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ULTRAVIOLET-INACTIVATION OF CONIDIA FROM HETEROKARYONS OF *NEUROSPORA CRASSA* CONTAINING UV-SENSITIVE MUTATIONS

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SUMMARY

The effect of three UV-sensitive mutations of Neurospora crassa, upr-1, uvs-4 and uvs-6, on the ultraviolet-inactivation of conidia from two-component heterokaryons was investigated.

In two-component heterokaryons with wild-type sensitivity to radiation inactivation, all three conidial fractions exhibited similar ultraviolet-inactivation curves. Each UV-sensitive mutation studied uniquely modified the ultraviolet-inactivation curves of conidia from two-component heterokaryons.

In heterokaryons heterokaryotic for upr-1, the upr-1 mutation was recessive and the repair function determined by the wild-type allele was functional to some degree in homokaryotic upr-1 conidia. All three conidial fractions of heterokaryons containing upr-1 in both components showed increased sensitivity to ultraviolet light.

The uvs-4 mutation was recessive and resulted in conidia with increased UV-sensitivity only when included in both components of a heterokaryon. Homokaryotic uvs-4 conidia, which arose from heterokaryons containing both uvs-4 and wild-type components, exhibited wild-type survival. Therefore, as with upr-1, there was a carryover of the repair capability to conidia which were genetically UV-sensitive.

The uvs-6 mutation, when included in one component of a two-component heterokaryon, resulted in increased UV-sensitivity of both heterokaryotic and homokaryotic uvs-6 conidia. When both components contained uvs-6, the UV-sensitivity of all three conidial fractions

was increased and all showed similar inactivation curves. Thus, as with upr-1 and uvs-4, there was a carryover of the wild-type repair capability to genetically uvs-6 conidia.

Heterokaryon tests for complementation between two non-allelic UV-sensitive mutations showed that in heterokaryotic conidia, complete complementation occurred between upr-1 and uvs-4.

INTRODUCTION

The recognition and partial elucidation of enzymatic systems capable of repairing damage in deoxyribonucleic acid has been a major accomplishment of radiation biology. DNA repair systems were first detected and have since been studied primarily in strains of prokaryotic organisms which show increased sensitivity to the lethal effects of ultraviolet light (UV). Repair systems in a eukaryote have also been investigated extensively in the fungus Neurospora crassa. Presently there are seven mutant strains (uvs-1, uvs-2, uvs-3, uvs-4, uvs-5, uvs-6, upr-1) of N. crassa which exhibit an increased sensitivity to the lethal effects of UV(5,19,20,21,22,23).

Worthy and Epler(26) have demonstrated an operative system of excision-repair in N. crassa. In a further investigation of the UV-sensitive mutants of N. crassa, Worthy and Epler(27) showed that all seven UV-sensitive strains exhibited photoreactivation. With regard to excision-repair, they found uvs-2 and upr-1 strains to be repair-deficient, uvs-1, uvs-5, and uvs-6 strains to be repair-sufficient and the uvs-3 strain to exhibit minimal excision capability. They did not examine the uvs-4 mutation.

Studies by de Serres(6) and de Serres and Schüpbach(8) determined that six of the UV-sensitive strains (uvs-1 was not studied) fell into three classes with regard to the rate at which mutations were induced by UV; (1) strains sensitive to mutation induction, upr-1, uvs-2, (2) strains resistant to mutation induction, uvs-3, uvs-4, uvs-5, and (3) strains with wild-type mutation induction, uvs-6.

In the present study, the number of strains required to study each UV-sensitive mutation precluded the study of all seven UV-sensitive mutations. Therefore, on the basis of the above mentioned mutation induction studies, three UV-sensitive mutations were chosen for investigation, upr-1, uvs-4, and uvs-6; one from each class.

In order to investigate the effects of these UV-sensitive mutations on conidial inactivation, the mutations were incorporated into two-component heterokaryons. Two-component heterokaryons, which produce one heterokaryotic and two homokaryotic conidial fractions, have been used in radiation inactivation studies for many years. Atwood(1,2) studied the inactivation of conidia from two-component heterokaryons using both X-rays and UV. He found, as expected with nuclear inactivation, that X-rays resulted in a rapid decrease in the proportion of heterokaryotic conidia. In contrast, UV resulted in little or no reduction in the proportion of heterokaryotic conidia.

The difference in the X-ray and UV-inactivation of heterokaryotic conidia can be best explained according to Atwood, on the assumption that UV-damaged nuclei can recover if at least one undamaged nucleus is present in the conidium(2). This proposal by Atwood of a possible

interaction between nuclei to alter the effect of UV had also been suggested by Norman(16,17) to explain why nuclei in multinucleate conidia were more resistant to UV-inactivation than nuclei in uninucleate conidia. In view of our present understanding of enzymatic dark repair of UV-induced DNA damage, it is possible that the nuclear interaction and recovery processes described by Atwood and Norman were manifestations of these repair systems.

This study was undertaken to investigate the role of DNA repair systems in the UV-inactivation of conidia. The first objective of the present investigation was to determine the effects of three different UV-sensitive mutations (upr-1, uvs-4, uvs-6) on the UV-inactivation of the three conidial fractions produced by a two-component heterokaryon of Neurospora crassa. The second objective was to determine by the analysis of inactivation curves the extent to which repair functions specified by nuclei of wild-type sensitivity were available to UV-sensitive nuclei within the same cytoplasm.

Inactivation curves also provided evidence of the repair capability available to genetically UV-sensitive conidia produced by heterokaryons containing both genetically UV-sensitive and resistant nuclei.

MATERIALS AND METHODS

Strains

The genetic compositions of component strains used to form two-component heterokaryons are presented in Table 1. Table 2 shows the pair of component strains contained in each two-component heterokaryon. All 74-UT strains were derived from crosses of 74-OR100-3a

TABLE I

DESIGNATION AND GENETIC COMPOSITION OF COMPONENT STRAINS

Strain Numbers	Linkage Group and Genotype								
	I					III	IV	V	VI
74-UT1-23A	A	+	+	al-2	+	+	cot-1	inos	pan-2
74-UT1-40A	A	+	+	al-2	nic-1	+	cot-1	+	pan-2
74-UT2-52A	A	upr-1	+	al-2	nic-1	+	cot-1	+	pan-2
74-UT2-119A	A	upr-1	+	al-2	+	+	cot-1	inos	pan-2
74-UT5-20A	A	+	+	al-2	+	uvs-4	cot-1	inos	pan-2
74-UT5-42A	A	+	+	al-2	nic-1	uvs-4	cot-1	+	pan-2
74-UT8-7A	A	+	uvs-6	al-2	nic-1	+	cot-1	+	pan-2
74-UT8-8A	A	+	uvs-6	al-2	+	+	cot-1	inos	pan-2

