6000 ergs mm^{-2}) at a distance of about 50 cms. The dish was gently agitated at intervals to ensure even exposure of the whole suspension to the UV light. The surviving fraction was of the order of 1×10^{-5} when the suspension was spread on suitably supplemented MM.

2. Mutagenesis by NTG.

The spore pellet was re-suspended in 5ml of tris-maleic acid buffer at pH 7.0 and 5 mg of NTG was added and quickly dissolved

by agitation on a Whirlimixer. The suspension was incubated at 37°C for 30 minutes, and then centrifuged at about 1500 g for ten minutes to collect the spores. The supernatant containing the bulk of the unused NTG was discarded and the pellet re-suspended in 10ml of sterile distilled water. The fraction surviving was in the region of 1×10^{-2} to 1×10^{-3}, when the suspension was spread on either supplemented MM or CM.

3. Screening the survivors of mutagenesis for *uvs* mutants.

A trial plating of the mutagen-treated spore suspension was made on suitably supplemented plates of MM (or CM after some of the NTG treatments) by the method which yields accurate colony counts after three days (Section II C 2 iv). The remaining spore suspension was meanwhile stored at 4°C. The colony count was used to estimate a dilution of the spore suspension which would yield between 150 and 250 isolated colonies in a subsequent main plating. Between 12 and 24 plates were usually inoculated at this dilution and then incubated for four to five days, when the colonies were well sporulating. Two replicas of each plate were made with a velvet pad to plates of similar composition. The second replica was a control of satisfactory replication of each colony, and the first was exposed to a dose of UV determined empirically to have little effect on the replication of wild-type colonies (it killed approximately 50% of the spores). It was expected that the replication of any *uvs* mutant would be visibly affected. The two replicas were compared after two days incubation and any colonies replicating to the control but not at all (or weakly) to the irradiated plate were picked off to

fresh plates and re-tested by the same technique. Twenty-three *uvs* mutants were isolated in this way.

B. Frequencies of *uvs* mutants amongst the survivors of mutagenesis.

Table 12 lists the *uvs* mutants isolated from each experiment, the parent strain from which they were obtained, the mutagen, the number of colonies screened and the plating medium.

Eleven *uvs* mutants were obtained after UV mutagenesis amongst about 16,450 colonies on supplemented MM; approximately 1 per 1,500. For NTG mutagenesis the results may be divided according to the plating medium. Platings on supplemented MM yielded 5 mutants amongst about 30,350 colonies; approximately 1 per 6,600. Plating on CM yielded 6 mutants amongst 15,550 colonies; approximately 1 per 2,600. From these figures UV would appear to have been the better mutagen, when the plating medium was MM (on CM the viability of UV treated spore suspensions was much reduced). However, it is now known that the conditions used for the NTG mutagenesis were far from optimal (Delić, Hopwood and Friend, manuscript in preparation). Their results show that for general mutagenesis (to auxotrophy) under optimal conditions NTG is a very potent mutagen, many times more effective than UV. In my experiments NTG gave a better yield on CM than on supplemented MM. There was no obvious reason for this difference, except that in the five experiments in which platings were made from the same spore suspension on both MM and CM, the following results, averaged for the five suspensions

TABLE 12 The Origins of the *uvs* Mutations.

Mutations	Parent	Mutagen	Plating medium	Approximate number of Colonies Screened
uvs-1	749	UV	MM	1000
uvs-2	916	NTG	MM	800
-	916	UV	MM	2250
-	749	UV	MM	550
uvs-3 uvs-4 uvs-5	749	UV	MM	2250
-	749	UV	MM	2550
uvs-6 uvs-7	916	UV	MM	2300
uvs-8 uvs-9 uvs-10	916	UV	MM	2300
-	916	UV	MM	1250
uvs-11 uvs-13	916	UV	MM	2000
-	A3(2)	NTG	MM	5400
-	A3(2)	NTG	MM	2550
-	A3(2)	NTG	MM	1400
-	A3(2)	NTG	MM	1850
uvs-14	A3(2)	NTG	MM	2900
-	A3(2)	NTG	MM	4100
uvs-15	A3(2)	NTG	MM	2150
uvs-16 uvs-17 uvs-19			CM	2600
-	A3(2)	NTG	MM	3300
uvs-18			CM	3600
-	A3(2)	NTG	MM	2850
-			CM	3750
-	A3(2)	NTG	MM	1500
-			CM	2750
uvs-22 uvs-23	A3(2)	NTG	MM	1550
uvs-20 uvs-21			CM	2850
uvs-24	A3(2)	UV	MM	*

* Originally picked on the basis of mutant spore colour; subsequently found to be *uvs*.

were obtained.

The colony counts made from trial platings on MM were 93% of those made from similar platings on CM. However, in the subsequent main plating, the approximate colony counts found on MM were only 69% of those on CM when 12 plates of MM and 12 plates of CM were plated at the same dilution. The CM counts averaged about 275 colonies per plate. This greater loss of viability on MM after storage of the suspensions may have preferentially included *uvs* mutants.

Up to four mutants were isolated from the same spore suspension and these therefore could have been members of a clone of spontaneous mutants. However, in every case where mutants could have been clonal, at least two independent mutations were defined, by different mapping locations or complementation results. This leaves *uvs* mutants D3 and D5, C8 and C10, A15 and A19, C16 and C17, and A22 and A23 as possibly having originated from the same spontaneous mutant clones.

C. UV survival curves of wild-type strains and their *uvs* mutants.

UV survival curves were determined for representative mutants of each of the genes, *uvsA*, *uvsC* and *uvsD*, for each mutant mapping in the lower half of the map *uvsB6*, *uvs-13*, *uvs-21* , and for the three uvs^+ strains from which the mutants were obtained.

1. Experimental procedure.

A spore pellet was prepared as described in Section II C 3 from a confluent slant culture of MM supplemented with proline, arginine, cystine, uracil (the growth requirements of 749 and its *uvs* derivatives), histidine, homoserine and phenylalanine (the growth requirements of 916 and its *uvs* derivatives), inoculated with a single colony and incubated for five days. This medium was chosen because certain strains sporulated better on supplemented MM than on CM and it was always supplemented with all the growth factors to maximise uniformity of growth conditions. The spore pellet was re-suspended in 2.5 ml of sterile distilled water, centrifuged again and the supernatant discarded. This ensured that the concentration of any UV absorbing compounds dissolved from the slant was very low in the final spore suspension. The spore pellet was re-suspended in a final volume of 10 ml of sterile distilled water and placed in a glass Petri dish covered by a metal lid. The suspension formed a layer 1.5 to 2mm deep in the bottom of the dish with a concentration of viable plating units on MM of between about 10^6 and 10^8 units per ml. The irradiation and sampling was then carried out in the same way and under the same conditions as have already been described for the $T2H^+$ bacteriophage survival curves, except that 0.5 or 1.0 ml samples were taken at the appropriate intervals and then treated as follows. The samples were diluted in ten-fold dilution steps in distilled water and plated in supplemented MM (appropriate to the strain) by the technique described in Section II C 2 iv, which yields accurate viable counts. The irradiation and plating was carried out at room temperature (20 to 25°C) under light from a Philips 35W SOX sodium vapour lamp.

Plates were incubated in the dark and colonies were counted each day from the third or fourth up to the seventh day if necessary, since at the lower survival levels many survivors were late in yielding visible colonies, presumably due to slower growth rates or delayed germination. Normal unirradiated spores yielded visible colonies after three days. Counts were taken as final when no further colonies appeared after an additional day's incubation. The UV dose was plotted linearly on the abscissa and the surviving fraction logarithmically on the ordinate.

2. *Uvs*$^+$ survival curves.

The experimental data are given in Table 13 and survival curves plotted from them in Figure 6. The three *uvs*$^+$ survival curves showed a "shoulder" of increasing slope to survival levels of about 2×10^{-1}, which was followed by nearly exponential inactivation of the spores (i.e. a region of the curve with nearly constant slope) to survival levels of about 5×10^{-4} and finally "tailing"; that is the slope of the curve decreased, presumably due to a more resistant component of the spore population constituting an increasing proportion of the survivors.

The four experiments on A3(2) gave a reproducible curve. This was not so for 916 or 749. 916, on which four experiments were performed, showed four curves, within which the main difference was the extent of the shoulder, and the main similarity was the slope of the part of the curves showing exponential inactivation. 749, on which three experiments were performed, showed a more constant shoulder, but a variable exponential slope.

Some of these differences may have resulted from the fact that

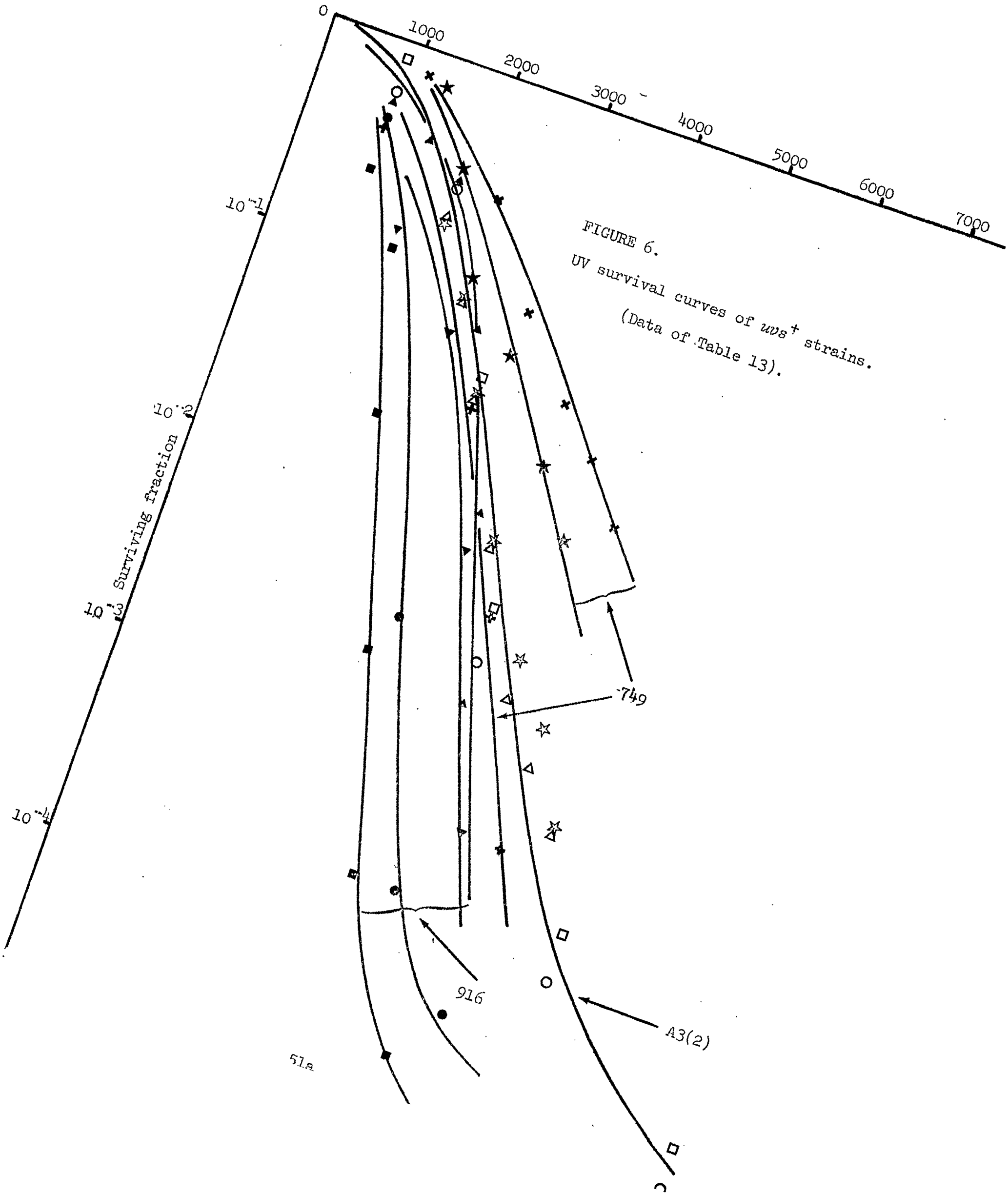


FIGURE 6.

UV survival curves of *uvs*$^{+}$ strains.

(Data of Table 13).

TABLE 13 Data for *uvs*$^{+}$ survival curves (plotted in Figure 6).

Strain A3(2) wild-type

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Open circles a	1 Open circles b	2 Open squares a	2 Open squares b	3 Open erect triangles a	3 Open erect triangles b	4 Open stars a	4 Open stars b
0	121×10^{6}	1.0	246×10^{6}	1.0	257×10^{6}	1.0	114×10^{6}	1.0
900	683×10^{5}	5.6×10^{-1}	199×10^{6}	8.1×10^{-1}	-	-	-	-
1800	313×10^{5}	2.6×10^{-1}	-	-	493×10^{5}	1.9×10^{-1}	207×10^{5}	1.8×10^{-1}
2250	-	-	-	-	220×10^{5}	8.5×10^{-2}	105×10^{5}	9.2×10^{-2}
2700	-	-	998×10^{4}	4.1×10^{-2}	841×10^{4}	3.3×10^{-2}	425×10^{4}	3.7×10^{-2}
3375	-	-	-	-	203×10^{4}	7.9×10^{-3}	931×10^{3}	8.2×10^{-3}
3600	29×10^{4}	2.4×10^{-3}	105×10^{4}	4.3×10^{-3}	-	-	-	-
4050	-	-	-	-	456×10^{3}	1.8×10^{-3}	316×10^{3}	2.8×10^{-3}
4500	-	-	-	-	251×10^{3}	9.8×10^{-4}	167×10^{3}	1.5×10^{-3}
4950	-	-	-	-	137×10^{3}	5.3×10^{-4}	671×10^{2}	5.9×10^{-4}
5400	14×10^{3}	1.2×10^{-4}	501×10^{2}	2.0×10^{-4}	-	-	-	-
7200	26×10^{2}	2.2×10^{-5}	830×10	3.4×10^{-5}	-	-	-	-
No. of samples counted at each dose	3		3		2		2	

Legend to Tables containing data for survival curves of *S. coelicolor* strains.

a) Average of the number of colonies counted per replicate sample x dilution factor. b) Surviving fraction. For some experimental points, colony counts were obtained at two dilutions. Values between 100 and 1,000 were chosen to calculate the surviving fraction. These limits were necessary since with decreasing colony counts the precision of the calculated surviving fraction decreases rapidly relative to the true value (Meynell and Meynell, 1965, p.166); and masking and overgrowth of colonies becomes an increasingly important source of error with increasing colony density. However, when only high or low counts were available, these were used.

TABLE 13 (continued) Data for *uvs*$^{+}$ survival curves (plotted in Figure 6)

Strain 916 *hisA1 mthB2 pheA1 strA1*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Closed circles		2 Closed squares		3 Erect closed triangles		4 Inverted closed triangle	
	a	b	a	b	a	b	a	b
0	423×10^{4}	1.0	126×10^{4}	1.0	444×10^{3}	1.0	278×10^{4}	1.0
900	182×10^{4}	4.3×10^{-1}	298×10^{3}	2.4×10^{-1}	222×10^{3}	5.0×10^{-1}	-	-
1350	-	-	149×10^{3}	1.2×10^{-1}	171×10^{3}	3.9×10^{-1}	393×10^{3}	1.4×10^{-1}
1800	-	-	264×10^{2}	2.1×10^{-2}	1300×10^{2}	2.9×10^{-1}	-	-
2250	-	-	-	-	-	-	1656×10^{2}	6.0×10^{-2}
2475	-	-	232×10	1.8×10^{-3}	327×10^{2}	7.4×10^{-2}	-	-
2700	122×10^{2}	2.9×10^{-3}	-	-	-	-	-	-
3150	-	-	23×10	1.8×10^{-4}	509×10	1.1×10^{-2}	195×10^{2}	7.0×10^{-3}
3600	78×10	1.8×10^{-4}	-	-	661×1	1.5×10^{-3}	-	-
4050	-	-	40×1	3.2×10^{-5}	-	-	1144×1	4.1×10^{-4}
4500	254×1	6.0×10^{-5}	-	-	-	-	-	-
No. of samples counted at each dose	2		2		2		2	

TABLE 13 (continued) Data for *uvs*$^{+}$ survival curves (plotted in Figure 6).

Strain 749 *proA1 argA1 cysD18 uraA1*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Plus a	1 Plus b	2 Cross a	2 Cross b	3 Closed Star a	3 Closed Star b
0	188 x 10^{4}	1.0	877 x 10^{3}	1.0	489 x 10^{3}	1.0
900	729 x 10^{3}	3.9 x 10^{-1}	-	-	-	-
1125	-	-	636 x 10^{3}	7.3 x 10^{-1}	-	-
1350	-	-	-	-	335 x 10^{3}	6.9 x 10^{-1}
1800	-	-	-	-	1586 x 10^{2}	3.2 x 10^{-1}
2250	-	-	238 x 10^{3}	2.7 x 10^{-1}	512 x 10^{2}	1.1 x 10^{-1}
2700	555 x 10^{2}	3.0 x 10^{-2}	-	-	-	-
2925	-	-	836 x 10^{2}	9.5 x 10^{-2}	283 x 10^{2}	5.8 x 10^{-2}
3600	711 x 10	3.8 x 10^{-3}	377 x 10^{2}	4.3 x 10^{-2}	1091 x 10	2.2 x 10^{-2}
4050	-	-	237 x 10^{2}	2.7 x 10^{-2}	524 x 10	1.1 x 10^{-2}
4500	728 x 1	3.9 x 10^{-4}	1357 x 10	1.5 x 10^{-2}	-	-
No. of samples counted at each dose.	2		2		2	

each experiment was performed on a different clone of spores. This had arisen from a single spore or small hyphal fragment, which had grown first to form a single colony, which was then inoculated to grow a confluent culture on a slant, which yielded the spore suspension. Clones of "modifiers" affecting UV sensitivity may have arisen at any stage during this growth, from a single spore through to the confluent slant culture. The size of any "modified" clone in the final spore suspension would affect its survival curve. Such modifiers would be presumed not to have occurred appreciably in A3(2).

Another source of variability in the survival curves may have been the different degrees of sporulation achieved before a spore suspension was prepared. A3(2) sporulated readily under a variety of conditions, and may have attained maximum sporulation for each experiment. However, strains such as 916 and 749, which contain a number of auxotrophic markers, did not sporulate so readily and the different experimental suspensions could have contained different proportions of spores and hyphal fragments. Hyphal fragments would have contained many nuclei, each having similar sensitivity to the single nuclei of spores. A number of lethal hits would have been required to inactivate a given hyphal fragment, depending upon the number of nuclei it contained. Therefore, the spore suspension as a whole would have appeared more resistant at low doses than at higher doses when nearly all the surviving members of the population, both spore and hyphal, would have contained only one viable nucleus each and therefore would have had the same effective sensitivity. Thus the proportion of hyphal fragments would be expected to affect the extent of the shoulder of the curve (however, see the results for *uvs-13*).

Whatever the causes of these variations between experiments involving 916 and 749, they were relatively small, and all of the survival curves performed on *uvs*$^+$ strains showed greater resistance to UV than those performed on the least sensitive mutant.

3. Survival curves of strains mutant in *uvsA*, *uvsC* or *uvsD*.

The data for strains mutant in one of these three closely linked genes (see Section IV C 3) are presented in Tables 14, 15, 16, respectively, and the survival curves plotted from them in Figures 7, 8, 9 respectively. They will be considered together.

The variations in sensitivity amongst the strains so far studied, which were mutant in gene *A* or gene *C*, were within similar limits. The two strains mutant in gene *D*, were closely similar in sensitivity, and this was within the range defined by the mutants in genes *A* or *C*.

All these mutants had survival curves with characteristics like those of the *uvs*$^+$ curves. They retained a shoulder, which was followed by a region showing exponential inactivation, followed by "tailing" of the curves at survival levels between about 1×10^{-3} and 1×10^{-4}. The feature in which they all differed from the *uvs*$^+$ curves was in the much steeper slope in the exponential parts of the curves.

4. Survival curves of mutants *uvsB6* and *uvs-21*.

The data for these mutants are presented in Table 17 and their survival curves in Figure 10. Their survival curves were almost identical, and also had the general features of the *uvs*$^+$ curves, retaining a shoulder, an exponential region, and a "tail". They were less sensitive than any of the *uvsA*, *uvsC* or *uvsD* mutants and the tailing appeared to commence earlier, at survival levels of about 1×10^{-2}.

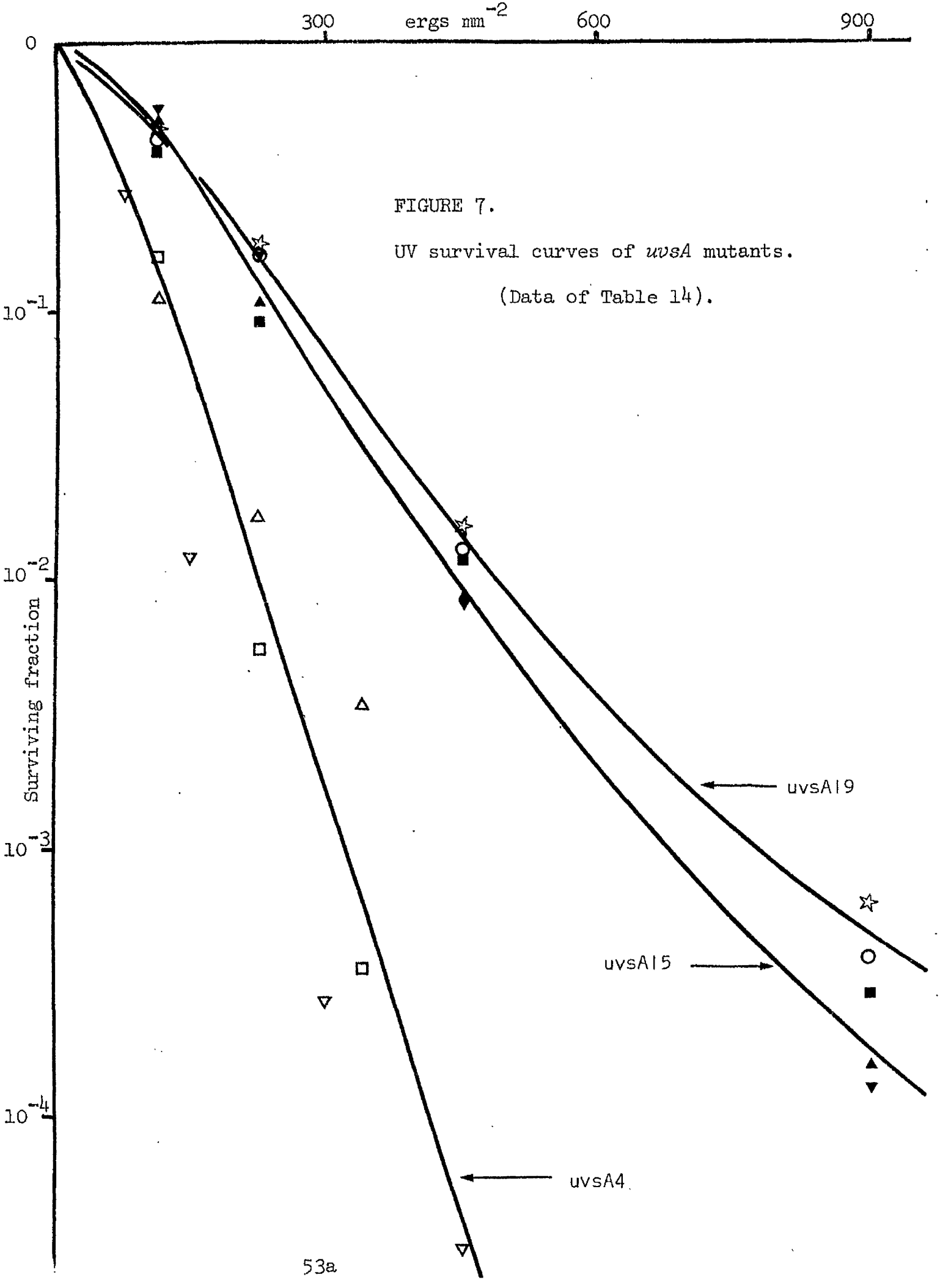


FIGURE 7.

UV survival curves of *uvsA* mutants.

(Data of Table 14).

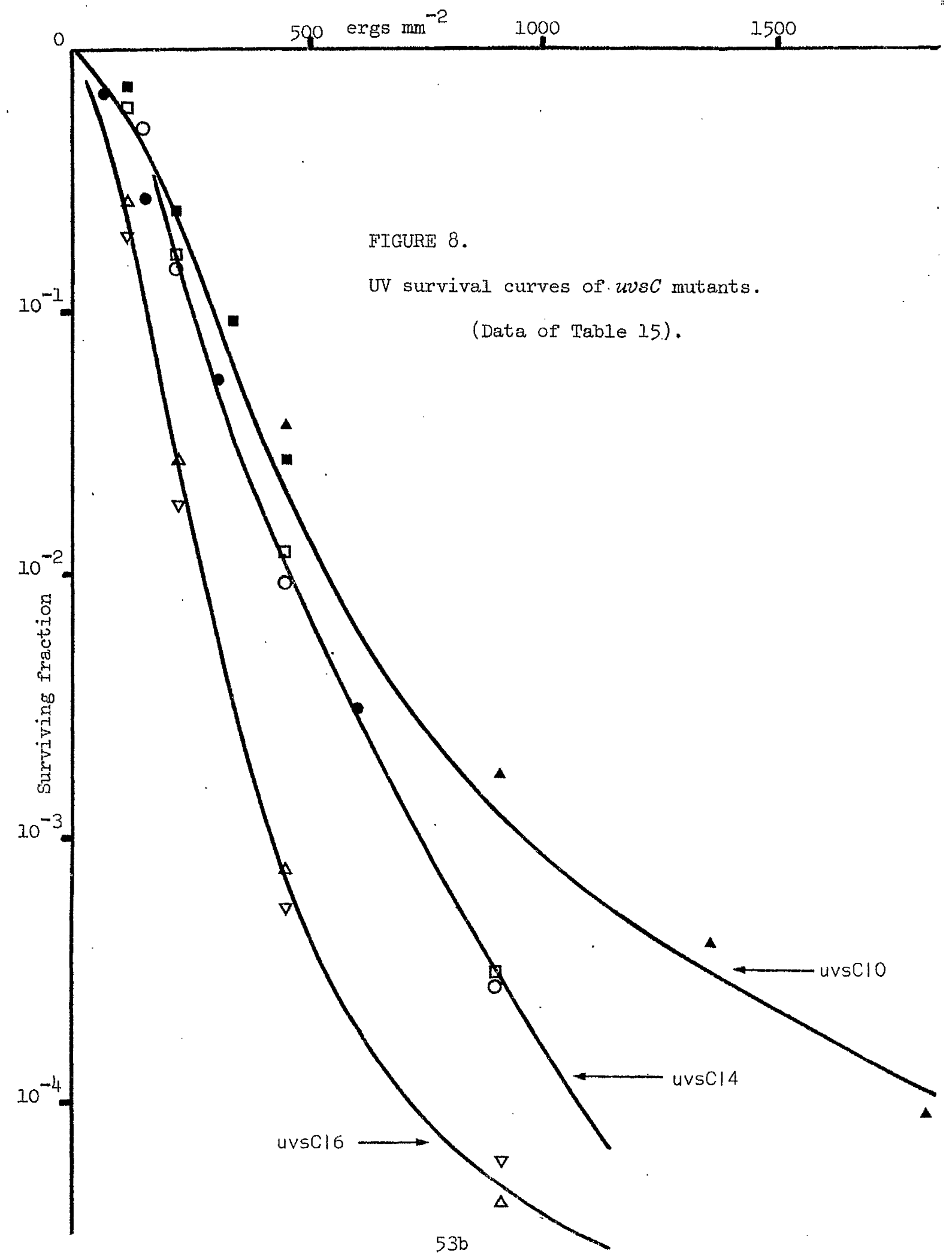


FIGURE 8.

UV survival curves of *uvsC* mutants.

(Data of Table 15).

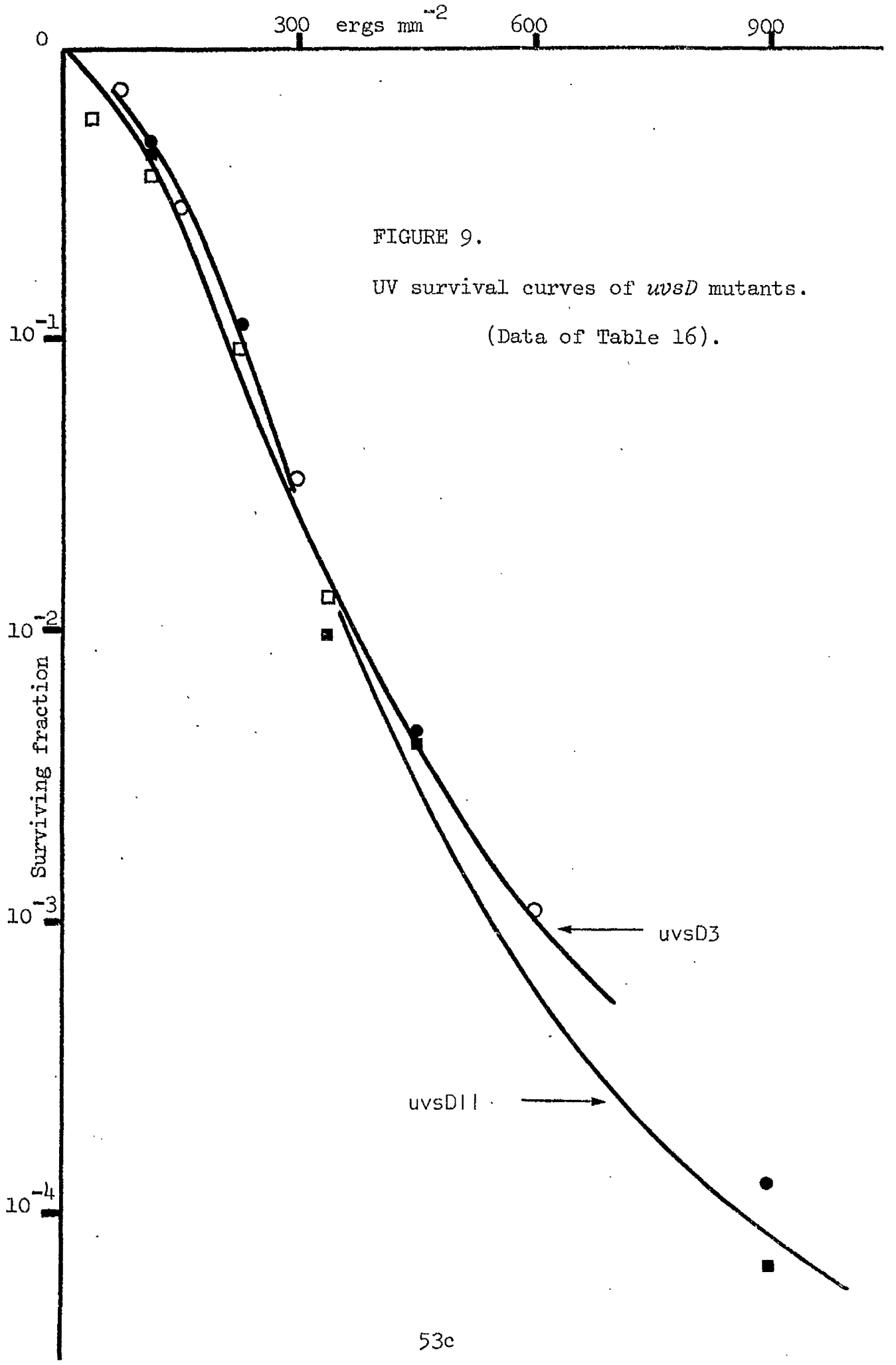


FIGURE 9.

UV survival curves of *uvsD* mutants.

(Data of Table 16).

