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Inactivation of Carotenoid-Producing and Albino Strains of *Neurospora crassa* by Visible Light, Blacklight, and Ultraviolet Radiation

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Suspensions of *Neurospora crassa* conidia were inactivated by blacklight (BL) radiation (300 to 425 nm) in the absence of exogenous photosensitizing compounds. Carotenoid-containing wild-type conidia were less sensitive to BL radiation than albino conidia, showing a dose enhancement factor (DEF) of 1.2 for dose levels resulting in less than 10% survival. The same strains were about equally sensitive to shortwave ultraviolet (UV) inactivation. The kinetics of BL inactivation are similar to those of photodynamic inactivation by visible light in the presence of a photosensitizing dye (methylene blue). Only limited inactivation by visible light in the absence of exogenous photosensitizers was observed. BL and UV inactivations are probably caused by different mechanisms since wild-type conidia are only slightly more resistant to BL radiation (DEF = 1.2 at 1.0% survival) than are conidia from a UV-sensitive strain (*upr-1, uvs-3*). The BL-induced lethal lesions are probably not cyclobutyl pyrimidine dimers since BL-inactivated *Haemophilus influenzae* transforming deoxyribonucleic acid is not photoreactivated by *N. crassa* wild-type enzyme extracts, whereas UV-inactivated transforming deoxyribonucleic acid is photoreactivable with this treatment.

The lethal and mutagenic effects of visible, near-ultraviolet (near-UV; 360 to 400 nm), and shortwave UV (<300 nm) radiation on cells and transforming deoxyribonucleic acid (DNA) are well known (2, 3, 7, 8, 12-14, 16, 26). Carotenoid pigments have been shown to protect both photosynthetic and non-photosynthetic organisms against damage by visible and near-UV radiation (4, 8, 9). Air-borne fungal spores, such as those of *Neurospora crassa*, receive substantial doses of visible and near-UV radiation from sunlight. Since *N. crassa* synthesizes carotenoid pigments in abundance when exposed to visible light, these pigments might be expected to protect this species against potentially damaging radiation. The lethality of visible, near-UV, and shortwave UV radiation to conidia of carotenoid-producing and albino strains of *N. crassa* is the subject of this report.

MATERIALS AND METHODS

Fungal strains. The following strains were used in the experiments described below: wild-type 74-OR23-1A (Fungal Genetic Stock Center [FGSC] no. 987), an albino (*al-2*, 15300) thiamine-requiring auxotroph (locus and allele unknown) given to M.L.S. by Noreen E. Murray; and prototrophic UV-sensitive strain *upr-1,uvs-3* (no allele number, ALS11; FGSC no. 2288).

Bacterial strains. The source of the streptomycin-sensitive recipient of *Haemophilus influenzae* used for the transformation experiments was mentioned previously (18, 19).

Media. The medium used for routine culturing of *Neurospora* strains was medium N (24) supplemented as required with thiamine (1 μg/ml). In irradiation experiments for which colony counts were required, plating was done on "inhibitory" medium N (21).

Visible light and BL dose-response curves. Cultures used to prepare conidial suspensions were grown on agar slants for 7 days at 31 C. Conidial suspensions were prepared in 0.1% Tween either in distilled water-saline (0.9% NaCl)-phosphate buffer (3) or in medium N without a carbon source. Suspensions were filtered through sterile cheesecloth to remove conidial chains and mycelial fragments. Suspensions were adjusted to 1.9×10^6 or 9.5×10^6 conidia per ml based on hemacytometer counts. Suspensions were irradiated in Pyrex culture tubes (16 by 160 mm) with sterile metal closures. Control tubes were wrapped with aluminum foil. Irradiated and control tubes were positioned between four fluorescent lamps in two parallel banks. Blacklight (BL) lamps (GE40BLB, integral filter) were used as a source of near-UV radiation with a range of emission from 300 to 425 nm

and maximum emission at 350 nm. These lamps emit 97% of their radiation between 300 and 400 nm. Cool White (CW) lamps (KEN-RAD 40CW/3) were used as a visible-light source; these lamps emit approximately 96% of their radiation between 400 and 700 nm and 4% in the range 300 to 400 nm (5). The dose rate for BL irradiation was 4.2×10^2 ergs/mm^2 per s as measured at the position of the irradiated suspensions by a Blak-Ray meter (model J-227) with a model J-222 near-UV-sensitive photovoltaic cell (Ultraviolet Products Inc.). The same meter equipped with a model J-225 shortwave UV-sensitive photovoltaic cell was used to estimate the shortwave UV dose, which was approximately 7.0 ergs/mm^2 per s at the level of the irradiated suspension. The dose rate for CW irradiation was 8.6×10^2 ergs/mm^2 per as measured by a YSI-Kettering model 65 radiometer. Irradiated suspensions were stirred continuously by means of a magnetic stirrer and a small Teflon-coated flea. Dark control suspensions were stirred continuously or intermittently. Suspensions were maintained at 17 to 19 C during irradiation. At intervals fractions were withdrawn from both the control and irradiated suspensions, diluted where appropriate, and plated. Dilution and plating were done under yellow fluorescent lighting (KEN-RAD "Gold") to avoid photoreactivation (21). Plates were incubated 3 to 5 days in the dark at 31 C before being counted. Counts represent the average of three plates.