TABLE 2

COMPONENT STRAINS OF
TWO-COMPONENT HETEROKARYONS

Heterokaryon Number	Genotype and Strain Number	
	Component I (nic-1, al-2, cot-1, pan-2)	Component II (al-2, cot-1, inos, pan-2)
HUT-1	74-UT1-40A	74-UT1-23A
HUT-2	74-UT2-52A	74-UT1-23A
HUT-4	74-UT1-40A	74-UT2-119A
HUT-5	74-UT2-52A	74-UT2-119A
HUT-10	74-UT8-7A	74-UT1-23A
HUT-11	74-UT1-40A	74-UT8-8A
HUT-12	74-UT8-7A	74-UT8-8A
HUT-13	74-UT5-42A	74-UT1-23A
HUT-14	74-UT1-40A	74-UT5-20A
HUT-15	74-UT5-42A	74-UT5-20A
HUT-20	74-UT2-52A	74-UT5-20A
HUT-21	74-UT5-42A	74-UT2-119A

(a nic-1 al-2 cot-1 inos pan-2) to the A al-2 cot-1 pan-2 radiation-sensitive strains of de Serres (6 and manuscript in preparation). All derived strains are thus isogenic with 74A-OR and heterokaryon-compatible.

Genetic markers used are as follows:

A,a - mating type
al-2 - albino
nic-1 - nicotinamide requirement
cot-1 - colonial, temperature sensitive
inos - inositol requirement
upr-1 - ultraviolet light sensitive
pan-2 - pantothenic acid requirement
uvs-4 - ultraviolet light sensitive
uvs-6 - ultraviolet light sensitive
+ - wild-type alleles

Media

Three basal media, Vogel's, Fries', and Westergaard's, were utilized in this study. Vogel's Medium N(24) was used initially for culturing but due to poor growth of many ascospore isolates, Fries' Basal Medium(12) was used in subsequent work. Westergaard's Medium(7) was modified from that described by Westergaard and Mitchell (25). This medium was used only as a crossing medium.

To obtain good colonial morphology on plates, a combination of L-sorbose, D-glucose and D-fructose was used as the carbon source in all plating media as suggested by Brockman and de Serres(4). Isolation media used for obtaining conidia were routinely supplemented with 2.0% sucrose and 2.0% agar. Vitamin supplements were provided in the following proportion: pantothenic acid - 10 mg/l, nicotinamide - 10 mg/l, inositol - 8 mg/l.

Heterokaryon Formation

To form heterokaryons conidial suspensions of the two parental strains were prepared in sterile water (5 X 10^5 conidia/ml) and mixed on the surface of the media in petri plates. Three plates, prepared for each combination, were placed at 30°C and the growth front was marked on the bottom of the plates after 24 and 48 hours incubation. The heterokaryon exhibiting the most vigorous growth was selected and subcultured on slants containing heterokaryon isolation medium. These subcultures were incubated at room temperature for seven days and then permanent stock cultures were made by placing conidial suspensions onto silica gel stock tubes prepared by a procedure described by Perkins(18) and modified by Brockman and de Serres(3).

Ultraviolet-Inactivation Experiments

Conidia for UV-inactivation experiments with homokaryotic strains were obtained from four, seven-to-ten-day old cultures on 13 X 100 mm culture tubes inoculated with silica gel crystals. Conidia from heterokaryotic strains were obtained from the same type of cultures except that six single-colony isolates were used as inocula. To obtain these single-colony isolates, two to six crystals of silica gel from the heterokaryon stock tubes were placed in 2 ml sterile water and vortexed. Two, ten-to-one serial dilutions were made of this conidial suspension. One milliliter of each dilution was then plated in 20 ml Fries plating medium. The plates were incubated at 30°C for 48 hours. At this time, six of the largest colonies were transferred to heterokaryon isolation medium. These single-colony isolates were grown for seven to ten

in the following proportion [illegible] ml

10 mg/l inositol - [illegible] ml

days at room temperature. Conidia from all six tubes were pooled for each experiment in order to obtain more uniform nuclear ratios.

Conidia were harvested and filtered through cotton to remove mycelial fragments and conidial chains and to produce a suspension of conidia of more uniform size. Conidial suspensions were counted with a hemocytometer and adjusted to a concentration of 2×10^6 conidia per milliliter.

Thirty milliliters of conidial suspension, stirred with a teflon-coated stirring bar, was UV-irradiated in a sterile 100 X 15 mm glass petri plate. Serial dilutions of the treated suspensions were made to obtain a dilution in which one milliliter contained approximately 200 colony-forming units.

For the plating of homokaryotic strains, the properly supplemented plating medium was inoculated with 0.3 ml, 0.7 ml and 1.0 ml of the appropriate dilution to give three plates at each dose. Plates were incubated at 30°C for 48 to 72 hours in the dark. In order to minimize photoreactivation, all treatments and platings were done in subdued light.

Plating of heterokaryotic strains was more involved. Since two-component heterokaryons contain two genotypically different nuclei and most of the conidia contain two or more nuclei, conidia are of three genotypes. Conidia may be homokaryotic for either of the component nuclei or they may be heterokaryotic containing both

both types of nuclei. To determine the survival of each of the three conidial fractions, the conidia from heterokaryons were plated on three different media at each dose. Survival of heterokaryotic conidia was calculated from the numbers of colonies on minimal plates. At each dose, the number of colonies on the minimal plates was subtracted from the number of colonies on the inositol or nicotinamide supplemented plates. The differences were the surviving numbers of each homokaryotic conidial fraction. Conidia from UV-sensitive strains characteristically exhibit low viability and UV-irradiation at low doses often results in increased conidial germination. In these experiments the highest survival was considered 100%, therefore in some cases 100% survival occurs at low doses and survival at zero dose is less than 100%. Plates were handled in the same manner as described for homokaryotic strains.

Through the use of this technique of differential plating, a dose-effect curve for the survival of each conidial fraction was obtained. The effect of the UV-sensitive mutations on survival of these fractions was determined by incorporating the UV-sensitive mutations into one or both of the component nuclei.

Ultraviolet dose-effect experiments were carried out using relative doses of 0, 2, 4, and 6 minutes exposure to ultraviolet light. At an incident dose rate of approximately 11 ergs mm^{-2} sec^{-1} these exposures correspond to absolute doses of about 0, 1320, 2640, and 3960 ergs mm^{-2}. These doses may be converted to energy fluence by dividing the numbers by 10 and changing the units to Joules per square meter ($J\ m^{-2}$).

