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The genetic implications
of u.v. light exposure and liquid-holding post-treatment in
the yeast *Saccharomyces cerevisiae*

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SUMMARY

After liquid-holding treatment in saline, cultures of yeast exposed to u.v. light showed an increased resistance to a second exposure to u.v. irradiation. This increase in resistance during a second series of u.v. exposures was correlated with an increase in the induction of intragenic recombinants. In contrast, no increase in the frequency of the intergenic recombinants or mutations to prototrophy could be detected during the second u.v. dose range. The results obtained could not be explained by the induction of meiosis during liquid holding or by changes in the timing of cell division after u.v. exposure.

A model of u.v. repair in yeast is postulated in which liquid-holding treatment results in changes in the proportions of lesions repaired by excision repair and recombination repair (respectively).

1. INTRODUCTION

The storage of u.v. inactivated wild-type cells of the yeast *Saccharomyces cerevisiae* in saline or distilled water before plating upon growth media results in increased cell survival compared with plating immediately after u.v. exposure (Patrick, Haynes & Uretz, 1964).

In bacteria the effect, which has been called liquid-holding recovery, is generally demonstrable only in some radiation-sensitive strains whose genetic defect has been ascribed to a change in the activity of the repair system termed recombination repair (REC) (Ganeson & Smith, 1968). This repair system is said to operate by the replication of a DNA region containing a u.v.-induced lesion, thus resulting in single-strand breaks. These regions may then recombine to produce intact regions and thus viable cells (Howard-Flanders, 1968). A reduction in cell viability after liquid holding has been demonstrated in some UVS mutants of *Saccharomyces cerevisiae* (Parry & Parry, 1969) and in wild-type cultures of the fission yeast *Schizosaccharomyces pombe* (Harm & Haefner, 1968).

Liquid-holding treatment has also been shown to have a number of genetic effects in *Saccharomyces cerevisiae*. Incubation in saline after u.v. exposure results in increased yields of mitotic intragenic recombinants and decreased yields of mitotic intergenic recombinants and mutations to prototrophy (Parry & Cox, 1968).

Patrick & Haynes (1968) have demonstrated an additional property of liquid-holding treatment in *Saccharomyces cerevisiae*, namely that cultures exposed to a second dose range of u.v. light after liquid holding show an increased resistance to u.v. light but not to X-rays. This property has been described as 'repair resistance'.

In this publication we report upon the genetic effects of the repair resistance phenomenon in cultures of yeast wild type for u.v. sensitivity. In these cultures the rates of mitotic intra- and intergenic recombination, mutation to prototrophy and cell viability were determined.

2. MATERIALS AND METHODS

(i) *Strains*

The cultures used in the experiments described were of the following genotypes:

(1) a WT prototrophic haploid culture

(2) $\dfrac{a\ +}{\alpha\ +}$ prototrophic diploid culture

(3) $\dfrac{a\ ade\,20}{\alpha\ ade\,20}$ auxotrophic diploid culture

(4) $\dfrac{a\ ade\,20}{\alpha\ \ +\ \ }$ prototrophic diploid, heterozygous at *ade-2* locus

(5) $\dfrac{a\ ade\,20}{\alpha\ ade\,21}$ auxotrophic diploid, heteroallelic at *ade-2* locus

ade 20 and *ade 21* are alleles of the gene *ade 2*, which is on fragment I (Mortimer & Hawthorne 1963). Diploids 3 and 5 form red colonies on complete medium and require adenine for growth. Diploids 2 and 4 and haploid 1 form white colonies on complete medium and are adenine-independent.

(ii) *Media*

The complete medium used (YC) was described by Cox & Bevan (1962). It is a yeast extract and peptone medium with 4 % (w/v) glucose, pH 6·7; when required the medium was solidified with Oxoid Agar No. 3. The minimal medium (YNB) was Difco Yeast Nitrogen Base without amino acids, solidified with Oxoid Agar No. 1 and supplemented with growth factors as necessary.

The sporulation medium used in some experiments was an acetate-raffinose medium as described by Pomper, Daniels & McKee (1954).

(iii) *U.v. treatment*

Cultures were grown for 3 days at 28 °C on solid YC medium, washed off and suspended in saline at a titre of approximately 10^7 cells/ml and shaken vigorously. These cultures were at the end of the log phase of growth and contain less than 5 % budded cells. The source of u.v. light was a Hanovia Model 11A Mercury discharge tube generating almost monochromatic radiation at 2357 Å. Cultures were exposed to a dose rate of 22 ergs/mm²/sec monitored by a calibrated photocell and voltmeter. 10 ml portions of the culture were exposed to u.v. in an agitated

open Petri dish. Cultures whose u.v. sensitivities were to be compared were in all cases irradiated at equal volumes of the same dilution. All manipulations were carried out in red light to avoid photoreactivation.

(iv) *Liquid-holding treatment*

Samples of u.v.-treated suspensions and untreated controls were stored in saline in light-proof bottles at 28 °C for 7 days.

In one series of experiments u.v.-treated cultures were suspended in aerated sporulation medium at 28 °C for periods of 48 h. This treatment results in the induction of meiosis as shown by the observed presence of spore-tetrads.

(v) *Growth in liquid culture*

In experiments to determine the timing of cell division after u.v. exposure, samples of treated cultures were grown in liquid YC at 28 °C in the dark for periods of up to 6 h. Samples were removed at 30 min intervals and the cell number determined by the use of a haemocytometer.