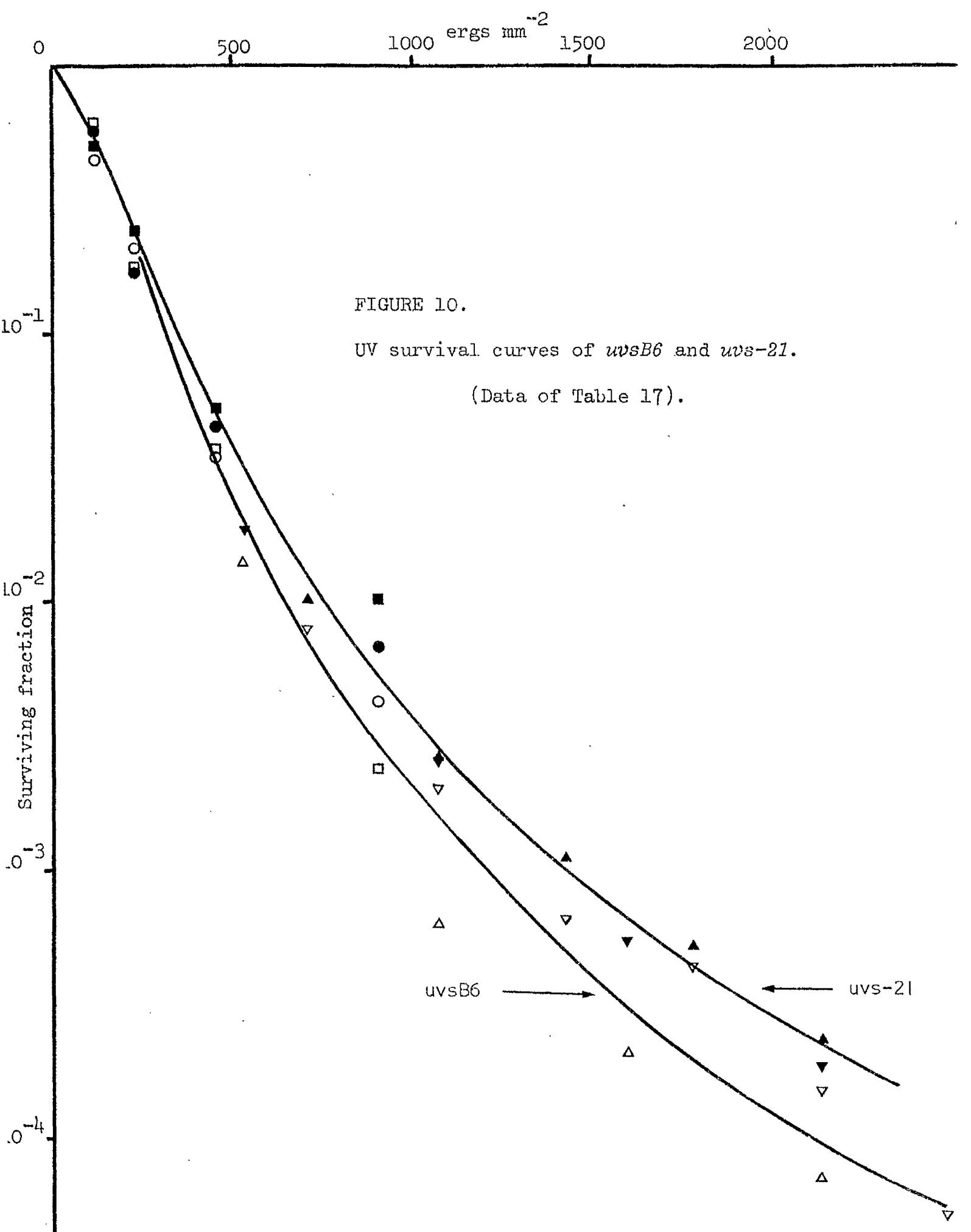


FIGURE 10.

UV survival curves of *uvsB6* and *uvs-21*.

(Data of Table 17).

TABLE 14 Data for survival curves of *uvsA* mutants (plotted in Figure 7).

Strain V2 *proA1 argA1 cysD18 uraA1 uvsA4*

Dose	Experiment No. and symbol in Figure					
	1		2		3	
	Open squares		Inverted open triangles		Erect open triangles	
ergs mm^{-2}	a	b	a	b	a	b
0	76×10^{3}	1.0	515×10^{3}	1.0	308×10^{3}	1.0
75	-	-	139×10^{3}	2.7×10^{-1}	-	-
112.5	122×10^{2}	1.6×10^{-1}	-	-	345×10^{2}	1.1×10^{-1}
150	-	-	642×10	1.2×10^{-2}	-	-
225	42×10	5.5×10^{-3}	-	-	521×10	1.7×10^{-2}
300	-	-	146×1	2.7×10^{-4}	-	-
337.5	27×1	3.6×10^{-4}	-	-	106×10	3.4×10^{-3}
450	-	-	17×1	3.3×10^{-5}	-	-
No. of samples counted at each dose	3		3		3	

TABLE 14 (continued) Data for survival curves of *uvsA* mutants (plotted in Figure 7).

Strain V57 *uvsA15*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Inverted closed triangles a	1 b	2 Erect closed triangles a	2 b	3 Closed squares a	3 b
0	343×10^{5}	1.0	383×10^{5}	1.0	296×10^{5}	1.0
112.5	199×10^{5}	5.8×10^{-1}	208×10^{5}	5.4×10^{-1}	112×10^{5}	3.8×10^{-1}
225	536×10^{4}	1.6×10^{-1}	436×10^{4}	1.1×10^{-1}	269×10^{4}	9.1×10^{-2}
450	289×10^{3}	8.4×10^{-3}	336×10^{3}	8.8×10^{-3}	358×10^{3}	1.2×10^{-2}
900	445×10	1.3×10^{-4}	628×10	1.6×10^{-4}	85×10^{2}	2.9×10^{-4}
No. of samples at each dose	3		3		3	

Strain V61 *uvsA19*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Open stars a	1 b	2 Open circles a	2 b
0	128×10^{6}	1.0	115×10^{6}	1.0
112.5	637×10^{5}	5.0×10^{-1}	532×10^{5}	4.6×10^{-1}
225	226×10^{5}	1.8×10^{-1}	180×10^{5}	1.6×10^{-1}
450	204×10^{4}	1.6×10^{-2}	150×10^{4}	1.3×10^{-2}
900	84×10^{3}	6.6×10^{-4}	46×10^{3}	4.0×10^{-4}
No. of samples at each dose	3		3	

TABLE 15 Data for survival curves of *uvsC* mutants (plotted in Figure 8)

Strain V13 *hisA1 mthB2 pheA1 strA1 uvsC10*

Dose	Experiment No. and symbol in Figure: 1, Closed circles		2, Closed squares		3, Closed triangles	
ergs mm^{-2}	a	b	a	b	a	b
0	284×10^{5}	1.0	293×10^{5}	1.0	301×10^{5}	1.0
75	193×10^{5}	6.8×10^{-1}	-	-	-	-
112.5	-	-	217×10^{5}	7.4×10^{-1}	-	-
150	809×10^{4}	2.8×10^{-1}	-	-	-	-
225	-	-	719×10^{4}	2.5×10^{-1}	-	-
300	1565×10^{3}	5.5×10^{-2}	-	-	-	-
337.5	-	-	275×10^{4}	9.4×10^{-2}	-	-
450	-	-	771×10^{3}	2.6×10^{-2}	1133×10^{3}	3.8×10^{-2}
600	905×10^{2}	3.2×10^{-3}	-	-	-	-
900	-	-	-	-	533×10^{2}	1.8×10^{-3}
1350	-	-	-	-	125×10^{2}	4.2×10^{-4}
1800	-	-	-	-	280×10	9.3×10^{-5}
No. of samples at each dose	3		3		3	

TABLE 15 (continued) Data for survival curves of *uvsC* mutants (plotted in Figure 8)

	Strain V56 *uvsC14*				Strain V58 *uvsC16*			
	Experiment No. and symbol in Figure							
Dose	1		2		1		2	
	Open circles		Open squares		Open erect triangles		Open inverted triangles	
ergs mm^{-2}	a	b	a	b	a	b	a	b
0	282×10^{6}	1.0	154×10^{6}	1.0	426×10^{5}	1.0	369×10^{5}	1.0
112.5	-	-	886×10^{5}	5.8×10^{-1}	117×10^{5}	2.7×10^{-1}	752×10^{4}	2.0×10^{-1}
135	147×10^{6}	5.2×10^{-1}	-	-	-	-	-	-
225	426×10^{5}	1.5×10^{-1}	239×10^{5}	1.6×10^{-1}	115×10^{4}	2.7×10^{-2}	708×10^{3}	1.9×10^{-2}
450	292×10^{4}	1.0×10^{-2}	185×10^{4}	1.2×10^{-2}	323×10^{2}	7.6×10^{-4}	210×10^{2}	5.7×10^{-4}
900	831×10^{2}	2.9×10^{-4}	483×10^{2}	3.1×10^{-4}	178×10	4.2×10^{-5}	230×10	6.2×10^{-5}
No. of samples at each dose	3		3		3		3	

TABLE 16 Data for survival curves of *uvsD* mutants (plotted in Figure 9)

Strain V1 *proA1 argA1 cysD18 uraA1 uvsD3* Strain V21 *hisA1 mthB2 pheA1 strA1 uvsD11*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Open circles a	1 Open circles b	2 Open squares a	2 Open squares b	1 Closed circles a	1 Closed circles b	2 Closed squares a	2 Closed squares b
0	304×10^{3}	1.0	24×10^{3}	1.0	433×10^{5}	1.0	117×10^{5}	1.0
37.5	-	-	14×10^{3}	5.7×10^{-1}	-	-	-	-
75	220×10^{3}	7.2×10^{-1}	-	-	-	-	-	-
112.5	-	-	86×10^{2}	3.6×10^{-1}	207×10^{5}	4.8×10^{-1}	509×10^{4}	4.4×10^{-1}
150	864×10^{2}	2.8×10^{-1}	-	-	-	-	-	-
225	-	-	218×10	9.1×10^{-2}	469×10^{4}	1.1×10^{-1}	-	-
300	100×10^{2}	3.3×10^{-2}	-	-	-	-	-	-
337.5	-	-	32×10	1.3×10^{-2}	-	-	112×10^{3}	9.6×10^{-3}
450	-	-	-	-	194×10^{3}	4.5×10^{-3}	49×10^{3}	4.2×10^{-3}
600	322×1	1.1×10^{-3}	-	-	-	-	-	-
900	-	-	-	-	567×10	1.3×10^{-4}	80×10	6.8×10^{-5}
No. of samples at each dose	3		3		3		3	

TABLE 17 Data for survival curves of *uvsB6* and *uvs-21* (plotted in Figure 10)

Strain V9 *hisA1 mthB2 pheA1 strA1 uvsB6*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Open circles		2 Open squares		3 Open erect triangles		4 Open inverted triangles	
	a	b	a	b	a	b	a	b
0	215×10^5	1.0	50×10^4	1.0	412×10^5	1.0	386×10^5	1.0
112.5	973×10^4	4.5×10^{-1}	306×10^3	6.1×10^{-1}	-	-	-	-
225	452×10^4	2.1×10^{-1}	880×10^2	1.8×10^{-1}	-	-	-	-
450	750×10^3	3.5×10^{-2}	186×10^2	3.7×10^{-2}	-	-	-	-
531	-	-	-	-	584×10^3	1.4×10^{-2}	-	-
708	-	-	-	-	-	-	306×10^3	7.9×10^{-3}
900	92×10^3	4.3×10^{-3}	119×10	2.4×10^{-3}	-	-	-	-
1062	-	-	-	-	261×10^2	6.3×10^{-4}	785×10^2	2.0×10^{-3}
1416	-	-	-	-	-	-	254×10^2	6.6×10^{-4}
1593	-	-	-	-	849×10	2.1×10^{-4}	-	-
1770	-	-	-	-	-	-	171×10^2	4.4×10^{-4}
2125	-	-	-	-	291×10	7.1×10^{-5}	584×10	1.5×10^{-4}
2478	-	-	-	-	-	-	201×10	5.2×10^{-5}
No. of samples at each dose	3		3		2		2	

TABLE 17 (continued) Data for survival curves of *uvsB6* and *uvs-21* (plotted in Figure 10)

Strain V63 *uvs-21*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Closed circles a	1 Closed circles b	2 Closed squares a	2 Closed squares b	3 Closed erect triangles a	3 Closed erect triangles b	4 Closed inverted triangles a	4 Closed inverted triangles b
0	155×10^{6}	1.0	236×10^{6}	1.0	161×10^{6}	1.0	115×10^{6}	1.0
112.5	876×10^{5}	5.7×10^{-1}	118×10^{6}	5.0×10^{-1}	-	-	-	-
225	259×10^{5}	1.7×10^{-1}	565×10^{5}	2.4×10^{-1}	-	-	-	-
450	694×10^{4}	4.5×10^{-2}	1226×10^{4}	5.2×10^{-2}	-	-	-	-
531	-	-	-	-	-	-	211×10^{4}	1.8×10^{-2}
708	-	-	-	-	161×10^{4}	1.0×10^{-2}	-	-
900	1045×10^{3}	6.8×10^{-3}	246×10^{4}	1.0×10^{-2}	-	-	-	-
1062	-	-	-	-	423×10^{3}	2.6×10^{-3}	285×10^{3}	2.5×10^{-3}
1416	-	-	-	-	175×10^{3}	1.1×10^{-3}	-	-
1593	-	-	-	-	-	-	616×10^{2}	5.4×10^{-4}
1770	-	-	-	-	815×10^{2}	5.1×10^{-4}	-	-
2125	-	-	-	-	369×10^{2}	2.3×10^{-4}	203×10^{2}	1.8×10^{-4}
No. of samples at each dose	3		3		2		2	

UvsB was defined by the mutation *uvs-6*; since the survival curve of *uvs-21* was similar to that of *uvsB6* and the two mutations were closely linked, *uvs-21* may lie in the same gene, but there was no definitive evidence for this possibility.

5. The survival curve of *uvs-13*.

The data are presented in Table 18 and the survival curve plotted in Figure 11.

The survival curve of *uvs-13* was unique in that it completely lacked a shoulder, showing exponential survival from the origin. In addition the slope of its exponential curve was very close to that of the exponential part of the curve for 916, since the D37 for the curve of *uvs-13* in Figure 11 = 315 ergs mm^{-2} and the average value for the exponential parts of the curves of 916 in Figure 6 = 322 ergs mm^{-2}. Thus it differed from the curve of its uvs^{+} ancestor only in lacking the shoulder. Since *uvs-13* differs phenotypically in this way from all the other *uvs* mutants, it has provisionally defined gene *uvsE*.

The absence of a definite shoulder also indicates that the presence of hyphal fragments in a spore suspension does not confer an appreciable shoulder on a survival curve obtained for that suspension. Suspensions of this *uvsE13* strain sometimes contained an appreciable proportion of hyphal fragments. These constituted an increasing proportion, up to an estimated 90%, of the plating units as the initial unirradiated viable count decreased from about 10^{8} to 10^{6} per ml; that is when suspensions were prepared from progressively less well sporulating slants.

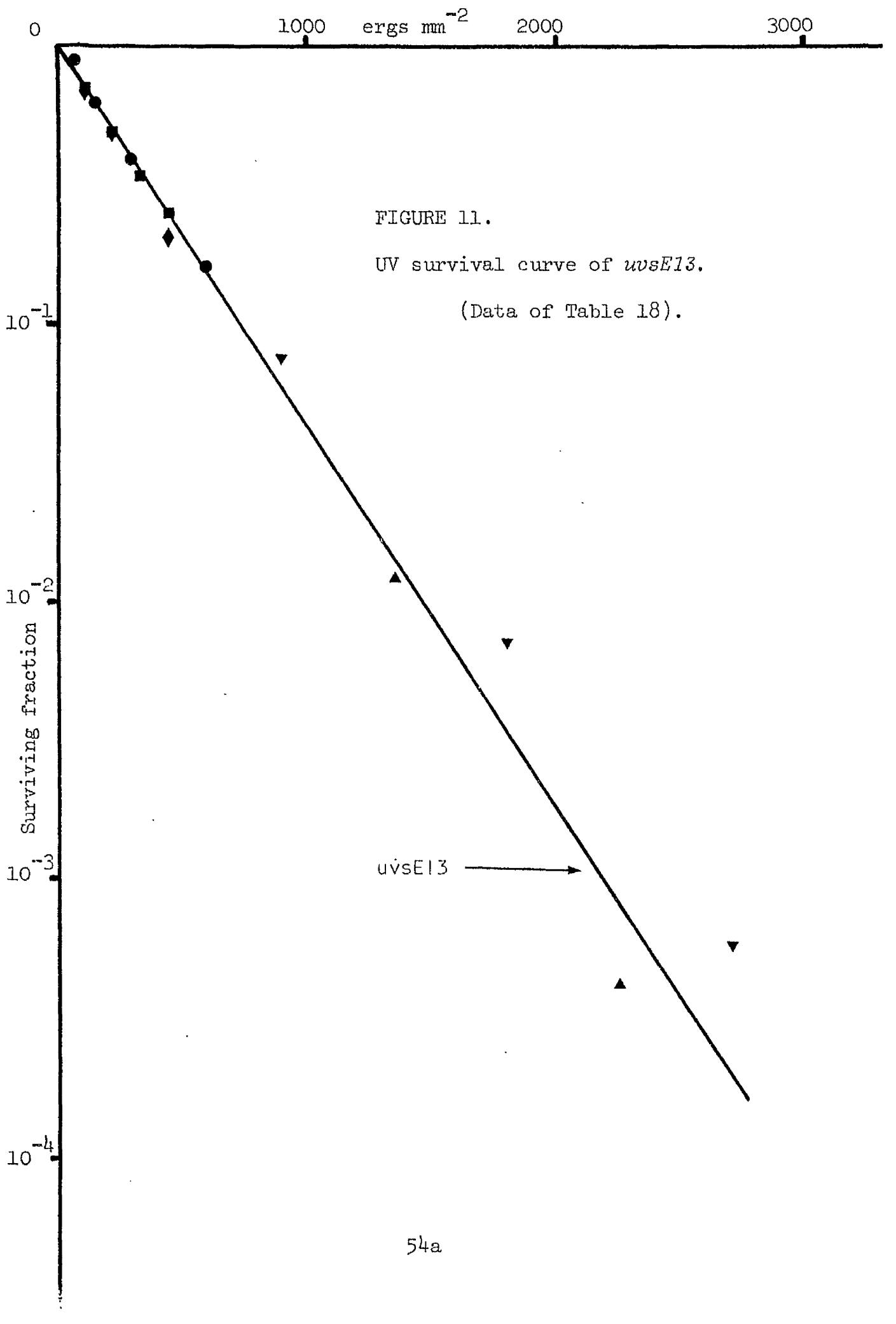


FIGURE 11.

UV survival curve of *uvsE13*.

(Data of Table 18).

TABLE 18 Data for the survival curve of *uvsE13* (plotted in Figure 11).

Strain V25 *hisA1 mthB2 pheA1 strA1 uvsE13*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Closed circles		2 Closed squares		3 Closed erect triangles		4 Closed inverted triangle	
	a	b	a	b	a	b	a	b
0	131×10^{5}	1.0	310×10^{5}	1.0	583×10^{3}	1.0	306×10^{5}	1.0
75	118×10^{5}	9.0×10^{-1}	-	-	-	-	-	-
112.5	-	-	216×10^{5}	7.0×10^{-1}	-	-	211×10^{5}	6.9×10^{-1}
150	821×10^{4}	6.3×10^{-1}	-	-	-	-	-	-
225	-	-	1525×10^{4}	4.9×10^{-1}	-	-	147×10^{5}	4.8×10^{-1}
300	513×10^{4}	3.9×10^{-1}	-	-	-	-	-	-
337.5	-	-	1069×10^{4}	3.4×10^{-1}	-	-	-	-
450	-	-	764×10^{4}	2.5×10^{-1}	120×10^{3}	2.1×10^{-1}	622×10^{4}	2.0×10^{-1}
600	2018×10^{3}	1.6×10^{-1}	-	-	-	-	-	-
900	-	-	-	-	-	-	233×10^{4}	7.6×10^{-2}
1350	-	-	-	-	697×10	1.2×10^{-2}	-	-
1800	-	-	-	-	-	-	221×10^{3}	7.2×10^{-3}
2250	-	-	-	-	242×1	4.2×10^{-4}	-	-
2700	-	-	-	-	-	-	176×10^{2}	5.8×10^{-4}
No. of samples at each dose	3		3		3		2	

IV. THE GENETICS OF *UVS* MUTATIONS

A. Primary mapping of *uvs* mutations

The approximate location of a new genetic marker in *S. coelicolor* may be readily found by the analysis of 50 to 100 recombinants selected from the progeny of a cross between two strains, one of which carries the new mutation (Hopwood, 1967a).

1. Characteristics of the cross.

There were three main requirements of a cross intended to give an approximate location to a new genetic marker.

Firstly, the markers of which the location was already known were distributed as evenly as possible around the circular genetic map of *S. coelicolor*. The "silent" regions imposed limitations on this distribution, see Figure 1. Usually at least six such markers were used.

Secondly, the parent strains were chosen to be complementary in genotype, so that all the markers in the cross were heterozygous and their alleles could segregate in the recombinant progeny.

Thirdly, two points of selection, one against each parent, were applied to the progeny of the cross. This was necessary since in almost all crosses between two strains of *S. coelicolor*, recombinant progeny constituted less than 1% of the total spore and hyphal units harvested from the cross. To select these from amongst the majority of parental genotypes, an allele of each parent was selected against, either by omitting a nutrient required for growth by one of the parents from the supplemented MM on which the cross was to be plated, or by

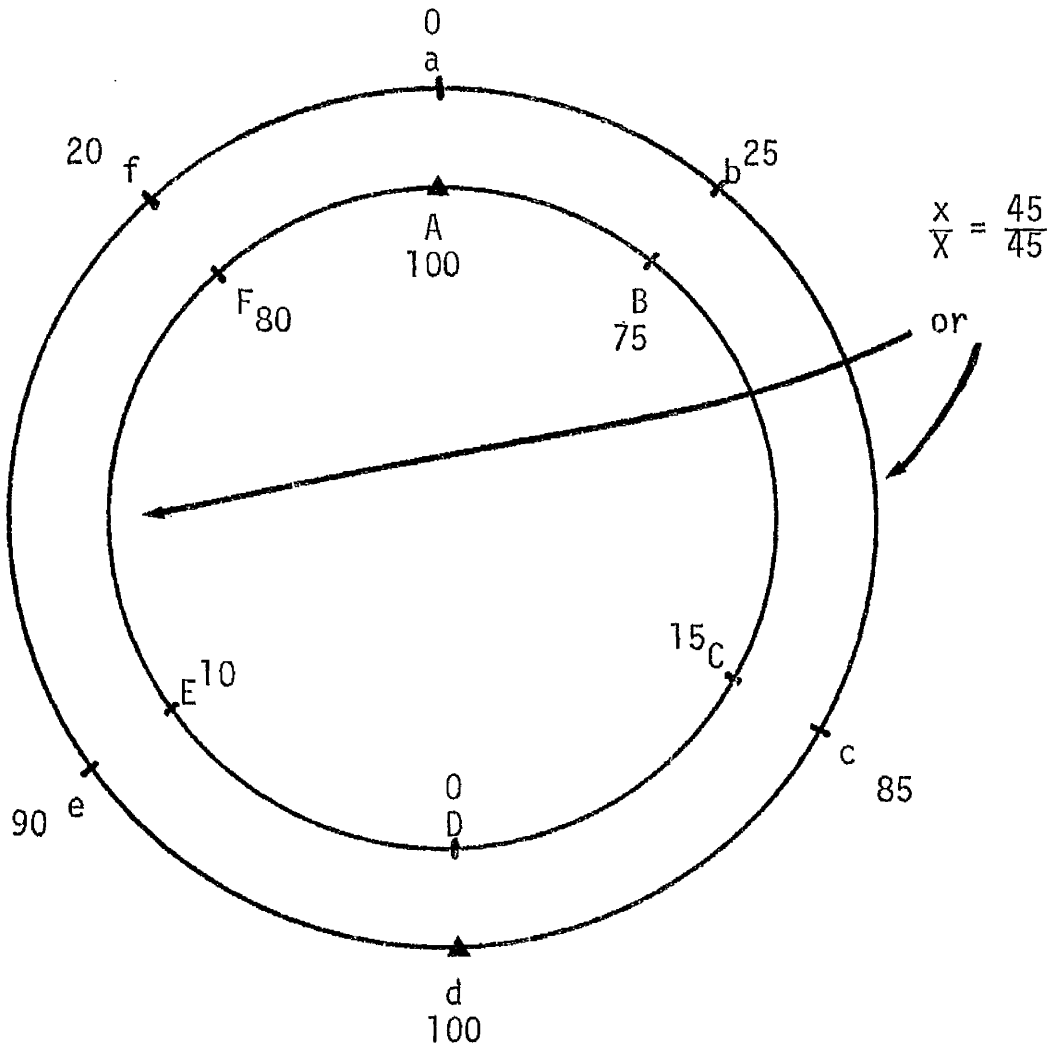
adding an antibiotic (dihydrostreptomycin sulphate) to the plating medium, at a concentration preventing growth of one of the parents. This also selected against those classes of recombinants which did not combine both selected markers. The remaining markers in the cross were kept unselected by adding the appropriate nutrients to the plating medium, or omitting from it an antibiotic. These two points of selection were chosen to be diametrically situated, thus bisecting the genetic map.

For each recombinant viable on the selective medium, at least one crossover (or a higher odd number) must have occurred in each of the two arcs defined by the selected markers. Therefore, the diametrical selections resulted in a distribution of crossovers as even as possible around the genetic map. Incompleteness of the zygotes and any polarity within the zygote population tends to distort this distribution (Hopwood, 1967a) the first of these factors would tend to concentrate the frequency of crossing-over in the regions adjacent to both the selected markers, and the latter would preferentially limit this concentration to one or other of them.

2. Rationale for the location of the new mutation.

A hypothetical cross is illustrated in Figure 12. The two concentric circles represent the genomes of the two parents carrying the alleles, *a*, *b*, *c*, *d*, *e*, and *f* or *A*, *B*, *C*, *D*, *E*, and *F*. The numbers represent the percentage frequencies with which they are found in the progeny. The two selected alleles indicated by triangles, occur with a frequency of 100% and the counterselected alleles, 0%. At any point on the map, the sum of the percentage frequencies must equal 100%. The alleles of the new marker are indicated by *x* and *X*. Recombinants

FIGURE 12. Hypothetical cross for illustrating the location of a new mutation amongst known markers.



See text for explanation.

viable on the selective plating medium (selecting *A d* recombinants) arise by an odd number of crossovers in each sector of the map defined by the selected markers. Most arise by one in each of these sectors; two crossovers in all. These will be referred to as simple crossover classes. Those arising by some multiple of two crossovers will be called multiple crossover classes. As a consequence, the allele frequencies of the known markers of each parent form two continuous series, starting with a frequency of 100% for the selected marker of each parent, at *A* or *d*, and descending along each arc of the genome, to meet at 0% for the counter selected markers of each parent at *a* or *D*.

The allele frequencies of $^{x}/_{X}$ will fit into the gradient of the allele frequencies of the known markers in two alternative positions, between $^{b}/_{B}$ and $^{c}/_{C}$ or between $^{e}/_{E}$ and $^{f}/_{F}$. The actual location is chosen from these two possible locations by trying each in turn, and adopting the location which maximises the number of recombinants in the simple crossover classes. Certain critical classes of recombinants will be simple in the right location but multiple in the wrong location.

3. Experimental procedure.

A cross was made between two suitable strains on a CM slant (Section II C 2 a) and incubated for three or four days. A suspension was then prepared from it (Section II C 3) and recombinants selected by spreading aliquots of the suspension at 10^{0}, 10^{-1}, and 10^{-2} dilutions on suitably supplemented MM plates (Section II C 2 a ii). After three days' incubation one of these plates usually yielded 50 - 100 sporulating recombinant colonies. In order to analyse readily the genotype of a random sample of these recombinants, 50 were individually

picked and inoculated to a fresh plate of the same selective MM in an assymetric rectangular grid pattern. This "master plate" was incubated for two or three days until the recombinant patches were sporulating and used to make replicas with a velvet pad (not more than three replicas from one velvet) to a series of plates, consisting of the following: two plates which were identical to the selective medium of the master plate, one of these being irradiated with about 1200 ergs mm^{-2} to differentiate *uvs* from *uvs*$^+$ recombinants and a set in which each plate lacked a different one of the growth factors present in the master plate. If antibiotic resistance was a non-selected marker in the cross then an additional plate containing all the supplements of the master plate plus the antibiotic at a suitable concentration was included. The series of plates was incubated for two days, when the genotype of each of the 50 recombinant patches was determined from its ability or inability to grow on each plate. The wild type genotype was indicated by growth on any plate lacking a supplement or which had been irradiated, and lack of growth on a plate containing an antibiotic. The mutant genotype was indicated by lack of growth on plates lacking a supplement or which had been irradiated, and growth in the presence of the antibiotic.

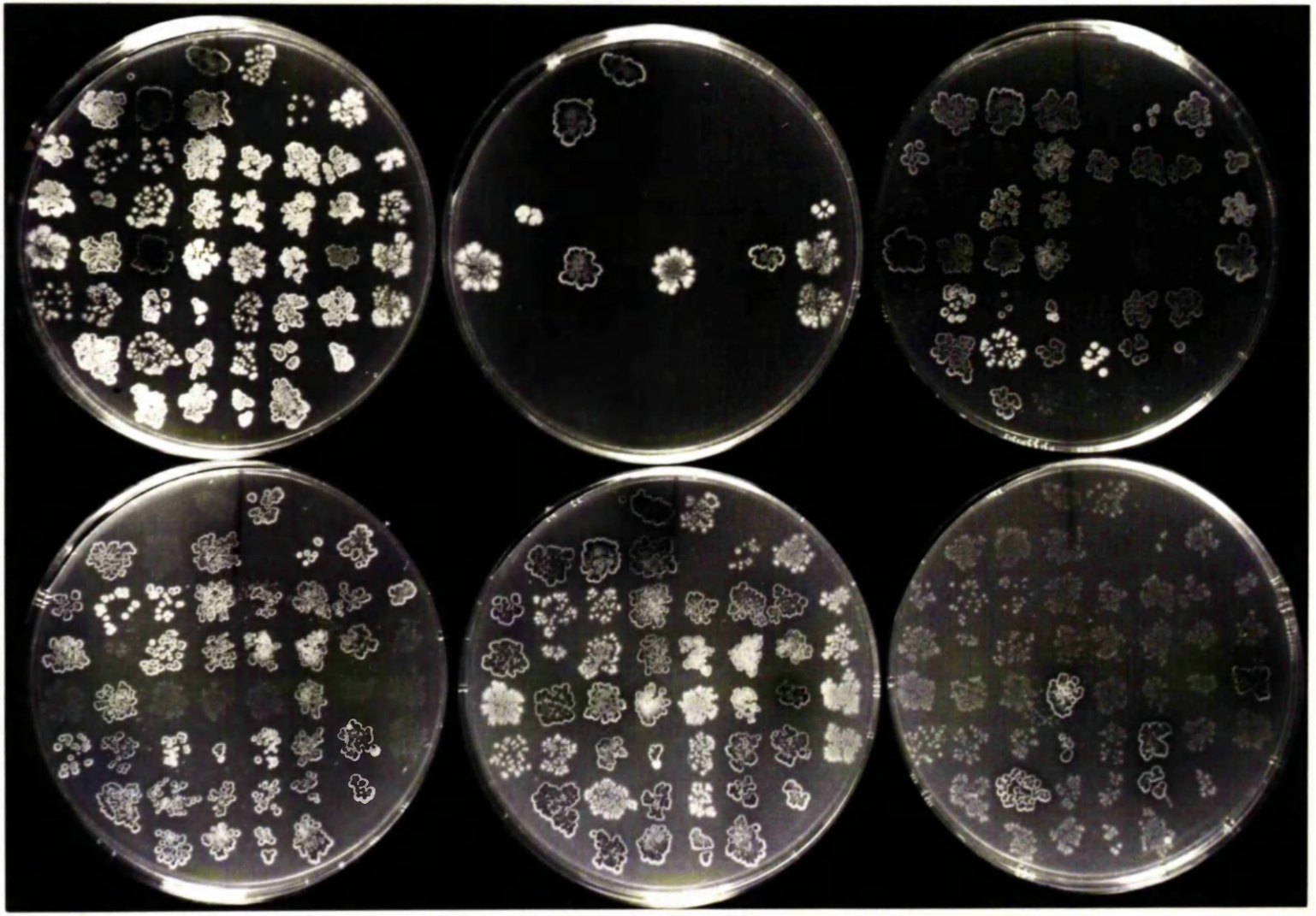
4. Results.

Crosses were made as follows: *uvs* strains derived from 749 *proA1 argA1 cysD18 uraA1* were crossed with 916 *hisA1 mthB2 pheA1 strA1*; *uvs* strains derived in 916 were crossed with 749; and *uvs* strains derived from A3(2) were crossed with 876 *proA1 hisC9 argA1 cysC3 pheA1 strA1*. The selection for *his*$^+$ *strA1* recombinants was similar in each case since *hisA* and *hisC* were closely linked. The three types

FIGURE 13a The replica plates for the cross of *uvsA24* x 876.