Plates were counted after 48 to 72 hours incubation at 30°C. All visible coloinies were counted. Colonies ranged from 0.5 mm to 5.0 mm in diameter but conidia from most heterokaryons produced uniform colonies from 3.0 mm to 5.0 mm in diameter. Survival of conidia was judged as the ability to form colonies of the above mentioned size under the conditions employed.

UV Source

A General Electric 15 watt G15T8 germicidal lamp was positioned 12 inches above the irradiated suspensions. Incident energy, 11 ergs mm^{-2} sec^{-1}, was measured by a Jagger meter(14).

Nuclear Counts

The average number of nuclei per conidium was determined for each heterokaryon. The procedure used was that of Heubschman(13) modified only in the mounting substance. Diaphane was used in place of xylol clarite. Counts were made on cultures identical to those used in UV-inactivation experiments.

RESULTS AND DISCUSSION

Nuclear Counts

To insure that differences in UV-sensitivity of heterokaryons were not due to differences in the number of nuclei contained in the conidia, the mean number of nuclei per conidium was determined for each heterokaryon studied. Nuclear counts for all heterokaryons averaged between 2.1 and 2.8 nuclei per conidium. The means were all near the 2.6 nuclei per conidium reported by Huebschman(13) for a heterokaryon growing on minimal medium.

Figure 1. Ultraviolet-inactivation of heterokaryon component strains.

A. Components with wild-type sensitivity:

□ 74-UT1-23A

△ 74-UT1-40A

B. Components containing uvs-6:

□ 74-UT8-8A

△ 74-UT8-7A

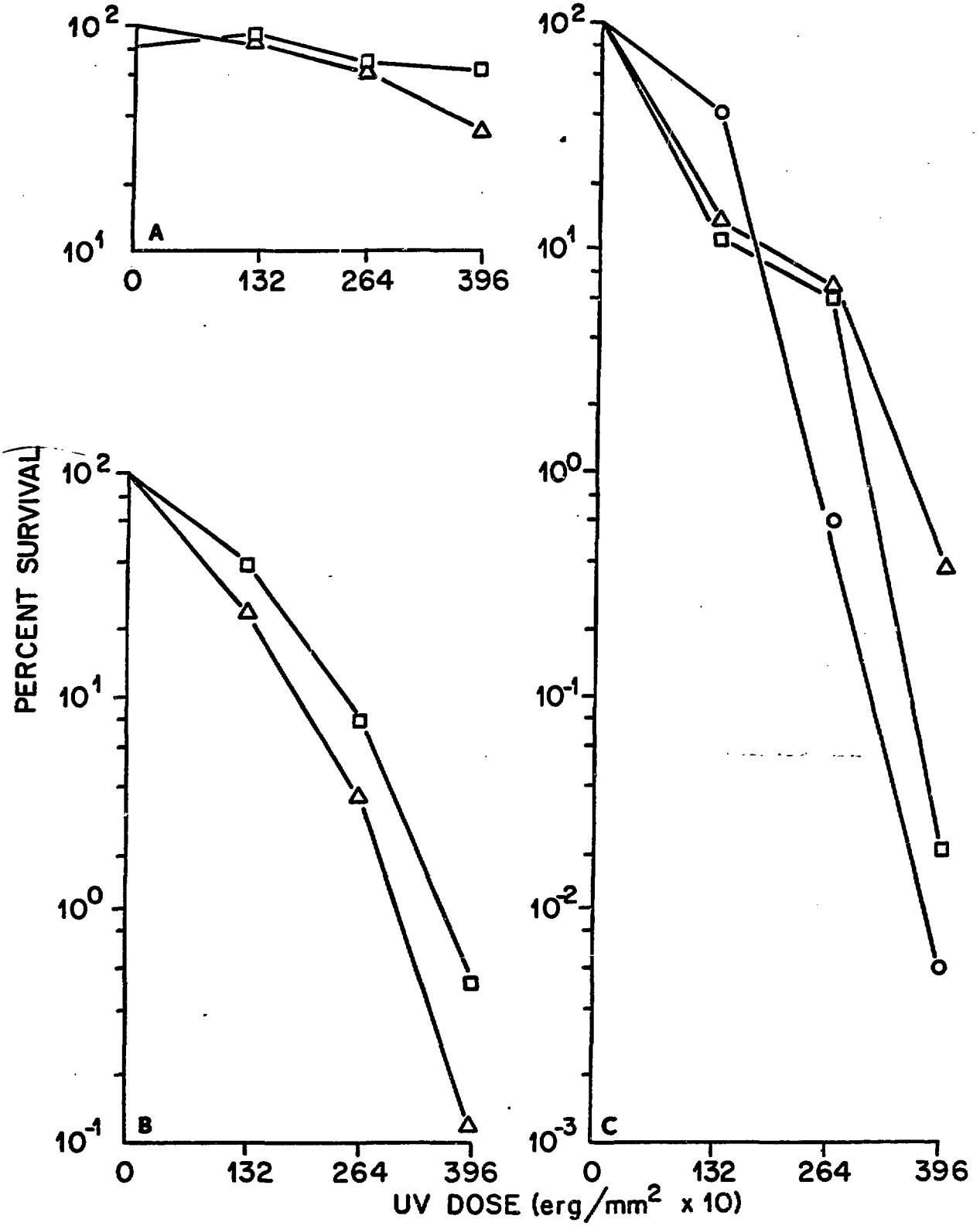
C. Components containing upr-1 or uvs-4:

□ 74-UT2-119A (upr-1)

△ 74-UT2-52A (upr-1)

○ 74-UT5-20A (uvs-4)

for a heterokaryon on


PERCENT SURVIVAL
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
A
B
C
0
132
264
396
UV DOSE (erg/mm^2 x 10)

Homokaryotic Strains

These UV-inactivation experiments were carried out to determine the UV-survival characteristics of the strains to be used as heterokaryon components. Data from these experiments were also used to determine whether or not the survival characteristics of the UV-sensitive mutations were altered by differences in nutritional markers or by their presence in heterokaryons.

Ultraviolet-inactivation curves were determined for seven of the eight strains of Neurospora used in this study as heterokaryon components. Data were not obtained for the nicotinamide-requiring uvs-4 strain (74-UT5-42-A) as growth was poor and sufficient conidia were not available. When observed in a heterokaryon, however, the behavior of 74-UT5-42A was nearly identical to that of the inos, uvs-4 strain 74-UT5-20A. This suggested that the two uvs-4 component strains exhibited the same UV-inactivation characteristics.