(vi) *Incubation and scoring*

All plated cultures were incubated at 28 °C in the dark and scored after 5 days growth. Survival was scored by counting colony-forming units on YC medium. Mitotic intragenic recombination and mutation was scored by counting adenine independent colonies on adenineless YNB. The frequency of adenine recombination was determined by performing appropriate dilutions to give a per plate count of at least 100 recombinants per 10^7 cells plated. The yield of recombinants obtained varied between $120/10^7$ and $500/10^5$ plated cells. Mutation to prototrophy was determined by performing appropriate dilutions giving at least 20 prototrophs per plate. All data was expressed as the mean of at least five separate plates per dilution. Mitotic intergenic recombination was scored by the detection of red colonies due to the formation of recessive homozygotes at the adenine-2 locus by mitotic crossing over in an adenine-2 heterozygote. The data is expressed as the mean frequency of homozygous colonies detected in a count of at least 1000 viable colonies after 5 days growth.

(vii) *Experiments*

The yeast cultures described were given the following treatments, after which survival, mutation rate and the induction of mitotic inter- and intragenic recombination were determined:

(1) U.v. exposure alone.

(2) U.v. + 7 days liquid holding.

(3) U.v. + 7 days liquid holding + 2nd u.v. exposure series.

(4) U.v. + 7 days liquid holding + 2nd u.v. + 7 days liquid holding.

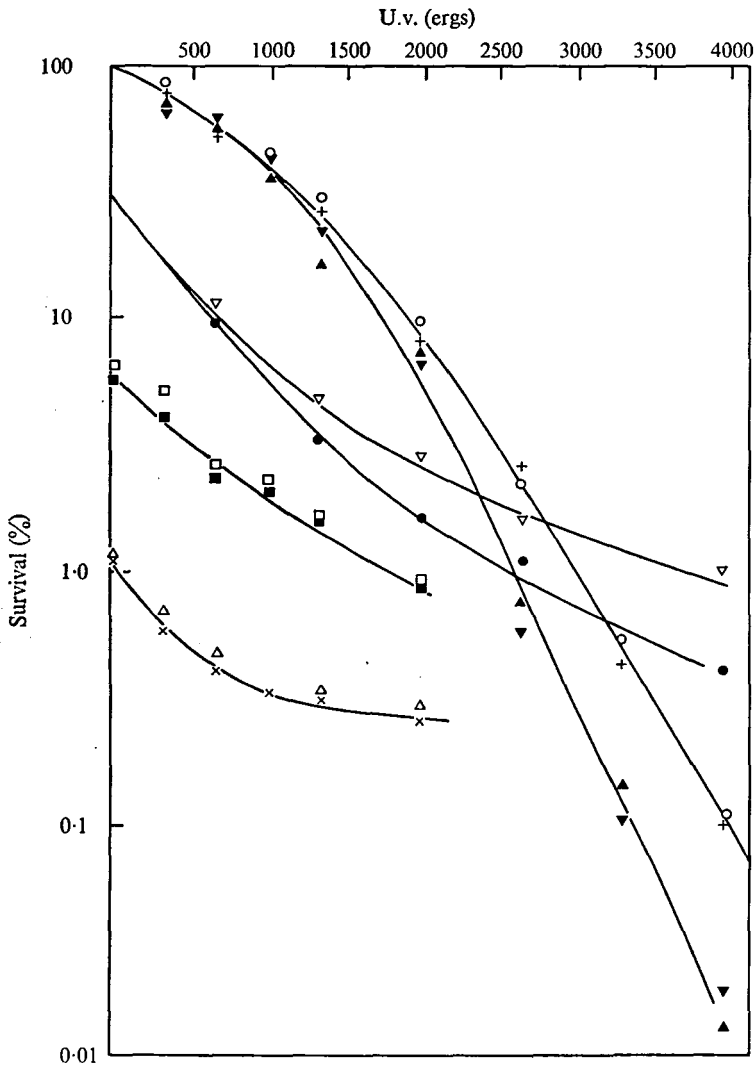All experiments were performed at least twice and representative results are presented here.