50 recombinants selected to be *hisA*$^{+}$ *strA1* on MM + proline, arginine, cystine, phenylalanine and streptomycin were inoculated to a master plate of the same composition. After 2 days growth this was replicated to the series of plates, which had also been incubated for 2 days, shown in the photograph.

Control UV irradiated - proline



- arginine - cystine - phenylalanine

FIGURE 13b The recombinant genotypes recorded for the cross *uvsA24* x 876.

			pro arg phe	*pro cys phe uvs*			
	phe uvs	*arg phe*	*phe uvs*	Failed on master plate	*cys phe uvs*	*cys phe uvs*	
phe uvs	*pro cys phe uvs*	*pro cys phe uvs*	*phe uvs*	*phe uvs*	*phe uvs*	*phe uvs*	*cys phe uvs*
pro phe uvs	*pro arg cys phe*	*cys phe uvs*	*phe uvs*	*pro phe uvs*	*pro phe uvs*	*pro phe uvs*	mixed
arg cys phe	*phe uvs*	*arg phe*	*arg uvs*	*pro arg cys phe*	*pro phe uvs*	*pro arg phe*	mixed
pro cys phe uvs	*cys phe uvs*	mixed	*uvs*	*pro cys phe uvs*	*uvs*	*phe uvs*	*pro arg cys phe*
	phe uvs	*cys uvs*	*phe uvs*	*cys phe uvs*	*uvs*	*pro phe uvs*	
		phe uvs	*pro phe uvs*	*pro phe uvs*	*pro phe uvs*		

Only mutant genotypes were recorded from the replica plates illustrated in Figure 13a.

of crosses satisfied the requirements for primary mapping in the even distribution of known markers, the complementarity of parental markers and the diametrically situated points of selection.

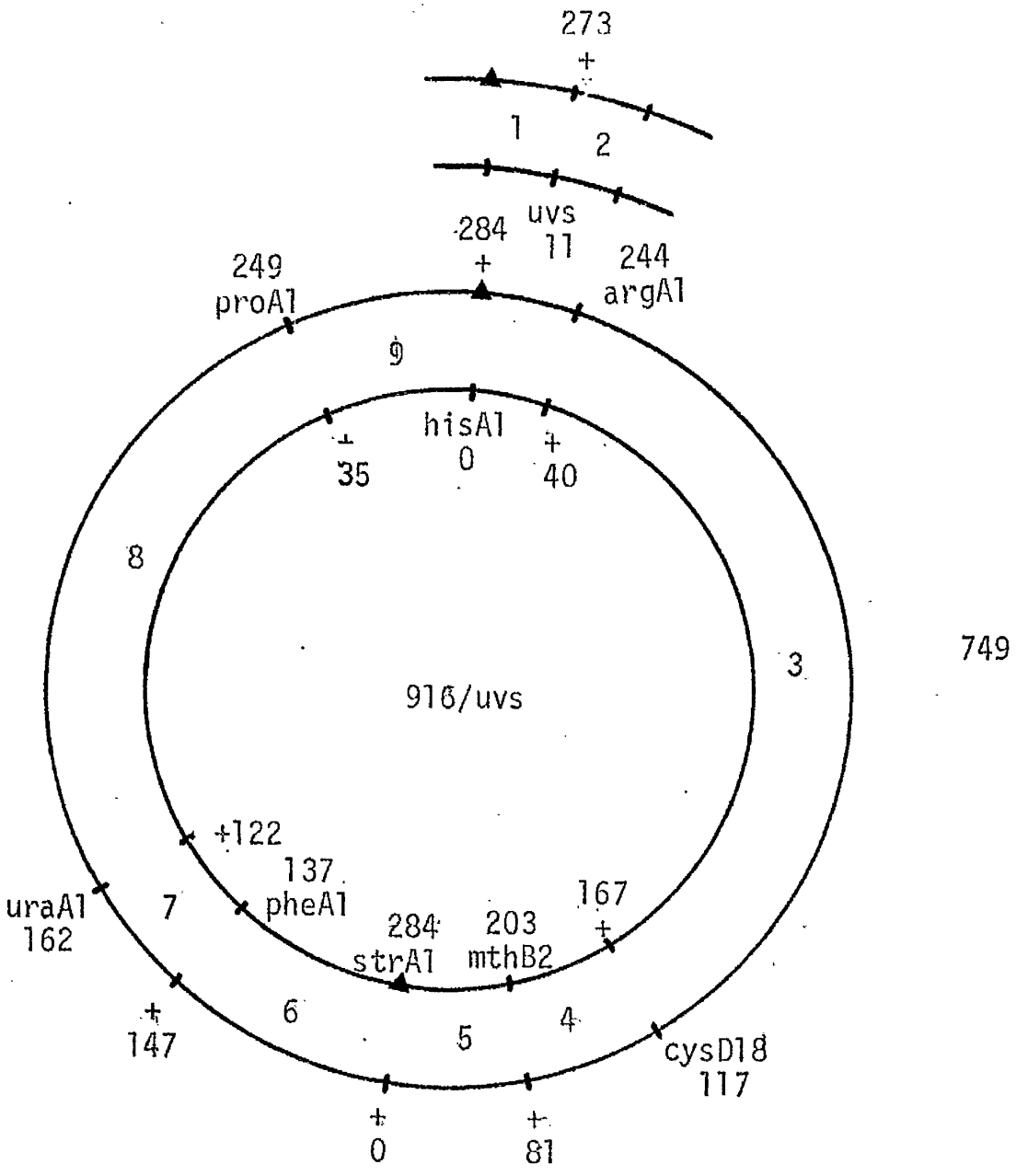
A set of replica plates after two days incubation is shown in Figure 13a for the cross *uvsA24* x 876. Figure 13b shows the genotypes recorded from their growth responses of each of the 50 recombinant patches represented on the plates. See also Table 22 and Figure 17.

In each cross, only 41 to 50 recombinants were analysed. This was insufficient in many cases to allow unambiguous location of the *uvs* mutation. Therefore the data from 23 crosses are pooled and analysed in five groups constructed as follows.

The similarity of parental marker arrangements provided three initial groupings: 916/*uvs* crossed with 749; 749/*uvs* crossed with 916; A3(2)/*uvs* crossed with 876. The first and the third of these were subdivided, since individual analysis of each cross in these two groups showed the mutations to be located in one of two regions of the map. On the assumption that the independent mutations within a group were in the same gene or one of a number of closely linked genes (clustering was already known to occur in *S. coelicolor*; Section I A 1), analysis of the pooled data would indicate its location. This assumption was confirmed when complementation tests and finer mapping were performed.

The data for the five groups are presented in Tables 19, 20, 21, 22, and 23 and analysed in Figures 14, 15, 16, 17, and 18 respectively. Table 24 summarises the probable location of each group of *uvs* mutations, based upon the analysis of the five groups of pooled data. Thus, *uvs-1*, *uvs-2*, *uvs-3*, *uvs-4*, *uvs-5*, *uvs-7*, *uvs-8*, *uvs-9*, *uvs-10*, *uvs-11*, *uvs-14*, *uvs-15*, *uvs-16*, *uvs-17*, *uvs-18*, *uvs-19*, *uvs-20*, *uvs-22*,

FIGURE 14. Analysis of the primary mapping crosses between 916/*uvs* strains and 749: *uvs* located near *his*. (Data of Table 19).

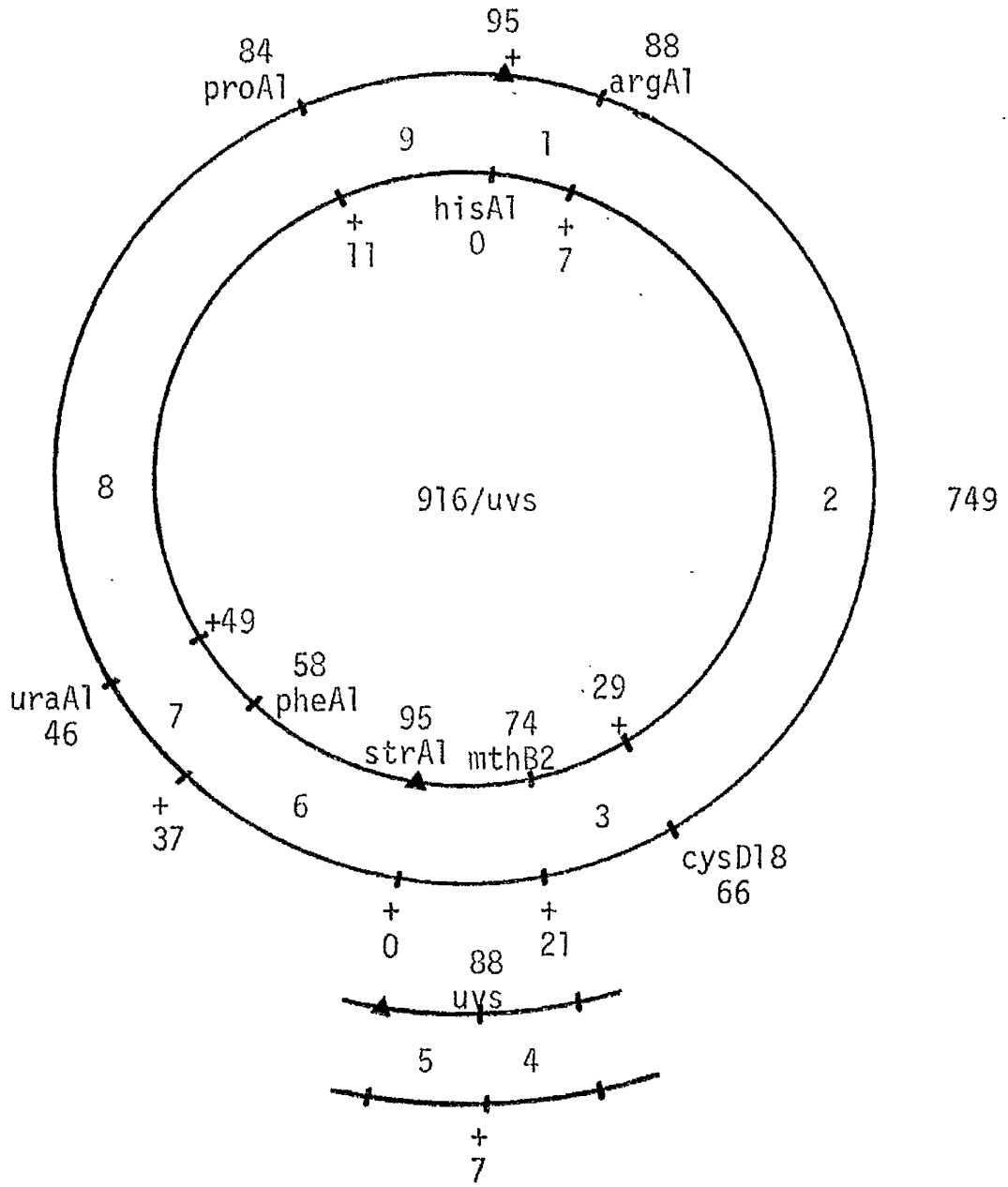


The two concentric circles represent the parental genomes, with the marker arrangements indicated.

Numbers adjacent to alleles indicate their frequencies amongst the sample of recombinants analysed.

Numbers between the circles indicate intervals referred to in the relevant table.

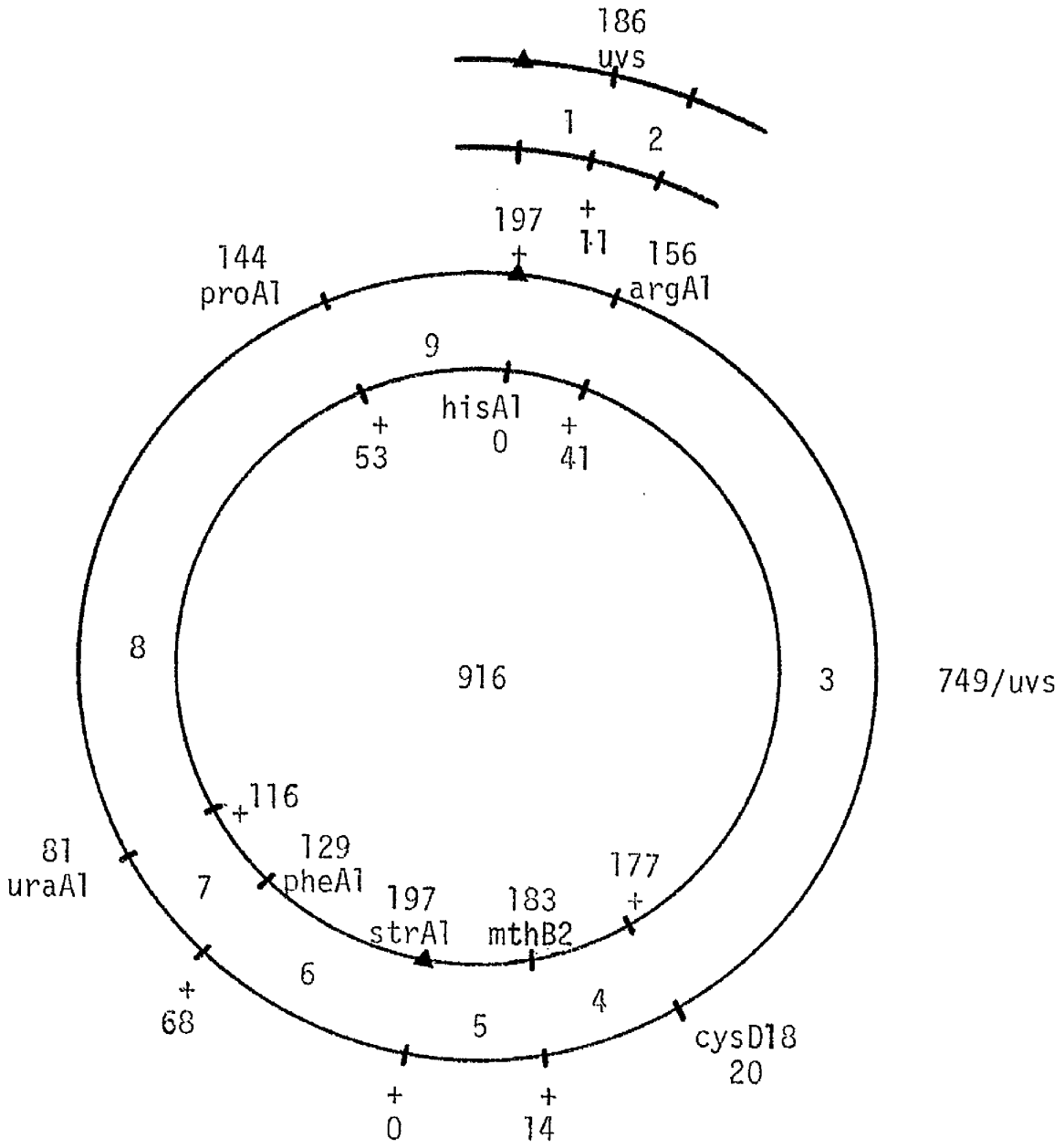
FIGURE 15. Analysis of the primary mapping crosses between 916/*uvs* strains and 749: *uvs* located near *str*. (Data of Table 20).



See legend to Figure 14.

FIGURE 16. Analysis of the primary mapping crosses between 749/*uvs* strains and 916: *uvs* located near *his*.

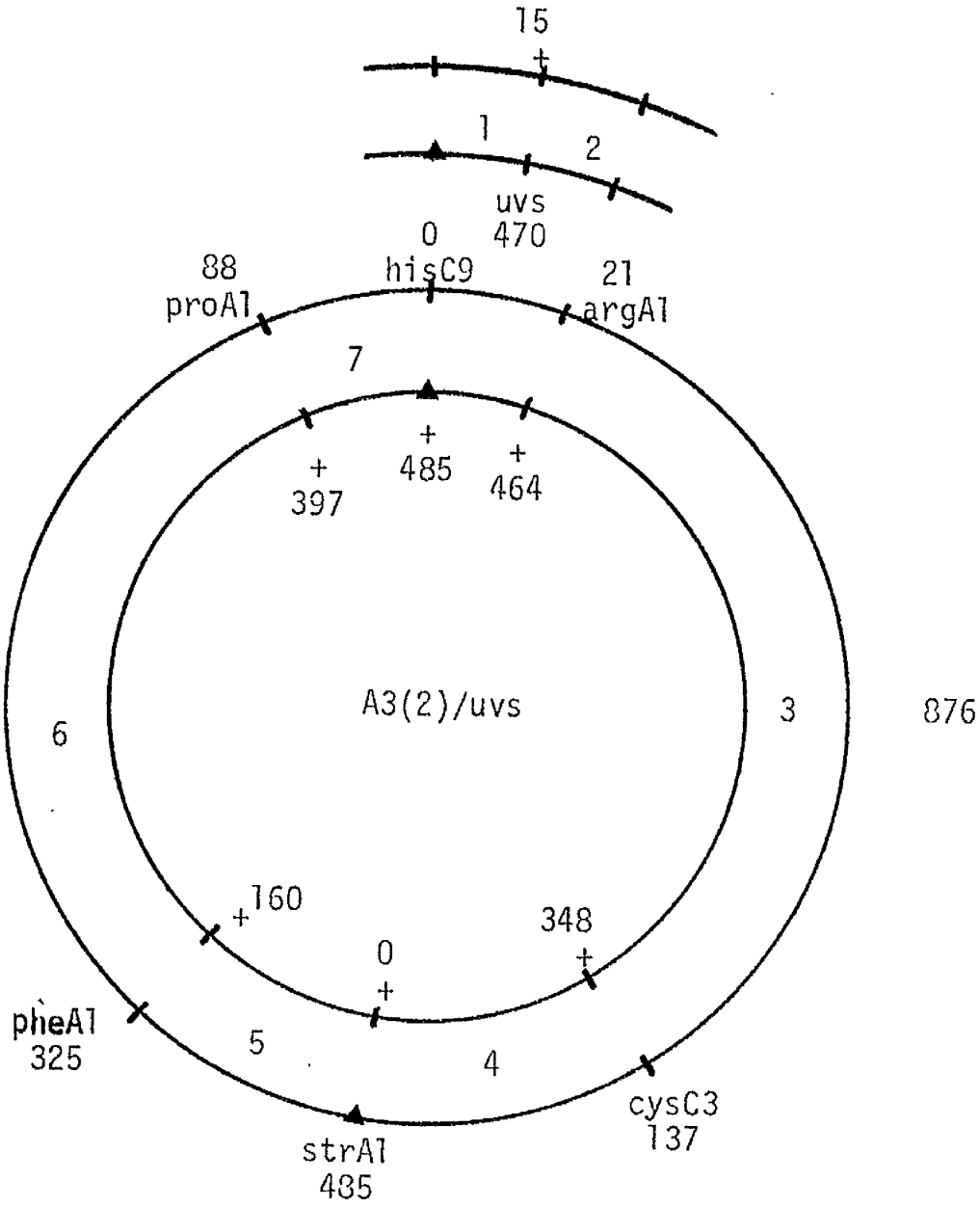
(Data of Table 21).



See legend to Figure 14.

FIGURE 17. Analysis of the primary mapping crosses between A3(2)/*uvs* and 876: *uvs* located near *his* .

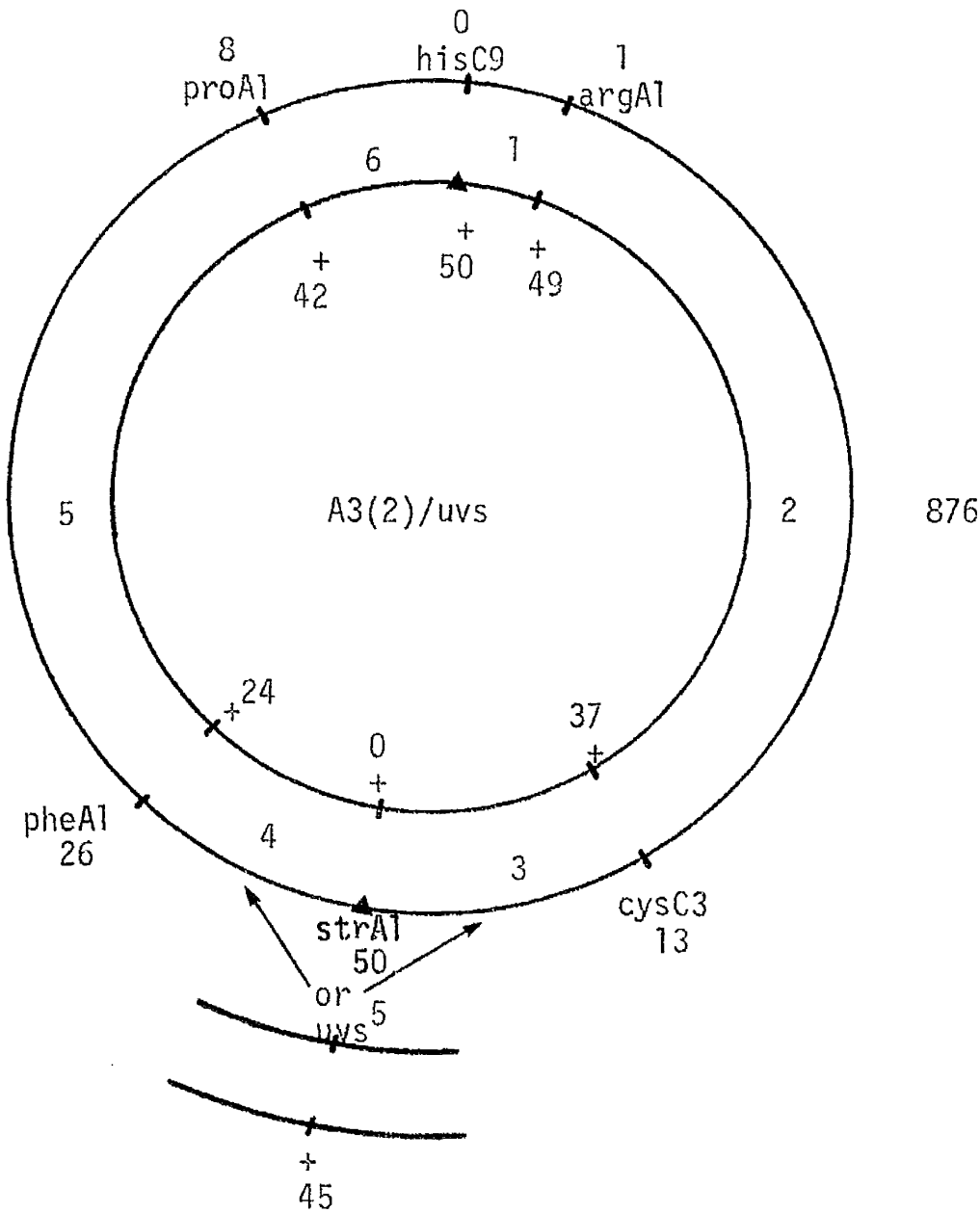
(Data of Table 22).



See legend to Figure 14.

FIGURE 18. Analysis of the primary mapping cross between an A3(2)/*uvs* strain and 876: *uvs* located near *str*.

(Data of Table 23).



See legend to Figure 14.

TABLE 19. Primary mapping of 916/*uvs* mutants in crosses with 749: *uvs* located near *his*.

(Data analysed in Figure 14).

Genotype (a)	Crossover intervals in Figure	Mutations (b)						Total (c)
		uvs-2	*uvs-7*	*uvs-8*	*uvs-9*	*uvs-10*	*uvs-11*	
hom phe	2,9	5	0	0	3	2	2	12
pro hom phe	2,8	3	2	0	2	1	1	9
pro hom phe ura	2,7	2	0	0	0	0	1	3
pro hom ura	2,6	1	1	0	1	0	0	3
arg hom phe	3,9	4	4	1	4	0	2	15
pro arg hom phe	3,8	13	6	6	9	7	8	49
pro arg hom phe ura	3,7	2	3	0	1	0	0	6
pro arg hom ura	3,6	5	13	12	15	6	6	57
arg cys hom phe	4,9	0	1	0	0	0	0	1
pro arg cys hom phe	4,8	0	0	1	0	2	3	6
pro arg cys hom phe ura	4,7	0	1	0	0	1	0	2
pro arg cys hom ura	4,6	0	1	9	2	10	5	27
arg cys phe	5,9	0	1	1	0	0	0	2
pro arg cys phe	5,8	1	4	5	2	1	2	15
pro arg cys phe ura	5,7	0	0	1	0	3	1	5
pro arg cys ura	5,6	0	9	11	6	14	14	54
cys hom phe	2,3,4,9	1	0	0	0	0	0	1
pro arg phe	3,4,5,8	0	1	0	1	0	0	2
pro arg hom	3,6,7,8	0	0	0	1	0	0	1
pro arg ura	3,4,5,6	0	1	0	0	0	0	1
pro arg cys hom	4,6,7,8	0	0	0	0	1	0	1
pro cys ura	2,3,5,6	0	0	0	0	0	1	1
hom phe uvs	1,9	2	0	0	1	0	0	3
pro hom phe uvs	1,8	1	0	0	1	0	1	3
pro hom phe ura uvs	1,7	1	0	0	0	0	0	1
pro hom ura uvs	1,6	0	0	1	1	0	0	2
cys hom phe uvs	1,3,4,9	0	0	0	0	0	1	1
pro cys phe uvs	1,3,5,8	0	0	0	0	0	1	1
Total recombinants		41	48	48	50	48	49	284

(a) Wild-type alleles omitted.

(b) Numbers given represent the frequencies of the genotypes in Column 1 amongst the recombinants recorded in individual crosses containing different *uvs* mutations.

(c) The allele frequencies shown in the relevant figure were calculated from the pooled data of this column.

TABLE 20. Primary mapping of 916/*uvs* mutants in crosses with 749: *uvs* located near *str*.

(Data analysed in Figure 15).

Genotype (a)	Crossover intervals in Figure	Mutations (b) *uvs-6*	Mutations (b) *uvs-13*	Total (c)
hom phe uvs	1,9	1	3	4
pro hom phe uvs	1,8	2	0	2
pro hom ura uvs	1,6	1	0	1
arg hom phe uvs	2,9	1	1	2
pro arg hom phe uvs	2,8	9	15	24
pro arg hom phe ura uvs	2,7	3	3	6
pro arg hom ura uvs	2,6	8	17	25
arg cys hom phe uvs	3,9	3	0	3
pro arg cys hom phe uvs	3,8	2	1	3
pro arg cys hom phe ura uvs	3,7	1	0	1
pro arg cys hom ura uvs	3,6	0	2	2
arg cys phe uvs	4,9	1	0	1
pro arg cys phe uvs	4,8	5	1	6
pro arg cys phe ura uvs	4,7	1	0	1
pro arg cys ura uvs	4,6	4	2	6
arg phe uvs	2,3,4,9	0	1	1
pro arg hom phe	2,4,5,8	1	0	1
pro arg cys phe	5,8	2	0	2
pro arg cys phe ura	5,7	1	0	1
pro arg cys ura	5,6	2	1	3
Total recombinants		48	47	95

See legend to Table 19.

TABLE 21. Primary mapping of 749/*uvs* mutants in crosses with 916: *uvs* located near *his*.

(Data analysed in Figure 16).

Genotype (a)	Crossover intervals in Figure	Mutations (b)				Total (c)
		uvs-1	*uvs-3*	*uvs-4*	*uvs-5*	
hom phe uvs	2,9	6	5	4	0	15
pro hom phe uvs	2,8	4	6	0	0	10
pro hom phe ura uvs	2,7	0	1	1	0	2
pro hom ura uvs	2,6	2	1	2	1	6
arg hom phe uvs	3,9	8	6	9	6	29
pro arg hom phe uvs	3,8	8	15	13	12	48
pro arg hom phe ura uvs	3,7	2	4	2	3	11
pro arg hom ura uvs	3,6	12	5	7	20	44
arg cys hom phe uvs	4,9	0	0	1	1	2
pro arg cys hom phe uvs	4,8	1	0	1	0	2
pro arg cys hom phe ura uvs	4,7	0	0	1	0	1
pro arg cys hom ura uvs	4,6	1	0	0	2	3
pro arg cys phe uvs	5,8	0	0	1	0	1
pro arg cys phe ura uvs	5,7	0	0	1	0	1
pro arg cys ura uvs	5,6	3	0	4	2	9
pro arg phe uvs	3,4,5,8	0	2	0	0	2
hom phe	1,9	1	3	2	0	6
pro hom phe	1,8	0	1	0	0	1
pro arg hom ura	1,2,3,6	1	0	0	1	2
pro arg cys ura	1,2,5,6	0	0	0	1	1
hom ura	1,6,8,9	1	0	0	0	1
Total recombinants		50	49	49	49	197

See legend to Table 19.

TABLE 22. Primary mapping of A3(2)/*uvs* mutants in crosses with 876: *uvs* located near *his*. (Data analysed in Figure 17).

Genotype (a)	Crossover intervals in Figure	Mutations (b)										Total (c)
		uvs-14	*uvs-15*	*uvs-16*	*uvs-17*	*uvs-18*	*uvs-19*	*uvs-20*	*uvs-22*	*uvs-23*	*uvs-24*	
arg cys phe	1,6	0	0	0	1	0	0	0	0	0	1	2
arg cys phe pro	1,7	0	0	0	0	1	0	1	0	0	3	5
arg cys uvs	2,5	0	0	0	0	2	0	0	0	0	0	2
arg cys phe uvs	2,6	0	0	0	0	1	0	2	0	0	1	4
arg cys phe pro uvs	2,7	0	0	0	1	0	0	0	0	0	0	1
cys uvs	3,5	2	1	1	3	6	0	4	5	1	1	24
cys phe uvs	3,6	7	8	4	11	6	9	11	3	8	6	73
cys phe pro uvs	3,7	2	2	0	1	3	3	5	0	2	5	23
uvs	4,5	24	16	8	12	6	15	1	25	16	3	126
phe uvs	4,6	10	17	26	14	18	19	15	10	17	13	159
pro phe uvs	4,7	4	3	8	2	5	2	7	4	6	9	50
cys phe	1,2,3,6	0	1	0	0	0	0	1	0	0	0	2
phe pro	1,2,4,7	0	0	0	0	1	0	0	0	0	0	1
arg phe	1,3,4,6	0	0	0	0	0	0	1	0	0	2	3
arg phe pro	1,3,4,7	0	0	0	0	0	0	0	0	0	2	2
arg uvs	2,3,4,5	1	0	0	0	0	0	0	0	0	1	2
cys pro uvs	3,5,6,7	0	0	0	0	0	0	1	0	0	0	1
uvs pro	4,5,6,7	0	1	2	0	0	0	0	2	0	0	5
Total recombinants		50	49	49	45	49	48	49	49	50	47	485

See legend to Table 19.

TABLE 23. Primary mapping of an A3(2)/*uvs* mutation crossed with 876: *uvs* located near *str*.

(Analysed in Figure 18).

Genotype (a)	Crossover intervals in Figure	Mutation (b)
pro arg cys phe	1, 6	1
cys	2, 4	3
cys phe	2, 5	7
pro cys phe	2, 6	2
+	3, 4	15
uvs		5
phe	3, 5	12
pro phe	3, 6	4
pro	3, 4, 5, 6	1
Total recombinants		50

See legend to Table 19.

TABLE 24. Summary of primary mapping locations.

Crosses	Derivatives of 916 x 749		Derivatives of 749 x 916	Derivatives of A3(2) x 876	
Group of mutations for which recombination data was pooled.	*uvs-2 uvs-7 uvs-8 uvs-9 uvs-10 uvs-11*	*uvs-6 uvs-13*	*uvs-1 uvs-3 uvs-4 uvs-5*	*uvs-14 uvs-15 uvs-16 uvs-17 uvs-18 uvs-19 uvs-20 uvs-22 uvs-23 uvs-24*	*uvs-21*
Data from Table:	19	20	21	22	23
Analysed in Figure:	14	15	16	17	18
uvs/*uvs*$^+$	$^{11}/_{273}$	$^{88}/_{7}$	$^{186}/_{11}$	$^{470}/_{15}$	$^{5}/_{45}$
Two locations by allele frequencies	*proA* to *hisA* / *hisA* to *argA*	*pheA* to *strA* / *strA* to *mthB*	*proA* to *hisA* / *hisA* to *argA*	*proA* to *hisA* / *hisA* to *argA*	*pheA* to *strA* / *strA* to *cysC*
Informative recombinants	11 *uvs*	7 *uvs*$^+$	11 *uvs*$^+$	15 *uvs*$^+$	5 *uvs*
Multiple crossovers amongst informative recombinants	7 / 0	4 / 1	4 / 3	7 / 3	0 / 0
Probable location	*hisA uvs argA*	*strA uvs mthB*	*hisA uvs argA*	*hisA uvs argA*	*pheA uvs cysC*

uvs-23 and *uvs-24* were located near *hisA/C*, probably between *hisA/C* and *argA*, *uvs-6* and *uvs-13* near *strA*, probably between *strA* and *mthB*, and *uvs-21* also near *strA*.