Strains with wild-type sensitivity in Figure 1A exhibited a reduction in survival to about 50% at the six minute exposure (approximately 3960 ergs/mm^2).

Components containing uvs-6, seen in Figure 1B, showed survival curves which were shouldered and were much more sensitive to inactivation than wild-type. Survival at six minutes exposure was below 1.0%. Figure 1C shows that the upr-1 strains both exhibited the same inactivation curve with a survival of approximately 0.1% at the six minute dose. Both strains exhibited a plateau in their survival curves between the two minute and four minute doses. The

uvs-4 strain, Figure 1C, exhibited a shouldered curve to the two minute dose and exponential survival thereafter. Survival at six minutes exposure was less than .01%.

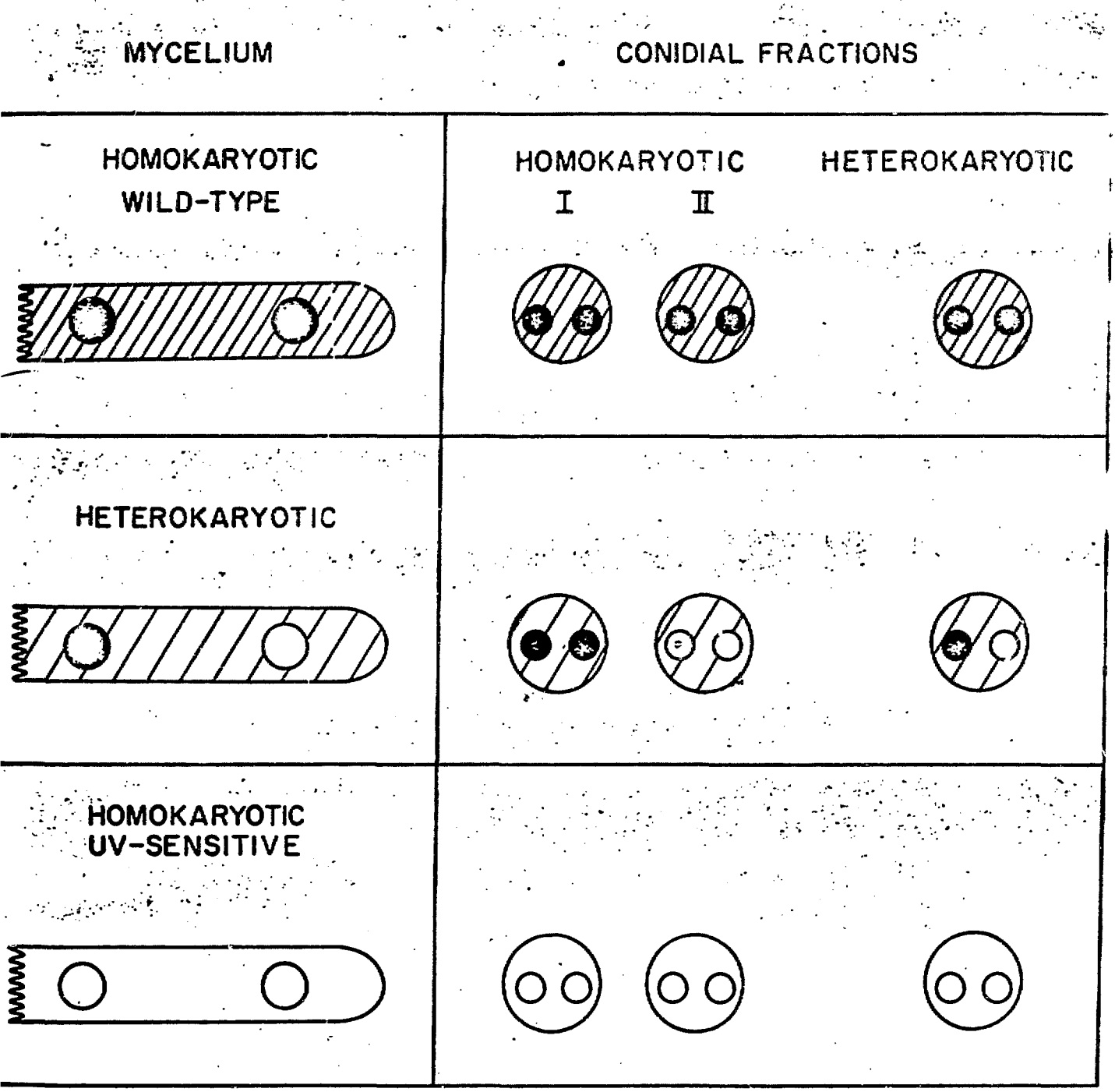
In wild-type, upr-1 and uvs-6 strains, UV-sensitivity was not greatly altered by differences in nutritional markers. In all cases, the sensitivity of the component strains was very similar to the sensitivity of the corresponding homokaryotic conidial fractions produced by heterokaryons containing the UV-sensitive markers in both components. Therefore the sensitivity of UV-sensitive mutants was not altered by inclusion in two-component heterokaryons.

Heterokaryons

The UV-inactivation of conidia from two-component heterokaryons was studied in heterokaryons with wild-type UV-sensitivity and in heterokaryons containing UV-sensitive mutations. Three UV-sensitive mutations were studied. Three two-component heterokaryons were formed for the study of each UV-sensitive mutation; one with the mutation in both components, one with the mutation in component I, and one with the mutation in component II.

The genetic and cytoplasmic composition of these heterokaryons and the three conidial fractions produced by each are represented in Figure 2. As can be seen, if wild-type gene products are present in the cytoplasm, they are included in all conidia produced by the heterokaryon regardless of the conidial genotype. With regard to the wild-type gene products of genes affecting UV-sensitivity, this means that genetically UV-sensitive conidia from heterokaryons containing a wild-type allele should contain some repair factor and be

GENOTYPIC AND CYTOPLASMIC COMPOSITION OF CONIDIA DERIVED FROM TWO-COMPONENT HETEROKARYONS HETEROKARYOTIC AND HOMOKARYOTIC FOR MUTATIONS TO UV-SENSITIVITY



more UV-resistant than genetically identical conidia produced by heterokaryons containing only UV-sensitive alleles.

Statistical Analysis

UV-inactivation experiments on all heterokaryons were repeated four times and the mean survival for each conidial fraction was calculated. The mean survivals of the three conidial fractions of each heterokaryon were compared at the six minute dose by a Duncan's New Multiple Range test(15). Results of statistical analyses are presented in Table 3.