Fig. 1. Cell viability after u.v. exposure before and after liquid-holding treatment in haploid culture 1. ▲, Culture plated immediately after u.v. exposure. ▼, Culture held in saline for 7 days, followed by u.v. exposure then plated immediately. ○, Culture irradiated with u.v. then held in saline for 7 days before plating. +, Cultures held for 7 days in saline before u.v. exposure followed by 7 days in saline before plating. ●, 1320 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure followed by immediate plating. ▽, 1320 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure followed by 7 days in saline before plating. ■, 2640 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure, followed by immediate plating. □, 2640 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure, followed by 7 days in saline before plating. ×, 3960 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure followed by immediate plating. △, 3960 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure, followed by 7 days in saline before plating.
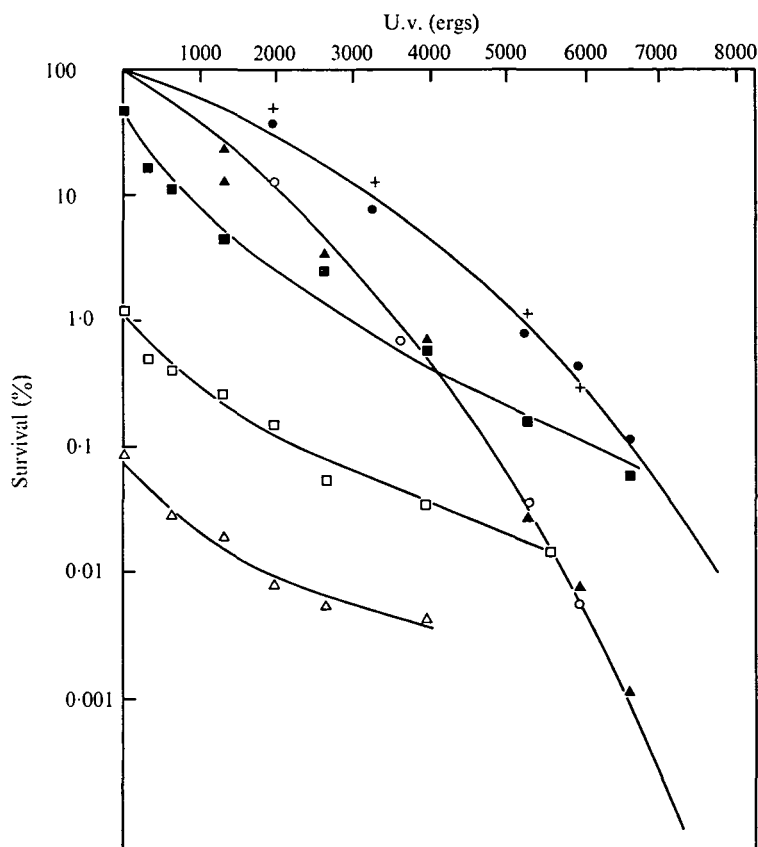
Fig. 2. Cell viability after u.v. exposure before and after liquid-holding treatment in diploid culture 2. ▲, Culture plated immediately after u.v. exposure. ○, Culture held for 7 days in saline, followed by u.v. exposure then plated immediately. +, Culture irradiated with u.v. then held in saline for 7 days before plating. ●, Culture held in saline for 7 days before u.v. exposure, followed by 7 days in saline before plating. ■, 2640 ergs/mm² of u.v., followed by 7 days in saline, then 2nd u.v. exposure, followed by immediate plating. □, 5280 ergs/mm² of u.v. followed by 7 days in saline, then 2nd u.v. exposure, followed by immediate plating. △, 6600 ergs/mm² of u.v., followed by 7 days in saline, then 2nd exposure, followed by immediate plating.

3. RESULTS

The presence of repair resistance after post-u.v. liquid-holding treatment in both haploid and diploid yeast cultures is shown by the results expressed in Figs. 1 and 2. The results presented indicate that liquid-holding treatment in saline after u.v. exposure results in increased resistance to a 2nd u.v. dose range as shown by the change in slope of the second compared with the first survival curve. The characteristics of each survival curve are expressed in Table 1, where the slope of each curve is shown as a numerical value, i.e. the dose of u.v. in ergs required to produce a reduction of one log cycle in cell viability.
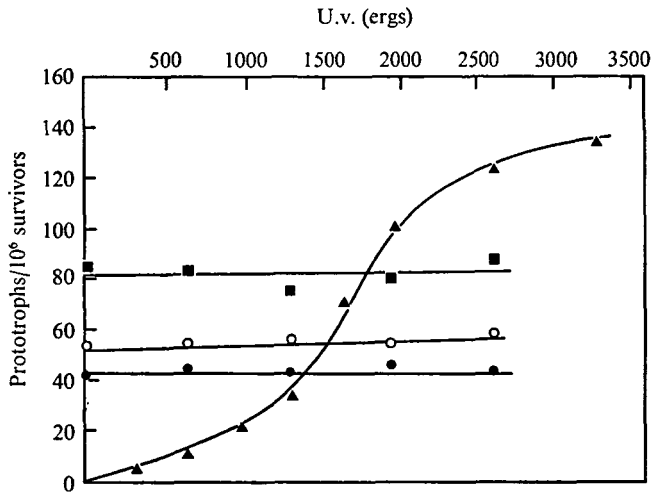
Fig. 3. Increase in mutations to prototrophy in homozygous adenine requiring diploid 3 before and after liquid-holding treatment. ▲, Culture plated immediately after u.v. exposure. ●, 1320 ergs/mm² of u.v., followed by 7 days in saline, followed by 2nd u.v. exposures, then plated immediately. ○, 1980 ergs/mm² of u.v., followed by 7 days in saline, followed by 2nd u.v. exposures, then plated immediately. ■, 2640 ergs/mm² of u.v., followed by 7 days in saline, followed by 2nd u.v. exposures, then plated immediately.

Table 1. *The effects of liquid holding treatment upon u.v. survival in haploid and diploid yeast cultures*

(Each result describes the characteristics of a u.v. survival curve produced by irradiation after the treatment listed in column 2. Where biphasic curves were obtained, the result listed describes only the slope of the most sensitive cell fraction.)