The conclusions from these data for individual crosses were necessarily tentative when it came to deciding between the two possible locations indicated by the allele frequencies, that is, the order of the *uvs* mutations relative to *hisA/C* or *strA*, due to the infrequency of critical genotypes. The allele frequencies of *uvs/uvs*$^{+}$ which were calculated from the pooled data did however clearly indicate that all the *uvs* mutations fell into two groups, one containing 20 mutations, located between *proA* and *argA*, the other containing three mutations located between *pheA* and *mthB* or *cysC*.

It must be said that when considering the crosses individually, in only one cross (that involving the *uvs-5* strain) was the conclusion based on the pooled data positively contradicted. In this case, the allele frequencies located *uvs-5* either between *proA* and *hisA* or *argA* and *cysC* (nearer *argA*) with two multiple crossover recombinants in either case.

The next steps were to attempt a complementation test within the two groups of mutations, and then to locate more precisely one or a few representative mutations of each gene found to exist.

B. Genetic complementation amongst the mutations located near *hisA*.

Twenty mutations were located near *hisA*, probably between *hisA* and *argA*, and the question to be asked was whether these mutations were all changes in the same or in different genes.

A test for complementation requires that part of the life cycle of the organism involves a diploid (or partially diploid) stage during which the phenotype of cells may be observed. The diploid is constructed to be heterozygous (in *trans*) for two independent mutations, each exhibiting a similar phenotype when monosomic. Such a situation exists in *S. coelicolor*, in the form of heteroclone genomes, which give rise to heteroclone colonies when grown under appropriate selective conditions.

1. Rationale for the complementation test.

Zygotes in *S. coelicolor* are partially diploid, containing a whole chromosome derived from one parent, and a partial chromosome derived from the other parent (Hopwood, 1967a). The fragment is of random length, averaging about $^{1}/_{6}$th of the genome, and with ends at random positions. Such a zygote and its progeny are illustrated in Figure 2. The zygote can give rise to haploids by an even number of crossovers, usually two, anywhere in the heterozygous region, and a recombinant fragment which is presumably lost. A single crossover, or any odd number of crossovers, yields a terminally repeated linear heteroclone genome which is still heterozygous to the same extent as the zygote. A further crossover, or any other odd number of crossovers, in the heteroclone genome will yield a haploid, and this process is the normal consequence for heteroclone genomes in the progeny of any cross, unless a special selection which selects against the haploids is

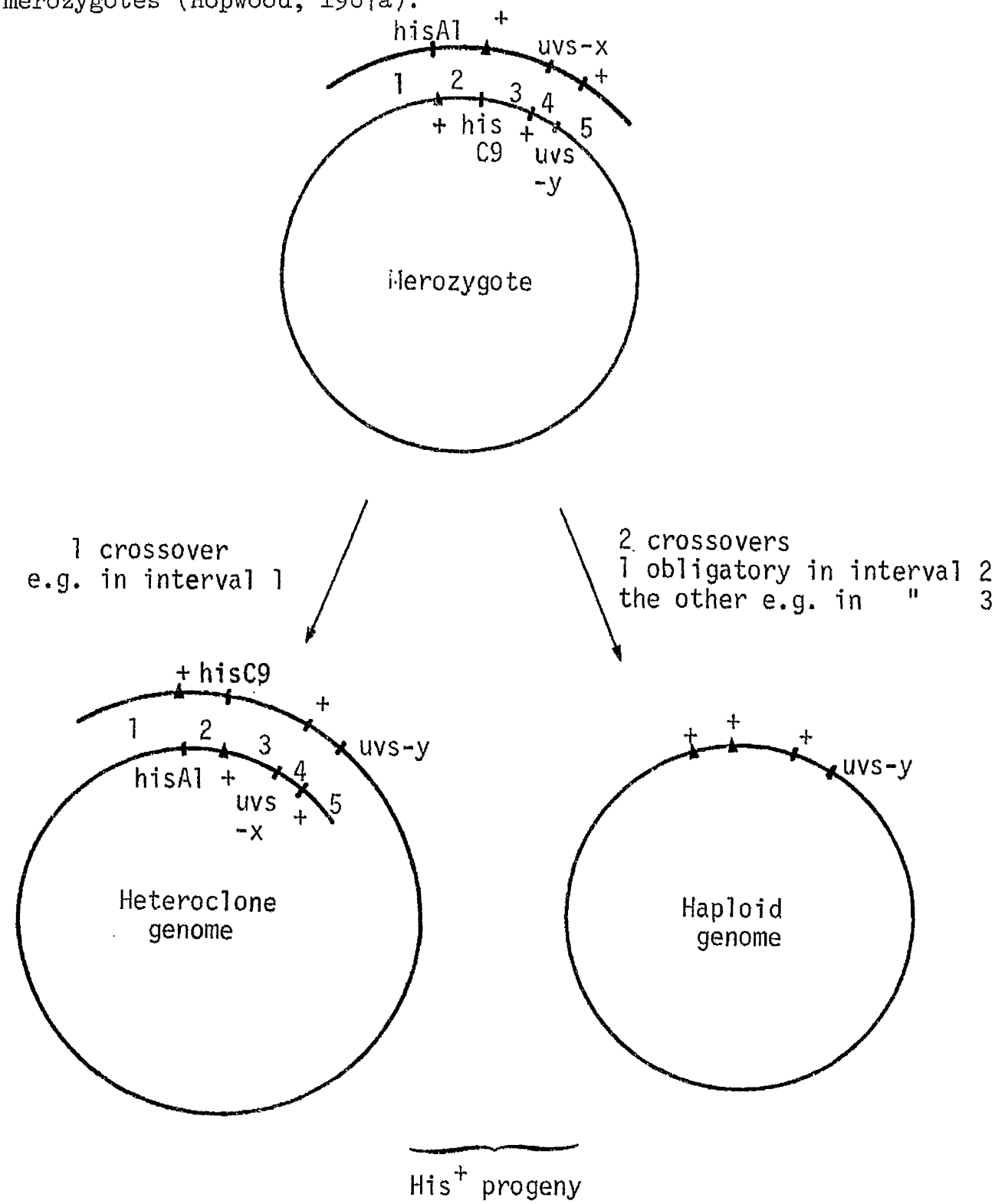
applied to these progeny. If selection is made against two closely linked markers in different genes such as *hisA1* and *hisC9* (i.e. the progeny of a cross between a parent *hisA1*, and a parent *hisC9* are plated on medium lacking histidine), then only progeny phenotypically His^+ are able to grow (Figure 19). These will consist of two types, both arising only from zygotes heterogeneous for *hisA* and *hisC*; true his^+ haploid genomes which arise by an even number of crossovers of which one must be in interval 2 (as illustrated in Figure 19 the other is in interval 3), and heteroclone genomes which arise by an odd number of crossovers anywhere in the heterozygous region. The latter will be His^+ because of complementation between $hisA^+$ and $hisC^+$. Haploid cells will grow into uniform, circular colonies whereas heteroclone cells, except for those which segregate a His^+ haploid clone early in development, by a further crossover in 2, will grow into irregular heteroclone colonies of variable size.

Heteroclone genomes cannot be directly selected for heterozygosity of *uvs* mutations in the way that auxotrophic mutations may be selected. However, since the twenty *uvs* mutations to be tested were located near to the *hisA hisC* region, heteroclone genomes selected in the way already described were almost always heterozygous for the region controlling UV sensitivity.

Consider a cross made between a strain *hisA1 uvs-x* and a strain *hisC9 uvs-y* from which the progeny were plated on medium lacking histidine (see Figure 19). Amongst these the viable progeny were either His^+ heteroclones or haploid cells, which had arisen from the zygotes as already described. Since interval 4 is small (see Section IV C) most haploids carried either *uvs-y* or *uvs-x* and were therefore

FIGURE 19. Illustration of the rationale for the complementation tests.

Based on the model for the origin of haploid and heteroclone genomes from merozygotes (Hopwood, 1967a).



See text for explanation

Uvs$^-$; haploids both *his*$^+$ and *uvs*$^+$ were rare. Colonies with uniform circular haploid appearance constituted approximately five per cent of the total colonies appearing in a cross with selection against *hisA1* and *hisC9* (Hopwood, 1967a). Since, however, heteroclone genomes were almost always heterozygous at the *uvs* region as well as at the *his* region, their UV sensitivity depended upon both *uvs-x* and *uvs-y*. If *uvs-x* and *uvs-y* were in different genes, complementation could occur, and these cells were Uvs$^+$. If however *uvs-x* and *uvs-y* were in the same gene, complementation could not occur (assuming no intragenic complementation), and these cells were Uvs$^-$.

Two classes of results were therefore expected, depending on the UV sensitivity of heteroclone plating units. In both classes, haploid cells would be UV sensitive. However, in the crosses in which complementation occurred, and since haploids were a small minority, the total His$^+$ progeny of the cross should have exhibited approximately wild-type sensitivity, whereas in the crosses in which complementation did not occur, the total His$^+$ progeny would almost all have been of mutant sensitivity.

2. Experimental procedure.

Half the twenty *uvs* mutations were obtained in *hisA1* strains either directly or by recombination with *hisA1* strains. The remaining *uvs* mutants were crossed with a *hisC9* strain and *uvs hisC9* strains isolated. Crosses were made in all possible pairwise combinations between *uvs hisA1* strains and *uvs hisC9* strains. 0.1ml samples of the spore suspensions from the crosses were spread in duplicate, undiluted and at an approximate ten-fold dilution (Section II C 2 iii), on MM agar plates lacking histidine but containing any other nutrients required

by either parent. One of each set of duplicate plates was exposed to a dose of UV (approximately 1200 ergs mm^{-2}) sufficient to kill approximately 50% of cells with wild-type sensitivity but killing more than 99% of cells with mutant sensitivity. All the plates were incubated for three days, when total colony counts were made.

For each cross, the number of colonies counted on the irradiated plate was expressed as a percentage of the number of colonies counted on the unirradiated control plate. Counts obtained at different dilutions for the same cross were added together, provided that counts could be obtained for both control and irradiated plates at each dilution.

3. Results.

The results obtained from up to three crosses between each pair of strains are presented in Table 25; they may be divided unambiguously into two classes. One class contains those crosses where the survival after irradiation was of the order of one per cent or less, indicating non-complementation, the highest survival in this class being less than or equal to 3.2%. The second class contains those crosses in which the survival after irradiation was between 82.4% and 14.3% indicating complementation, with an average value of 48.6% in this class. This was in good agreement with the value expected in fully complementing combinations derived as follows: 5% of viable progeny were haploid and Uvs^-; the remaining 95% were heteroclone cells almost all of which would have been Uvs^+, therefore the overall survival should approximately equal $100 - 5 \times {}^{50}/_{100} = 47.5\%$.

On the basis of these results, the twenty *uvs* mutations were divided into three complementation groups defining genes *uvsA*,

TABLE 25. The results of complementation tests between *uvs* mutations.

uvs mutation in *hisA1* parent		*uvs* mutation in *hisC9* parent								
		uvs A15 □	*uvs A19* □	*uvs A22* □	*uvs A23* □	*uvs A24* △	*uvs C14* □	*uvs C16* □	*uvs C17* □	*uvs D18* □
uvsA2 ■	a	0	0	1	4	0	191	47	86	17
	b	224	499	342	1286	504	346	60	166	36
	c	<0.45	<0.21	0.29	0.31	<0.2	55.2	78.3	51.8	47.2
uvsA4 ▲	a	0	0	0	0	0	50	16	39	96
	b	218	367	72	111	∝	178	43	137	264
	c	<0.46	<0.28	<1.4	<0.91	0	28.1	37.2	28.5	36.4
uvsA9 ■	a	0	0	1	0	0	187	18	134	15
	b	80	994	256	798	101	350	50	337	36
	c	<1.25	<0.11	0.39	<0.13	<1.0	53.4	36	39.8	41.7
uvsA20 ▼	a	0	0	0	0	0	116	1	17	61
	b	66	195	80	176	88	247	7	34	144
	c	<1.6	<0.52	<1.25	<0.57	<1.2	47.2	14.3	50	42.4
uvsC7 ■	a	18	74	50	34	101	1	0	0	12
	b	77	228	92	72	157	460	35	98	20
	c	23.4	32.5	54.3	47.2	64.3	0.22	<2.9	<1.1	60
uvsC8 ■	a	15	123	63	217	22	0	0	3	36
	b	65	205	120	275	42	1247	32	1083	45
	c	23.1	60	52.5	78.9	52.4	<0.08	<3.2	0.28	80
uvsC10 ■	a	118	799	51	82	79	0	1	0	59
	b	228	1278	136	247	173	400	51	301	109
	c	51.8	62.5	37.5	33.2	45.7	<0.25	2.0	<0.34	54.1
uvsD1 ▲	a	63	430	203	188	112	207	26	147	0
	b	189	1072	462	386	136	426	50	248	35
	c	33.3	40.1	43.9	48.7	82.4	48.6	52	59.3	<2.9
uvsD3 ▲	a	33	340	71	367	69	29	28	183	0
	b	112	716	156	563	108	106	49	310	95
	c	29.5	47.5	45.5	65.2	63.9	27.4	57.1	59.0	<1.1
uvsD5 ▲	a	196	318	148	131	72	495	21	74	0
	b	415	677	327	231	93	1400	39	160	85
	c	47.2	47.0	45.3	56.7	77.4	35.4	53.8	46.3	<1.2
uvsD11 ■	a	209	500	88	96	37	253	24	119	0
	b	663	759	213	240	68	443	47	235	93
	c	31.5	65.9	41.3	40.0	54.4	57.1	51.1	50.6	<1.1

TABLE 25 (continued).

NOTES.

a) Number of colonies on irradiated plate(s).

b) Number of colonies on unirradiated plate(s).

c) % survival $(\frac{a}{b} \times 100.)$

Results for crosses showing no complementation are in heavy type.
Results for crosses showing complementation are in light type.

□ *uvsA15, A19, A22, A23, C14, C16, C17,* and *D18* strains also contained *proA1 strA1.*

△ *uvsA24* strain also contained *strA1* and a spore colour mutation C73.

■ *uvsA2, A9, C7, C8, C10* and *D11* strains also contained *mthB2 pheA1 strA1.*

▲ *uvsA4, D1, D3* and *D5* strains also contained *pheA1.*

▼ *uvsA20* strain also contained *strA1.*

uvsC, *uvsD*. Crosses involving *uvs* mutations within a gene showed non-complementation, and crosses involving *uvs* mutations assigned to different genes showed complementation in every case. There was no indication that the results observed were due to the occurrence of intragenic complementation. If this had been the case, and all the mutations were in the same gene, a frequent class of *uvs* mutants would have been expected to show non-complementation with all of the other *uvs* mutants. This group would have contained the mutants coding for no protein at all, or one so grossly altered that it was incapable of complementation. Thus the results obtained were entirely consistent with the existence of three genes controlling UV sensitivity in this region.

C. Fine mapping of representative mutations of *uvsA*, *uvsC* and *uvsD*, and *uvsB6*, *uvsE13* and *uvs-21*.

1. Rationale.

Ordering of mutations relative to other markers within a small region of the map can serve two purposes. If two independent mutations with similar phenotypes can be located on either side of a known gene, which has an unrelated phenotype, then this is evidence for two separate genes which should be supported by any complementation data available. Alternatively, if a number of different genes, defined by complementation data, cannot be separated by any of the known markers located in this region, then this is a preliminary indication of a gene cluster, perhaps an operon, and the possibility of co-ordinate gene control.

The characteristics of a cross designed to order a new marker

relative to other known markers, all located within a fairly short region of the map, differ in one respect from those of a cross designed to give a first approximate location to the new marker, in that the two points of selection should be chosen to define the short section of the map within which the new marker is known to lie. The genotypes of the two parents should again be complementary for the known ordered markers in the short region, and ideally these should be evenly distributed. The ratio of allele frequencies for the new marker will locate it as before with respect to the known markers in a position which should minimise the number of multiple crossover recombinants.

2. Experimental procedure.

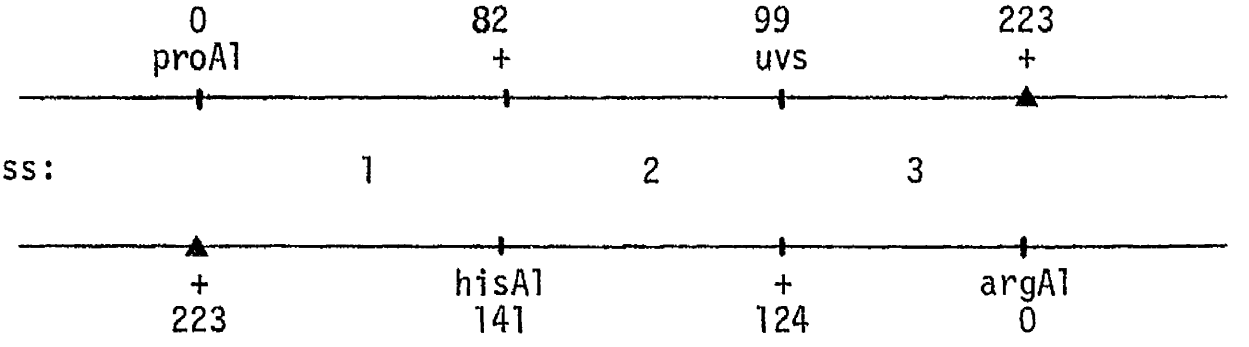
Crosses were performed and analysed as described in Section IV A 3.

3. Results for *uvsA*, *uvsC* and *uvsD*.

The primary mapping described earlier had located the *uvsA*, *uvsC* and *uvsD* group of mutations between *proA* and *argA*, probably between *hisA* and *argA*. The following crosses were performed and analysed in order to confirm the location with respect to *hisA*. *ProA1 uvsA4*, *proA1 uvsC7* and *proA1 uvsD3* strains were obtained by recombination, and were each crossed with strain 26 *hisA1 argA1* and *proA*$^+$ *argA*$^+$ recombinants selected.

The crosses, the results and their analysis are illustrated in Table 26. The allele ratio for the pooled results, $uvs^+/uvs = {}^{124}/_{99}$, clearly located *uvs* between *hisA* and *argA*. This location was supported by each of the individual crosses analysed alone. It required four of the observed recombinants to occur by multiple crossovers, whereas the other possible order, *proA uvs hisA argA*, required 21 multiple crossover recombinants. Therefore the order was *proA hisA (uvsA uvsC uvsD) argA*.

TABLE 26. The ordering of *uvsA*, *uvsC* and *uvsD* relative to *hisA*.



Genotype	Crossover interval	Mutations (a) *uvsA4*	*uvsC10*	*uvsD3*	Total (b)
+ uvs	1	23	38	17	78
his uvs	2	7	6	8	21
his +	3	59	43	18	120
+ +	1,2,3	2	0	2	4
Total Recombinants		91	87	45	223

The strains used were V17 *proA1 mthB2 uraA1 strA1 uvsA4*, V41 *proA1 cysD18 uraA1 uvsC10*, and V20 *proA1 mthB2 uraA1 strA1 uvsD3*, each crossed with 26 *hisA1 argA1*.

Markers not included in the table were located outside the region of interest, were unselected in the crosses and were ignored.

For simplicity only the region of the parental chromosomes in which the *uvs* mutations were already located is represented by the straight lines and the markers placed equidistant. Their actual locations may be seen from Figure 1.

(a) Numbers given represent the frequencies of the genotypes in Column 1 amongst the recombinants recorded in crosses containing the *uvs* mutations indicated.

(b) The allele frequencies shown in the figure above the table were calculated from the data of this column.

The following crosses were performed to obtain a more precise location for *uvsA*, *uvsC*, and *uvsD* in the region *hisA argA*. *ArgA1 uvs* recombinants were isolated for the mutants *uvsA4*, *uvsC7*, *uvsD1*, *uvsD3* and *uvsD5*. These recombinant strains were crossed with 948 *hisA1 ammA5 serA1*. The order of the markers involved in the cross was *hisA - ammA - serA - argA*, with the gene for UV sensitivity expected to fall somewhere within this series. The recombinants were selected to be $hisA^+$ $argA^+$.

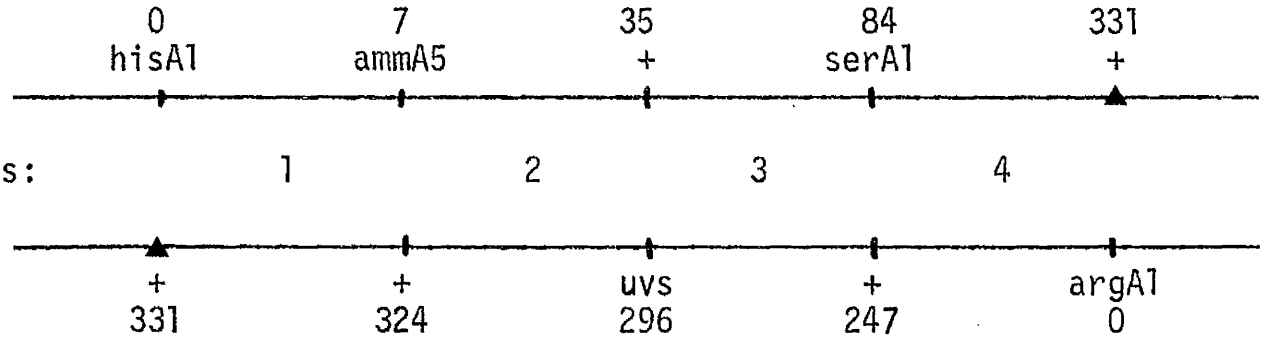
The crosses, their results and their analysis are presented in Table 27. The allele frequencies of $^{uvs^+}/_{uvs} = {}^{35}/_{296}$ for the pooled results clearly located *uvs* between *ammA* and *serA*. The data for the individual crosses all supported this location, which required only 5 observed recombinants to arise by multiple crossovers for this arc of the map. Any other order required many more multiple crossover recombinants, 33 for the order *hisA uvs ammA serA argA* and 51 for the order *hisA ammA serA uvs argA*. The order of the genes was therefore *hisA ammA* (*uvsA uvsC uvsD*) *serA argA*.

4. Results for *uvsB6*, *uvsE13* and *uvs-21*.

A complementation test of the type used to discriminate different genes amongst the top group of 20 mutations has not yet been successfully applied to these three mutations, *uvs-6*, *uvs-13*, *uvs-21*. *Uvs-6* was used to define gene *uvsB*.

Since the primary mapping had not conclusively ordered these mutations with respect to *strA*, this was done for each mutation as follows. Strains of genotype *mthB2 uvsB6*, *cysD18 uvsE13*, and *pheA1 uvs-21* were obtained by recombination. The *uvsB6* strain was crossed with strain 59 *hisD3 argA1 uraA1* and $mthB^+$ $uraA^+$ recombinants

TABLE 27. The ordering of *uvsA*, *uvsC* and *uvsD* relative to *ammA* and *serA*.



Genotype	Crossover interval	Mutations (a) *uvsA4*	*uvsC7*	*uvsD3, 5, 1*	Total (b)
amm + ser	1	2	0	3	5
+ + ser	2	2	13	13	28
+ uvs ser	3	11	17	23	51
+ uvs +	4	79	61	103	243
+ + +	2,3,4	1	0	1	2
amm uvs +	1,2,4	0	0	2	2
Total recombinants		95	91	145	331

The strains used were: V22 *argA1 mthB2 pheA1 strA1 uvsA4*, V145 *argA1 uvsC7*, V7 *argA1 mthB2 pheA1 strA1 uvsD1*, V19 *argA1 mthB2 pheA1 strA1 uvsD3* and V23 *argA1 cysD18 mthB2 pheA1 strA1 uvsD5* each crossed with 948 *hisA1 ammA5 serA1*.

See also legend to Table 26.

selected; the *uvsE13* strain was crossed with strain 36 *hisD3 pheA1* and *cysD*$^+$ *pheA*$^+$ recombinants selected; and the *uvs-21* strain was crossed with 59 *hisD3 argA1 uraA1* and *hisD*$^+$ *pheA*$^+$ recombinants selected.

The parental marker arrangements of these crosses, the results and their analysis are illustrated in Tables 28, 29 and 30 for *uvsB6*, *uvsE13* and *uvs-21* respectively.

The allele frequencies $^{uvsB6}/_{uvs^+} = {}^{16}/_{81}$, $^{uvsE13}/_{uvs^+} = {}^{17}/_{31}$ and $^{uvs-21}/_{uvs^+} = {}^{36}/_{52}$, located each *uvs* mutation between *strA* and *hisD*. This location required a minimum number of multiple crossover recombinants, one for the *uvsB6* cross, none for the *uvsE13* cross, and one for the *uvs-21* cross.

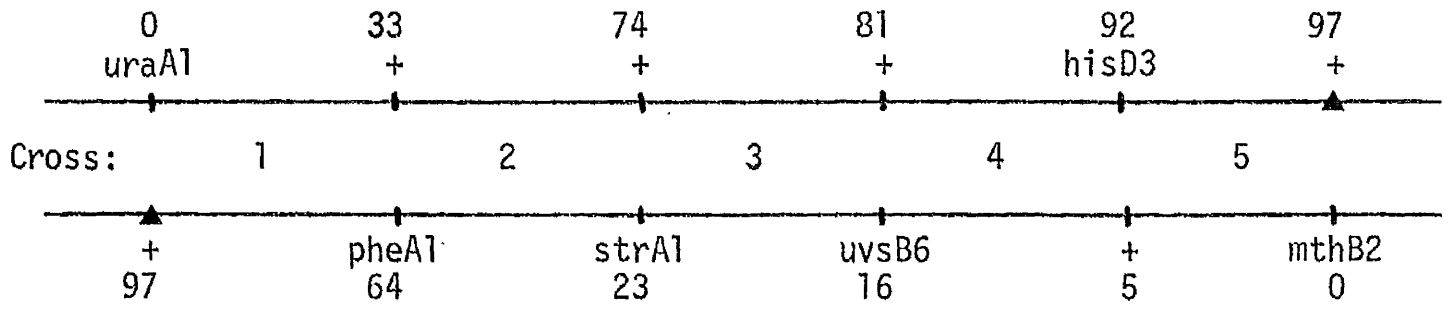
Finally these mutations were ordered relative to *strA guaA hisD* by crossing them with strain 255 *argA1 guaA1*.

The results for *uvsB6* and *uvs-13* will be considered together since the crosses were similar with selection for *strA1 mthB*$^+$ recombinants and they had a similar location. The cross with *uvs-21* will be considered separately since the wild-type allele of a different gene, *cysC* was used instead of *mthB*$^+$ as one of the points of selection.

The results for *uvsB6* and *uvs-13* are presented in Table 31, and for *uvs-21* in Table 32. The known order of all the markers between *strA* and *cysC* involved in the crosses described was *strA-guaA-hisD-mthB-cysC*. Region 3 was therefore longer in the cross with *uvs-21*.

For *uvsB6* and *uvs-13* the allele ratios of $^{uvs^+}/_{uvs} = {}^{48}/_{225}$ located *uvs* very close to *guaA* between *guaA* and *mthB*. Amongst the recombinants analysed in these crosses there was only one observed recombinant in each cross, of genotype *guaA*$^+$ *hisD*$^+$, which gave information on their order with respect to *guaA* amongst 162 and 111

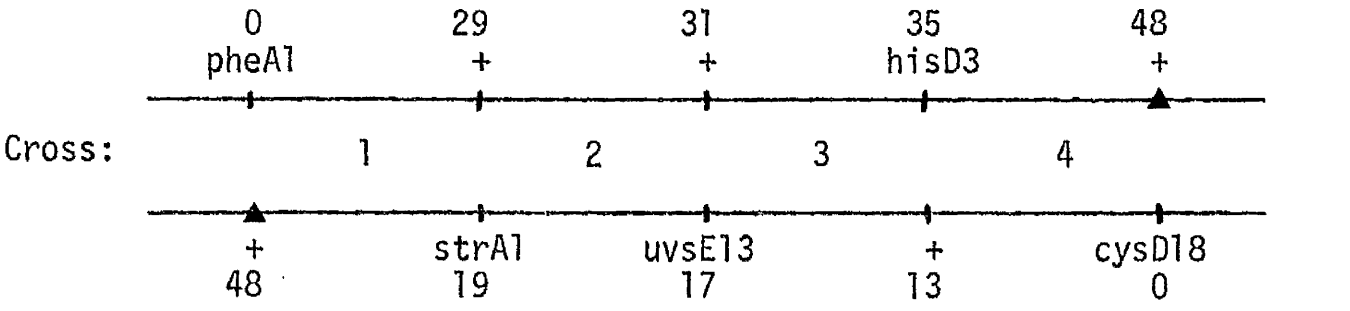
TABLE 28. The ordering of *uvsB6* relative to *strA*.



Genotype (c)	Crossover interval	Mutation (a) (b) *uvsB6*
his	1	32
phe his	2	42
phe str his	3	6
phe str uvs his	4	11
phe str uvs	5	5
str his	1,2,3	1
Total recombinants		97

The strains used were V30 *proA1 mthB2 pheA1 strA1 uvsB6* crossed with 59 *hisD3 argA1 uraA1*.

TABLE 29. The ordering of *uvsE13* relative to *strA*.



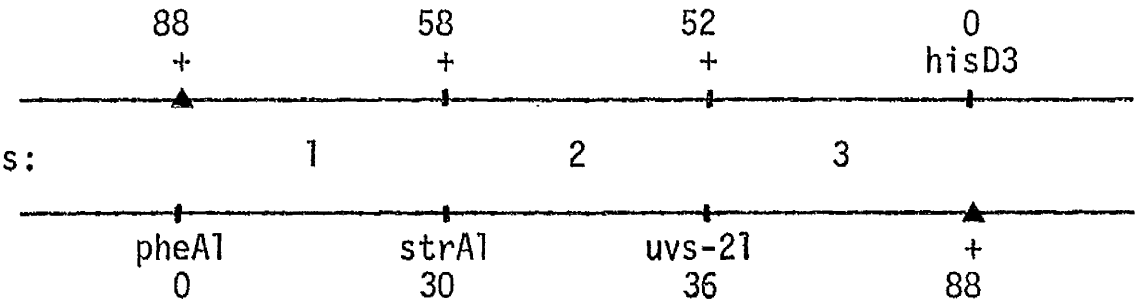
Genotype (c)	Crossover interval	Mutation (a) (b) *uvsE13*
his	1	29
str his	2	2
str uvs his	3	4
str uvs	4	13
Total recombinants		48

The strains used were V47 *proA1 argA1 cysD18 uraA1 strA1 uvsE13* crossed with 36 *hisD3 pheA1*.

(c) Wild-type alleles omitted.

See also legend to Table 26.

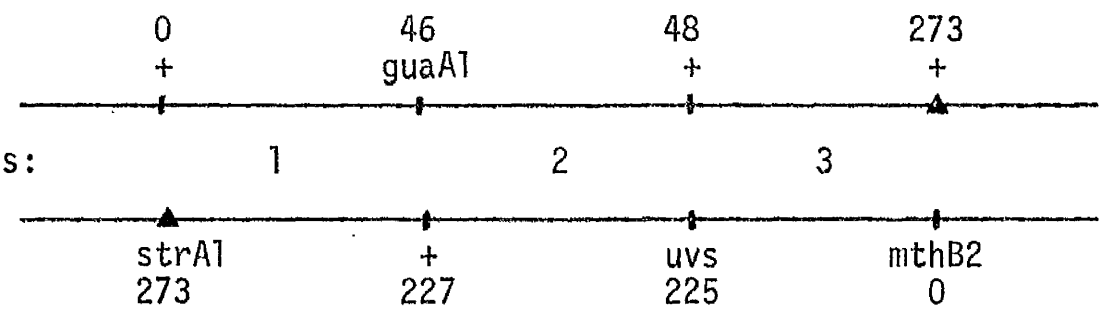
TABLE 30. The ordering of *uvs-21* relative to *strA*.



Genotype	Crossover interval	Mutation (a) (b) *uvs-21*
str uvs	1	29
+ *uvs*	2	7
+ +	3	51
str +	1,2,3	1
Total recombinants		88

The strains used were V123 *proA1 cysC3 pheA1 strA1 uvs-21* crossed with 59 *hisD3 argA1 uraA1*.

TABLE 31. The ordering of *uvsB6* and *uvsE13* relative to *guaA* .