Heterokaryon with Wild-type Sensitivity

HUT-1 is a heterokaryon which contains no mutations resulting in sensitivity to UV. As is shown in Figure 3A, all three conidial fractions exhibited similar survival curves. These results were in agreement with the previous experiments of Atwood(1).

upr-1 Effects

The effects of upr-1 on UV-inactivation of conidia from two-component heterokaryons are shown in Figures 3B-D. When upr-1 was present in only one component (HUT-2, HUT-4), the conidial fraction homokaryotic for upr-1 exhibited increased UV-sensitivity. Survival of upr-1 conidia at the six minute exposure was about 10% compared to about 30% in homokaryotic wild-type conidia. Sensitivity of heterokaryotic conidia was like wild-type which indicated that the upr-1 mutation was recessive.

Figure 3. Ultraviolet-inactivation of heterokaryons containing upr-1.

A. HUT-1

○ Heterokaryotic conidia.

△ Homokaryotic nic-1 conidia.

□ Homokaryotic inos conidia.

B. HUT-4

○ Heterokaryotic conidia.

△ Homokaryotic nic-1 conidia.

□ Homokaryotic inos, upr-1 conidia.

C. HUT-2

○ Heterokaryotic conidia.

△ Homokaryotic nic-1, upr-1 conidia.

□ Homokaryotic inos conidia.

D. HUT-5

○ Heterokaryotic conidia.

△ Homokaryotic nic-1, upr-1 conidia.

□ Homokaryotic inos, upr-1 conidia.

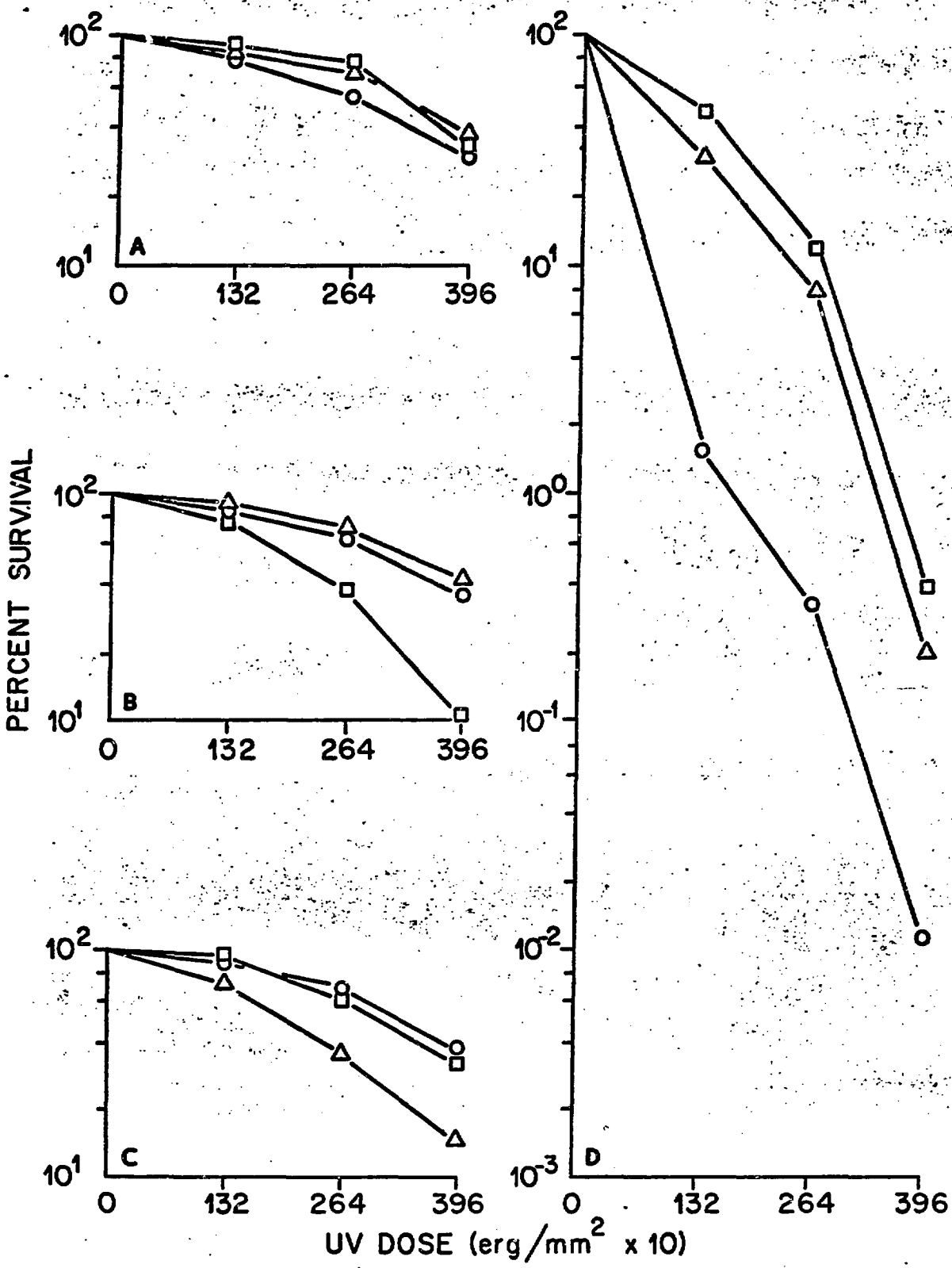

PERCENT SURVIVAL
10^2
10^1
A
0
132
264
396
10^2
10^1
B
0
132
264
396
10^2
10^1
C
0
132
264
396
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
D
0
132
264
396
UV DOSE (erg/mm^2 x 10)

TABLE 3

COMPARISONS OF MEAN SURVIVALS AT SIX MINUTE UV DOSE
F RATIOS AND RESULTS OF DUNCAN'S NEW MULTIPLE RANGE TEST

Heterokaryon	F Ratio		Mean Percent Survival of Conidial Fractions[1]		
	Block	Treatment	Heterokaryotic	nic-1	inos
HUT-1	7.13*	2.42	30^{a}	37^{a}	31^{a}
HUT-2	0.60	7.70*	36^{a}	15^{b}	32^{a}
HUT-4	1.30	8.10*	30^{a}	41^{a}	10^{b}
HUT-5	3.46	9.81*	$.012^{a}$	0.29^{b}	0.41^{b}
HUT-10	1.07	6.14*	23^{a}	23^{a}	40^{b}
HUT-11	2.89	3.68	20^{a}	37^{b}	26^{ab}
HUT-12	1.52	0.81	0.25^{a}	0.27^{a}	0.44^{a}
HUT-13	2.19	2.78	26^{a}	21^{a}	38^{a}
HUT-14	7.28*	8.47*	26^{a}	40^{b}	38^{b}
HUT-15	0.80	0.10	$.0025^{a}$	$.0023^{a}$	$.0040^{a}$
HUT-20	0.97	6.71*	24^{a}	10^{b}	14^{b}
HUT-21	4.67	20.30**	24^{a}	15^{b}	14^{b}

[1] Means on a line followed by the same letter are not significantly different at the .05 level of significance as determined by Duncan's New Multiple Range Test.