Strain	Treatment given to culture before the final series of u.v. exposures	Survival curve expressed as ergs of u.v. required to give a 1 log cycle reduction in cell viability
n	Control	700
	7 days liquid holding	700
	1320 ergs u.v. + liquid holding	1350
	2640 ergs u.v. + liquid holding	2100
	3960 ergs u.v. + liquid holding	2000
2n	Control	900
	7 days liquid holding	900
	2640 ergs u.v. + liquid holding	1600
	5280 ergs u.v. + liquid holding	2200
	6600 ergs u.v. + liquid holding	2200

A number of the survival curves obtained after liquid-holding treatment are biphasic, suggesting the presence of cell fractions of differing sensitivity. The comparisons of survival shown in Table 1 are based upon the slope of the survival curve for the most sensitive cell fraction. In Figs. 1 and 2 it can be seen that in cultures given only one series of u.v. exposures before plating a period of liquid
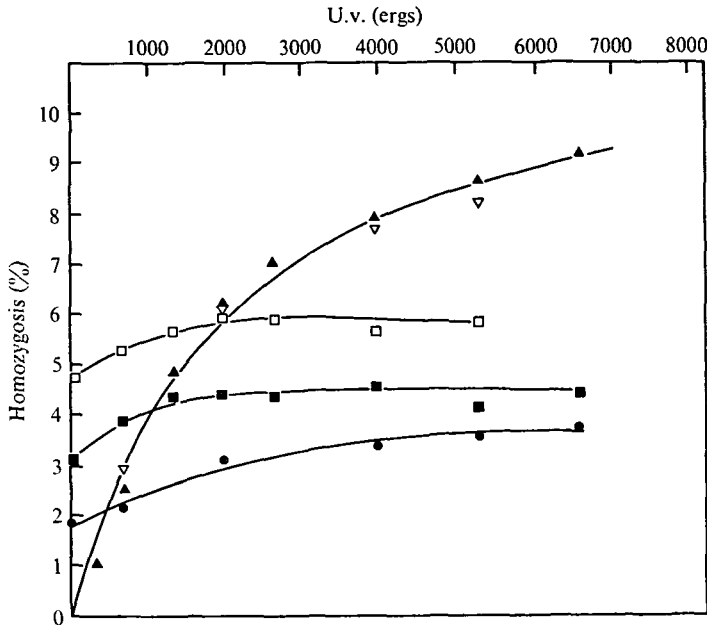
Fig. 4. Frequency of recessive homozygous recombinants after u.v. exposure before and after liquid-holding treatment in heterozygous diploid 4. ▲, Culture plated immediately after u.v. exposure. ▽, Culture held for 7 days in saline, followed by u.v. exposure and immediate plating. ●, 1320 ergs/mm² of u.v., then held in saline for 7 days, followed by 2nd u.v. exposure and plated immediately. ■, 2640 ergs/mm² of u.v. then held in saline for 7 days, followed by 2nd u.v. exposure and plated immediately □, 5280 ergs/mm² of u.v., then held in saline for 7 days, followed by 2nd u.v. exposure and plated immediately.

holding treatment led to an increase in cell viability as shown by comparison of the results of (liquid holding + u.v.) and (liquid holding + u.v. + liquid holding). In both haploid and diploid cultures a significant increase in viability was produced by the second period of liquid holding. This result can also be seen in the haploid culture after the treatment (60 sec u.v. + liquid holding + u.v.) but not at the higher exposures (120 sec u.v. + liquid holding + u.v.) and (180 sec u.v. + liquid holding + u.v.). At the doses giving higher levels of resistance no significant effect of a final period of liquid holding could be detected.

The effect of liquid-holding treatment upon u.v.-induced mutation from auxotrophy to prototrophy has been determined in diploid cultures homozygous at the adenine-2 locus and are expressed in Fig. 3. The results demonstrate that during the first u.v. dose range the frequency of prototrophs per survivor increased to a maximum of approximately 130 prototrophs/10^6 survivors at 3300 ergs of u.v. Liquid-holding treatment after u.v. exposure resulted in a reduction in the yield of prototrophs to a maximum of approximately 95 prototrophs/10^6 survivors. Cultures which had been exposed to the treatment (u.v. + liquid holding + u.v.) are also shown. In none of these cultures, all of which showed repair resistance for survival, could any increase in prototrophy frequency be detected during the
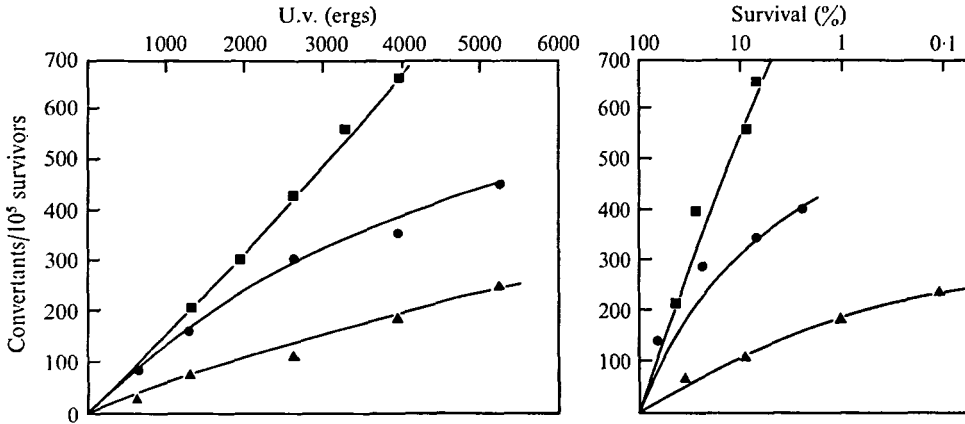
Fig. 5. Frequency of prototrophic recombinants/10⁵ survivors after u.v. exposure with and without liquid-holding treatment in the heteroallelic diploid 5. The data is expressed to show the increase in prototrophs per unit of u.v. light and per survivor. ▲, Culture plated immediately after u.v. exposure. ●, 1320 ergs/mm² of u.v., then held in saline for 7 days, followed by 2nd u.v. exposure and plated immediately. ■, 2640 ergs/mm² of u.v., then held in saline for 7 days, followed by 2nd u.v. exposure and plated immediately.