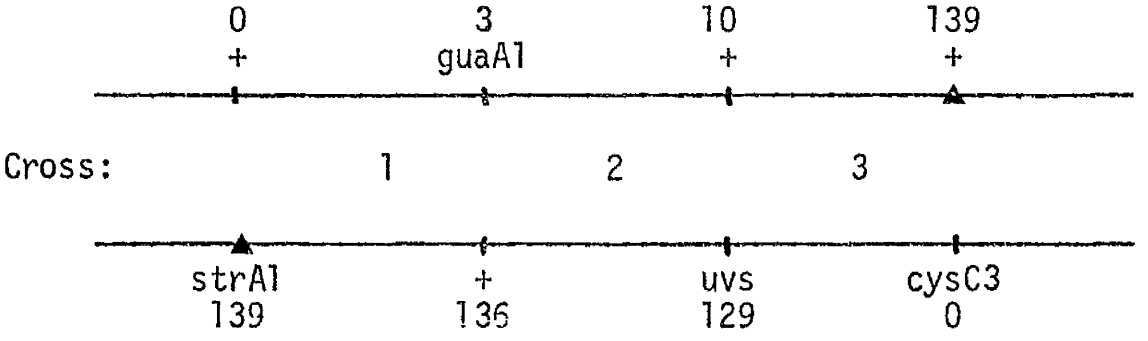


Genotype	Crossover interval	Mutations (a) *uvsB6*	Mutations (a) *uvs-13*	Total (b)
gua +	1	26	20	46
+ +	2	1	1	2
+ *uvs*	3	135	90	225
Total recombinants		162	111	273

The strains used were V9 *hisA1 mthB2 pheA1 strA1 uvsB6*, V25 *hisA1 mthB2 pheA1 strA1 uvsE13* crossed with 255 *argA1 guaA1*.

See legend to Table 26.

TABLE 32. The ordering of *uvs-21* relative to *guaA*.



Genotype	Crossover interval	Mutation (a)(b) *uvs-21*
gua +	1	3
+ +	2	7
+ *uvs*	3	129
Total recombinants		139

The strains used were V133 *proA1 cysC3 pheA1 strA1 uvs-21* crossed with 255 *argA1 guaA1*.

See legend to Table 26

recombinants observed, respectively. This location requires no multiple crossover classes in the recombinants observed for the region *strA-mthB*. The evidence for *uvs-21* was stronger; the allele ratio for $^{uvs+}/uvs$ = $^{10}/129$ clearly located *uvs-21* between *guaA* and *cysC*. This location again required no multiple crossover classes amongst the recombinants observed for the region *strA - cysC*.

The overall order for all three *uvs* mutations was concluded to be *strA-guaA-(uvsB6, uvsE13, uvs-21)-hisD-mthB-cysC*.

D. Ordering of the genes *uvsA*, *uvsC* and *uvsD*.

1. Experimental procedure.

Pairwise crosses were made between representative mutations of each of the genes *uvsA*, *uvsC* and *uvsD*, in both coupling arrangements with respect to the outside markers *hisA1* and *argA1*, selecting *his*$^+$ *arg*$^+$ recombinants. The proportion of *uvs*$^+$ haploid recombinants amongst the progeny of each cross was determined. For each pair of crosses, depending upon the order of the *uvs* markers in the region between *hisA* and *argA*, one cross was expected to yield *uvs*$^+$ recombinants by a single crossover in this region, and with higher frequency than the other in which three crossovers were required.

ArgA$^+$ *hisA*$^+$ recombinants were selected from 0.1ml aliquots plated at accurate dilutions (Section II C 3) of 10^0, 10^{-1} and 10^{-2}. These trial platings were incubated for two days, when recombinant colonies were counted, the spore suspensions having been stored at 4^oC meanwhile. A suitable dilution was calculated from the results of these counts which would yield approximately 200 recombinant colonies per plate; 12 such plates were prepared for each cross. These plates were

incubated for five or six days, when well-sporulating colonies had developed. Two replicas of each plate were made with a velvet pad to plates of the same medium. The first of each pair of replicas was exposed to a dose of UV which would kill approximately 50% of *uvs*$^{+}$ spores transferred to the replica but more than 99% of *uvs* spores. The second replica was kept as a control of successful replication of each colony. The pairs of replicas were incubated together for two days, when the control replica was counted to give the number of recombinant colonies successfully screened. Then a comparison of each pair of plates was made, and any replica patches which appeared to represent confluent (Uvs^{+}) growth on the irradiated plate were inoculated to a defined patch of a new plate of the same medium (20 patches per plate). These putative *uvs*$^{+}$ recombinants were again tested for Uvs^{+} phenotype by comparison of the replica plates and the number confirmed as *uvs*$^{+}$ was recorded.

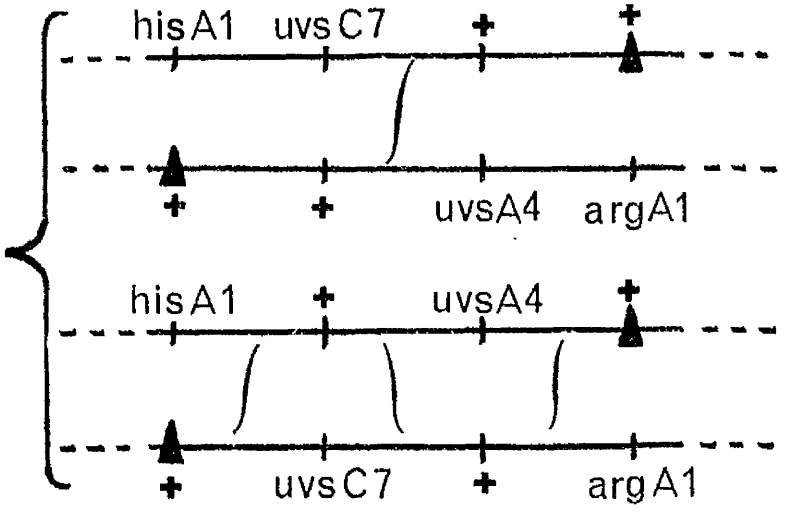
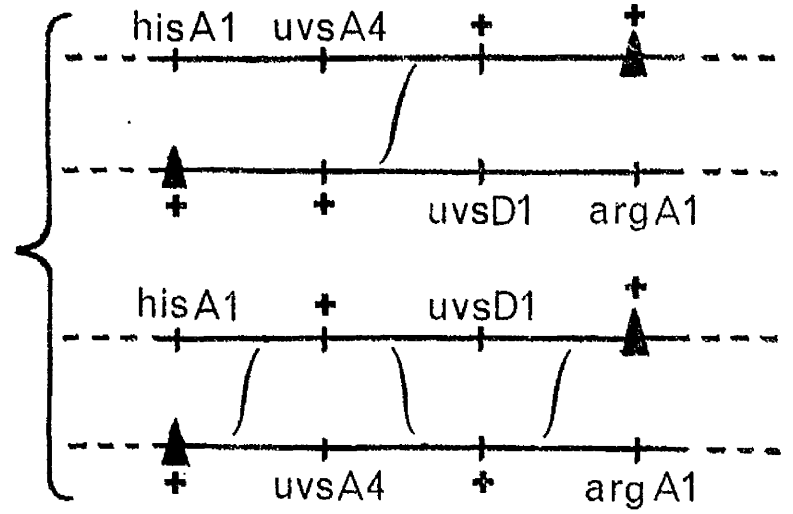
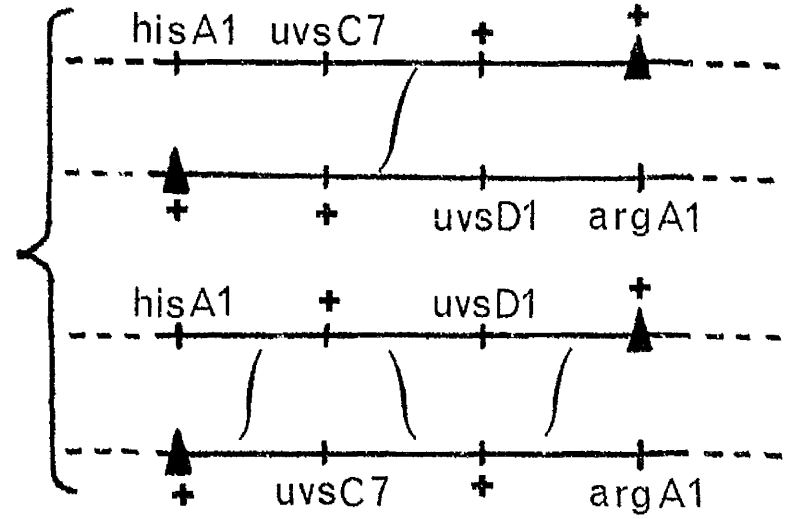
2. Results.

The coupling relationships of the parents in the crosses, the number of colonies screened, and the numbers of *uvs*$^{+}$ recombinants amongst these are presented in Table 33.

It can be seen from the results that, for each pair of reciprocal crosses, one yielded eight to seventeen times more *uvs*$^{+}$ recombinants than the other. These results were consistent with the orders of genes illustrated in Table 33 for each cross and an overall gene order of *hisA-uvsC-uvsA-uvsD-argA*.

One possible source of error in this experiment may lie in the fact that not all the recombinants were tested for their UV sensitivity; only those that could replicate successfully were screened, and this

TABLE 33. The ordering of the genes *uvsA*, *uvsC* and *uvsD*.

CROSS	RECOMBINANTS TESTED	%*uvs*$^+$
	1909	5.6
	2285	0.7
	4197	0.24
	3809	<0.03
	1646	2.0
	1643	0.12

/ indicate the half-crossovers required to give *uvs*$^+$ recombinants.

See text for explanation.

proportion varied according to their density on the plates. Although this was always a large majority of the total recombinants, the possibility remains that those not tested because of their failure to replicate did not represent a random sample with respect to UV sensitivity, so that the results might have been distorted. This gene order is therefore presented with this reservation.

E. The analysis of strain V60 as a double mutant.

1. The detection of two levels of UV sensitivity in recombinants of V60.

During the course of studying survival curves of the mutant strains, some *uvs* recombinant strains were also studied. One of these, a recombinant from a cross involving strain V60 *uvs-18*, which was the most sensitive mutant strain obtained, exhibited a UV sensitivity much lower than that of V60, but greater than that of uvs^+ strains. The data for the survival curves of this recombinant strain, which was designated V115 *proA1 hisC9 strA1 uvsD18*, and V60 *uvs-18* are presented in Table 34 and the curves plotted in Figure 20 together with a representative curve of a uvs^+ strain. V115 was used in the complementation tests already described, and *uvs-18* was found to be a mutation in gene *D*. However, this mutation, *uvsD18*, was clearly not responsible for the whole of the greater sensitivity of strain V60 when compared with uvs^+ strains. It was postulated that V60 contained a second mutation (conferring UV sensitivity) which was designated *uvs-25* and which might be separable from *uvsD18* by recombination. The fact that V60 contained two mutations would, if proven, explain why this particular apparently single step mutant strain was appreciably more sensitive

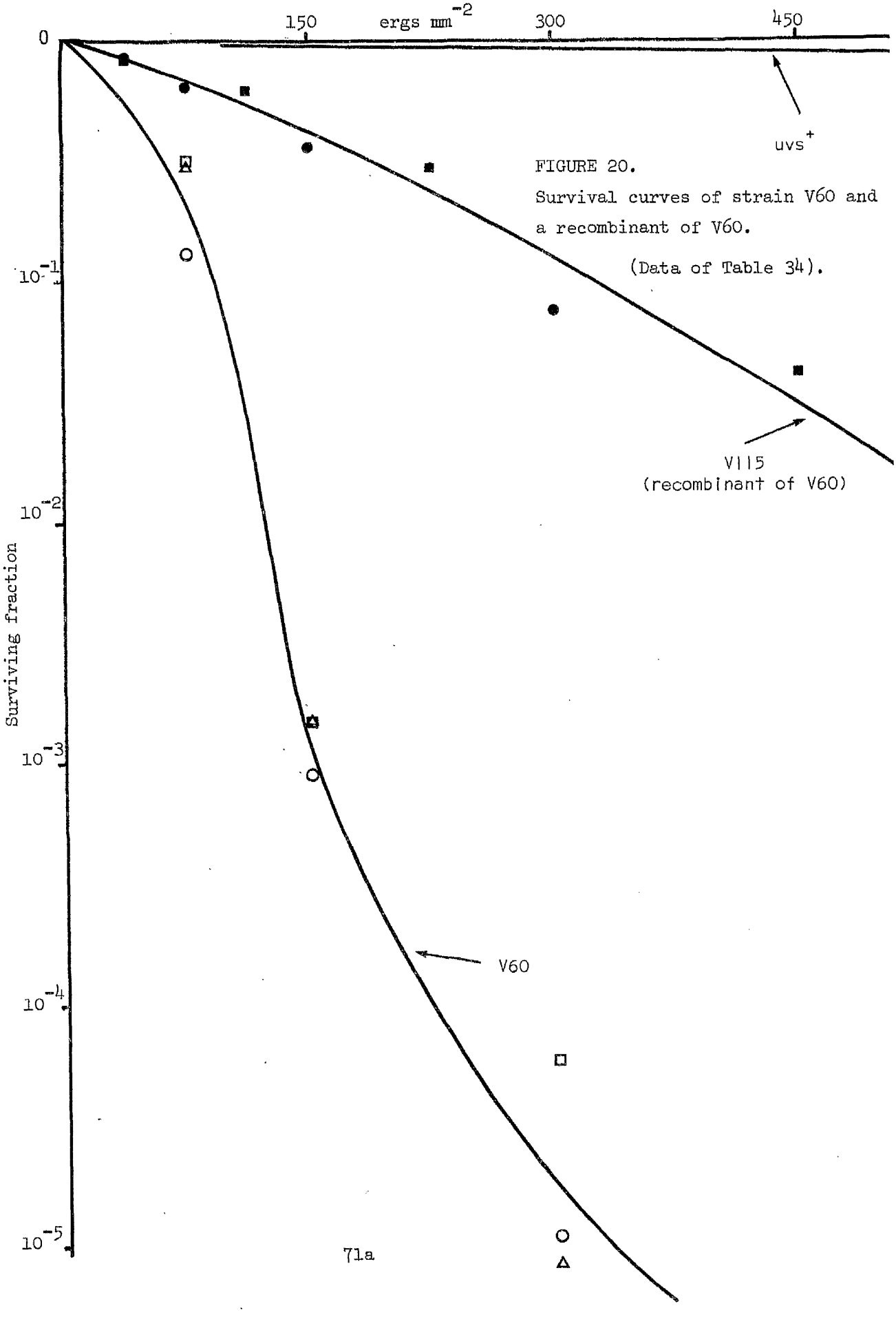


FIGURE 20.
Survival curves of strain V60 and a recombinant of V60.

(Data of Table 34).

TABLE 34. Data for the survival curves of strain V60 and a recombinant of V60 (plotted in Figure 20).

Strain V60 *uvsD18 uvsF25.*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure 1 Open circles a	b	2 Open squares a	b	3 Open erect triangles a	b
0	154×10^{4}	1.0	130×10^{4}	1.0	231×10^{4}	1.0
75	195×10^{3}	1.3×10^{-1}	399×10^{3}	3.1×10^{-1}	683×10^{3}	3.0×10^{-1}
150	139×10	9.0×10^{-4}	196×10	1.5×10^{-3}	347×10	1.5×10^{-3}
300	17×1	1.1×10^{-5}	64×1	4.9×10^{-5}	20×1	8.7×10^{-6}
No. of samples at each dose	3		3		3	

Strain V115 *proA1 hisC9 strA1 uvsD18.*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure 1 Closed circles a	b	2 Closed squares a	b
0	357×10^{4}	1.0	676×10^{5}	1.0
37.5	303×10^{4}	8.5×10^{-1}	568×10^{5}	8.4×10^{-1}
75	233×10^{4}	6.5×10^{-1}	-	-
112.5	-	-	422×10^{5}	6.2×10^{-1}
150	1300×10^{3}	3.6×10^{-1}	-	-
225	-	-	2020×10^{4}	3.0×10^{-1}
300	2749×10^{2}	7.7×10^{-2}	-	-
450	-	-	2865×10^{3}	4.2×10^{-2}
No. of samples at each dose	3		3	

See legend to Table 13.

than the rest.

In order to test whether or not the appearance of strains with the sensitivity of V115 was an unusual event, a cross was made between V60 *uvsD18 uvs-25* and 876 *proA1 hisC9 argA1 cysC3 pheA1 strA1* and a sample of the recombinants selected to be *pro*$^+$ *strA1* was analysed for auxotrophic markers as described in Section IV A 3.

Classification of the UV sensitivity of the recombinants was done using three replica plates with the same composition as the master plate. The first replica was exposed to about 150 ergs mm^{-2}, the second to about 900 ergs mm^{-2} and the third kept as an unirradiated control of satisfactory replication. The dose of 150 ergs mm^{-2} should have killed more than 99% of spores which were *uvsD18 uvs-25*, but not more than 70% of spores which were *uvsD18* only, and a considerably smaller proportion of spores which were *uvs*$^+$; this dose should therefore have distinguished *uvsD18 uvs-25* strains from the others. The dose of 900 ergs mm^{-2} should have killed more than 99% of spores which were *uvsD18* and even more of spores which were *uvsD18 uvs-25*, but less than 50% of spores which were *uvs*$^+$; it should therefore have distinguished *uvs*$^+$ recombinants from both *uvsD18 uvs-25*$^+$ and *uvsD18 uvs-25* recombinants. The characterisation of the recombinants, by their growth response on the three replicas, is summarised below.

	UV dose ergs mm^{-2}.		
Presumed Genotype	0	150	900
uvsD18 uvs-25	+	-	-
uvsD18 uvs-25$^+$	+	+	-
uvsD$^+$ *uvs-25*$^+$	+	+	+

where + indicates growth apparently unaffected by the irradiation

and - indicates absent or poor growth compared with that at 0 ergs mm^{-2}.

The classification of UV sensitivity was first carried out on replicas of the original master plate, then repeated on secondary master plates made by subculturing recombinant patches from the original master plate to larger defined areas of a fresh plate of the same composition (20 recombinants per plate) to clarify the classification. As a final test of this classification, two recombinants of each mutant UV sensitivity group were isolated and purified by streaking and the survival of a spore suspension measured at two low doses of UV, sufficient to distinguish the levels of sensitivity of strains like V115 from that of strains like V60. Spore suspensions were prepared and treated for UV survival measurements by the methods described in Section III C 1, except that a general inoculum from a stock slant was used to inoculate the large slant from which the spore suspension was prepared. The results for the four isolated recombinants, their classification from replica plates and survival levels of strains V60 and V115 at the same UV doses are presented in Table 35a, and clearly confirm the classification made from the irradiated replica plates.

2. The location of *uvs-25*.

Analysis of the results obtained for this cross were complicated, since although four UV sensitivity genotypes could theoretically appear in the recombinant progeny, only three phenotypic classes could be distinguished: those due to the *uvsD18 uvs-25*, *uvsD18 uvs-25*$^+$ and *uvsD*$^+$ *uvs-25*$^+$ genotypes. *UvsD*$^+$ *uvs-25* recombinants were either absent from the progeny or present but classified as one of the three other groups. If they were absent, then analysis of the recombinants observed would give a true location for *uvs-25*. If they were present,

TABLE 35. The classification of strains as *uvs*$^+$, *uvsD18 uvsF*$^+$ or *uvsD18 uvsF25*.

(a) Strains from V60 *uvsD18 uvsF25* x 876 *uvs*$^+$ cross.

Genotype from replica plates		ergs mm^{-2} 0	150	300	*Uvs* genotype from UV survival
pheA1 strA1 uvsD18 uvsF25	i	1065 x 10^3	714 x 10	11 x 1	
	ii	1.0	5.5 x 10^{-3}	1.0 x 10^{-5}	*uvsD18 uvsF25*
cysC3 pheA1 strA1 uvsD18 uvsF25	i	363 x 10^3	55 x 10	3 x 1	
	ii	1.0	1.5 x 10^{-3}	6.9 x 10^{-6}	*uvsD18 uvsF25*
pheA1 strA1 uvsD18 uvsF$^+$	i	1449 x 10^4	4220 x 10^3	6980 x 10^2	
	ii	1.0	2.9 x 10^{-1}	4.8 x 10^{-2}	*uvsD18 uvsF*$^+$
cysC3 pheA1 strA1 uvsD18 uvsF$^+$	i	860 x 10^3	420 x 10^3	716 x 10^2	
	ii	1.0	4.7 x 10^{-1}	8.1 x 10^{-2}	*uvsD18 uvsF*$^+$
Strain V60	ii	1.0	1.5 x 10^{-3}	2.3 x 10^{-5}	
Strain V115	ii	1.0	4.1 x 10^{-1}	1.2 x 10^{-1}	

(b) Strains from V60 *uvsD18 uvsF25* x V155 *uvsD18 uvsF*$^+$ cross.

uvsD18 uvsF25	i	144 x 10^4	68 x 10^2	7 x 10	
	ii	1.0	4.7 x 10^{-3}	4.5 x 10^{-5}	*uvsD18 uvsF25*
uvsD18 uvsF25	i	387 x 10^4	202 x 10^2	12 x 10	
	ii	1.0	5.2 x 10^{-3}	3.0 x 10^{-5}	*uvsD18 uvsF25*
cysC3 uvsD18 uvsF25	i	195 x 10^3	11 x 10	2 x 1	
	ii	1.0	5.7 x 10^{-4}	1.0 x 10^{-5}	*uvsD18 uvsF25*
cysC3 uvsD18 uvsF$^+$	i	442 x 10^3	422 x 10^2	711 x 10	
	ii	1.0	1.4 x 10^{-1}	1.6 x 10^{-2}	*uvsD18 uvsF*$^+$
uvsD18 uvsF25	i	156 x 10^4	1030 x 10	138 x 1	
	ii	1.0	6.6 x 10^{-3}	8.7 x 10^{-5}	*uvsD18 uvsF25*
cysC3 uvsD18 uvsF$^+$	i	306 x 10^3	370 x 10^2	261 x 10	
	ii	1.0	1.2 x 10^{-1}	8.5 x 10^{-3}	*uvsD18 uvsF*$^+$

(c) Strains from V157 *uvsD*$^+$ *uvsF25* x V155 *uvsD18 uvsF*$^+$ cross.

pheA1 strA1 uvsD18 uvsF25	i	451 x 10^3	145 x 10	9.0 x 1	
	ii	1.0	3.2 x 10^{-3}	1.9 x 10^{-5}	*uvsD18 uvsF25*
pheA1 strA1 uvsD18 uvsF25	i	50 x 10^3	33 x 10	2 x 1	
	ii	1.0	6.6 x 10^{-3}	4.0 x 10^{-5}	*uvsD18 uvsF25*

i. Average of the number of colonies counted per replicate sample x dilution factor. Two replicates were counted for all samples.

ii. Surviving fraction. See text for explanation.

but misclassified then the following table indicates whether or not the allele ratios of *uvsD18*/*uvsD*$^+$ and *uvs-25*/*uvs-25*$^+$ would be correct.

	uvsD$^+$ *uvs-25* classified as:		
Allele ratios	*uvsD18 uvs-25*	*uvsD18 uvs-25*$^+$	*uvsD*$^+$ *uvs-25*$^+$
uvsD18/*uvsD*$^+$	incorrect	incorrect	correct
uvs-25/*uvs-25*$^+$	correct	incorrect	incorrect

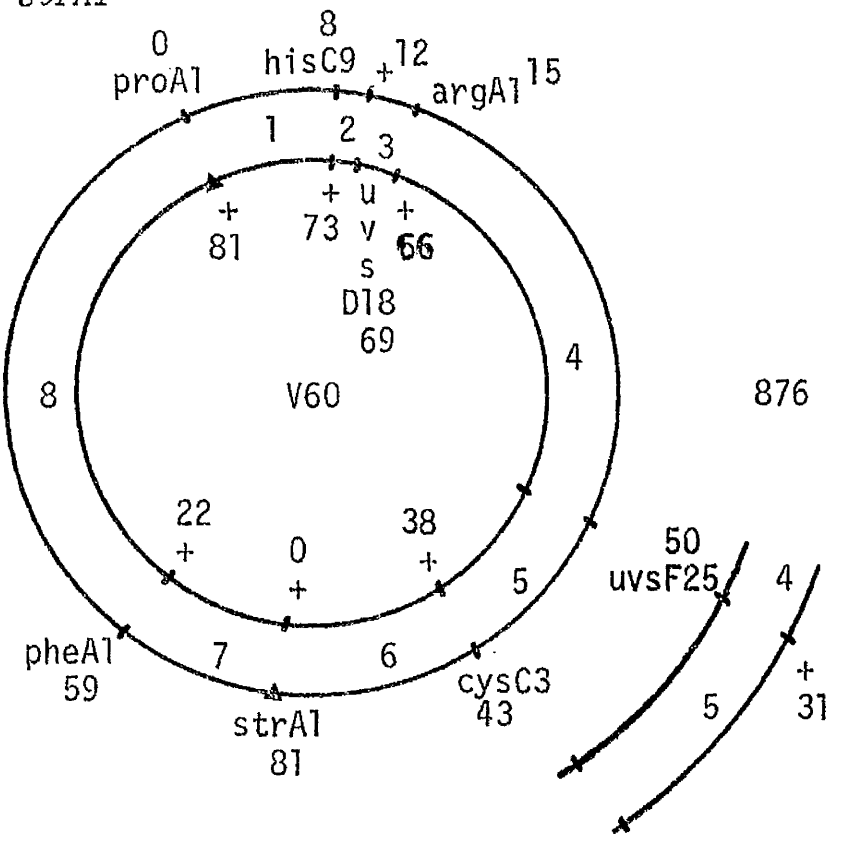
The data for the cross are presented in Table 36 and analysed in Figure 21. Analysis of the recombinants as a whole, Figure 21a, located *uvsD18*/*uvsD*$^+$ correctly between *hisA* and *argA*; therefore *uvsD*$^+$ *uvs-25* was either classified as *uvsD*$^+$ *uvs-25*$^+$ or it was sufficiently infrequent not to cause mislocation of *uvsD*. In the first case, the *uvsD18 uvs-25* and *uvsD18 uvs-25*$^+$ classes would have been correctly classified and we could locate *uvs-25*/*uvs-25*$^+$ by considering only *uvsD18* recombinants (equivalent to introducing *uvsD18* as an additional point of selection) as in Figure 21b. In the latter case, analysis of all the recombinants would give at least an approximate location for *uvs-25*, Figure 21a. In fact both analyses gave the same location for *uvs-25*, between *cysC* and *argA*. This location was compatible with the recombinant genotype *uvsD*$^+$ *uvs-25* being a rare multiple crossover class.

In order to observe an unambiguous segregation of *uvs-25* two strains both carrying *uvsD18* (V60 *uvsD18 uvs-25* and V155 *cysC3 pheA1 strA1 uvsD18)* were crossed, enabling *uvs-25* to be mapped in the usual way.

The two levels of UV sensitivity (due to *uvsD18 uvs-25*$^+$ or *uvsD18 uvs-25* recombinants) were classified on three replica plates exposed to 0, 150 and 900 ergs mm^{-2} as before. This classification was repeated

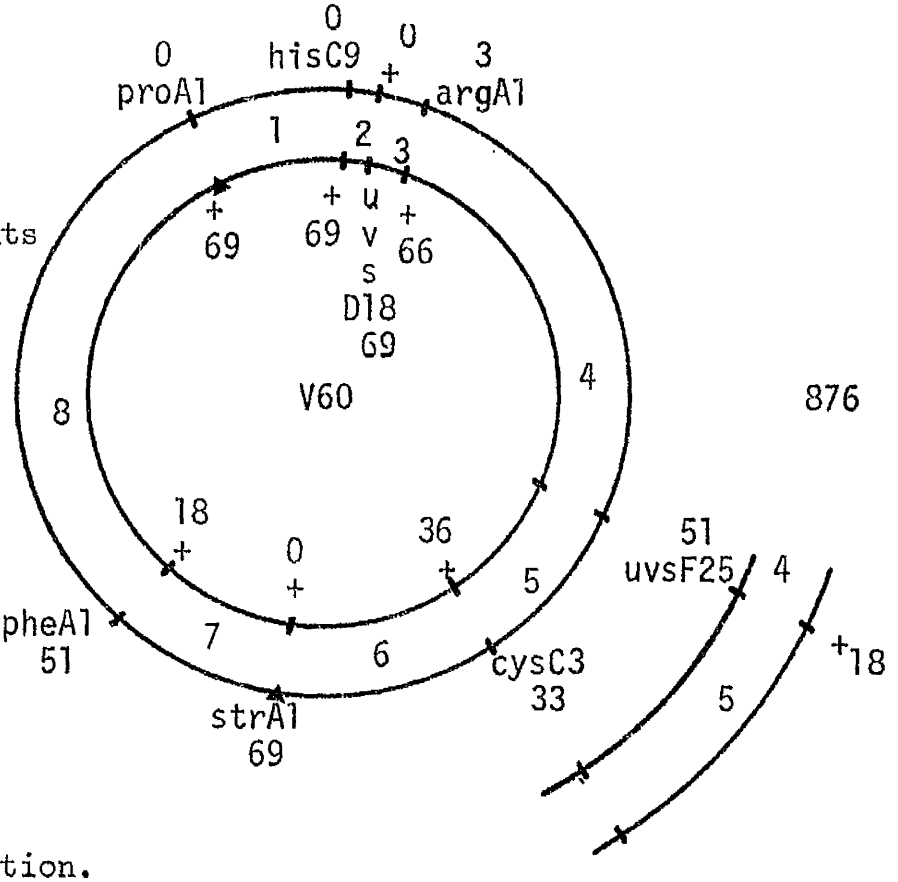
FIGURE 21. Location of *uvsF25* in a cross heterozygous for *uvsD* and *uvsF*. (Data of Table 36).

(a) Analysis of *proA*$^+$ *strA1* recombinants.



Possible locations of *uvsF25*.	Multiple crossover recombinants
cysC argA	6
pheA proA	9

(b) Analysis of *proA*$^+$ *strA1 uvsD18* recombinants only.



Possible Locations of *uvsF25*	Multiple crossover recombinants
cysC argA	4
pheA proA	5

See text for explanation.

See also legend to Figure 14.

TABLE 36. The frequencies of recombinants observed in a cross heterozygous for *uvsD* and *uvsF*. (Data analysed in Figure 21).

Genotype	Crossover intervals	Number
his arg cys phe	1, 8	4
his arg cys	1, 7	3
arg cys phe	2, 8	2
arg cys	2, 7	1
uvsD18 arg cys phe	3, 8	1
uvsD18 cys phe	4, 8	12
uvsD18 cys	4, 7	4
uvsD18 uvsF25 cys phe	5, 8	10
uvsD18 uvsF25 cys	5, 7	6
uvsD18 uvsF25 phe	6, 8	25
uvsD18 uvsF25	6, 7	7
his arg phe	1, 5, 6, 8	1
arg phe	2, 5, 6, 8	1
uvsD18 uvsF25 arg phe	3, 4, 6, 8	2
uvsD18	4, 5, 6, 7	1
uvsD18 phe	4, 5, 6, 8	1
Total recombinants		81

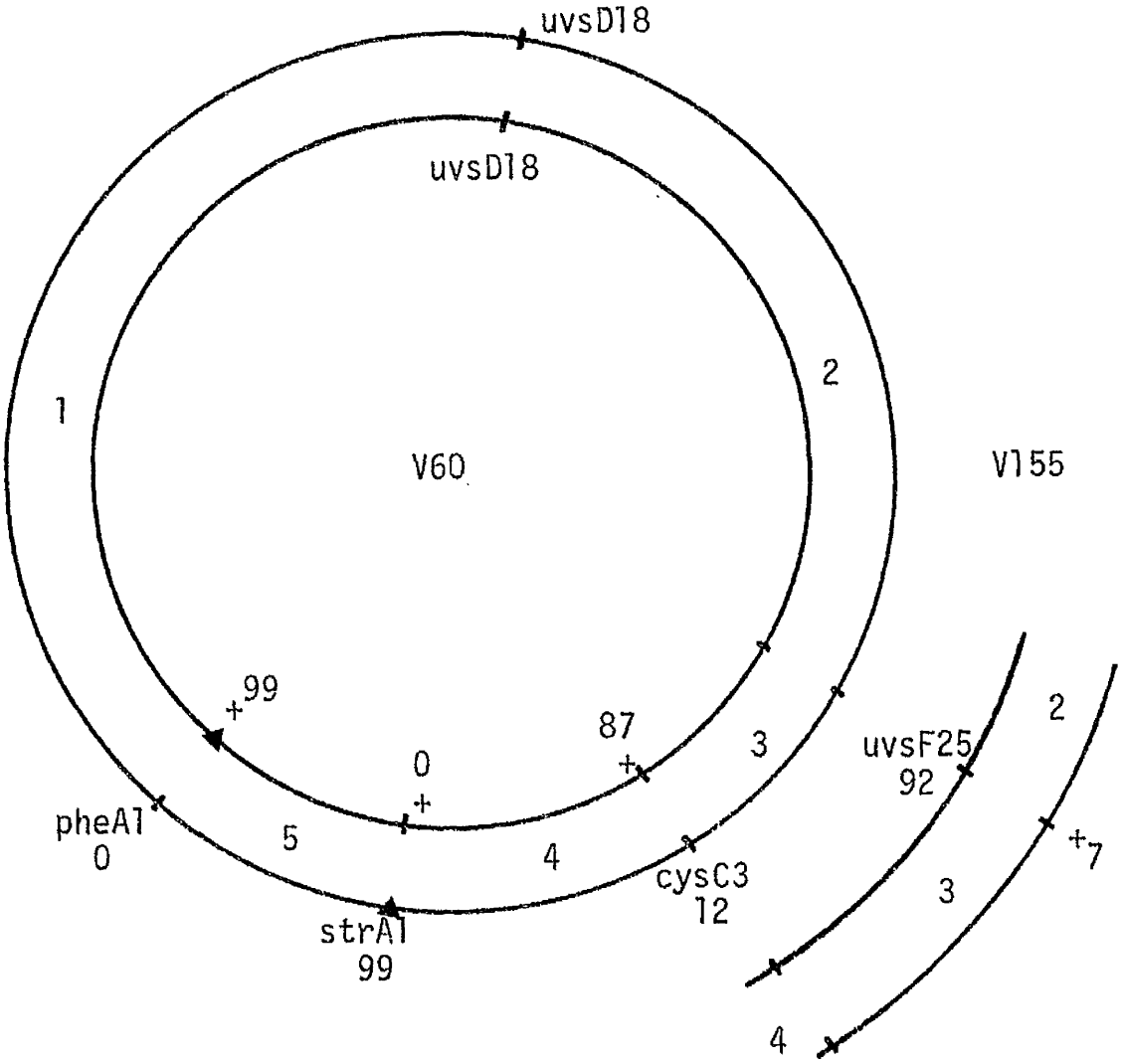
from secondary master plates streaked with 20 recombinant patches, and six recombinants were purified and their sensitivity checked by determining the surviving fraction of a spore suspension exposed to 0, 150 and 300 ergs mm^{-2}. The results are presented in Table 35b together with their classification from irradiated replicas, and it can be seen that the two methods of assessing the sensitivity were in agreement. The data for the cross are presented and analysed in Figure 22. The ratio for $^{uvs-25}/_{uvs-25^+} = {}^{92}/_{7}$ located *uvs-25* in regions 1 or 2 of Figure 22. The location as illustrated near *cysC* was compatible with the polarity of the cross (the crossovers were concentrated on either side of *strA*) and the location already indicated by the previous cross. This location required none of the observed recombinants to be multiple crossover classes. The location of this mutation in a position distant from the genes *uvsA-E* defined another gene affecting UV sensitivity, designated *uvsF*.