* Significant at the .05 level of significance.

** Significant at the .01 level of significance.

recessive.

The upr-1 mutation, when present in both components (HUT-5) resulted in increased sensitivity of all three conidial fractions. This is shown in Figure 3D. The heterokaryotic conidia exhibited the greatest sensitivity with exponential survival at low doses followed by a plateau at higher doses. This plateau was seen in the upr-1 component strains and resembles the inflection in the UV-inactivation curve of E. coli B reported by Haynes(11). Homokaryotic fractions exhibited shouldered curves but with greater sensitivity than in HUT-2 or HUT-4.

The difference in sensitivity of the homokaryotic upr-1 conidial fraction (HUT-2 and HUT-4) from the genetically identical conidia from HUT-5 was extreme. These data indicated that upr-1 conidia from a heterokaryon containing both upr-1 and wild-type alleles possessed an increased level of repair capability. This was attributed to a carryover of the wild-type gene product through the cytoplasm to upr-1 conidia. This carryover was responsible for a difference in survival of upr-1 conidia at the six minute dose from less than 1.0% in HUT-5 to about 10% in HUT-2 and HUT-4.

In HUT-5, separation of the heterokaryotic and homokaryotic curves was similar to results obtained with ionizing radiation (Shelby, unpublished). As upr-1 strains have been shown to be excision-deficient(27), these results indicated that the recovery process suggested by Atwood(1) and by Norman(16,17) could be an excision-repair process. The loss of the ability to deal with UV-induced lesions could result in inactivation dose-response curves similar to those obtained with ionizing

radiation induces a large number of lesions not susceptible to repair.

uvs-4 Effects

Two-component heterokaryons which contained uvs-4 in only one component produced conidial fractions all of which exhibited wild-type UV-sensitivity. This is shown in Figure 4B-C. The wild-type sensitivity of heterokaryotic conidia suggests that uvs-4 is recessive which is in agreement with the results of Schroeder(20). Homokaryotic uvs-4 conidia from these two heterokaryons exhibited wild-type sensitivity demonstrating that complete repair capability was present in genetically UV-sensitive conidia.

Figure 4D shows that when present in both components of a heterokaryon, uvs-4 resulted in greatly increased sensitivity of all three conidial fractions. All three conidial fractions exhibited the short-shouldered survival curve reported by Schroeder for uvs-4 strains. HUT-15 exhibited very poor growth characteristics and colony morphology was adversely affected by UV-irradiation of conidia.

As with upr-1, there was a carryover of repair capability from the heterokaryon containing both UV-sensitive and wild-type alleles to the genetically UV-sensitive conidia produced. In contrast to upr-1, however, the repair capability was fully functional in uvs-4 conidia. This carryover of repair capability produced a striking comparison between the survivals of homokaryotic uvs-4 conidia. At the six minute exposure, the survival

Figure 4. Ultraviolet-inactivation of heterokaryons containing uvs-4.

A. HUT-1

- ○ Heterokaryotic conidia.
- △ Homokaryotic nic-1 conidia.
- □ Homokaryotic inos conidia.

B. HUT-14

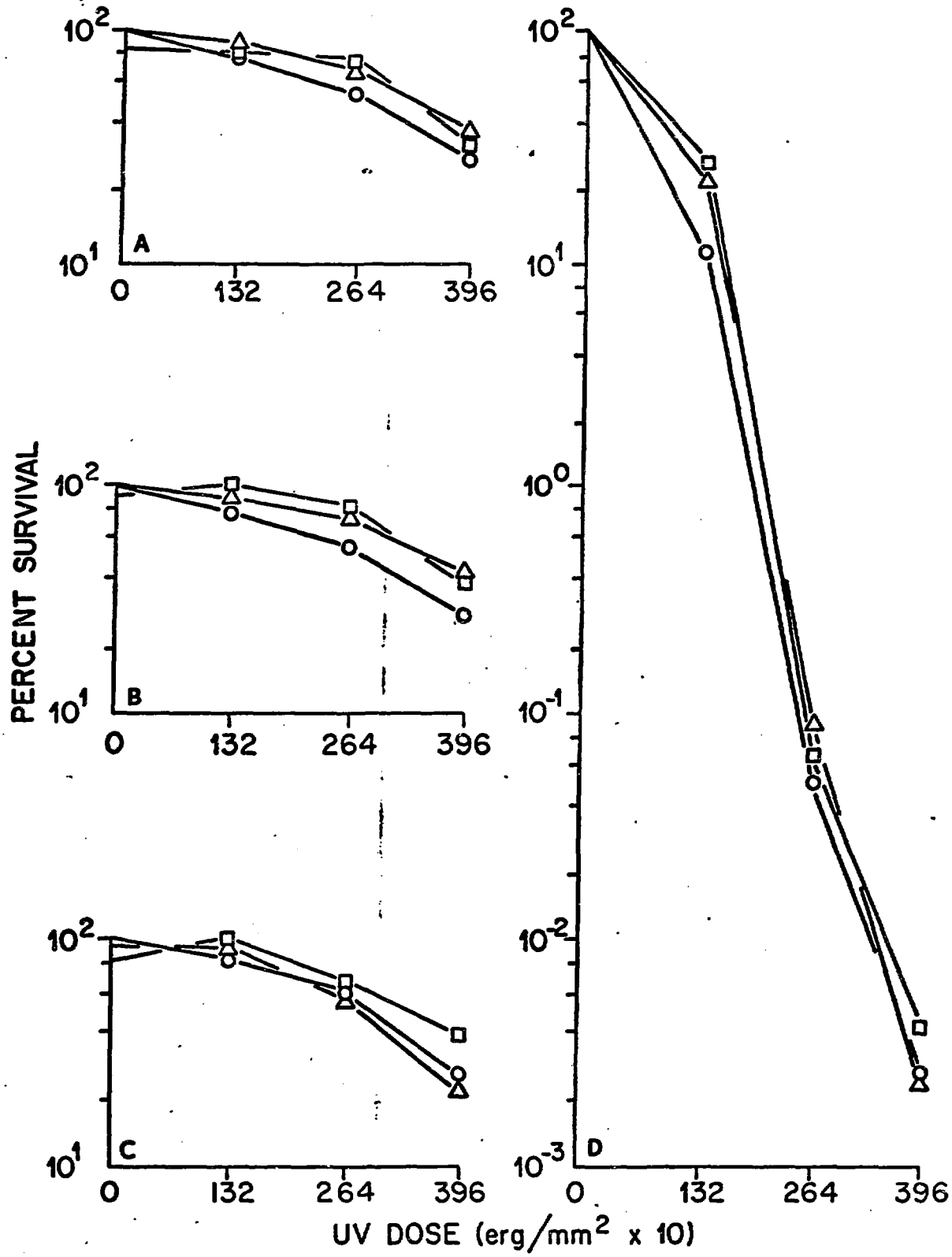
- ○ Heterokaryotic conidia.
- △ Homokaryotic nic-1 conidia.
- □ Homokaryotic inos, uvs-4 conidia.