Shortwave UV dose-response curves. The methods used for obtaining shortwave UV dose-response curves were identical to those described previously (3, 21).

Naphthalene filter. A solution of 3.9% naphthalene in absolute ethanol was used to filter the BL radiation in a single experiment (2). The tube containing the irradiated suspension was centered in a Pyrex beaker to which the filter solution was added. The effective thickness of the filter was 1.8 cm.

Dose-response curves for visible light in the presence of photosensitizing dye. The methods for obtaining dose-response curves for visible light in the presence of methylene blue (10 μg/ml) were described previously (19).

Preparation of Neurospora enzyme extract. Wild-type *N. crassa* crude enzyme extract was prepared as described previously (18).

BL and UV inactivation of transforming DNA. *H. influenzae* transforming DNA in sterile saline (0.16 μg/ml) was inactivated by BL, using the apparatus described above. Methods for inactivation of transforming DNA with shortwave UV radiation were identical to those described previously (18). Transforming DNA irradiated with UV or BL was either used directly to assay for residual transforming activity or treated with *Neurospora* enzyme extract after which residual transforming activity was assayed.

PR. The system used for photoreactivation (PR) treatment of inactivated transforming DNA is identical to that described previously (18).

H. influenzae transformation. The *H. influenzae* transforming DNA carrying the marker for streptomycin resistance (Str^r) was prepared as described by Marmur (10). The methods used for the preparation of competent cells and for the transformation for Str^r were identical to those described by Setlow et al. (15).

RESULTS

Effect of BL, CW, and UV on wild-type and albino N. crassa conidia. Dose-response curves for BL inactivation of conidia from a carotenoid-producing wild type and a colorless albino strain are shown in Fig. 1. There is a shoulder in the dose-response curves up to 2 h after which inactivation proceeds exponentially. Since no inactivation of wild-type or albino conidial suspensions in the unirradiated control tubes was observed, these points have not been presented to avoid complicating the graph. The albino strain is somewhat more sensitive than the wild type, with a constant dose enhancement factor (DEF) of 1.2 between 10^{-1} and

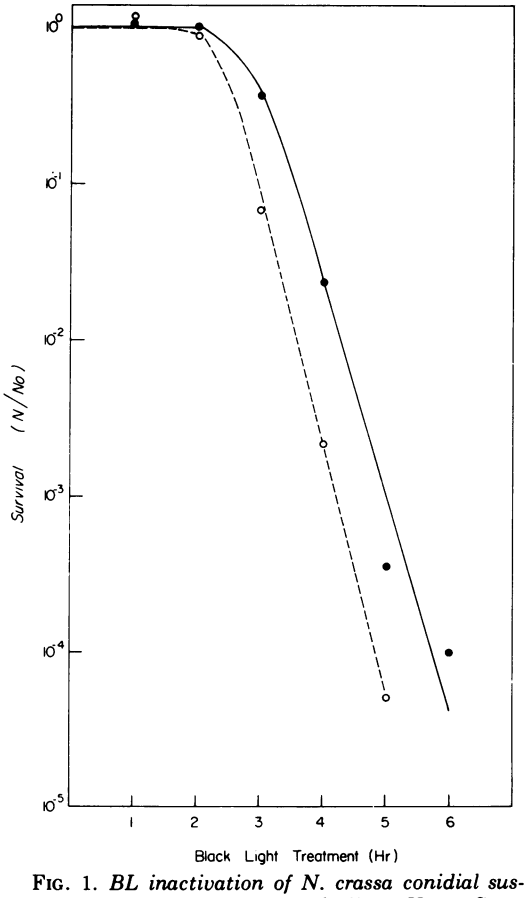

FIG. 1. *BL inactivation of N. crassa conidial suspensions in 0.033 M phosphate buffer, pH 7.0. Symbols: ●, wild type; ○, albino. N, Viable count after treatment; N_0, viable count before treatment.*

10^{-4} survival. The DEF is calculated by dividing the dose required to inactivate the more resistant of the two strains being compared at a particular survival level by the dose required to inactivate the more sensitive strain to the same survival level. When CW lamps were used for irradiation of wild-type and albino suspensions, survival of the wild type was approximately 80% after 5 h of CW irradiation and 25% after 9 h. There was no significant difference in the response of wild-type and albino strains after 5 h of CW irradiation. The limited inactivation observed can probably be accounted for by the near-UV component of the CW source. The conidia of the auxotrophic albino strain were plated on inhibitory minimal N supplemented with thiamine. Addition of thiamine to the plating medium for the wild type did not alter its response to BL.