second u.v. dose range. Thus in a culture exposed to conditions favourable for repair resistance no u.v.-induced mutation to prototrophy could be detected.

The frequency of u.v.-induced mitotic intergenic recombination scored by the production of homozygous red adenine requiring colonies in a white diploid culture heterozygous at the adenine-2 locus is shown in Fig. 4. The results obtained show a similar pattern of response to u.v. exposure and liquid-holding treatment as that shown by mutation to prototrophy. The frequency of homozygous colonies increased during the 1st u.v. dose range, reaching a maximum of 9·2 % homozygosis after 6600 ergs of u.v.; at doses greater than 6600 ergs, a reduction in homozygosis was observed. A period of liquid-holding treatment before plating resulted in a significant reduction in the frequency of homozygous colonies at all u.v. exposures tested. Cultures which were exposed to a second u.v. dose range after the treatment (u.v. + liquid holding) showed little or no increase in the yield of homozygous colonies. After 5280 ergs of u.v. + liquid-holding homozygous colony frequency increased from 4·8 % to a maximum of 6·0 %.

In direct contrast to the results obtained with regard to u.v.-induced mitotic intergenic recombination and mutation to prototrophy are those obtained with mitotic intragenic recombination shown in Fig. 5. Intragenic mitotic recombination, detected by a production of prototrophic colonies on selective medium in an auxotrophic yeast culture heteroallelic at the adenine-2 locus, increased during the 1st u.v. exposure, reaching a maximum of approximately 250 recombinant/10⁵ survivors after 5300 ergs of u.v. light giving a cell survival of 0·1 %. Cultures exposed to a second u.v. dose range after the treatments (u.v. + liquid holding) showed a significant further increase in the yield of recombinants both per erg of

Table 2. *The effect of modifying treatment upon the u.v.-induced frequency of adenine independent recombinants in a heterallelic diploid 20/21*

(The results indicate the yields of recombinants produced after u.v. exposures of 2000 ergs and at cell survival of 10%.)

Treatment	Increase in adenine recombinant frequency per 10^5 survivors	
	2000 ergs u.v.	10% survival
Control	105	110
7 days liquid holding	105	110
1320 ergs u.v. + liquid holding	235	320
2640 ergs u.v. + liquid holding	300	550

All results of recombination frequency are based upon the increase in adenine prototrophs with u.v. exposure and have been corrected for the frequency of prototrophs present in each culture before the various treatments.

u.v. and per survivor. The increase in intragenic recombinant frequency is shown by the data in Table 2, which illustrates the yield of adenine recombinants after the various treatments at a u.v. dose of 2000 ergs and at a cell survival of 10%.

The possibility that the differences in the yields of intra- and intergenic recombinants after u.v. irradiation and liquid holding treatment may be due to selective differences between recombinant and non-recombinant cells was tested in both diploids 4 and 5 in a series of experiments. In these experiments the following comparisons of u.v. sensitivity before and after liquid-holding treatment were made:

(1) Between heterozygous colonies of genotype *ade* 2/+ from the prototrophic diploid 4 and a sample of homozygous recessive auxotrophic recombinants derived from diploid 4.

(2) Between heteroallelic colonies of genotype *ade* 20/*ade* 21 from the auxotrophic diploid 5 and a sample of prototrophic recombinants derived from diploid 5.

The comparisons of the pairs of cultures both recombinant and non-recombinant are shown in Figs. 6(*a*, *b*). The results shown indicate that in both Expts 1 and 2 there is no indication of any significant differences in sensitivity between recombinant and non-recombinant cells either before or after liquid-holding treatment. The results therefore suggest that the observed yields of recombinant cells produced by u.v. irradiation and liquid-holding treatment are not the result of selective differences between recombinant and non-recombinant cells.

One possible explanation for the observed changes in the rates of mutation to prototrophy and intra- and intergenic mitotic recombination produced by liquid holding treatment is that they result from the onset of meiosis in diploid cultures during the period of post-u.v. incubation in saline. This possibility was tested by two series of experiments:

(1) By the examination of all cultures after liquid-holding treatment for the presence of spore tetrads and any of the observable manifestations of meiosis. This
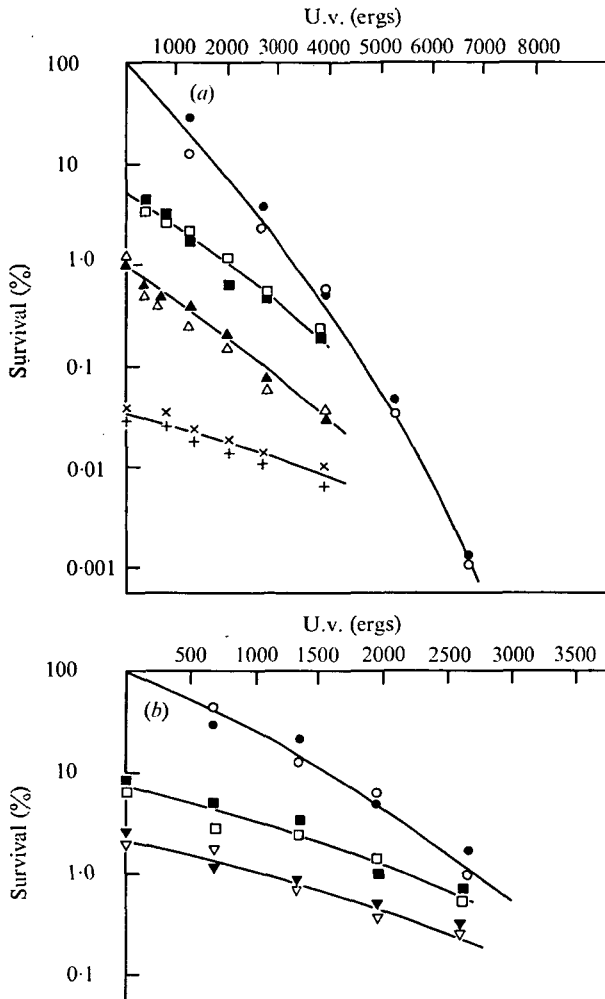
Fig. 6. Comparison of the u.v. sensitivities before and after liquid-holding treatment of parental diploid strains 4 and 5 with samples of recombinant colonies derived from them. Recombinant sample produced by the harvesting of 100 recombinant colonies produced after 330 sec u.v. exposure. Open symbols, *ade 2/ade 2*; close symbols, *ade 2/ +* .

(*a*) Comparison between cultures of genotype *ade 2/ +* and *ade 2/ade 2*. ●, Culture plated immediately after u.v. exposure. ■, 3960 ergs of u.v., then 7 days in saline, followed by 2nd u.v. exposure and immediate plating. ▲, 5280 ergs of u.v., then 7 days in saline, followed by 2nd u.v. exposure and immediate plating. ×, 6600 ergs of u.v., then 7 days in saline, followed by 2nd u.v. exposure and immediate plating.

(*b*) Comparison between the culture of genotype *ade 20/ade 21* and *ade 2/ +* . Closed symbols indicate parental strains and open symbols recombinant cultures. ●, Culture plated immediately after u.v. exposure. ■, 1980 ergs of u.v., then 7 days in saline, followed by 2nd exposure and immediate plating. ▼, 2640 ergs of u.v., then 7 days in saline followed by 2nd u.v. exposure and immediate plating.

Table 3. *The effects of incubation in sporulation medium after u.v. irradiation upon cell viability and upon mitotic intergenic recombination, scored by frequency of recessive homozygosis for adenine requirement*