C. HUT-13

- ○ Heterokaryotic conidia.
- △ Homokaryotic nic-1, uvs-4 conidia.
- □ Homokaryotic inos conidia.

D. HUT-15

- ○ Heterokaryotic conidia.
- △ Homokaryotic nic-1, uvs-4 conidia.
- □ Homokaryotic inos, uvs-4 conidia.


PERCENT SURVIVAL
10^2
10^1
A
0
132
264
396
10^2
10^1
B
0
132
264
396
10^2
10^1
C
0
132
264
396
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
D
0
132
264
396
UV DOSE (erg/mm^2 x 10)

of homokaryotic uvs-4 conidia from a heterokaryon containing uvs-4 and wild-type alleles was near 40% while genetically identical conidia from a heterokaryon containing only uvs-4 alleles was less than .01%. This was over a thousand-fold difference in the survival of genetically identical conidia.

uvs-6 Effects

Figures 5B and 5C show the UV-inactivation curves of conidia from two-component heterokaryons containing the uvs-6 mutation in only one component. In both HUT-10 and HUT-11, the homokaryotic uvs-6 conidia and heterokaryotic conidia exhibited slightly increased sensitivity to UV. The increased sensitivity of heterokaryotic conidia may be due to a low activity of the wild-type repair factor in the cytoplasm and/or transport limitations of the factor across nuclear membranes. It is apparent however that the wild-type repair factor if functional in the cytoplasm. A comparison of homokaryotic conidia from HUT-10 and HUT-11 with similar conidia from HUT-12 reveals a higher repair capability in the former. This is in agreement with results from upr-1 and uvs-4.

HUT-12 contains uvs-6 in both components and all three conidial fractions exhibited greatly increased sensitivity to UV-inactivation and similar inactivation curves.

Complementation

Studies on the interaction of repair systems between nuclei have recently been carried out in heterokaryons of human cells. DeWeerd-Kastelein et al(9) have demonstrated complementation

Figure 5. Ultraviolet-inactivation of heterokaryons containing uvs-6.

A. HUT-1

○ Heterokaryotic conidia.

△ Homokaryotic nic-1 conidia.

□ Homokaryotic inos conidia.

B. HUT-11

○ Heterokaryotic conidia.

△ Homokaryotic nic-1 conidia.

□ Homokaryotic inos, uvs-6 conidia.

C. HUT-10

○ Heterokaryotic conidia.

△ Homokaryotic nic-1, uvs-6 conidia.

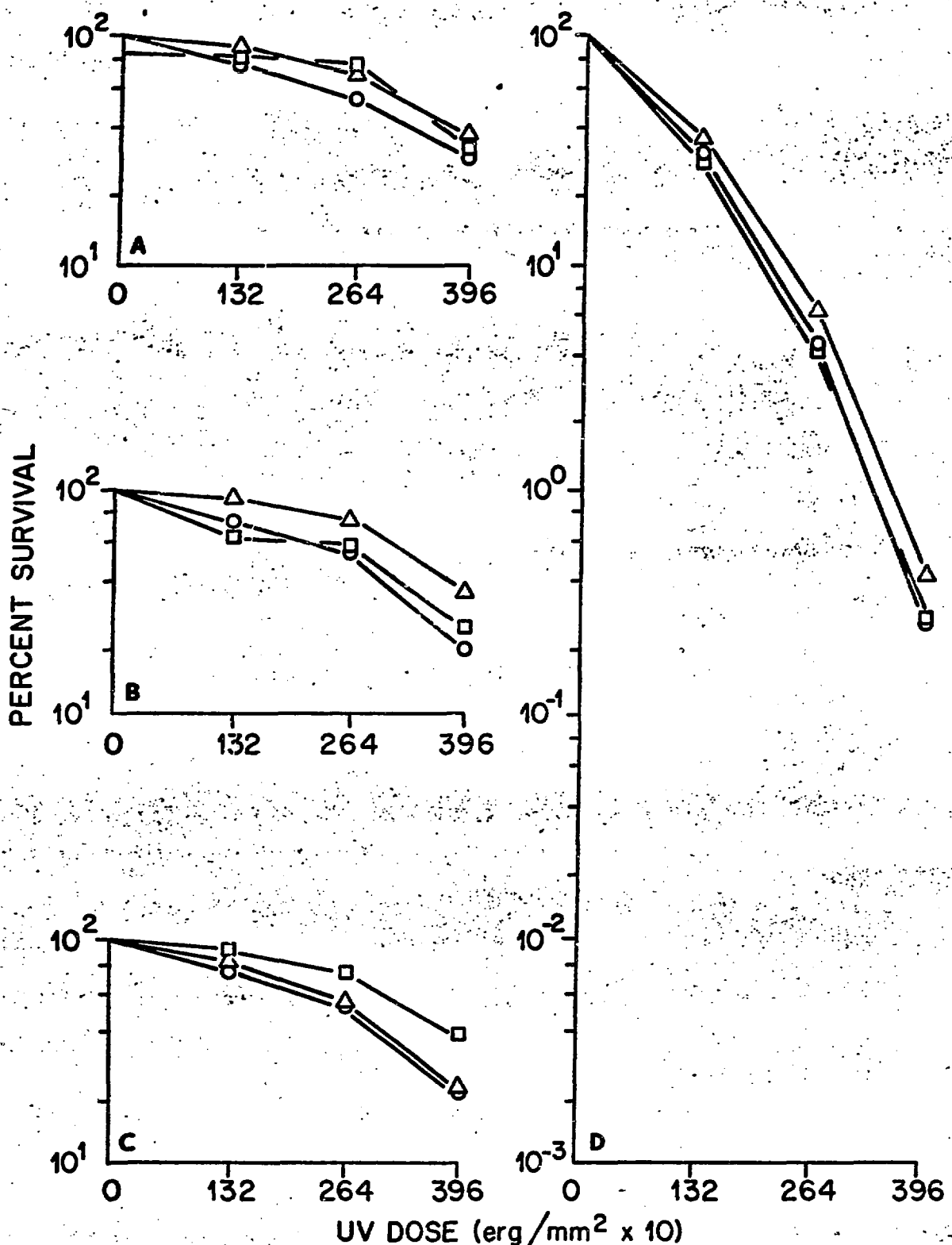
□ Homokaryotic inos conidia.