Effect of filtered BL on wild-type N. crassa conidia. The Pyrex tubes used for irradiation experiments absorb nearly all radiation below 300 nm. To establish further that BL inactivation was not the result of stray UV radiation, a naphthalene filter was used to eliminate radiation of wavelength below 325 nm (Fig. 2). The filtered BL was nearly as effective as unfiltered BL for inactivation of wild-type conidia (Fig. 3). The small difference in response can probably be attributed to the loss of BL radiation due to reflection by the beaker containing the filter and the absorbance of the filter solution in the region 300 to 330 nm.

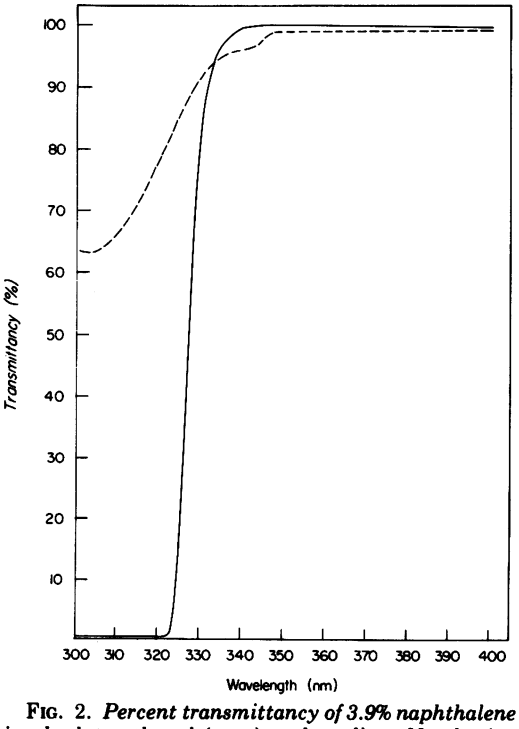

FIG. 2. *Percent transmittancy of 3.9% naphthalene in absolute ethanol (——) and medium N salts (no carbon sources) (-----), as measured by a Cary 14 recording spectrophotometer; path length, 1 cm.*

Effect of shortwave UV on the wild type and an albino strain of N. crassa. The dose-response curves for conidia from the wild type and an albino strain irradiated with shortwave UV are presented in Fig. 4. The curve for the wild type compares favorably with the one published previously (19). Clearly the absence of carotenoid pigments in the albino strain does not enhance the sensitivity of the strain to shortwave UV.

Effect of BL on the wild type and a UV-sensitive strain of N. crassa. In Fig. 5 dose-response curves are shown for BL-inactivated conidia derived from a UV-sensitive mutant (*upr-1,uvs-3;* 20) and for two concentrations of

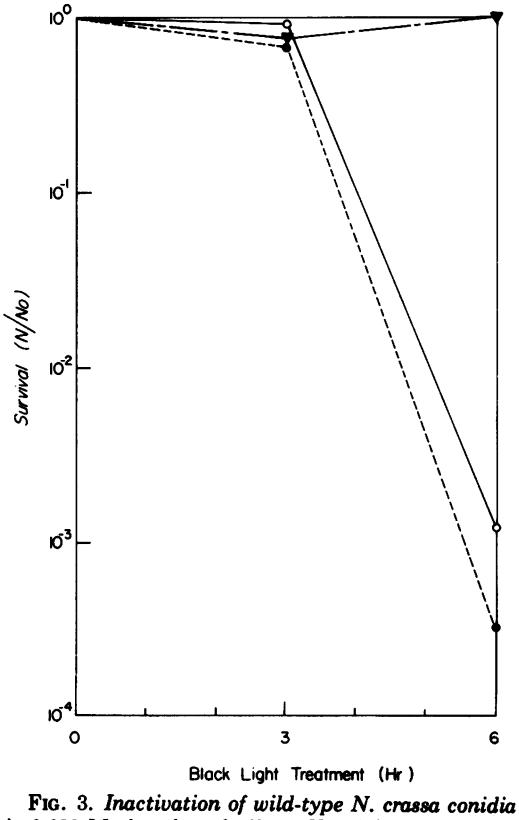

FIG. 3. *Inactivation of wild-type N. crassa conidia in 0.033 M phosphate buffer (pH 7.0) by filtered and unfiltered BL. Filtered BL passed through 1.8 cm of a 3.9% solution of naphthalene in absolute ethanol. This solution absorbs all radiation below 330 nm (see Fig. 2). Symbols: ○, filtered BL; ●, unfiltered BL; ▼, dark control.*