3. Isolation and characterisation of a strain *uvsD*$^+$ *uvsF25*.

A cross was made between V60 *uvsD18 uvsF25* and 999 *hisA1 argA1 cysD18 pheA1 tps-30 strA1*, and *strA1 cysD*$^+$ recombinants selected. The cross is illustrated in Figure 23. A sample of recombinants was analysed, and four *hisA1 argA1 pheA1 tps*$^+$ recombinant strains were purified. They should all have been *uvsD*$^+$, on the assumption that they were simple crossover recombinants, but might have shown segregation for *uvsF*$^+$ and *uvsF25*. None of these recombinants was apparently sensitive on replica plates exposed to doses of UV up to 1600 ergs mm^{-2}.

Two of these strains were crossed with V155 *cysC3 pheA1 strA1 uvsD18* and selection made for *cysC*$^+$ *argA*$^+$ recombinants (the cross is

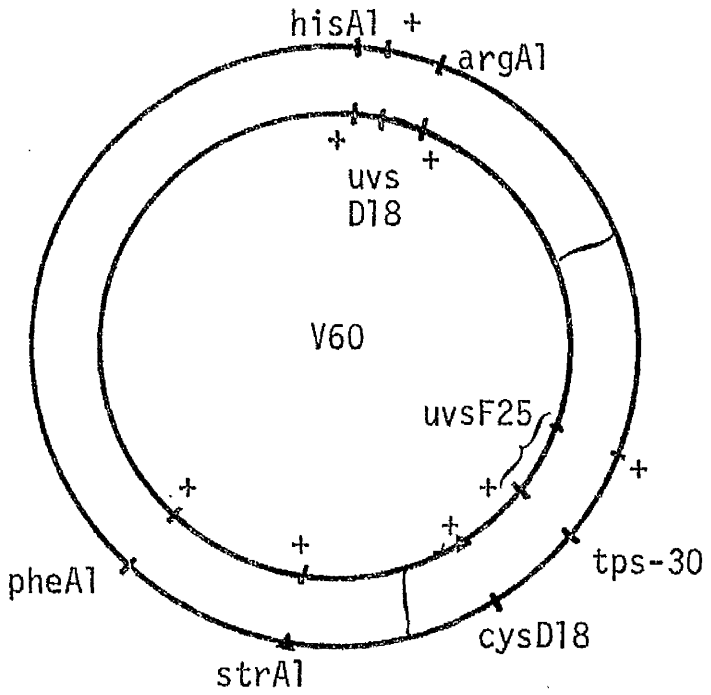
FIGURE 22. The location of *uvsF25* in a cross homozygous for *uvsD* and heterozygous for *uvsF*.



Genotype	Crossover intervals	Number
+ *uvsD18 uvs-25*	4,5	87
cys uvsD18 uvs-25	3,5	5
cys uvsD18 +	1 or 2,5	7
Total recombinants		99

See legend to Figure 14.

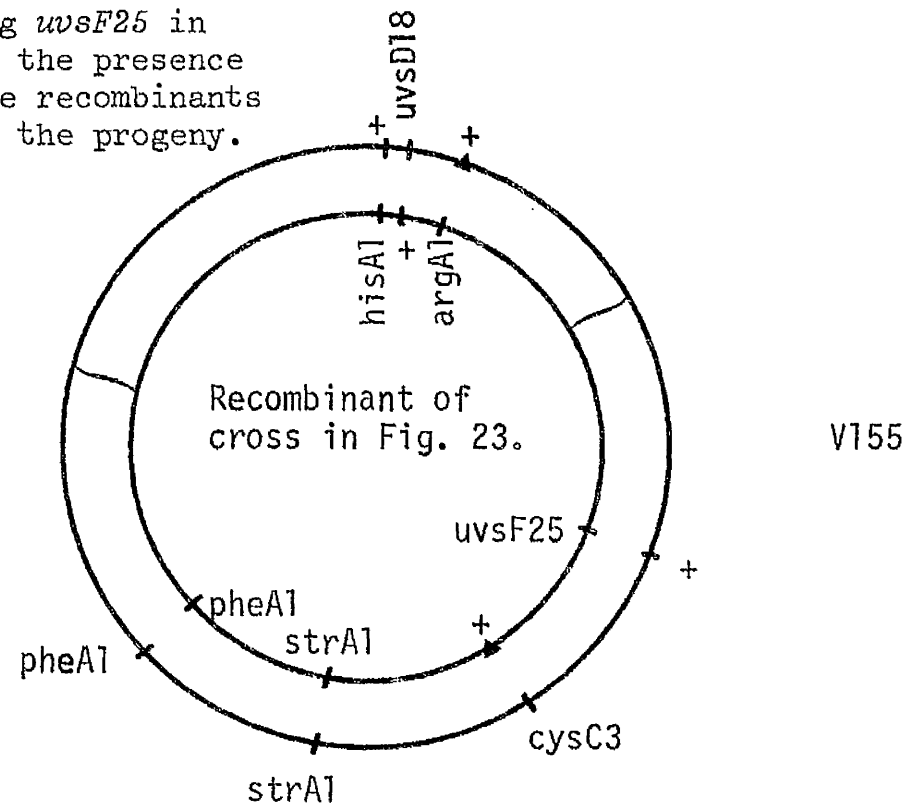
FIGURE 23. Cross for obtaining a *uvsF25 uvsD*$^{+}$ strain.



{indicates that *uvsF25* had not been ordered with respect to *tps-30*.

FIGURE 24.

Cross for detecting *uvsF25* in a parent strain by the presence of highly sensitive recombinants *(uvsD18 uvsF25)* in the progeny.



{indicate the intervals in which recombination would produce the desired recombinant.

illustrated in Figure 24); it was expected that if the mutation *uvsF25* had been present in either of the strains then highly sensitive *uvsD18 uvsF25* recombinant strains should have appeared in the progeny of the cross as simple crossover classes. This was so for one of the crosses; two recombinants classified from replicas of primary and secondary master plates as having sensitivity due to the genotype *uvsD18 uvsF25* were purified and showed the survival levels presented in Table 35c, when spore suspensions prepared for UV survival curves were exposed to 0, 150, and 300 ergs mm^{-2}. These results confirmed the presence of *uvsF25* in this recombinant derived from the V60 by 999 cross and this strain was designated V157 *hisA1 argA1 pheA1 strA1 uvsF25*. UV survival curves were obtained for this strain as described in Section III C 1. The results are presented in Table 37 and plotted and compared with the curve for A3(2) in Figure 25. Clearly the *uvsF25* mutation by itself did not enhance the sensitivity of a strain otherwise uvs^{+}.

4. The effect of *uvsF25* on the sensitivity of strains containing *uvsD3*, *uvsA4* or *uvsC10*.

UvsF25 was recognised by its enhancement of the sensitivity of strains bearing *uvsD18*. Crosses were performed to see if *uvsF25* sensitised strains carrying another mutation in *uvsD*, *uvsD3*, and also strains bearing mutations in the other genes closely linked to *uvsD*, *uvsA4* and *uvsC10*.

The marker arrangements of the parents and the points of selection are indicated in Figure 26. A sample of recombinants from each cross was characterised and apparently highly sensitive recombinants detected as previously described from irradiated replicas of primary

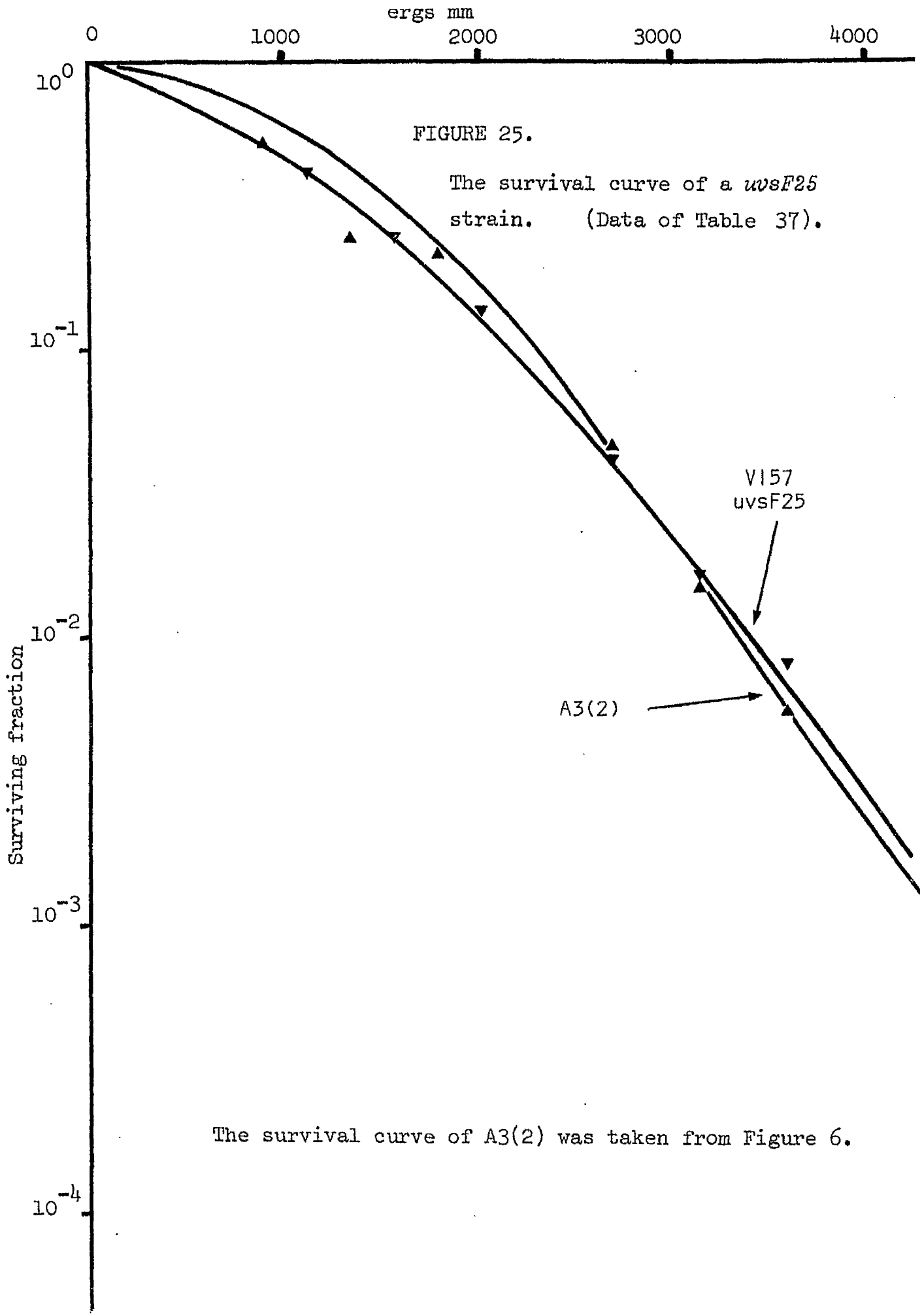


FIGURE 25.

The survival curve of a *uvsF25* strain. (Data of Table 37).

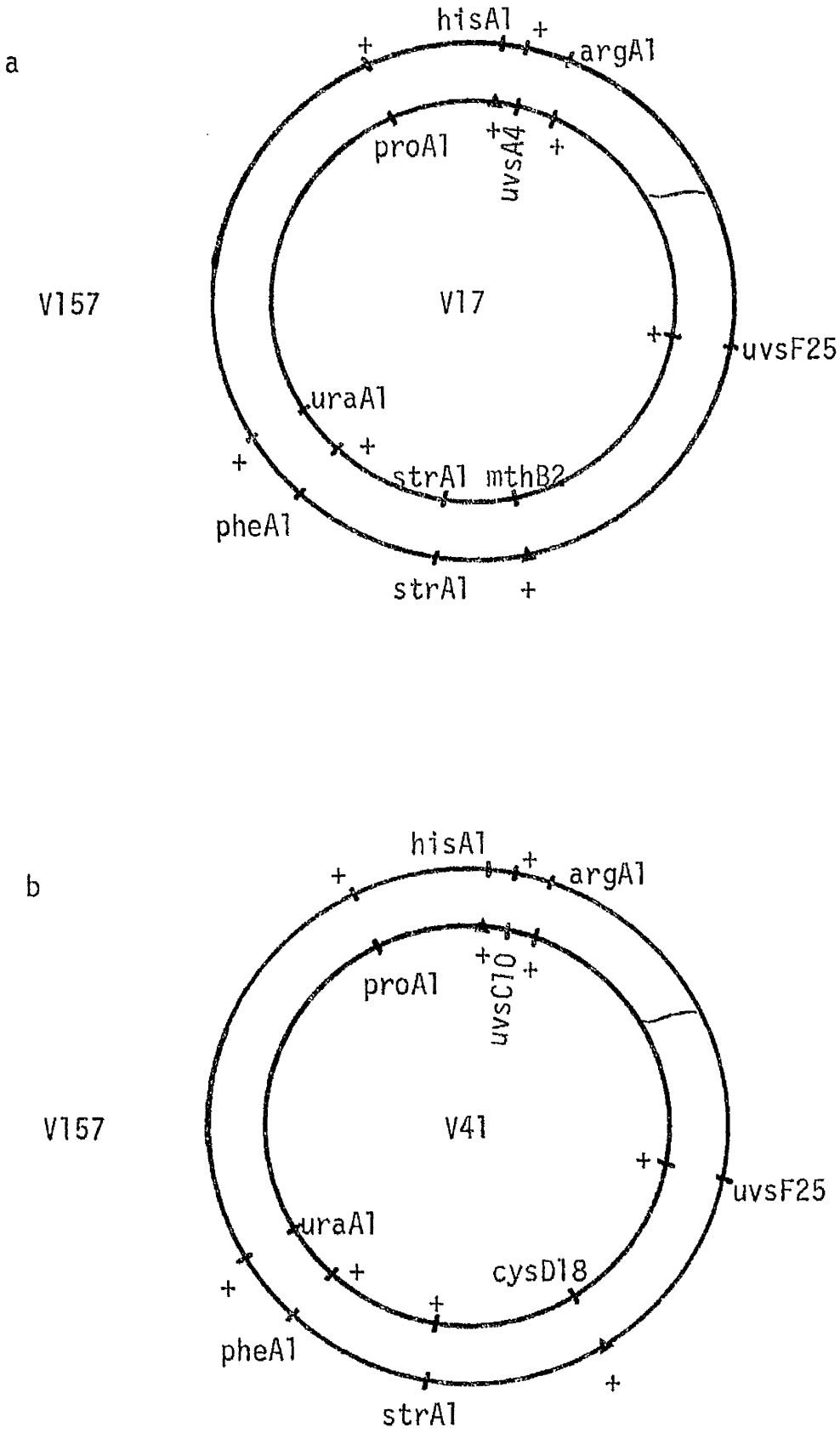
The survival curve of A3(2) was taken from Figure 6.

TABLE 37. Data for the survival curve of strain V157 *hisA1 argA1 pheA1 strA1 uvsF25*. (Plotted in Figure 25).

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1, Closed erect triangles, a	1, Closed erect triangles, b	2, Closed inverted triangles, a	2, Closed inverted triangles, b
0	644×10^{3}	1.0	127×10^{4}	1.0
900	341×10^{3}	5.3×10^{-1}	-	-
1125	-	-	539×10^{3}	4.3×10^{-1}
1350	164×10^{3}	2.5×10^{-1}	-	-
1575	-	-	318×10^{3}	2.5×10^{-1}
1800	139×10^{3}	2.2×10^{-1}	-	-
2025	-	-	172×10^{3}	1.4×10^{-1}
2700	303×10^{2}	4.7×10^{-2}	527×10^{2}	4.2×10^{-2}
3150	935×10	1.5×10^{-2}	2143×10	1.7×10^{-2}
3600	363×10	5.6×10^{-3}	1069×10	8.4×10^{-3}
No. of samples at each dose	2		2	

See legend to Table 13.

FIGURE 26. Crosses for obtaining *uvsA4 uvsF25*, *uvsC10 uvsF25* and *uvsD3 uvsF25* strains.

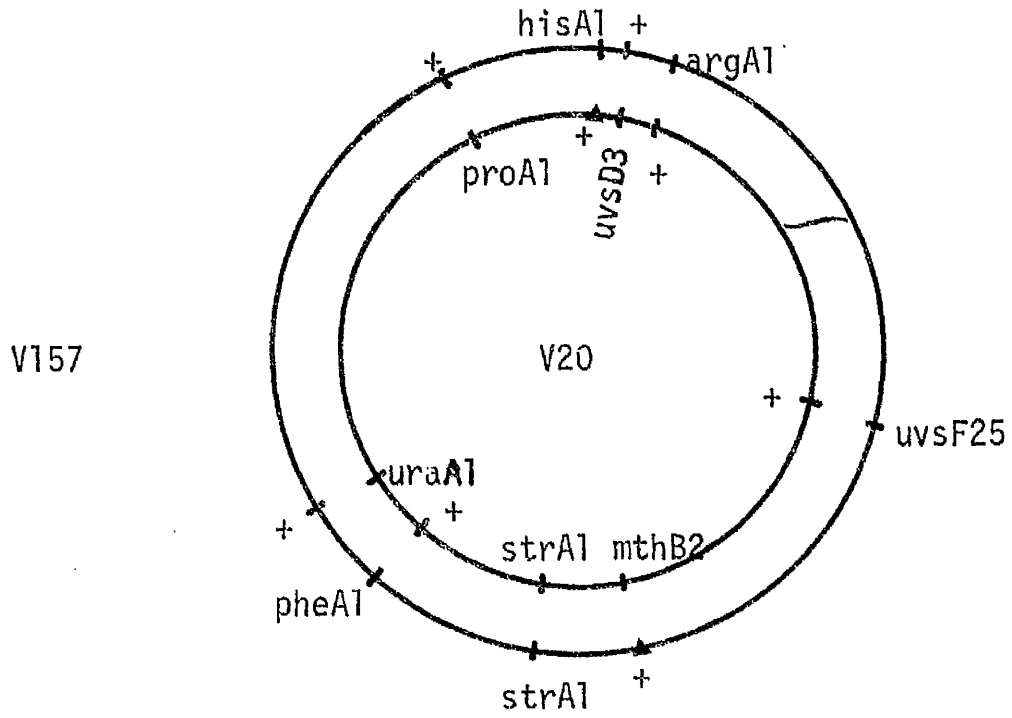


(indicates the interval in which recombination was required to give the desired recombinant.

FIGURE 26 (continued).

Crosses for obtaining *uvsA4 uvsF25*, *uvsC10 uvsF25* and *uvsD3 uvsF25* strains.

c



)indicates the interval in which recombination was required to give the desired recombinant.

and secondary master plates were purified. These were prepared as spore suspensions for UV survival curves, and their sensitivity measured for two doses of UV. The results are presented in Table 38a, b, c, together with the survival levels of the parent strains at the same doses of UV. Highly sensitive strains were obtained from the crosses involving *uvsC10* and *uvsD3*, but of six recombinants tested from two crosses with the *uvsA4* strain none were of sensitivity similar to that of strain V60.

These results showed that *uvsF25* was not specific in its action on *uvsD18* but was effective in enhancing the UV sensitivity conferred by a mutation in *uvsC* and also a second mutation in *uvsD*. There was no conclusive evidence of the effect of *uvsF25* on mutations in *uvsA*, in the absence of proof that recombinants of the critical genotype *uvsA4 uvsF25* were obtained.

TABLE 38. The survival levels of recombinants classified as probably *uvsA4 uvsF25*, *uvsC10 uvsF25* or *uvsD3 uvsF25* on irradiated replica plates compared with those of the parent strains.

Genotype from replica plates		ergs mm^{-2} 0	118	177	236	354	*Uvs* genotype from UV survival
(a) Recombinants from V157 x V17 cross.							
proA1 uraA1 strA1 uvsA4 uvsF25	i	347×10^5	-	465×10^4	-	303×10^3	
	ii	1.0	-	1.3×10^{-1}	-	8.7×10^{-3}	*uvsA4 uvsF*$^+$
proA1 uraA1 strA1 uvsA4 uvsF25	i	260×10^5	-	359×10^4	-	166×10^3	
	ii	1.0	-	1.4×10^{-1}	-	6.4×10^{-3}	*uvsA4 uvsF*$^+$
proA1 uraA1 strA1 uvsA4 uvsF25	i	334×10^5	759×10^4	-	137×10^4	-	
	ii	1.0	2.3×10^{-1}	-	4.1×10^{-2}	-	*uvsA4 uvsF*$^+$
proA1 pheA1 strA1 uvsA4 uvsF25	i	1070×10^4	352×10^4	-	483×10^3	-	
	ii	1.0	3.5×10^{-1}	-	4.6×10^{-2}	-	*uvsA4 uvsF*$^+$
proA1 pheA1 uraA1 strA1 uvsA4 uvsF25	i	1048×10^3	142×10^3	-	121×10^2	-	
	ii	1.0	1.4×10^{-1}	-	1.2×10^{-2}	-	*uvsA4 uvsF*$^+$
proA1 pheA1 strA1 uvsA4 uvsF25	i	1124×10^4	631×10^4	-	763×10^3	-	*uvsA4 uvsF*$^+$
	ii	1.0	5.6×10^{-1}	-	6.8×10^{-2}	-	
(b) Recombinants from V157 x V41 cross.							
uraA1 uvsC10 uvsF25	i	805×10^3	752×10	-	32×1	-	
	ii	1.0	9.3×10^{-3}	-	4.0×10^{-5}	-	*uvsC10 uvsF25*
pheA1 uraA1 strA1 uvsC10 uvsF25	i	302×10^3	66×10	-	26×1	-	
	ii	1.0	2.2×10^{-3}	-	8.6×10^{-5}	-	*uvsC10 uvsF25*

(continued overleaf)

TABLE 38 (continued)

Genotype from replica plates		ergs mm^{-2} 0	118	177	236	354	*Uvs* genotype from UV survival
(c) Recombinants from V157 x V20 cross.							
proA1 uraA1 strA1 uvsD3 uvsF25	i	197×10^{4}	941×10^{3}	-	161×10^{3}	-	
	ii	1.0	4.8×10^{-1}	-	8.4×10^{-2}	-	*uvsD3 uvsF*$^{+}$
proA1 uraA1 strA1 uvsD3 uvsF25	i	215×10^{4}	899×10^{2}	-	762×1	-	
	ii	1.0	4.2×10^{-2}	-	6.6×10^{-4}	-	*uvsD3 uvsF25*
proA1 uraA1 strA1 uvsD3 uvsF25	i	893×10^{3}	224×10^{3}	-	188×10^{2}	-	
	ii	1.0	2.4×10^{-1}	-	2.0×10^{-2}	-	*uvsD3 uvsF*$^{+}$
Strain							
V157 *hisA1 argA1 pheA1 strA1 uvsF25*	ii	1.0	5.0×10^{-1}	3.2×10^{-1}	2.0×10^{-1}	6.6×10^{-2}	
V17 *proA1 mthB2 uraA1 strA1 uvsA4*	ii	1.0	2.8×10^{-1}	1.1×10^{-1}	3.7×10^{-2}	3.9×10^{-3}	
V41 *proA1 cysD18 uraA1 uvsC10*	ii	1.0	1.7×10^{-1}	-	2.1×10^{-2}	-	
V20 *proA1 mthB2 uraA1 strA1 uvsD3*	ii	1.0	4.0×10^{-1}	-	1.1×10^{-1}	-	

See legend to Table 35.

V. FURTHER STUDIES ON UV SURVIVAL.

A. Survival curve studies on strains containing two mutations affecting UV sensitivity.

1. Rationale.

It should be possible, by the use of double mutant strains, to obtain information as to whether two UV sensitive mutations affect related or unrelated functions. If a strain contained a mutation which eliminated one function in a pathway of genetically determined functions leading to repair of potentially lethal lesions in that strain, then a further mutation eliminating another of the functions in the same pathway would not be expected to increase the sensitivity of the strain. If, however, the second mutation eliminated a function in another pathway, able to repair the same or different potentially lethal damage, then the strain containing both mutations would be expected to be more sensitive than two other strains, each containing only one of the mutations. However, if one or both singly mutant strains carried a mutation only partially eliminating a function, both functions acting in the same pathway, then the double mutant might be expected to be more sensitive than one or both of the strains containing only one of the mutations, but its sensitivity should not exceed that of a singly mutant strain which completely lacked one of the functions of the pathway.

2. Preparation and confirmation of double mutant strains.

Three double mutant strains were prepared with mutations in *uvsA* and *uvsB*, *uvsC* and *uvsB*, *uvsD* and *uvsB* respectively.

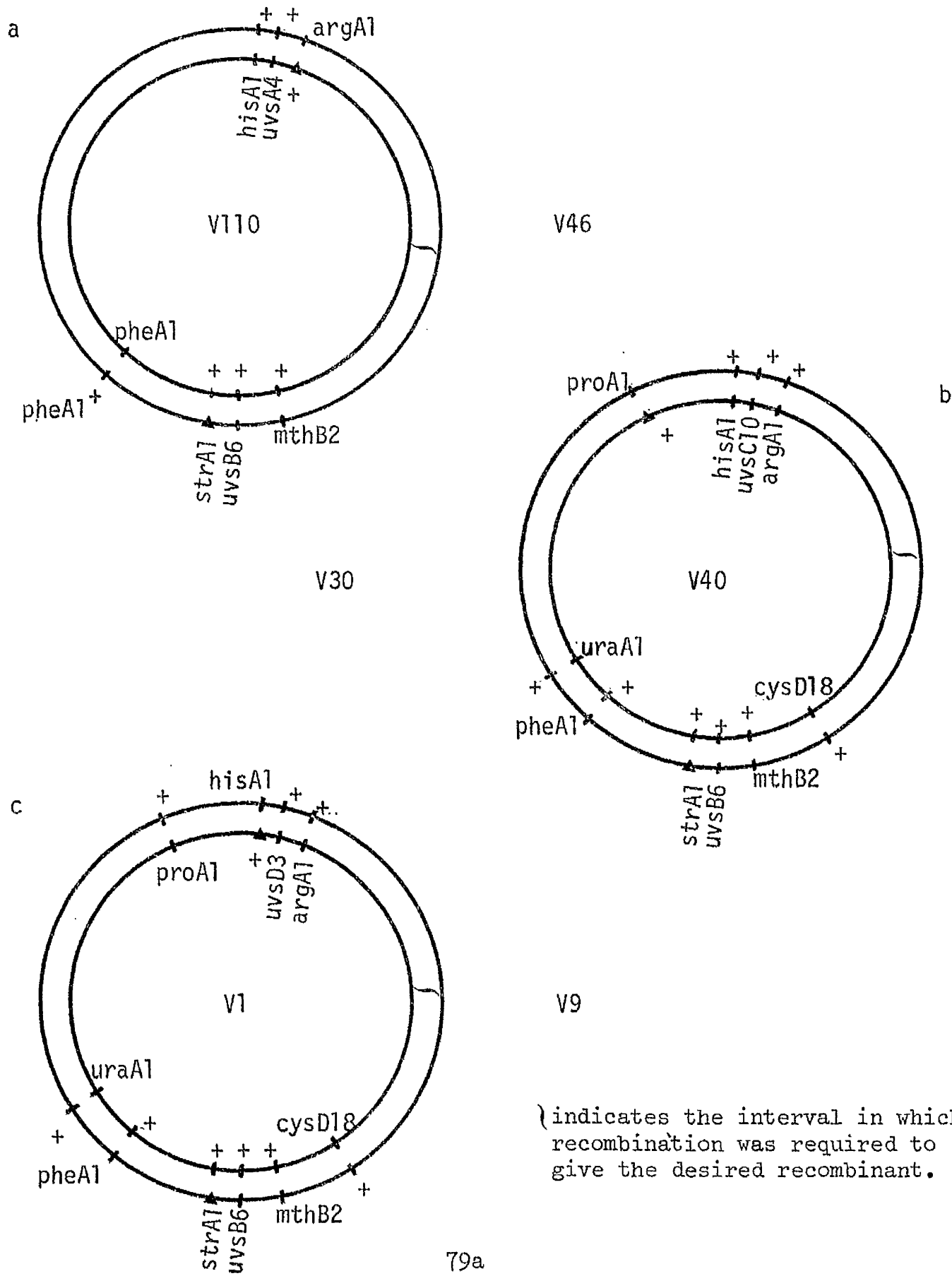
Since *uvsA*, *uvsC* and *uvsD* mapped distantly from *uvsB*, these

double mutants were readily obtained by recombination from the three preparation crosses illustrated in Figure 27. The strains V124 *hisA1 mthB2 pheA1 strA1 uvsA4 uvsB6*, V121 *hisA1 argA1 cysD18 mthB2 pheA1 strA1 uvsB6 uvsC10* and V134 *argA1 mthB2 pheA1 strA1 uvsB6 uvsD3* were isolated from these crosses and purified by streaking. In all three cases, the strains were detected as UV sensitive from replica plates exposed to UV doses of about 1200 ergs mm^{-2}; sensitivity to this dose could have been due to the presence of only one *uvs* mutation in these strains. However, the marker combinations of parental and recombinant strains were chosen in such a way that the recombinants would have been members of rare multiple crossover classes if they had not carried both *uvs* markers; a marker on either side of each *uvs* mutation was present in the chosen recombinants. The three recombinant strains were checked for the presence of each of the two *uvs* mutations that they were presumed to contain by the following tests designed to re-isolate and prove the identity of each separate *uvs* mutation.

Crosses were made between each of the presumed double mutant strains and another suitably marked uvs^+ strain; recombinants were selected from the progeny in such a way that they would all be likely to be uvs^+ for one of the *uvs* mutant sites in each doubly mutant parent but some at least would contain the other *uvs* mutation. A separate cross was required for each component of each presumed double mutant making six crosses in all.

