ENVIRONMENTAL MODIFICATIONS OF THE RESPONSE OF *NEUROSPORA* TO ULTRAVIOLET RADIATION

A DISSERTATION

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BY

CAROLYN MCFALL DESHA, B.A., M.S.

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Texas Woman's University

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We hereby recommend that the dissertation prepared under our supervision by Carolyn McFall DeSha entitled "Environmental Modifications of the Response of Neurospora to Ultraviolet Radiation" be accepted as fulfilling this part of the requirements for the Degree of Doctor of Philosophy.

Committee:

Robert Fuerst
Chairman

Accepted:

Dean of Graduate Studies

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INTRODUCTION

Mutagenic and lethal effects of ultraviolet light have been studied for many years on selected strains of bacteria. Neurospora crassa has only been used to a very limited extent for these types of studies. Since Neurospora provides an excellent tool for investigations of actions of UV light on both asexual and sexual growth, the research reported in this dissertation has been undertaken. The effect of ultraviolet light on Neurospora crassa Em5297a was modified either by using visible light after irradiation, or by exposure to selected gas atmospheres either prior to or following irradiation. Oxygen and other gases were used to test for enhancement of, or protection against, UV irradiation induced damage on mycelial growth and perithecia formation. In addition, the effect of monochromatic light in the range of 350 to 900 nanometers on sexual or asexual growth of Neurospora was determined. Since different investigators used various designations for the measure of wavelength, the decision was reached to convert all of these units to nanometers (nm) for purposes of this dissertation.

A review of the literature has shown that one of the earliest studies cited by many investigators involving inhibition of microbial growth due to ultraviolet light was

reported by Ward (1893). Yet it was not until Gates (1929) published the first action spectrum for the bacteriocidal activity of UV radiation that the effects of various wavelengths of light in the UV spectrum could be compared. An action spectrum is defined as a plot of the reciprocal of the dose required for a given effect versus wavelength of light. Samples of purine and pyrimidine bases and aromatic amino acids were tested for their absorption of light. The same wavelength range was employed to study the effect of light on Staphylococcus aureus and Escherichia coli. A close correlation was obtained between the spectrum of the DNA bases and the bacterial spectrum indicating that the DNA, specifically the DNA bases, were the critical target for UV radiation. This investigation (Gates, 1929) indicated for the first time the role and importance of nucleic acids in living systems. In studies by Hollaender (1941), UV radiation, at 190 to 380 nm, was found to stimulate the production of mutations. Another band of radiation, 350 to 490 nm, produced no increase in mutation rate but caused instead a division delay in E. coli cells (Hollaender and Emmons, 1941).

Setlow (1960) found that nucleic acids in E. coli absorbed more light from the spectrum extending from 240 to 280 nm than other cellular constituents. Absorption by proteins was exhibited at wavelengths below 230 nm. It was stated in the review article by Kanazir (1969) that the

protein in the protein-DNA complex present in the cell protected the DNA against radiation-induced damage. In mammalian in vitro studies, Weiss and Wheeler (1964) compared the RNA synthesizing activity of DNA and DNP (DNA coupled with protein). These workers reported that for radiation doses up to 12,000 R, the DNP showed a 30% reduction in RNA synthesis, whereas the free DNA suffered a 98% reduction in synthesizing ability. Higher levels of ionizing radiation seemed to stimulate RNA synthesis in the irradiated DNP as much as 40% above the nonirradiated DNP. Georgiev (1967) believes that the DNA and protein are not complexed along the entire length of the chain but are made up of complexed and noncomplexed areas which would help explain the results of Weiss and Wheeler (1964). Lower energy radiation would attack the noncomplexed areas, which would reduce synthesis, while the higher doses would tend to free the complexed areas thereby increasing RNA synthesis (Georgiev, 1967).

The DNA moiety seems to be the critical target for damage by UV as well as other forms of radiation. It has been found by Setlow and Carrier (1966) and Logan and Whitmore (1966) that UV radiation of DNA and polynucleotides of various purine and pyrimidine composition yield a variety of photoproducts which include dimers or hydrated bases which have arisen from the addition of water to the 5, 6 double bond of the purine or pyrimidine base. Dimerization