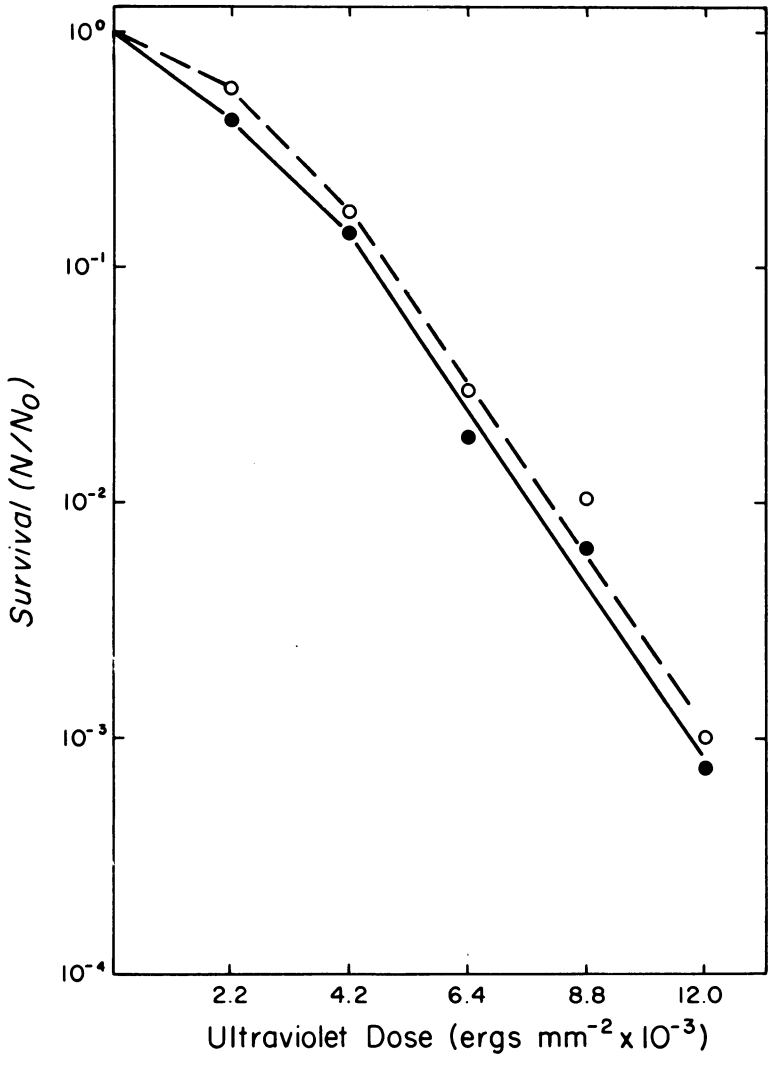

FIG. 4. *Inactivation of N. crassa conidia in 0.033 M phosphate buffer (pH 7.0) by shortwave UV. Symbols:* ●, *wild type;* ○, *albino.*

conidia derived from the wild type. Since conidia of the UV-sensitive mutant are only about 10% viable, a suspension approximately 10-fold more concentrated than that routinely used with the wild type had to be irradiated to ensure adequate plate counts at high BL doses. The greater BL sensitivity of the less-concentrated wild-type conidial suspension indicates that at higher conidial densities there must be a "shading effect" (6). The UV-sensitive mutant is only slightly more sensitive to BL than is the wild type at the same conidial density (DEF = 1.1 at 10^{-2} survival). This strain has greatly reduced capacity for PR and dark repair of shortwave UV-induced lesions when compared with the wild type (DEF = 20 at 10^{-2} survival; 20). These data suggest that inactivation by BL and shortwave UV proceed via different mechanisms. The conidia derived from the UV-sensitive mutant are only about 10% viable based on a comparison of hemacytometer versus viable counts, whereas wild-type conidia are at least 85% viable based on the same comparison. The apparent BL sensitivity of the UV-sensitive mutant conidia might be accounted for if the nonviable conidia in the total conidial population did not contribute to the shading effect to the same degree that viable conidia do. Perhaps the content of carotenoids in nonviable conidia is lower (bleached). If this were true, then the

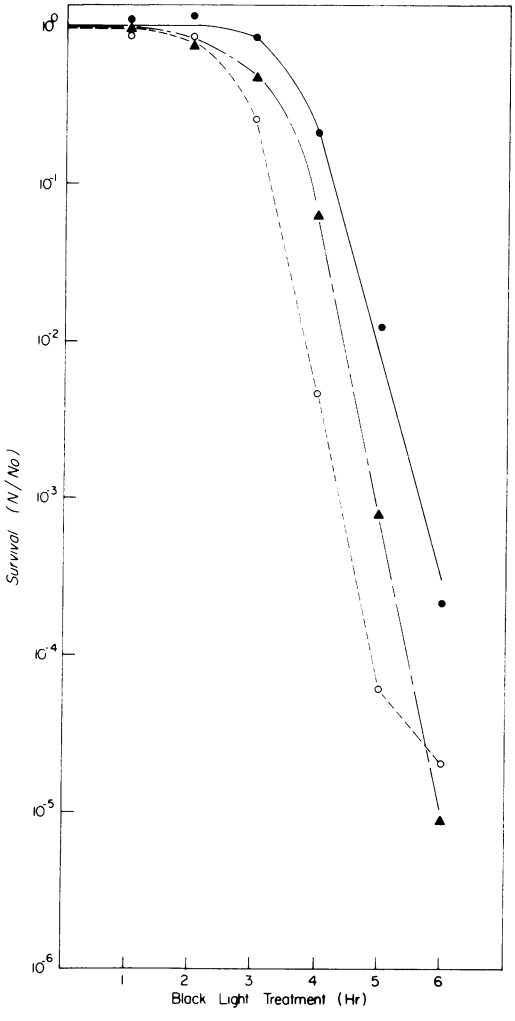

FIG. 5. *BL inactivation of wild-type and UV-sensitive N. crassa conidial suspensions in 0.033 M phosphate buffer, pH 7.0. Symbols:* ●, *wild type, 9.5 × 10⁶ conidia per ml;* ○, *wild type, 1.9 × 10⁶ conidia per ml;* ▲, *UV-sensitive (upr-1,uvs-3), 9.5 × 10⁶ conidia per ml.*

apparent BL sensitivity of the UV-sensitive mutant would simply be an artifact of the low conidial viability characteristic of this strain.