The strains and the selections applied in this first step of the analysis are illustrated in Figures 28, 29 and 30, together with the frequencies of Uvs^+ and Uvs^- recombinants observed in the progeny analysed. In every case, there was an appreciable proportion of

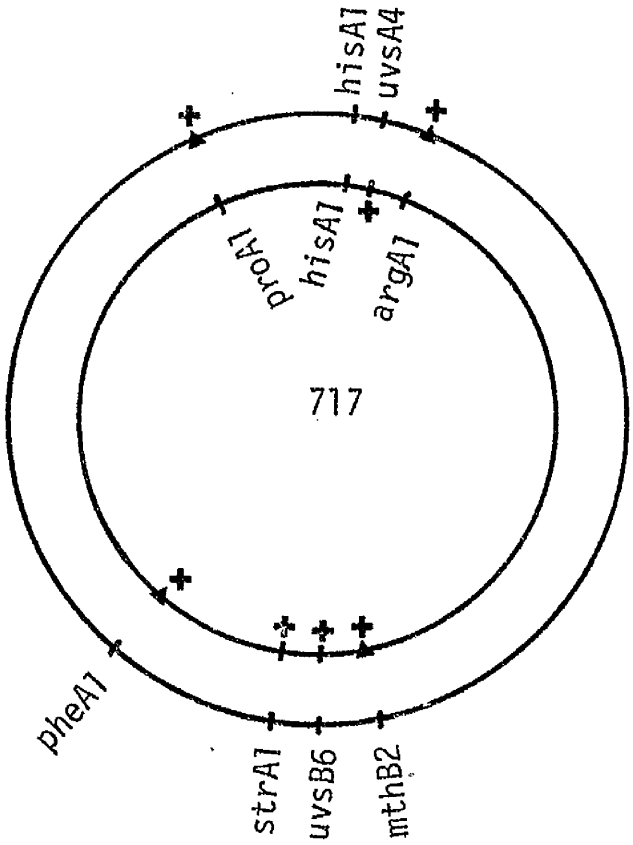
FIGURE 27. Crosses for obtaining *uvsA uvsB*, *uvsB uvsC* and *uvsB uvsD* strains.



) indicates the interval in which recombination was required to give the desired recombinant.

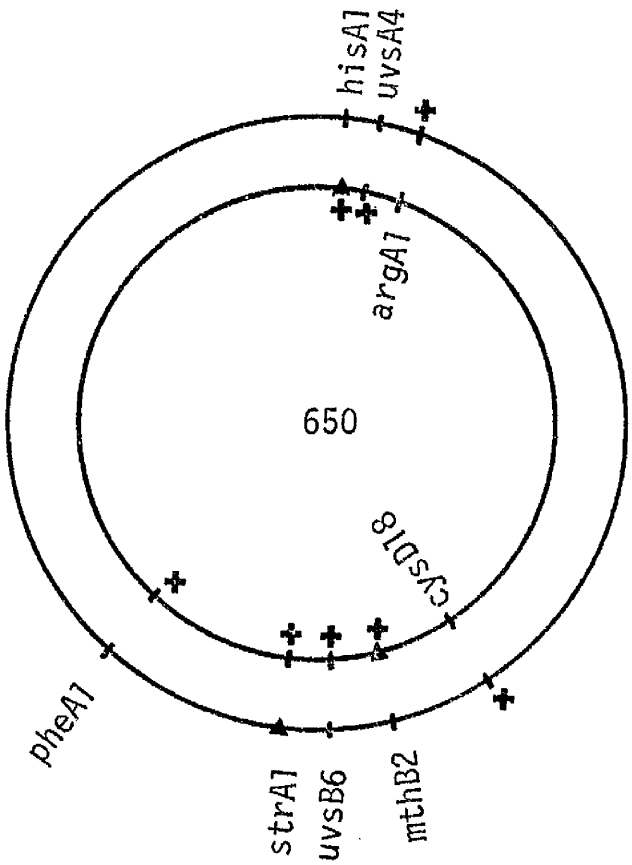
FIGURE 26. Segregation of *uvs* recombinants at two loci in different crosses from a presumed *uvsA uvsB* strain.

a



$$\frac{uvs}{uvs^+} = \frac{600}{20}$$

b

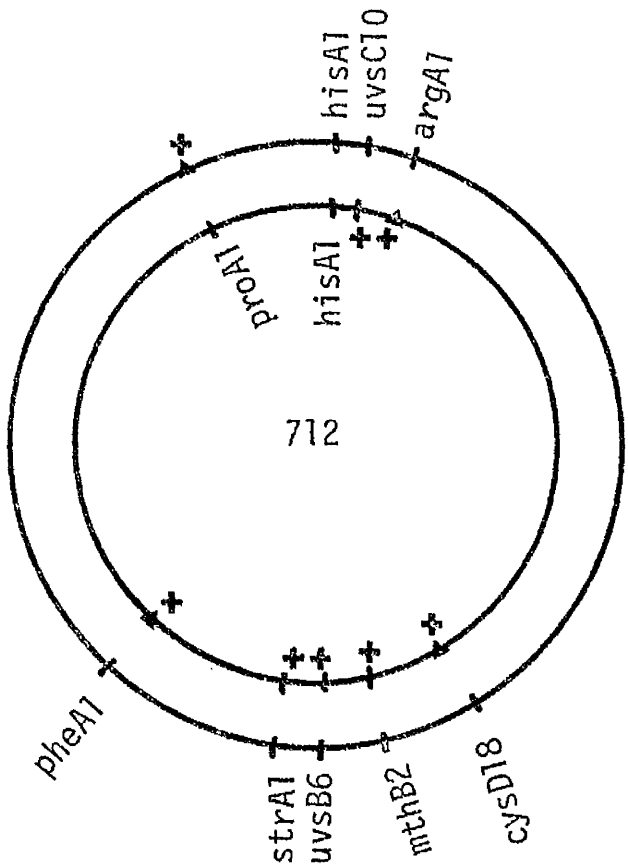


$$\frac{uvs}{uvs^+} = \frac{59}{28}$$

See text for explanation.

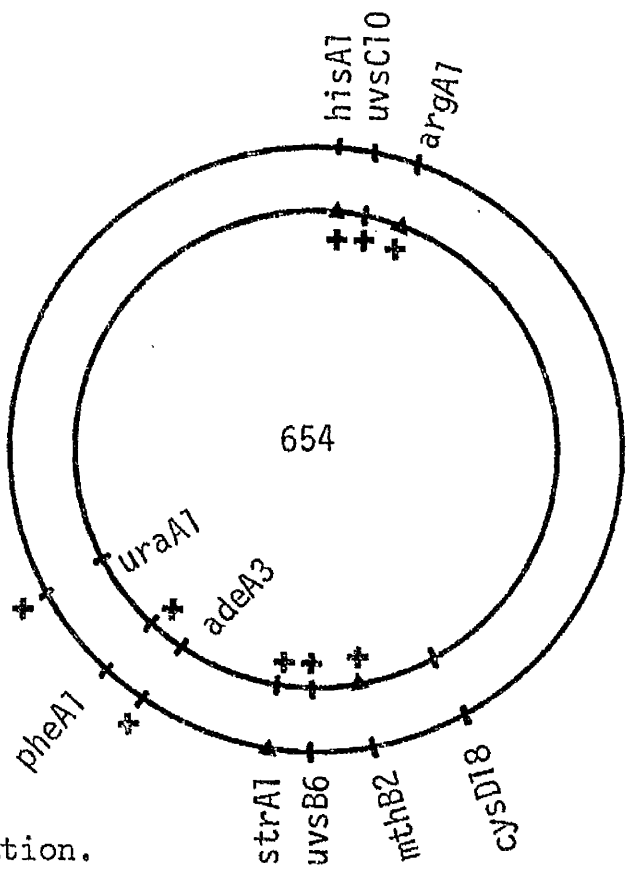
FIGURE 29. Segregation of *uvs* recombinants at two loci in different crosses from a presumed *uvsB uvsC* strain.

a



$\frac{uvs}{uvs^+} = \frac{46}{16}$

b

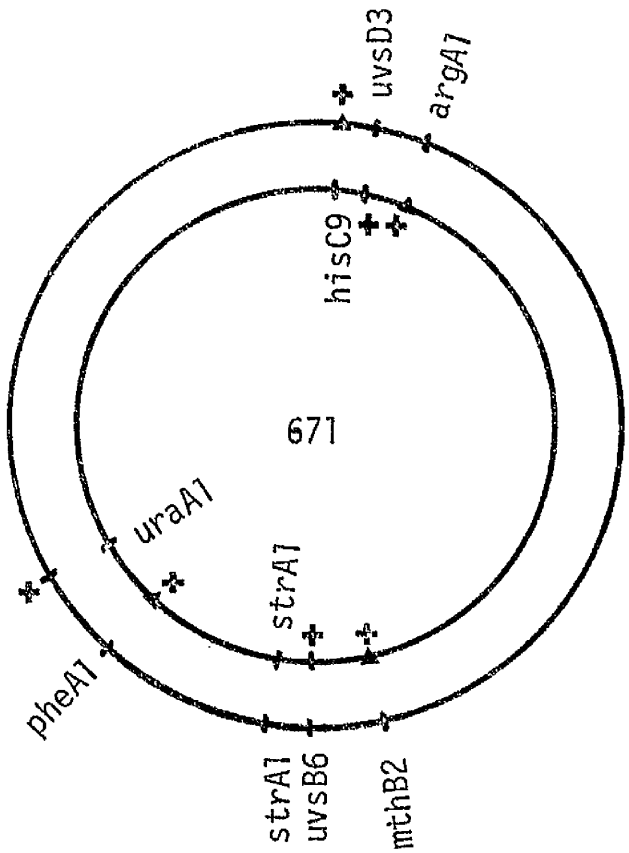


$\frac{uvs}{uvs^+} = \frac{8}{20}$

See text for explanation.

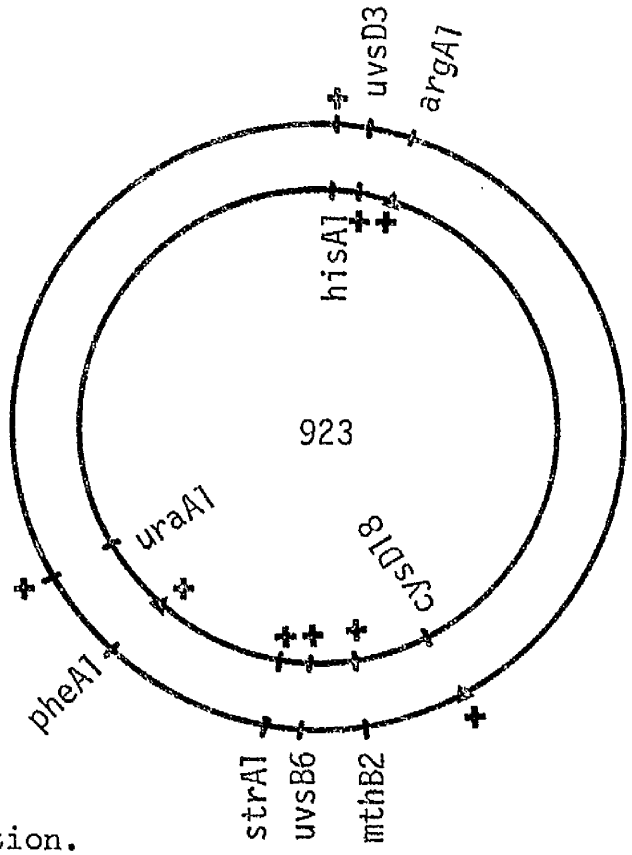
FIGURE 30. Segregation of *uvs* recombinants at two loci in different crosses from a presumed *uvsB uvsD* strain.

a



$$\frac{uvs}{uvs^+} = \frac{98}{145}$$

b



$$\frac{uvs}{uvs^+} = \frac{107}{163}$$

See text for explanation.

Uvs⁻ recombinants, as was expected.

One of the following strains was isolated and purified from each of the above crosses, V127 *hisA1 uvsA4*, V128 *hisA1 uvsC10*, V144 *uraA1 strA1 uvsD3*, V132 *argA1 cysD18 pheA1 strA1 uvsB6* (recombinant derivative of V124), V131 *adeA3 cysD18 uraA1 strA1 uvsB6* (recombinant derivative of V121), and V143 *hisA1 mthB2 uraA1 strA1 uvsB6* (recombinant derivative of V134). The identity of the *uvs* mutation in each strain was tentative at this stage, and the next step in the analysis was designed to confirm it.

V127 and V128 were crossed in turn with strains carrying representative mutations in *uvsA*, *C* and *D* (V107 *proA1 hisC9 strA1 uvsA22*, V104 *proA1 hisC9 strA1 uvsC17* and V115 *proA1 hisC9 strA1 uvsD18*); heteroclones were selected between $hisA^+$ and $hisC^+$ and the progeny tested for complementation by the method described in Section IV B. The results are presented in Table 39 and fully support the expectations that V127 contained a mutation in *uvsA* and V128 a mutation in *uvsC*.

The four remaining strains (V144 (*uvsD3*), V132, V131, V143 (*uvsB6*))were tested for the presence of their presumed *uvs* mutation by mapping them in an appropriate position. Crosses were made between V144 and 651 *hisA1 argA1 cysD18*, V132 and 681 *hisA1 mthB2 uraA1*, V131 and 922 *proA1 argA1 mthB2 pheA1 strA1*, V143 and 749 *proA1 argA1 cysD18 uraA1*. The parental marker arrangements and the analysis of these crosses are presented in Figure 31. In each case, one of the two locations indicated by the ratio of $^{uvs}/_{uvs^+}$ frequencies was that expected from the ancestry of the *uvs* mutation; the second possible location was never that of the other *uvs* mutation expected to be present in the double mutant strain from which the strain under study

TABLE 39. Identification by complementation that strain V127 was mutant in *uvsA* and strain V128 in *uvsC*.

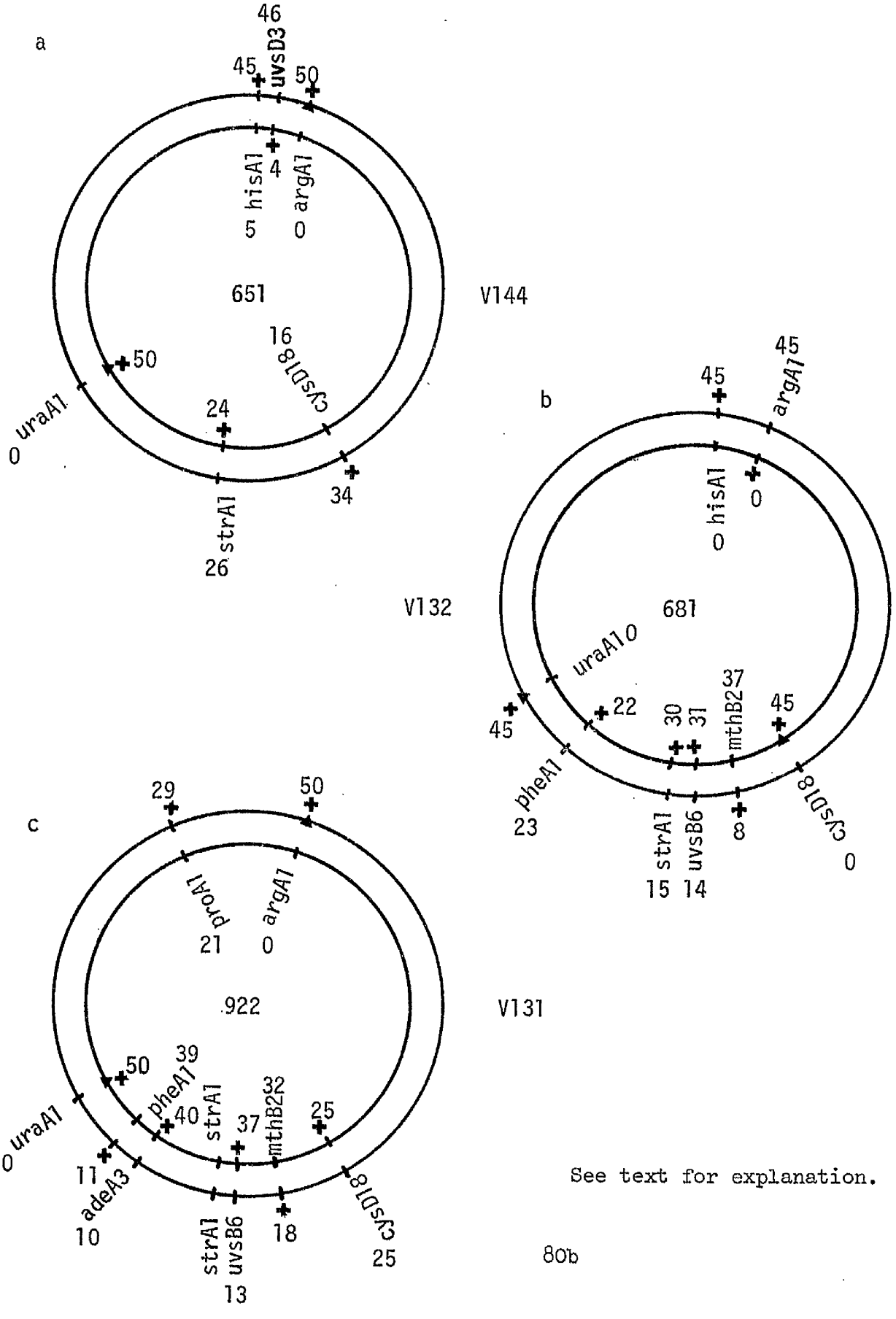
Cross	Colony Counts Unirradiated	Irradiated	% survival	Complementation
	a	b	$\frac{b}{a}$ x 100	
V127 *hisA1 uvsA4* x V107 *proA1 hisC9 strA1 uvsA22*	254	2	0.8	-
V127 *hisA1 uvsA4* x V104 *proA1 hisC9 strA1 uvsC17*	342	53	15.5	+
V127 *hisA1 uvsA4* x V115 *proA1 hisC9 strA1 uvsD18*	82	16	19.5	+
V128 *hisA1 uvsC10* x V107 *proA1 hisC9 strA1 uvsA22*	191	43	22.5	+
V128 *hisA1 uvsC10* x V104 *proA1 hisC9 strA1 uvsC17*	510	0	<0.2	-
V128 *hisA1 uvsC10* x V115 *proA1 hisC9 strA1 uvsD18*	32	5	15.5	+

+ indicates complementation.

- indicates non-complementation.

See text for explanation.

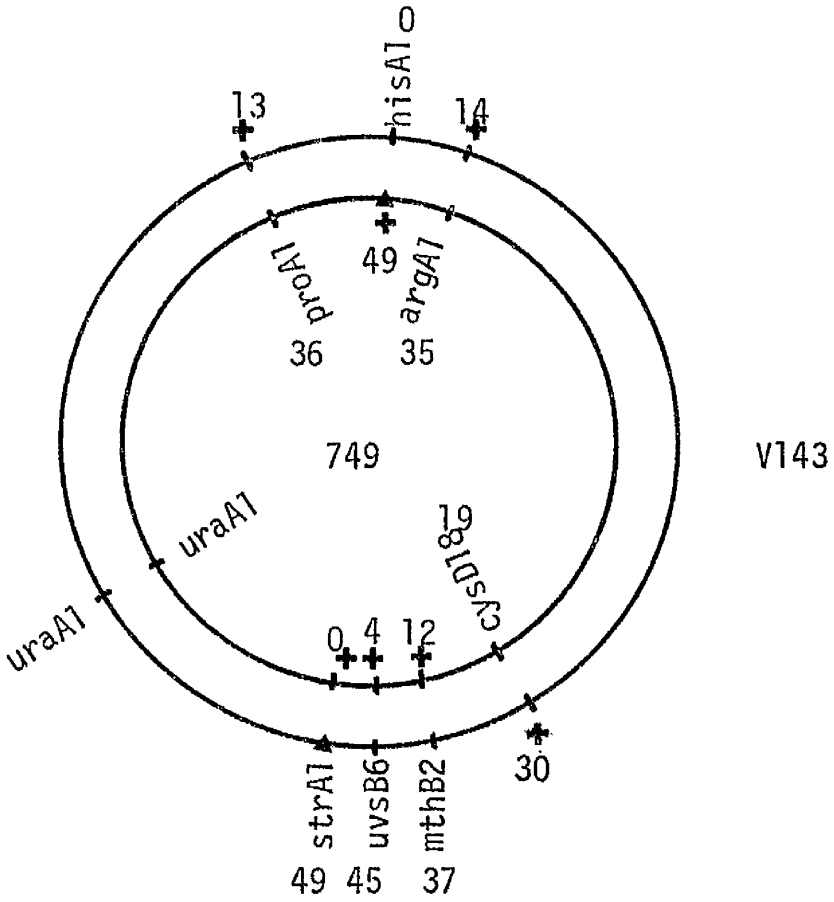
FIGURE 31. The location of *uvs* mutations carried by recombinant progeny of double *uvs* mutants.



See text for explanation.

FIGURE 31 (continued). The location of *uvs* mutations carried by recombinant progeny of double *uvs* mutants.

d



See text for explanation.

was derived.

Since the results had agreed with the expectations throughout, the strains V124, V121 and V134 were confirmed as double *uvs* strains carrying *uvsB6* in combination with mutations in *uvsA*, *uvsC* and *uvsD*.

3. Survival curves of the double mutants.

UV survival curves were obtained for each of these strains; the data are presented in Table 40, and the survival curves are plotted in Figure 32, together with survival curves for V2 *proA1 argA1 cysD18 uraA1 uvsA4*, V9 *hisA1 mthB2 pheA1 strA1 uvsB6*, V13 *hisA1 mthB2 pheA1 strA1 uvsC10* and V1 *proA1 argA1 cysD18 uraA1 uvsD3*. The survival curves for the three double mutants are clearly very similar and follow closely that for *uvsA4*. This was the most sensitive strain amongst those studied by survival curves which were mutant in any of the genes *uvsA*, *uvsC* or *uvsD*, apart from strain V60 which was shown to be a spontaneous double mutant (Section IV E).

4. Investigation of the number of *uvs* mutations in the original *uvsA4* mutant.

To investigate the possibility that the *uvsA4* strain was itself a double mutant, a cross was made between strain V2 *proA1 argA1 cysD18 uraA1 uvsA4* and strain 921 *hisA1 cysA15 mthB2 pheA1 strA1* and a sample of recombinants analysed which had been selected to be $mthB^+$ $pheA^+$ $argA^+$. The parental marker arrangements and the genotypes of 45 recombinants are illustrated in Figure 33.

The genotype $cysA^+$ *cysD18* was distinguished from *cysA15* $cysD^+$ and *cysA15 cysD18* recombinants by substituting sodium thiosulphate for cystine as a supplement in an additional plate in the series of replica plates for analysing the recombinants; $cysA^+$ *cysD18* strains

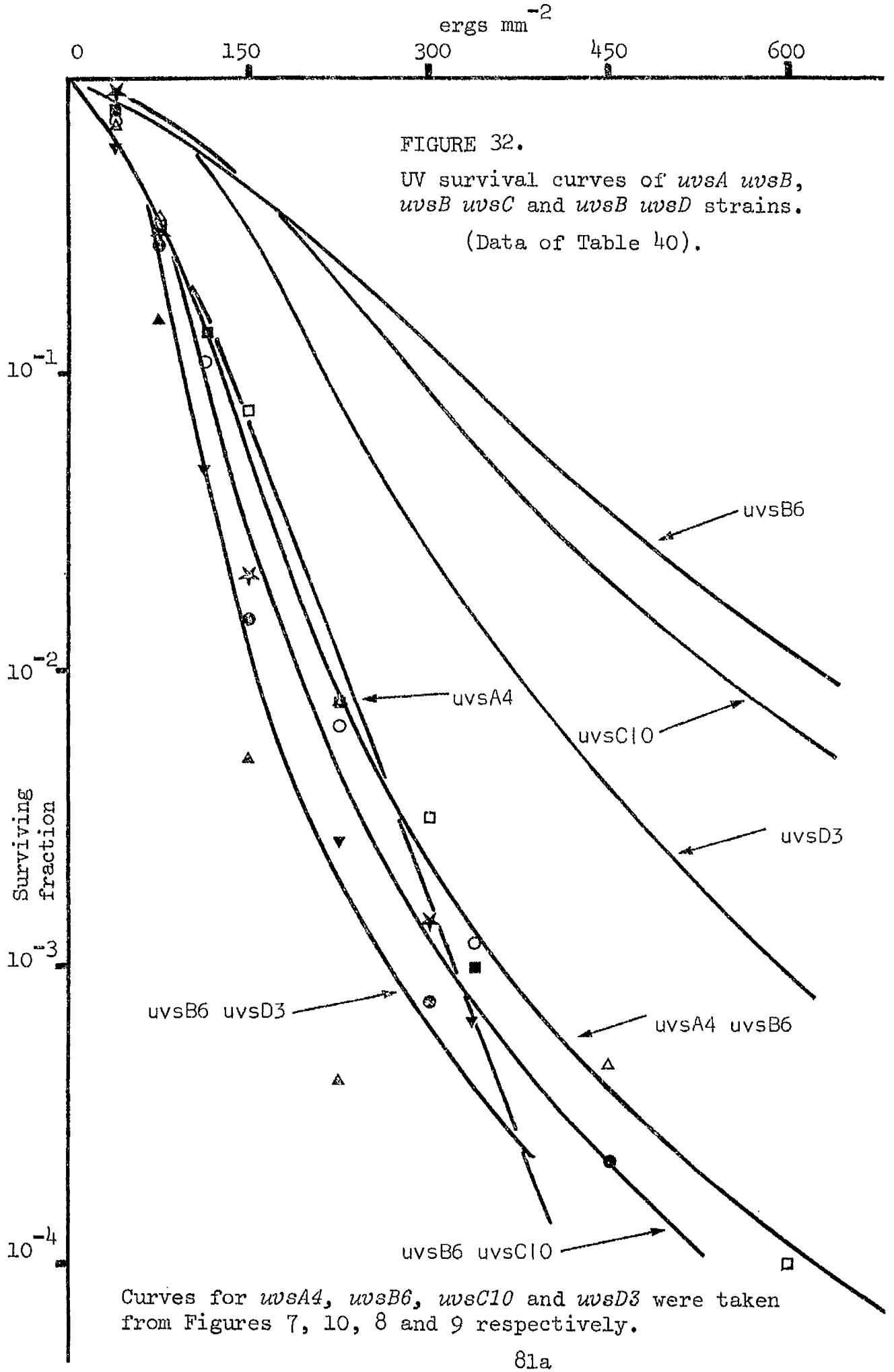


FIGURE 32.

UV survival curves of *uvsA uvsB*, *uvsB uvsC* and *uvsB uvsD* strains.

(Data of Table 40).

Curves for *uvsA4*, *uvsB6*, *uvsC10* and *uvsD3* were taken from Figures 7, 10, 8 and 9 respectively.

TABLE 40. Data for UV survival curves of *uvsA uvsB*, *uvsB uvsC* and *uvsB uvsD* strains. (plotted in Figure 32).

Strain V124 *hisA1 mthB2 pheA1 strA1 uvsA4 uvsB6.*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Open squares a	1 Open squares b	2 Open circles a	2 Open circles b	3 Open erect triangles a	3 Open erect triangles b
0	220×10^{5}	1.0	445×10^{4}	1.0	598×10^{4}	1.0
37.5	-	-	320×10^{4}	7.2×10^{-1}	414×10^{4}	6.9×10^{-1}
75	70×10^{5}	3.2×10^{-1}	-	-	205×10^{4}	3.4×10^{-1}
112.5	-	-	474×10^{3}	1.1×10^{-1}	-	-
150	168×10^{4}	7.6×10^{-2}	-	-	-	-
225	-	-	291×10^{2}	6.5×10^{-3}	473×10^{2}	7.9×10^{-3}
300	71×10^{3}	3.2×10^{-3}	-	-	-	-
337.5	-	-	513×10	1.2×10^{-3}	-	-
450	-	-	-	-	276×10	4.6×10^{-4}
600	22×10^{2}	1.0×10^{-4}	-	-	-	-
No. of samples at each dose	3		3		3	

Strain V121 *hisA1 argA1 cysD18 mthB2 pheA1 strA1 uvsB6 uvsC10.*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 Closed circles a	1 Closed circles b	2 Closed squares a	2 Closed squares b
0	197×10^{5}	1.0	315×10^{5}	1.0
37.5	-	-	249×10^{5}	7.9×10^{-1}
75	557×10^{4}	2.8×10^{-1}	-	-

(continued overleaf)

TABLE 40 (continued) Strain V121 *hisA1 argA1 cysD18 mthB2 pheA1 strA1 uvsB6 uvsC10* (continued).

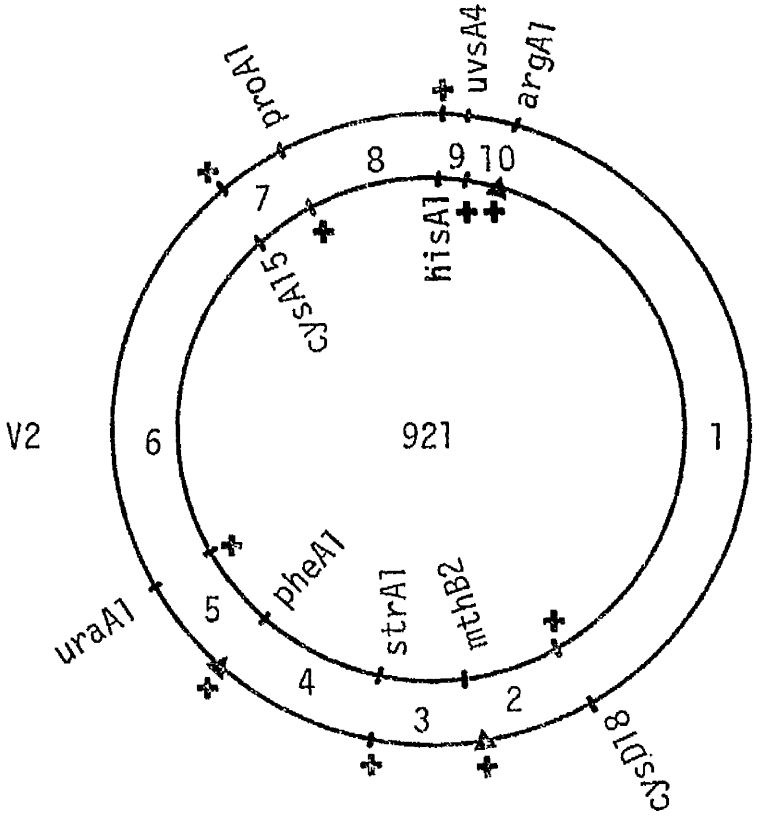
Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1, Closed circles		2, Closed squares	
	a	b	a	b
112.5	-	-	455×10^{4}	1.4×10^{-1}
150	291×10^{3}	1.5×10^{-2}	-	-
225	-	-	250×10^{3}	7.9×10^{-3}
300	149×10^{2}	7.6×10^{-4}	-	-
337.5	-	-	321×10^{2}	1.0×10^{-3}
450	434×10	2.2×10^{-4}	-	-
No. of samples at each dose	3		3	

Strain V134 *argA1 mthB2 pheA1 strA1 uvsD3 uvsB6.*

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1, Closed erect triangles		2, Closed inverted triangles		3, Stars	
	a	b	a	b	a	b
0	895×10^{3}	1.0	145×10^{4}	1.0	292×10^{3}	1.0
37.5	-	-	835×10^{3}	5.8×10^{-1}	256×10^{3}	8.8×10^{-1}
75	139×10^{3}	1.5×10^{-1}	-	-	912×10^{2}	3.1×10^{-1}
112.5	-	-	688×10^{2}	4.7×10^{-2}	-	-
150	436×10	4.9×10^{-3}	-	-	599×10	2.1×10^{-2}
225	354×1	4.0×10^{-4}	382×10	2.6×10^{-3}	-	-
300	-	-	-	-	407×1	1.4×10^{-3}
337.5	-	-	917×1	6.3×10^{-4}	-	-
No. of samples at each dose	3		3		3	

See legend to Table 13.

FIGURE 33. Cross designed to detect segregation of two *uvs* mutations in strain V2 if they were separable by recombination.



Genotype (a)	Crossover intervals	Number
(cysD) cysA his	1 or 2, 5	4
(cysD) ura cysA his	1 or 2, 6	9
cysD ura his	1, 7	5
cysD ura pro his	1, 8	8
cysD ura pro	1, 9	2
cysD ura pro uvs	1, 10	5
ura his	2, 7	2
ura pro his	2, 8	1
cysD ura	1, 7, 8, 9	2
(cysD) str cysA his	1 or 2, 3, 4, 5	3
str ura pro his	2, 3, 4, 8	1
str his	2, 3, 4, 5, 6, 7	1
Total recombinants		45

Parentheses indicate that the *cysD* allele in these strains was not determined due to the presence of *cysA15*.

(a) Wild-type alleles omitted.