| Incubation in sporulation medium before plating (h)	Control		2640 ergs of u.v.		3960 ergs of u.v.		5280 ergs of u.v.	
	Cell viability (%)	Homozygosis (%)	Cell viability (%)	Homozygosis (%)	Cell viability (%)	Homozygosis (%)	Cell viability (%)	Homozygosis (%)
0	100 ± 3·2	0·0	18·7 ± 1·5	3·5	2·35 ± 0·2	5·1	0·642 ± 0·03	8·4
3	—	—	16·7 ± 1·7	3·7	2·7 ± 0·21	8·3	0·993 ± 0·04	16·7
6	80·0 ± 3·3	0·22	22·4 ± 2·1	11·9	4·15 ± 0·274	14·0	0·82 ± 0·038	18·5
9	—	—	34·0 ± 2·1	14·0	5·75 ± 0·45	17·2	1·0 ± 0·03	13·4
24	87·5 ± 3·1	0·14	—	—	—	—	2·9 ± 0·56	24·0
48	98·9 ± 3·2	0·18	53·0 ± 3·08	11·9	25·6 ± 0·75	25·9	2·7 ± 0·07	27·3
2640 ergs + 6 h in sporulation medium followed by u.v.	22·4 ± 0·20	11·9	1·13 ± 0·143	21·0	0·52 ± 0·174	34·0	0·156 ± 0·02	37·0
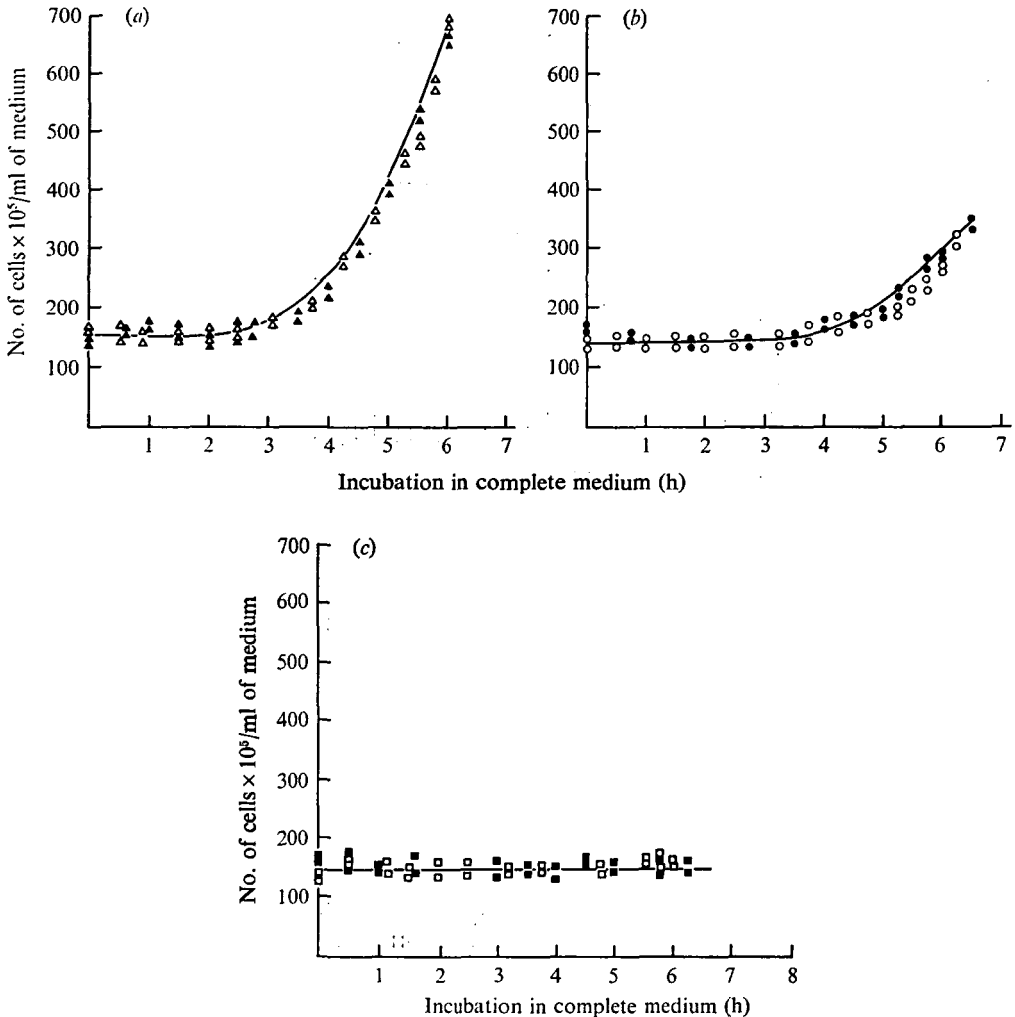
Fig. 7. A comparison of the effect of u.v. exposure and liquid-holding treatment upon cell division after incubation in liquid growth medium. Open symbols indicate incubation immediately after u.v. exposure. Closed symbols indicate incubation after a period of 7 days in saline. $\triangle$, $\blacktriangle$, Control; $\bigcirc$, $\bullet$, 1320 ergs/mm² of u.v.; $\square$, $\blacksquare$, 5280 ergs/mm² of u.v.

examination did not reveal the presence of any of the observable stages of meiosis in any of the u.v.-treated or control cultures.

(2) By the deliberate induction of meiosis after u.v. exposure by incubation in aerated sporulation medium in place of the usual storage in saline. The results of such a procedure upon the induction of homozygous red adenine-requiring colonies in a culture heterozygous at the adenine-2 locus may be seen in Table 3. The results presented indicate that the induction of meiosis resulted in a significant increase in the frequency of homozygous recessive colonies during which period cell viability also showed a significant increase. For example, a u.v. exposure of 2660 ergs/mm²

resulted in a cell survival of $2.35 \pm 0.2\%$ and a frequency of 5.1% recessive homozygosis; after incubation in sporulation medium survival increased to $5.57 \pm 0.45\%$ and the frequency of recessive homozygosis to 17.2%.

The results in Table 3 also show the effect of exposure to a second u.v. dose range after a first u.v. exposure of 2660 ergs/mm² followed by 6 h of incubation in sporulation medium. This treatment produces a further increase in the frequency of recessive homozygosis. Those measurements of recessive homozygosis, obtained after the induction of meiosis, are in direct contrast to those obtained after liquid holding in saline which resulted in a consistant reduction in the frequency of adenine homozygotes.

No attempt was made in the experiments utilizing sporulation medium to determine the percentage of adenine requiring colonies that were haploid.

The results of both series of experiments indicate that the observed effects of the liquid-holding treatment reported here cannot be explained by the induction of meiosis during the period of liquid holding in saline.

The effects of post-u.v. liquid-holding treatment upon the timing of cell division when cultures are placed in aerated liquid growth medium has also been determined. After cultures had been exposed to u.v. irradiation they were either incubated in medium immediately or after 7 days storage in saline. Representative results of these experiments are shown in Fig. 7. The results indicate that although increases in u.v. exposure produced a delay in the timing of the onset of cell division, no significant difference could be detected between cultures incubated immediately or after a period of liquid holding. These results suggested that the observations made here upon the genetic effects of liquid holding are not explicable on the basis of a differential timing of cell division after u.v. exposure and post treatments.

No attempt was made in these experiments to determine the timing of DNA synthesis after u.v. exposure, but such experiments are in progress and the results will be reported upon at a later date.