Effect of visible light on N. crassa conidia in the presence of photosensitizing dye. The dose-response curves for photodynamic inactivation of conidia derived from the wild type, the albino strain, and the UV-sensitive mutant are presented in Fig. 6. Each curve is characterized by a shoulder and a final slope which is probably exponential. The albino strain is more sensitive than the wild type to photodynamic killing (DEF = 1.3 to 1.4 from 10^{-1} to 10^{-4} survival). There is no difference in the response of the wild type and the UV-sensitive strain to photodynamic inactivation.

Modification of BL response of wild-type N. crassa conidia by medium N. In Fig. 7 the response of wild-type *N. crassa* conidial suspensions to BL irradiation in phosphate buffer is compared with that of suspensions irradiated in various other suspending media. No significant differences in response were observed for suspensions irradiated in distilled water, saline, 0.033 M phosphate buffer (pH 7.0), 0.033 M phosphate buffer (pH 5.8), or 0.1 M phosphate buffer (pH 7.0) (data for last two suspending media are not presented). However, suspensions of conidia in medium N salts (pH 5.8) showed approximately 30-fold greater survival after 6 h of BL irradiation. Medium N salts shows limited absorption of 300- to 330-nm radiation but to a much lesser extent than a 3.9% naphthalene solution (Fig. 2). When the naphthalene solution was used to filter BL radiation (Fig. 3), the response reduction was much less than that observed for irradiation in medium N salts. These data demonstrate that the protective capacity of medium N salts is not a result of simple absorption.

Inactivation of transforming DNA by UV and BL. Both UV and BL radiation were effective in activating the Str^r marker of *H. influenzae* transforming DNA (Table 1). UV-inactivated DNA was partially reactivated by treatment with *N. crassa* crude enzyme extract in the presence of visible light (Table 1). That this repair is enzymatic PR is demonstrated by the fact that boiled extract has no capacity for photorepair. BL-inactivated DNA was not photoreactivable by the *Neurospora* enzyme extract (Table 2). BL-inactivated DNA and UV-inactivated DNA incubated with crude enzyme extract in the dark were inactivated further. This inactivation was presumably due to the activity of a nuclease(s) in the enzyme extract since boiled extract did not inactivate DNA (Tables 1 and 2). This nuclease activity probably reduced the apparent PR sector for enzyme-treated, UV-inactivated DNA. Figure 8 shows the kinetics of presumed nuclease inactivation for UV- and BL-inactivated DNA.

DISCUSSION

Suspensions of *N. crassa* conidia in buffer were inactivated by visible light in the presence of photosensitizing dye or by BL without an exogenous photosensitizer. Dose-response curves for both processes had pronounced thresholds, and the albino strain was more sensitive than the wild type in both cases (Fig. 1