(and of course *cysA*$^+$ *cysD*$^+$ strains) can grow on this supplement as a source of sulphur. UV sensitivity was detected on three replica plates on the same medium as the master plate; one was kept as an unirradiated control, one exposed to about 1200 ergs mm^{-2} and another to about 1800 ergs mm^{-2}. It was hoped that this selection would allow any other possible *uvs* mutation located in the long arc between *hisA*$^+$ and close to *argA1* to enter recombinants separately from *uvsA4*, and that if the possible second mutation conferred UV sensitivity no greater than that conferred by *uvsE13*, which was the least sensitive mutation known to confer sensitivity by itself, the dose of 1800 ergs mm^{-2} would detect it. In fact five *uvs* recombinants were found amongst 45 recombinants analysed, which were all sensitive to the dose of 1200 ergs mm^{-2}. They were also *proA1 cysD18* and therefore almost certainly *uvsA4*. There was no indication that any other recombinants were UV sensitive although, between them, they contained regions of the V2 genome from *hisA*$^+$ round to *cysD18*, and presumably beyond, without including *uvsA4*. In particular two recombinants which were *cysD18 uraA1 proA1* were clearly of wild-type sensitivity. Therefore on this test strain V2 did not appear to be a double mutant.

5. Conclusions.

Since the constructed double mutants were no more sensitive than strain V2 it may be concluded that *uvsA*, *uvsB*, *uvsC* and *uvsD* probably act in the same pathway of repair of lethal UV damage. As a result we might expect some further mutations of all four genes to be as sensitive as *uvsA4*. In particular we might expect further mutations in *uvsB* to result in greater sensitivity than that of *uvsB6*, which conferred the least sensitivity in the *uvsA, B, C, D* group of mutations.

Thus most of the mutations in *uvsA*, *B*, *C* or *D* which have been studied by survival curves appeared to have retained residual activity in the UV repair system affected, since they showed less sensitivity than *uvsA4*.

B. Photoreactivation in *Streptomyces coelicolor*.

The first demonstration of photoreactivation in any organism was by Kelner (1949) who discovered that when visible light was administered to a spore suspension of *Streptomyces griseus* after it had been irradiated with UV, there was an increase in the survival of the irradiated spores; a large fraction of the lethal effect of the UV had been reversed. The action spectrum for photoreactivation in *S. griseus* was determined by Kelner (1951). Below a wavelength of 360 nm, post-irradiation light treatment had little effect. From 360 nm the light treatment had increasing effect up to a maximum at 450 nm, and then fell to zero effect above 525 nm. On the basis of this information, irradiation and plating for quantitative studies of the effect of UV on strains of *S. coelicolor* A3(2) were carried out in light from a sodium vapour lamp as described in Section III C 1. This lamp emits at 589 nm, well above the upper limit for photoreactivation found by Kelner, and therefore photoreactivation would have been avoided.

Later when photoreactivability of A3(2) was investigated, this strain was found to be incapable of classical photoreactivation.

1. Experimental procedure.

The procedure for determining the photoreactivability of a strain was a modification of the procedure for obtaining UV survival curves

(Section III C 1 a). The modification was as follows. After the UV irradiation and sampling at various doses, the various samples were divided, and one of each of these was incubated for 35 minutes at 30°C in the dark. The other was incubated for 35 minutes at 30°C in a temperature-controlled waterbath, illuminated by two Osram Series B, 500W, UMA9, photographic bulbs 22.6cm apart when placed against the sides of the bath. This treatment was known from a trial experiment to give maximal photoreactivation of K673. The different samples were diluted to the first dilution which was to be plated, before being exposed to one of the treatments at 30°C. This dilution varied with the UV dose and the post-irradiation treatment which the sample received. After the treatments at 30°C, the samples were further diluted as necessary and 0.1ml aliquots plated in duplicate at room temperature (approximately 20°C) in MM agar held at 50°C, supplemented with glucose, and with actidione at a concentration of 7.5ug/ml to inhibit possible fungal contamination. The plating was performed in light from the sodium vapour lamp, and subsequent incubation was in the dark. Thus two survival curves for each spore suspension were obtained with and without post-irradiation light treatment.

2. Results.

The photoreactivability of two wild-type strains, A3(2) and K673 was studied. The results for two experiments with each of these strains are presented in Table 41 and their survival curves in Figure 34. It was clear that K673 exhibited a classical response to photo-reactivating light with a constant dose reduction factor (DRF) of about 0.5; that is, the visible light treatment reversed the lethal effect of about half the UV dose. This DRF was similar to that found

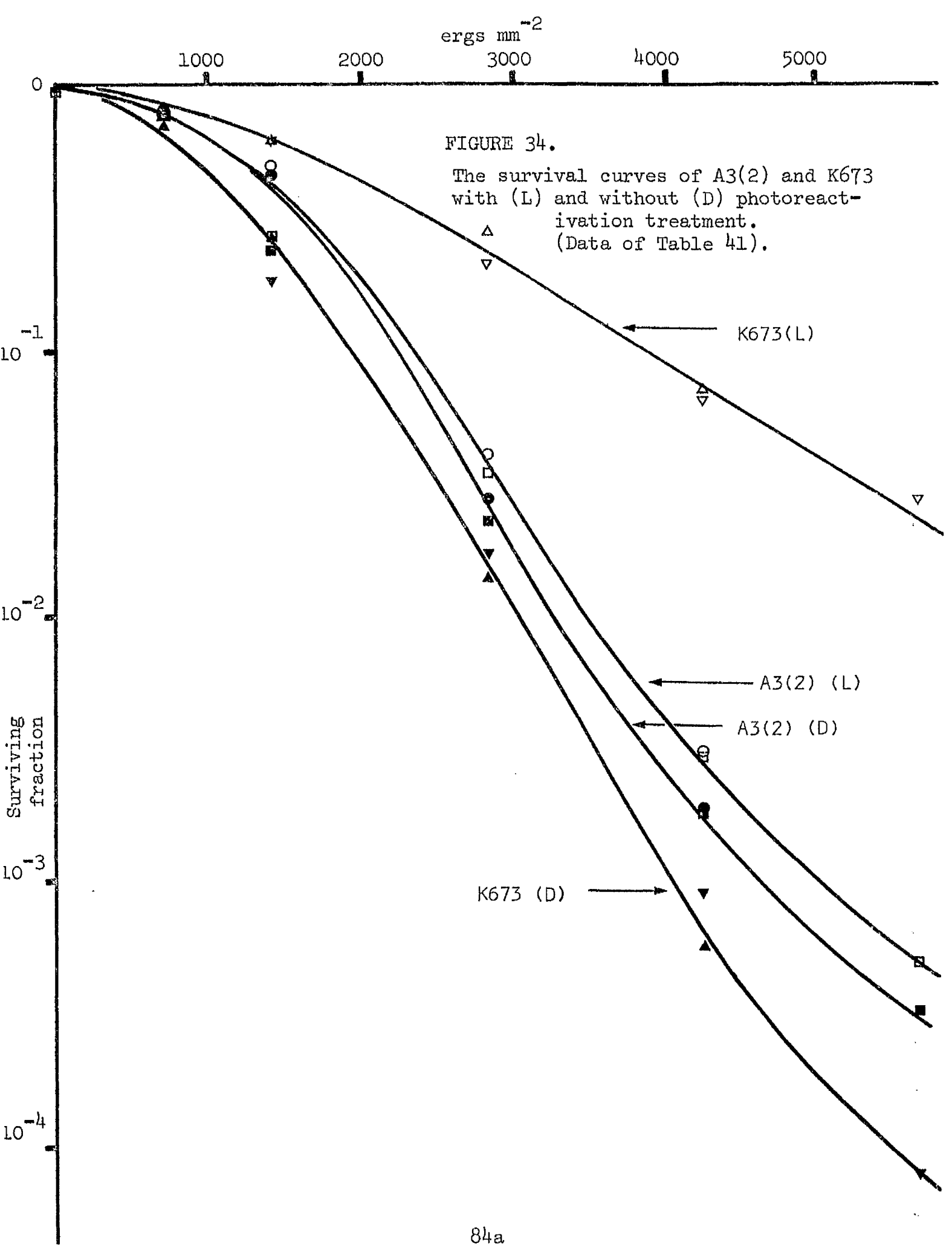


FIGURE 34.

The survival curves of A3(2) and K673 with (L) and without (D) photoreactivation treatment. (Data of Table 41).

TABLE 41. Data for UV survival curves of strains A3(2) and K673 without (D) and with (L) photoreactivation treatment (plotted in Figure 34).

Strain A3(2)

Dose ergs mm^{-2}	Experiment No. and symbol in Figure: 1 D Closed circles a	1 D Closed circles b	1 L Open circles a	1 L Open circles b	2 D Closed squares a	2 D Closed squares b	2 L Open squares a	2 L Open squares b
0	336×10^{6}	1.0	350×10^{6}	1.0	166×10^{6}	1.0	155×10^{6}	9.3×10^{-1}
708	279×10^{6}	8.3×10^{-1}	273×10^{6}	8.1×10^{-1}	-	-	-	-
1416	1529×10^{5}	4.6×10^{-1}	1690×10^{5}	5.0×10^{-1}	406×10^{5}	2.4×10^{-1}	447×10^{5}	2.7×10^{-1}
2832	9456×10^{3}	2.8×10^{-2}	13904×10^{3}	4.1×10^{-2}	382×10^{4}	2.3×10^{-2}	588×10^{4}	3.5×10^{-2}
4240	6384×10^{2}	1.9×10^{-3}	10568×10^{2}	3.1×10^{-3}	292×10^{3}	1.8×10^{-3}	482×10^{3}	2.9×10^{-3}
5664	-	-	-	-	564×10^{2}	3.3×10^{-4}	838×10^{2}	5.0×10^{-4}
No. of samples at each dose	2		2		2		2	

Strain K673

Dose ergs mm^{-2}	1 D Closed erect triangles a	1 D Closed erect triangles b	1 L Open erect triangles a	1 L Open erect triangles b	2 D Closed inverted triangles a	2 D Closed inverted triangles b	2 L Open inverted triangles a	2 L Open inverted triangles b
0	963×10^{5}	1.0	923×10^{5}	9.6×10^{-1}	422×10^{5}	1.0	438×10^{5}	1.0
708	669×10^{5}	7.0×10^{-1}	759×10^{5}	7.9×10^{-1}	-	-	-	-
1416	261×10^{5}	2.7×10^{-1}	600×10^{5}	6.2×10^{-1}	751×10^{4}	1.8×10^{-1}	260×10^{5}	6.2×10^{-1}
2832	1346×10^{3}	1.4×10^{-2}	265×10^{5}	2.8×10^{-1}	733×10^{3}	1.7×10^{-2}	874×10^{4}	2.1×10^{-1}
4240	549×10^{2}	5.7×10^{-4}	675×10^{4}	7.0×10^{-2}	375×10^{2}	8.9×10^{-4}	267×10^{4}	6.3×10^{-2}
5664	-	-	-	-	323×10	7.7×10^{-5}	1153×10^{3}	2.7×10^{-2}
No. of samples at each dose	2		2		2		2	

See legend to Table 13. Counts in excess of 2,500 were obtained by counting 1/8th or 1/16th of the plate and multiplying by the appropriate factor.

in a variety of other micro-organisms (Rupert, 1964). A3(2) did not exhibit such photoreactivation response; there was a slight but consistent increase in survival after high UV doses, due to the exposure to visible light, which was clearly not a classical photo-reactivation response; it may have been due to some phenomenon such as photo-protection in which exposure to visible light before the UV was as effective as exposure after the UV. In *E. coli* B the action spectrum for this was the same as that for induction of delayed cell division but it was different from that for photoreactivation (Jagger and Stafford, 1965).

C. Factors influencing UV survival.

1. Effect of genetic background on UV sensitivity.

It was shown in Section III that the three *uvs*$^+$ strains, from which *uvs* mutants were obtained, were similar but not identical in their UV sensitivities. When survival curves were obtained for some recombinant *uvs* strains, V17 *proA1 mthB2 uraA4 strA1 uvsA4*, V41 *proA1 cysD18 uraA1 uvsC10* and V20 *proA1 mthB2 uraA1 strA1 uvsD3*, it was possible to compare them with those of the original *uvs* strains. (These survival curves were also needed for comparison with the presumed *uvsA4 uvsF25*, *uvsC10 uvsF25* or *uvsD3 uvsF25* recombinants from crosses of V17, V41 or V20 with V157 (Section IV E 4)).

The results for these three strains are presented in Table 42 and their survival curves plotted in Figure 35 together with those for the original *uvsA4*, *uvsC10* and *uvsD3* mutant strains. The *uvsA4* and *uvsD3* survival curves were similar; the differing genetic background had little effect. However, the two strains containing *uvsC10*

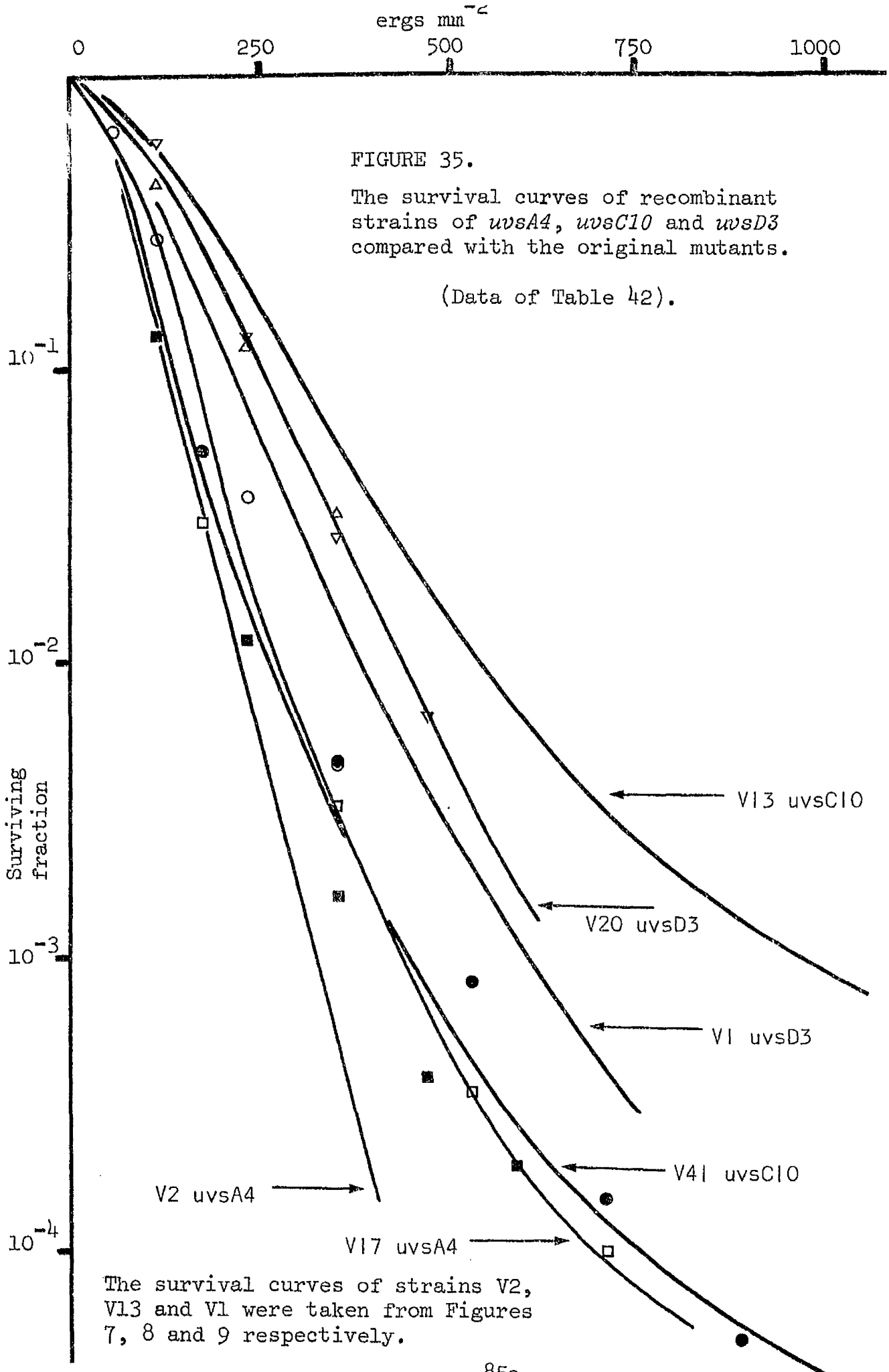


FIGURE 35.

The survival curves of recombinant strains of *uvsA4*, *uvsC10* and *uvsD3* compared with the original mutants.

(Data of Table 42).

The survival curves of strains V2, V13 and V1 were taken from Figures 7, 8 and 9 respectively.

TABLE 42. Data for UV survival curves of *uvsA4*, *uvsC10* and *uvsD3* recombinant strains (plotted in Fig. 35)

Dose ergs mm^{-2}	Strain V17 *proA1 mthB2 uraA1 strA1 uvsA4*				Strain V41 *proA1 cysD18 uraA1 uvsC10*			
	Experiment No. and symbol in Figure							
	1		2		1		2	
	Open circles		Open squares		Closed circles		Closed squares	
	a	b	a	b	a	b	a	b
0	572×10^5	1.0	214×10^4	1.0	678×10^4	1.0	148×10^5	1.0
59	385×10^5	6.7×10^{-1}	-	-	-	-	-	-
118	1593×10^4	2.8×10^{-1}	-	-	-	-	190×10^4	1.3×10^{-1}
177	-	-	649×10^2	3.0×10^{-2}	357×10^3	5.3×10^{-2}	-	-
236	2094×10^3	3.7×10^{-2}	-	-	-	-	177×10^3	1.2×10^{-2}
354	2545×10^2	4.5×10^{-3}	71×10^2	3.3×10^{-3}	314×10^2	4.6×10^{-3}	231×10^2	1.6×10^{-3}
472	-	-	-	-	-	-	581×10	3.9×10^{-4}
531	-	-	76×10	3.5×10^{-4}	554×10	8.2×10^{-4}	-	-
590	-	-	-	-	-	-	282×10	1.9×10^{-4}
708	-	-	22×10	1.0×10^{-4}	102×10	1.5×10^{-4}	-	-
885	-	-	-	-	35×10	5.0×10^{-5}	-	-
No. of samples at each dose	2		2		2		2	

Dose ergs mm^{-2}	Strain V20 *proA1 mthB2 uraA1 strA1 uvsD3*			
	1		2	
	Open erect triangles		Open inverted triangles	
	a	b	a	b
0	933×10^4	1.0	372×10^5	1.0
118	401×10^4	4.3×10^{-1}	186×10^5	5.0×10^{-1}
236	116×10^4	1.2×10^{-1}	481×10^4	1.3×10^{-1}
354	295×10^3	3.2×10^{-2}	1013×10^3	2.7×10^{-2}
472	-	-	2427×10^2	6.5×10^{-3}
No. of samples at each dose	2		2	

See legend to Table 13.

showed quite different curves, the recombinant *uvsC10* strain being more sensitive than the original strain. The cause of this difference was not investigated, but it may have been due to one of the modifiers postulated earlier to account for the variations in uvs^+ curves. The two *uvsC10* curves still fell within the range of sensitivities of all the other *uvsA*, *uvsC* and *uvsD* mutants studied.

2. Effect of the growth medium after irradiation on UV sensitivity.

Survival curves for different strains were performed under identical conditions on all strains except when growing survivors after the irradiation. Each strain was grown on MM supplemented only with its own growth requirements. Different supplements might have had differing effects on the viability of a given strain. One experiment on A3(2) was performed to test the likelihood of this. A spore suspension prepared in the usual way for a survival curve was exposed to 0, 2250 and 4500 ergs mm^{-2} and suitable serial dilutions of each sample were prepared. Six 1.0ml samples of each of the appropriate dilutions were pipetted into empty Petri dishes. Pairs of these samples were suspended in MM, or MM + Pro Arg Cys Ura, or MM + His Hom Phe and incubated. (These were the three most commonly used media in the experiments on survival curves in earlier sections). The results are presented in Table 43.

Although from these results the survival of A3(2) was higher on MM + His Hom Phe than on MM or on MM + Pro Arg Cys Ura, the increase in survival was proportionally the same irrespective of the UV dose, so that the surviving fractions calculated from the counts obtained on the three media were similar to one another. Therefore, the

different compositions of the plating media would not appear to have been an important cause of variations in the UV survival curves of the different mutants.

TABLE 43 The survival of irradiated *S. coelicolor* A3(2) when grown in the presence of different combinations of nutrients.

Composition of plating medium	ergs mm^{-2} 0	2250	4500
MM	205×10^{6}	140×10^{3}	133×10^{3}
	1.0	6.8×10^{-2}	6.5×10^{-4}
MM + His Hom Phe	227×10^{6}	218×10^{5}	136×10^{3}
	1.0	9.6×10^{-2}	6.0×10^{-4}
MM + Pro Arg Cys Ura	185×10^{6}	112×10^{5}	108×10^{3}
	1.0	6.1×10^{-2}	5.9×10^{-4}

VI. DISCUSSION.

The results presented in this thesis have established that *S. coelicolor* contains a number of genes in which mutations to greater UV sensitivity may be obtained. This implies that *S. coelicolor*, like many other protokaryotes, has mechanisms for preventing or repairing damage induced by UV. Mutants of this type have been most extensively characterised in strains of *Escherichia coli*, but the more limited studies that have been reported using other protokaryotes indicated that the genes shown to exist in *E. coli* are probably widespread amongst the protokaryotes (see Section I, B and C). Thus *S. coelicolor* may well have genes with similar functions to those discovered in *E. coli*. Since Clark and Margulies (1965) had shown that *rec* mutants of *E. coli* were also UV sensitive, it was hoped that some of the UV sensitive mutants obtained in *S. coelicolor* would also be *rec*, so that they could then be used to test a hypothesis for the origin of heteroclone genomes as described in Section I, A 3. However, despite the fact that none were apparently both UV sensitive and *rec*, other comparisons with *E. coli* and other protokaryotes were attempted.

The genetic map of *S. coelicolor* in Figure 1 includes the locations of genes shown to affect UV sensitivity. Three genes, *uvsA*, *uvsC* and *uvsD* are located in the short region between *ammA* and *serA*, three mutations (probably representing at least two genes, *uvsB* and *uvsE*), between *guaA* and *hisD*, and one gene, *uvsF*, between *cysC* and *argA*. The data for the order of the two genes *uvsB* and *uvsE* relative to *guaA* are very limited whereas those for *uvs-21* are more conclusive.

UvsA, *uvsC* and *uvsD* were defined by a complementation test

described in Section IV B. Attempts were made to apply a similar complementation test to the group of mutations *uvs-6*, *uvs-13* and *uvs-21* using pairs of complementing auxotrophic markers located near to this region, but so far without success since typical heteroclone progeny were not obtained. Further investigations are needed to clarify the reasons for this. However, *uvs-6* defined *uvsB* and *uvs-13* defined *uvsE* on the basis of phenotypic differences.

The genetic studies of UV sensitivity contribute to two characteristics of the distribution of genes with related functions already shown to exist in *S. coelicolor*. The two groups of genes, *uvsA*, *C*, *D* and *uvsB*, *E* are examples of clustering of related genes (Hopwood, 1965a, 1965c). The two groups are also approximately diametrically located and are members of the two *sequences* of functionally related genes or gene clusters composed of several approximately diametrically located pairs of related genes or clusters (Hopwood, 1967b). In *Escherichia coli* the members of two pairs of loci, *exr* (*E. coli* B) and *uvrA* (*E. coli* K12), and *recB* and *recC* have not so far been separated by other loci and therefore form clusters of related genes (see Figure 4). There are, in addition, other regions of the map where, with finer mapping, other clusters of phenotypically distinct but related genes may be shown to exist, for example, *uvrD* and *darA* or *uvs-1*, *-8*, *phr* and *fil*.

Since photoreactivation treatment has been shown to remove pyrimidine dimers from the DNA of photoreactivable cells (Setlow, 1966) it would be expected that photoreactivable strains which lacked a dark repair mechanism for dealing with pyrimidine dimers would still approach the sensitivity of wild-type strains under optimum conditions for photoreactivation. If the mutant strains were UV sensitive because

of inability to deal with damage other than pyrimidine dimers then photoreactivation treatment could not compensate for this deficient mechanism, and they would still be more UV sensitive than wild-type, despite photoreactivation treatment. Thus the photoreactivability of *uvs* mutants compared with *uvs*$^+$ strains enables deductions to be made as to whether the *uvs* mutants are deficient in a system involving elimination of the lethal effect of pyrimidine dimers or of some other kind of lesion. This indirect test of dimer eliminating ability was not applicable to strains of *S. coelicolor* A3(2), since A3(2) was not photoreactivable (see Section V b). Although another *S. coelicolor* strain, K673, was photoreactivable, crosses attempted between the two strains so far have proved infertile (D.A. Hopwood, personal communication), so that it was not possible to obtain a hybrid photoreactivable *uvs* strain by recombination.

Witkin (1963) showed that the addition of acriflavine at a concentration of 1ug/ml to the plating medium of UV irradiated *E. coli* B/r with incubation in the dark greatly increased the lethal effect of the UV, presumably by interfering with dark repair. However, there was no effect of acriflavine on the sensitivity of *S. coelicolor* A3(2) after UV irradiation, using acriflavine at a concentration of 1 ug/ml which did not affect the unirradiated viable count but caused the colony morphology to be more compact.

Metzger (1964) showed that dark repair in *E. coli* B and dark repair and Hcr of UV irradiated bacteriophage grown on *E. coli* K12 was also inhibited by caffeine using concentrations up to 1 mg/ml. Caffeine (at concentrations of 0.5 or 1.0 mg/ml) has also been shown to increase

UV induced lethality in the eukaryote *Schizosaccharomyces pombe* (Clarke, 1968). However, irradiation of *S. coelicolor* A3(2) suspensions and plating in medium with and without caffeine at 1.5 mg/ml (a concentration which did not affect the viability of unirradiated cells), showed no effect of the caffeine in enhancing the UV effect. Thus, it was not possible to implicate a dark repair system similar to that of other organisms by demonstrating its caffeine or acriflavine sensitivity.

The absence of a suitable bacteriophage with *S. coelicolor* as the host strain also prevented any attempt to detect a differential Hcr effect between the wild-type strains and any of the *uvs* mutants. Since Hcr ability has been shown to be correlated with dark repair *(uvr)* ability in *E. coli* (Howard-Flanders, Boyce and Theriot, 1966, for example) and other protokaryotes, the *uvs* mutants of *S. coelicolor* might in fact be Hcr^- and the uvs^+ strains Hcr^+. This possible similarity with an *E. coli* mechanism known to excise dimers would have implicated a dimer excising mechanism in *S. coelicolor*. The only alternative method would have been to attempt the demonstration of thymine dimer excision using cells labelled with H^3- thymidine by methods much as those of Setlow and Carrier (1964) and Boyce and Howard-Flanders (1964).

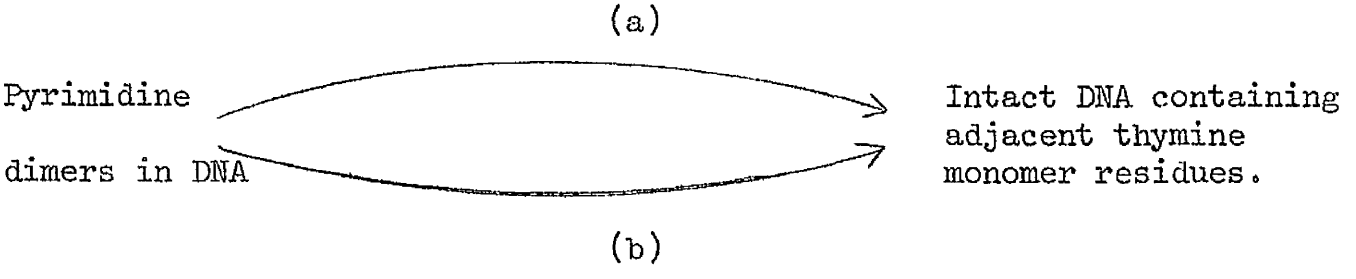
Thus in comparison with *E. coli*, all that can be said is that *E. coli* has at least four genes involved in dark excision repair of UV induced pyrimidine dimer damage and that *S. coelicolor* also has four genes having similar phenotypes that appear from double mutant studies to have related functions. These may be involved in the excision repair of dimers, but no real evidence of this was obtained.

Most *uvs* strains of *S. coelicolor* appeared to retain residual

repair activity since they were not as UV sensitive as the constructed double mutants nor the most sensitive single mutant in this group. They also appeared to retain another mechanism (or mechanisms) for overcoming UV induced damage since they were less sensitive than strains which contained some *uvsA* or *D* mutations as well as the UV sensitivity enhancer mutation *uvsF25*. Their survival curves were similar in shape to the *uvs*$^{+}$ survival curves, having a 'shoulder', an exponential region and a 'tail'. This was in contrast to the mutation *uvsE13* (provisionally defining gene *uvsE*) which had a survival curve lacking only the shoulder of that of its *uvs*$^{+}$ progenitor; its survival curve was exponential from the origin with the same slope as the exponential region of the *uvs*$^{+}$ curve. It would appear to have lost completely a repair mechanism which becomes saturated above about 1500 ergs mm^{-2}. The nature of this mechanism was not investigated.

One of the original *uvs* strains, V60, was found to be a spontaneous double mutant, containing a mutation in *uvsD*, *(uvsD18)* and another mutation, *uvs-25*, which was located distant from all the other mutations and defined the gene *uvsF*. The mutation *uvsF25* had no effect in a strain which was otherwise *uvs*$^{+}$. However, if the same strain contained the *uvsD* mutations, *uvsD3* or *uvsD18*, or the mutation *uvsC10*, the sensitivity of the double mutants was much greater than that conferred by the single *uvsC* or *uvsD* mutation. *UvsF25* was therefore an enhancer of the sensitivity conferred by these *uvsC* or *uvsD* mutations. Thus it would appear that the function affected by *uvsF25* was of no importance to the cell, provided *uvsC* and *uvsD* were functioning normally, when they could presumably cope with whatever was the deficiency due to

uvsF25. In the presence of defective *uvsC* and *uvsD* genes, which themselves conferred UV sensitivity upon a strain containing them, the state of *uvsF* was then of importance to the cell for overcoming the effects of UV. Thus there would seem to be at least two mechanisms affecting UV sensitivity in *S. coelicolor*. Since the effect of *uvsF25* on *uvsE13* was not known, these genes may or may not have related functions. If we accept, as seems likely, that UV in *S. coelicolor* as in other organisms induces pyrimidine dimers in its DNA and that these, unless dealt with by some mechanism, will be lethal to cells that contain them (it cannot be ruled out, however, that there are other minor products in DNA induced by UV), then alternative mechanisms of repair of the same lesions (pyrimidine dimers) seem more likely than separate pathways acting upon distinct lesions (unless these were different pyrimidine dimers, for example, cytosine-cytosine as opposed to cytosine-thymine or thymine-thymine dimers). The *uvr* and *phr* mechanisms certainly operate on more than one type of dimer (Howard-Flanders, Boyce and Theriot, 1966, Setlow, 1966).



The two candidates for (a) and (b) from the studies of *E. coli* would be dark repair and recombinational repair. In this organism a defect in either mechanism confers an increased UV sensitivity upon a strain containing the defect, but this may not necessarily be the case in other organisms. For example, we may postulate that the intact

mechanism involving the genes *uvsC* and *uvsD* (and *uvsA* and *uvsB*) has sufficient capacity to repair any UV damage not repaired by the defective mechanism, e.g. that caused by *uvsF25*; or that it is so efficient that the second mechanism does not normally act on UV induced defects, but that the reverse was not so. When both are defective, however, much less (if any) repair occurs and the cell is more sensitive even than a cell in which the least efficient mechanism is intact. Since crosses in which both parents carried the same mutation, using representative mutations of all the loci so far defined, have yielded recombinants, none of these loci were apparently concerned with recombination. However, this test for recombination in *S. coelicolor* would not have detected a reduced level of recombination because of the difficulty of making absolute comparisons of recombinant frequencies between different crosses. However, it is possible in organisms other than *E. coli* that the recombinational mechanism of repairing UV damage may not be involved in normal recombination, but that such a mechanism could involve steps which had counterparts in recombination; perhaps they could have had a common evolutionary origin.

From the results so far obtained with *uvs* mutants other approaches for the isolation of *rec* mutants would probably be more promising. As an indirect approach, isolation of mutations causing X-ray sensitivity might yield some mutants which were also *rec*, since three out of four loci in *E. coli* which control X-ray sensitivity also control recombination.

In conclusion, the discovery of UV sensitive mutants in *S. coelicolor* has provided the material for further comparative studies with other protokaryotes. Thus the mechanisms of UV repair could be

further investigated, for example, by a direct assay for thymine dimer excision; and the mechanisms of UV mutation and their relation to mutation by other mutagens could also be studied. Once again, this field has already been extensively investigated in *E. coli* using UV sensitive mutants.

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