4. DISCUSSION

The results of our experiments may be summarized as in Table 4.
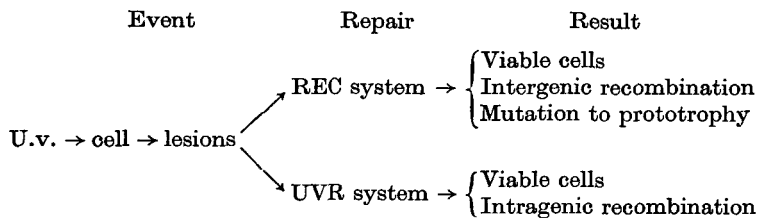
In the bacterium *Escherichia coli* K-12 the effects of post-u.v. liquid-holding treatment have been ascribed to an increase in the proportion of u.v.-induced lesions repaired by the UVR system and a reduction in those repaired by the REC system (Ganeson & Smith, 1968, 1969). By analogy with the bacterial situation we wish to propose the following model of u.v.-induced genetic change in vegetative cultures of the yeast *Saccharomyces cerevisiae*.

U.v.-light exposure at 2357 Å results in the production of pyrimidine dimers and other 'unknown' lesions. The induction of these lesions may result in cell death, or, after the action of the cellular repair system, mutation to prototrophy and mitotic recombination. Repair of u.v.-induced lesions may occur by either of two pathways – either by the replication across a lesion, followed by strand recombination to produce intact viable strands governed by the REC system, or by exision-repair governed by the UVR system. UVR repair has been postulated as an error-

Table 4. *Summary of experimental results*

Event	Treatment			
	U.v. ⟶ LH ⟶ u.v. ⟶ LH			
Cell death	Increase	Decrease	Increase lower per unit of u.v.	Decrease
Mitotic intergenic recombination	Increase	Decrease	No change	Decrease
Mitotic intragenic recombination	Increase	Increase	Increase greater per unit of u.v.	No data
Mutation to prototrophy	Increase	Decrease	No change	No data

Table 5

Event	Repair	Result
U.v. → cell → lesions	REC system →	Viable cells / Intergenic recombination / Mutation to prototrophy
	UVR system →	Viable cells / Intragenic recombination

free system, whereas REC repair, in contrast, is an error-prone mechanism (Witkin, 1969).

A general outline is shown in Table 5. It is proposed that those lesions which are repaired by the error-prone REC system may result in mitotic intergenic recombinants and mutations to prototrophy. In contrast, those lesions which are repaired by the error-free UVR system may result in mitotic intragenic recombinants. One feature of the model is that repair of a u.v. lesion by the UVR system has a higher probability of leading to cell viability than repair by the REC system.

The observed effects of post-u.v. liquid holding upon cell viability, mitotic recombination and mutation to prototrophy may be interpreted as a change in the proportion of lesions repaired by the REC system or the UVR system. It is proposed that after a period of liquid-holding treatment a lower proportion of u.v.-induced lesions are repaired by the REC system and a higher proportion repaired by the UVR system compared with direct plating. Thus after liquid-holding treatment this increase in UVR repair would result in increased viability and intragenic recombination and reduced intergenic recombination and mutation to prototrophy. After a period of liquid-holding treatment, the yeast culture responds to a second dose of u.v. irradiation by repair of u.v. lesions exclusively by the UVR system. This would result in an increased proportion of lesions being exposed to excision-repair with a resulting increase in the fraction of viable cells and an increase in the proportion of intragenic recombination. Since few, if any, of the lesions are repaired by the REC system little or no increase would be observed in the frequency of intergenic recombinants or mutation to prototrophy.

The change in the frequency of lesions repaired after liquid holding by the UVR and REC system respectively may be the result of a number of factors.

For example, a decay in the enzymes involved in the REC system may take place, or some other agency may result in the prevention of replication of the DNA helix beyond the position of a u.v. lesion, thus preventing the development of regions of newly synthesized DNA containing single strand gaps. The fact that intergenic recombinants, formed by REC repair, are reduced at high doses of u.v. suggests that the replication of dimer-containing regions may be inhibited under some conditions.

The proposed model of the response of yeast to u.v. light makes a number of predictions, perhaps the most interesting of which are that:

(1) Inter- and intragenic mitotic recombination induced by u.v. light is the result of repair by different pathways.

(2) In cells exposed to liquid-holding treatment a higher proportion of u.v. lesions are repaired by excision repair than in cells which are immediately plated on to growth medium.

The first prediction of the model will be open to testing when strains of yeast are available which are clearly known to be defective in either the UVR system or the REC system. In such strains the presence or absence of inter- and intragenic recombinants after u.v. exposure may indicate whether in fact the two genetic events are the result of different pathways of repair. Unfortunately we are not yet able to ascribe the defect of UVS yeast strains to any particular pathway with precision.

The second prediction is also open to test due to the different characteristics of the cellular DNA exposed to either UVR or REC repair after the 1st post-u.v. replication cycle. During immediate post-u.v. exposure to growth media, lesions are repaired by both UVR and REC repair, thus producing DNA containing single strand breaks as a common intermediate. In the case of REC repair single-strand lesions are the result of the replication of non-excised lesions. After liquid-holding treatment the resultant DNA contains no single-strand gaps before DNA replication as all repair has occurred by the UVR system. Theoretically such a situation may be resolved by examination of the physical properties of the DNA before and after liquid-holding treatment. Unfortunately the situation may be more difficult to resolve in a fungal system due to the absence of specific labelling techniques but the problem appears worthy of an attempted solution.

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