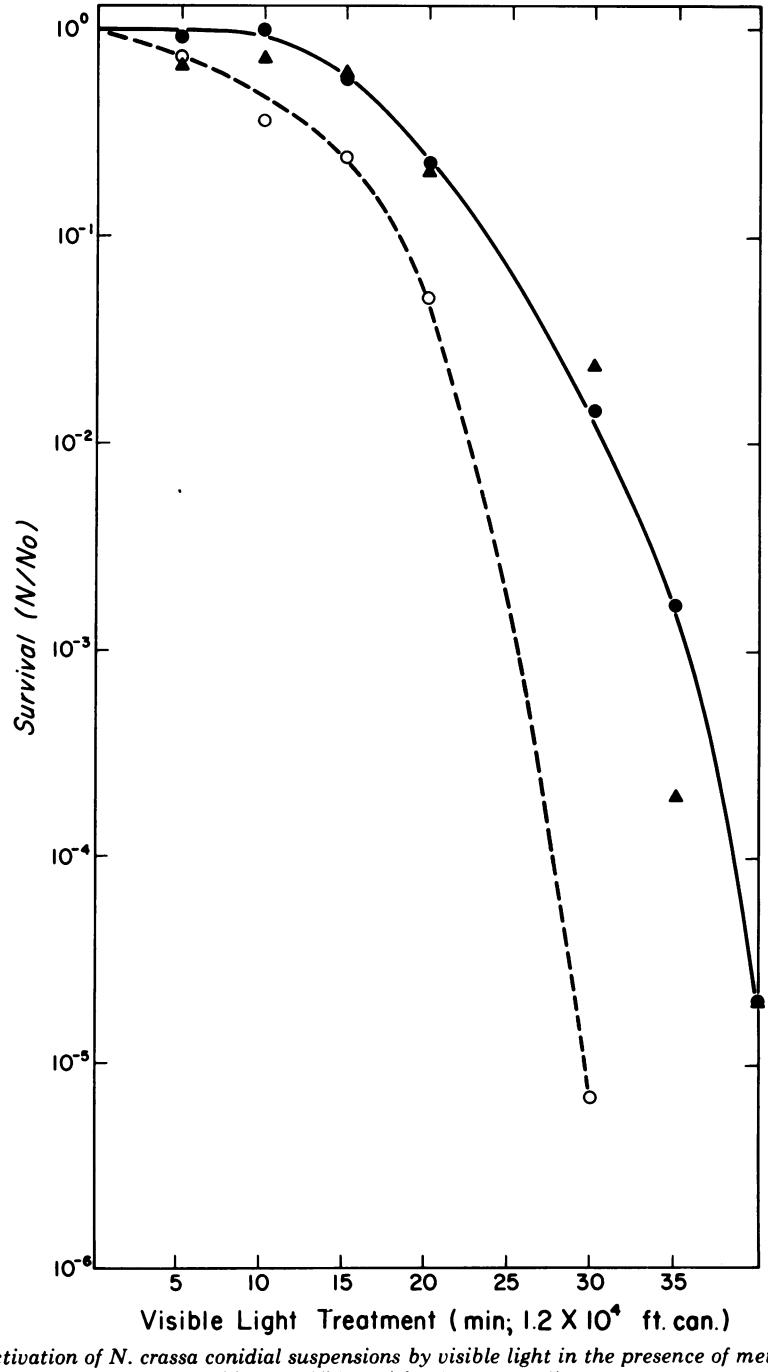

FIG. 6. *Inactivation of N. crassa conidial suspensions by visible light in the presence of methylene blue (10 μg/ml). Symbols: ●, wild type; ○, albino; ▲, UV sensitive (upr-1,uvs-3).*

and 6). We did not observe any difference in the sensitivity of wild-type and albino conidia to UV radiation (Fig. 4). This latter finding is contrary to the observations of Morris and Subden (11), although it is in agreement with the results of Kunisawa and Stanier (9), in which it was demonstrated that carotenoid pigments involved in protection against photodynamic damage in a chemoheterotrophic bacterium do not modify its response to UV.

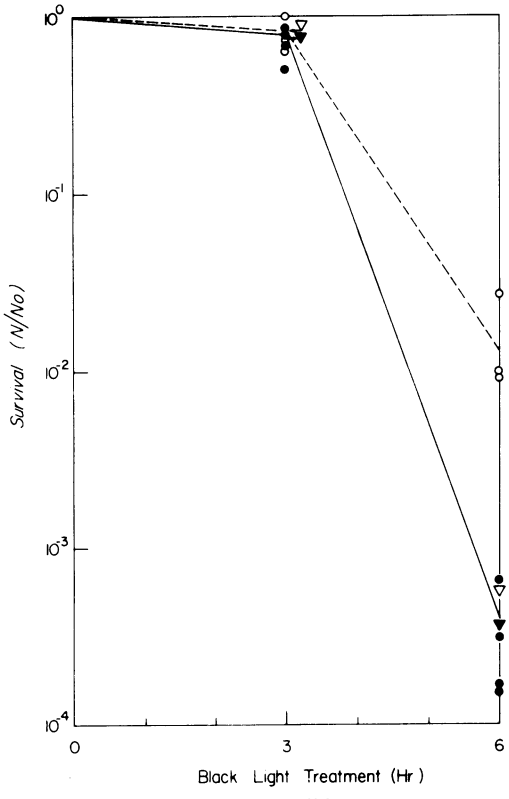

FIG. 7. *BL inactivation of wild-type N. crassa conidial suspensions in distilled water, saline (0.9% NaCl), phosphate buffer, and medium N salts. Symbols: ●, 0.033 M phosphate buffer, pH 7.0 (four experiments); ○, medium N salts (three experiments); ▼, distilled water; ▽, saline.*