D. HUT-12

○ Heterokaryotic conidia.

△ Homokaryotic nic-1, uvs-6 conidia.

□ Homokaryotic inos, uvs-6 conidia.


PERCENT SURVIVAL
A
B
C
D
0
132
264
396
UV DOSE (erg/mm² x 10)

between cell strains originating from different Xeroderma pigmentosum (XP) patients. This study showed that repair replication was completely restored in nuclei of heterokaryotic cells formed from two parental cell strains, neither capable of repair replication. Giannelli, Croll and Lewin(10) demonstrated that in heterokaryons between normal and XP cells, the XP nuclei undergo normal DNA repair synthesis.

In this study, two heterokaryons were formed to test complementarity of the non-allelic mutations, upr-1 and uvs-4. These two heterokaryons differed only in the exchange of the nutritional markers with which each UV-sensitive mutation was associated in each component. Ultraviolet-inactivation curves of conidia from these heterokaryons are shown in Figure 6. The two mutations exhibited complete complementarity in heterokaryotic conidia which showed wild-type survival. In both heterokaryons, the homokaryotic conidial fractions exhibited slight but significantly greater sensitivity than the heterokaryotic conidial fractions.

CONCLUSIONS

Data from this investigation show that with the three UV-sensitive mutations studied, repair factors determined by the wild-type alleles are all present and functional in the cytoplasm. These repair factors are capable of promoting repair of UV-induced damage in nuclei which lack the wild-type allele. In two-component heterokaryons, the carryover of this cytoplasmically mediated repair into genetically UV-sensitive conidia was partial as with upr-1 and uvs-6 or complete as with uvs-4. It seems likely that

Figure 6. Ultraviolet- inactivation of heterokaryons containing both upr-1 and uvs-4.

A. HUT-1

○ Heterokaryotic conidia.

△ Homokaryotic nic-1 conidia.

□ Homokaryotic inos conidia.

B. HUT-20

○ Heterokaryotic conidia.

△ Homokaryotic nic-1, upr-1 conidia.

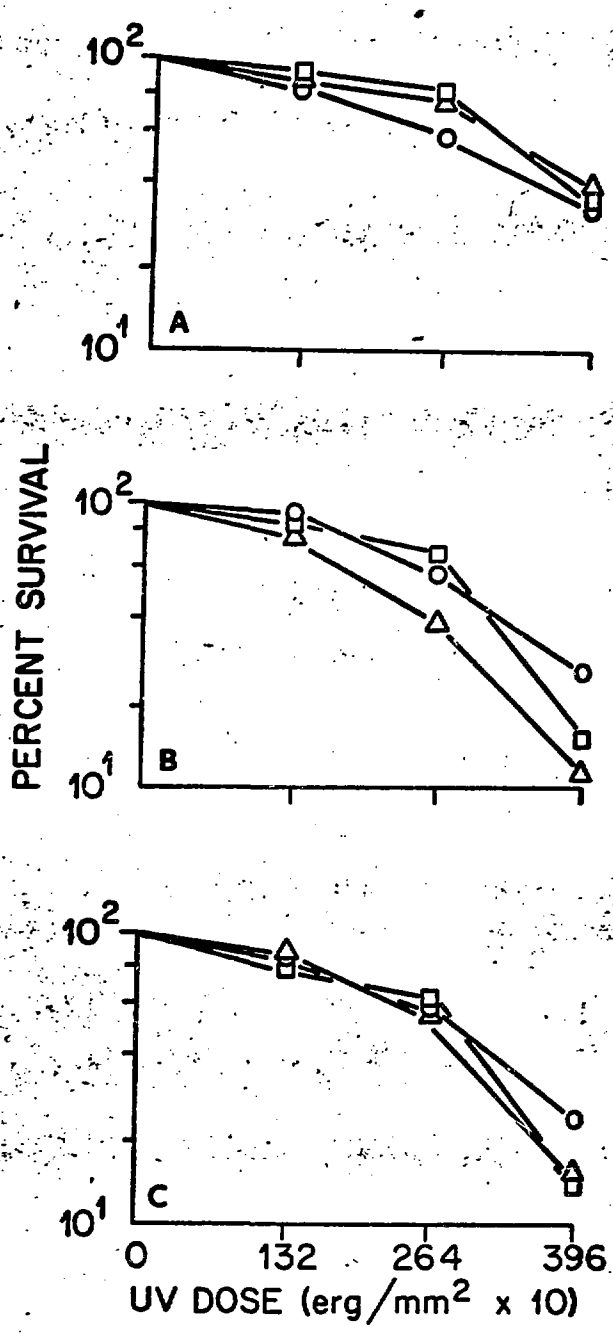
□ Homokaryotic inos, uvs-4 conidia.

C. HUT-21

○ Heterokaryotic conidia.

△ Homokaryotic nic-1, uvs-4 conidia.

□ Homokaryotic inos, upr-1 conidia.


10^2
10^1
A
PERCENT SURVIVAL
10^2
10^1
B
10^2
10^1
C
0
132
264
396
UV DOSE (erg/mm^2 x 10)

this availability of repair functions between nuclei is responsible for the recovery process hypothesized by Atwood(1).

Furthermore, the range of modifications in UV-inactivation curves shown to result from single gene differences points out the difficulties associated with studies of inactivation-kinetics of conidia from two-component heterokaryons. The range of changes in the shapes and slopes of inactivation curves is most certainly not due to changes in the number of "targets" in the cells, but to changes in the ability of the cells to repair UV-induced damage.

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