The similarity in the kinetics of inactivation of conidia by visible light in the presence of methylene blue and BL, together with the relative sensitivity of the albino strain to both methods of inactivation, suggests that BL inactivation is a photodynamic process (Fig. 1 and 6). If BL damage is mediated by an endogenous photosensitizer (1), the chromophore involved must be highly specific for near-UV radiation since little inactivation by CW was observed. Several candidates for a near-UV chromophore have been proposed (13, 25). That the BL-induced lethal lesions are not cyclobutyl pyrimidine dimers is suggested by the observation that PR does not enhance survival of *N. crassa* after BL irradiation (data not presented). This observation is consistent with the fact that BL-inactivated transforming DNA is not photoreactivable by *Neurospora* enzyme extracts, whereas UV-inactivated DNA is (Tables 1 and 2). Peak et al. (12–14) have suggested that near-UV and shortwave UV inactivation of transforming DNA occur via different mechanisms. However, one can argue that pyrimidine dimers are induced in the DNA of *N. crassa* conidia during BL irradiation but that inactivation and maximal PR occur concomitantly so that no recovery is observed with subsequent PR treatment (1). This possibility seems unlikely, however, since a UV-sensitive strain with greatly reduced capacity for PR (20) is only slightly more sensitive, if at all, than the wild type to BL inactivation (Fig. 5). A second possibility is that, in addition to inducing cyclobutyl pyrimidine dimers in DNA, BL also inactivates the PR enzyme for *N. crassa* as has been demonstrated for *Escherichia coli* (23). Consequently, PR would not be demonstrable after BL treatment.

There are at least three possible targets for BL inactivation of *N. crassa:* DNA, proteins, and/or a small molecule that can give rise to a toxic photoproduct. The ability of BL to inactivate the transforming principle suggests that DNA is a plausible target for BL-induced inactivation of *N. crassa* conidia (2, 12–14; Tables 1 and 2). If damage to DNA were involved in BL killing, one might expect to observe mutagenic effects of BL radiation. We often observed morphologically altered colonies resulting from BL-irradiated conidia even at nonlethal doses. Although these morphological variants might be attributed to the results of mutation, preliminary experiments have shown that BL radiation, unlike UV radiation, causes little or no reversion (back-mutation) of allele *37401* at the inositol locus of an auxotrophic strain of *N. crassa* (*inl,met-3;* FGSC 2159). However, mutagenic effects of near-UV radiation on bacteria have been observed (2, 26). Therefore, the absence of induced reversion at a single locus (*inl*) can not be taken to mean that BL is not mutagenic for all *N. crassa* loci.

BL killing could be caused by destruction of proteins. If this were the case, the observed threshold for inactivation might represent the dose required to completely inactivate an essential enzyme. Destruction of photoreactivating enzyme (23) and dark-repair enzymes (22) of *E. coli* by near-UV radiation has been demonstrated. However, considering the absorption characterisitics of DNA and protein, it seems unlikely that BL is responsible for the major fraction of inactivating events of these two classes of macromolecules. It is plausible that BL killing could result from photooxidation of small endogenous molecule(s) that is toxic in its interaction with DNA and proteins. If this were the case, the threshold of the BL dose-response curves could represent the dose required for

TABLE 1. *Effect of wild-type N. crassa crude enzyme extract on UV- and BL-treated Str H. influenzae transforming DNA*

Components of transformation mixture							
Competent cells[a]	DNA (0.016 μg)	UV-DNA[b] (0.016 μg)	BL-DNA[c] (0.016 μg)	Enzyme-treated UV-DNA[d] (0.016 μg)	Enzyme-treated BL-DNA[d] (0.016 μg)	Light	Str transformants (no./ml)
+	−	−	−	−	−	−	0
−	+	−	−	−	−	−	0
−	−	+	−	−	−	−	0
−	−	−	+	−	−	−	0
−	−	−	−	+	−	−	0
−	−	−	−	−	−	−	0
+	+	−	−	−	−	−	7.0×10^3
+	−	+	−	−	−	−	1.5×10^2
+	−	−	+	−	−	−	1.8×10^2
+	−	−	−	+	−	−	9.3×10^1
+	−	−	−	+	−	+	8.7×10^2
+	−	−	−	+[e]	−	−	1.9×10^2
+	−	−	−	+[e]	−	+	1.6×10^2
+	−	−	−	−	+	−	3.3×10^1
+	−	−	−	−	+	+	ND[f]
+	−	−	−	−	+[e]	−	1.9×10^2
+	−	−	−	−	+[e]	+	1.6×10^2

[a] Viable competent cells per milliliter = 3.8×10^8. It should be noted that the transforming DNA concentration used is below saturation (saturation ≅ 0.1 μg of DNA per transformation tube or greater).

[b] UV-DNA, UV-treated DNA. UV dose = 1.7×10^3 ergs/mm^2.

[c] BL-DNA, BL-treated DNA. BL dose = 1.1×10^7 ergs/mm^2.

[d] A 0.3-ml portion of crude wild-type enzyme extract (4.5 mg of soluble protein per ml) was mixed with UV- or BL-treated DNA (0.16 μg/ml). The mixture was either exposed to PR light (21) for 15 min at 37 C or held in the dark at 37 C.

[e] The crude *N. crassa* enzyme preparation was boiled for 5 min before being mixed with DNA.

[f] Not determined.

toxic photoproducts to reach lethal concentrations. There is precedence for the suggestion that BL may result in toxic photoproducts that interact with DNA, protein, or both, leading to lethality. Stoien and Wang (17) have demonstrated that tryptophan or tyrosine, in the presence of riboflavin, cause the toxicity of BL-irradiated tissue culture medium to mammalian cells. In addition, Webb and Lorenz (25) have shown that nutrient agar medium irradiated with BL is made toxic to some repair-deficient strain of *E. coli*. Yoakum (27) has demonstrated that UV photoproducts of L-tryptophan sensitize bacterial DNA to 365-nm radiation, increasing the yield of DNA single-strand breaks or alkali-labile bonds. Further support for the idea that lethality caused by BL is based on the formation of toxic photooxidation products comes from the observation that carotenoid pigments protect conidia of wild-type *N. crassa* from BL inactivation (Fig. 1). Carotenoids also protect conidia of the wild type from inactivation by visible light in the presence of a photosensitizer (Fig. 6).

Several possible mechanisms of carotenoid protection against aerobic photosensitization have been proposed (8). However, one can not rule out the possibility that this response modification is caused by purely physical factors as a result of the high density of conidia in the irradiated suspensions. At the concentrations used, radiation reaching the center of the tube had penetrated the equivalent of several layers of cells (6). Since carotenoid pigments absorb strongly in the near-UV and visible-light regions, wild-type conidia should be more highly absorbing of radiation at these wavelengths, thus diminishing the average fluence per cell of wild-type suspensions in BL and visible-light experiments. Such a response modification would probably not be ecologically significant since it is not based on the "quenching" of toxic photoproducts.

The protective capacity of medium N salts against BL inactivation has been described (Fig. 7). Attempts to isolate a single protective agent contained in this medium were unsuccessful. Biotin and citrate are the only organic

TABLE 2. *Effect of wild-type N. crude enzyme extract on blacklight BL-inactivated Str H. influenzae transforming DNA*

Components of transformation mixture					Str transformants (no./ml)
Competent cells[a]	DNA (0.016 μg)	BL-DNA[b] (0.016 μg)	Enzyme-treated BL-DNA[c] (0.016 μg)	Light	
+	−	−	−	−	0
−	+	−	−	−	0
−	−	+	−	−	0
−	−	−	+	−	0
−	−	−	+	+	0
−	−	−	+[d]	−	0
−	−	−	+[d]	+	0
+	+	−	−	−	4.9×10^3
+	−	+	−	−	2.3×10^2
+	−	−	+	−	6.8×10^1
+	−	−	+	+	5.0×10^1
+	−	−	+[d]	−	1.8×10^2
+	−	−	+[d]	+	1.9×10^2

[a] Viable competent cells per milliliter = 4.6×10^9. It should be noted that the transforming DNA concentration used is below saturation (saturation ≅ 0.1 μg of DNA per transformation tube or greater).

[b] BL-DNA, BL-inactivated DNA. BL dose = 1.1×10^7 ergs/mm^2.

[c] A 0.3-ml portion of crude wild-type enzyme extract (4.5 mg of soluble protein per ml) was mixed with BL-DNA (0.16 μg/ml). The mixture was either exposed to PR light (21) for 15 min at 37 C or held in the dark at 37 C.

[d] The crude *N. crassa* enzyme preparation was boiled for 5 min before being mixed with BL-DNA.

constituents of medium N salts and were judged to be the most likely candidates for a single protective agent. Omission of biotin or 10-fold enhancement of biotin concentration did not affect the survival of wild-type conidia irradiated in medium N, and no differences in the response of wild-type conidia irradiated in phosphate buffer, citrate buffer, or citrate-phosphate buffer were observed (data not presented). We suggest that the protective capacity of minimal N salts can best be explained physiologically. In N salts, the conidia may not germinate and grow, but perhaps can carry out minimal metabolism that can obviate, in some unknown way, the damage induced by BL. Whether there exists in *N. crassa* repair systems capable of dealing with BL lesions is not known, but it is an attractive possibility.

Both BL- and UV-treated *H. influenzae* transforming DNA are inactivated, with the same kinetics, by nucleases in crude enzyme extracts of *N. crassa* (Fig. 8). This suggests that BL does not induce lesions that are a unique substrate for certain nucleases in *N. crassa*.

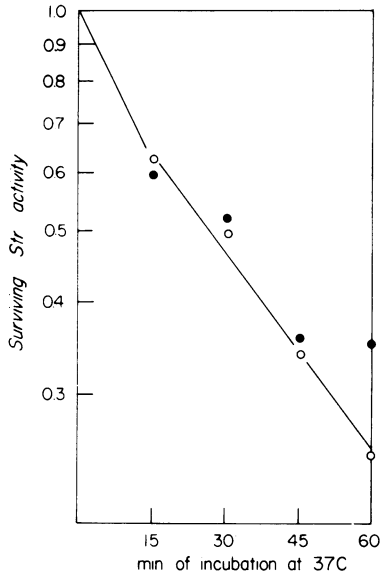

FIG. 8. *Surviving transforming activity after various periods of incubation, in the dark, of either UV-inactivated (0.021 initial surviving activity) or BL-inactivated (0.025 initial surviving activity) transforming DNA (0.16 μg/ml) with crude wild-type Neurospora enzyme extract (4.5 mg of protein per ml). Symbols: ●, BL-inactivated DNA; ○, UV-inactivated DNA.*

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