of pyrimidines occurs when two hydrated bases form a four carbon cyclobutane ring. Earlier, Sinsheimer and Hastings (1949) reported the absence of a peak at 260 nm in an aqueous solution of an UV irradiated pyrimidine. The addition of water to the double bond of the bases accounts for the loss of absorbance by the nucleotide. The primary photoproducts in irradiated DNA have been found to be thymine-thymine, cytosine-thymine, and cytosine-cytosine dimers (Setlow and Carrier, 1966). These dimers interfer with the nuclease activity of the cell and inhibit DNA synthesis in an in vitro situation. It has been noted in E. coli that the thymine dimers are more stable than the other dimers. At low doses of UV radiation, thymine dimers are believed to cause the majority of the mutagenic and lethal effects (Witkin, 1969, and Jagger, 1967). A photoreactivating enzyme isolated from yeast has been found to be capable of splitting thymine, uracil, and cytidine dimers from irradiated polynucleotides (Setlow and Carrier, 1966). All of these dimers may be important in evaluating radiation damage. Drake (1969) noted that UV light causes frameshift mutation (CG ———> AT) or base pair substitutions (C ———> T) in E. coli, bacteriophages T4 and s13, and possibly also in Neurospora. The C ———> T transition may be due to the formation of cytosine hydrate while the GC ———> AT change may be caused by the pyrimidine dimers which can be repaired by photoreactivation. Both frameshift

mutations and base pair substitutions are subject to photoreversal by white (visible) light (Drake, 1969).

The majority of ultraviolet irradiation studies have been performed on cells in solution, i.e., wet cells. It has been indicated in work reported by Webb and Tai (1968) that if E. coli cells are irradiated at 253 nm in a dry state, the lesions formed are non-photorepairable and are mutagenic or lethal to the organism. The range of wavelength of 320 to 400 nm (near-visible light), which has been found to activate the photoreactivating enzyme after UV irradiation, appears to be more damaging to the cells in this dry or semi-dry state than the UV light. Apparently damage incurred in dry or semi-dry cells is of a different type than the damage found in wet cells. Webb and Tai found that each E. coli strain irradiated in an aerosol of airborne cells with a specific amount of humidity resulted in one type of mutation. For example, E. coli B cells resulting from a synchronized 24 hr old culture, treated with 320 to 400 nm light at 60% humidity, were all streptomycin dependent cells. Webb and Tai (1969) believe that radiations between 320 and 400 nm are absorbed by protein associated with DNA in E. coli and energy absorbed by this protein is transmitted to the DNA. Webb and Tai theorized that these energy transfers would take place in a different way when the cells are dehydrated than if water was found in the cell. In addition, it is postulated that in the resting phase of

the bacterial cell, the cell membrane is in contact with a specific part of the DNA. This contact point of the DNA would vary with the growth phase of the organism thereby accounting for the similarity of mutants produced by organisms in the same growth phase in response to near visible light. Webb and Mawinska (1970) observed a similar effect in Streptococcus liquefaciens in response to black light, 320-400 nm, which is in the near visible region, but not to far UV.

It has been noted (Hewitt and Billen, 1964; Freese and Freese, 1966; and Hanawalt, 1966) that even in UV resistant bacteria, DNA synthesis is inhibited by UV radiation. Prior to inhibition of DNA synthesis, there is a period in which protein synthesis is delayed. During this lag period, enzymes are produced to repair the DNA if the strain is genetically competent for the dark repair system. UV sensitivity involves a reduction in the ability of a strain to repair the pyrimidine dimers produced as a result of UV radiation, or a reduction in the ability of a strain to tolerate these dimers (Witkin, 1969B).

Basic repair mechanisms may be modified to increase survival of the cell. Photoprotection involves pretreatment of the cells with either visible or infra-red radiation. Studies involving E. coli B indicate that this is an indirect effect (Jagger and Stafford, 1965). Jagger and Stafford believe that the initial photochemical reaction is not

enzymatic but triggers a chain of reactions which may include enzymatic steps which lead to the repair of the UV induced damage. There appears to be no temperature dependence in this reaction as is present in photoreactivation. In addition, photoprotection has been found to be effective against damage incurred due to nitrogen mustard treatment (Lakchaura and Clark, 1969). Liquid holding recovery (LHC) has been shown in bacteria, fungi, and yeasts (Roberts and Aldous, 1949; Patrick and Hayes, 1964; Terry, Kilbey, and Howe, 1967). LHC involves incubation in water or another liquid from an hour to several days which inhibits normal metabolism of the organism, allowing time for the cell to excise dimers present on the DNA chain (Harm, 1966). Witkin (1969A) found that excision repair, which is also termed dark recovery since it operates without benefit of any light source, can operate on both photoreactivable and nonphotoreactivable damage. Dark repair is not specific and may be involved in the repair of damage caused by a chemical such as nitrogen mustard (Witkin, 1969A). Minimal medium recovery (MMR) is a mechanism present in E. coli K-12, an organism which does not have the enzyme necessary for excision of dimers on the DNA (Ganesan and Smith, 1970). The authors feel that MMR reflects a type of repair independent of excision. Thermal reversal in Salmonella typhimurium which involves a heat treatment at 43 C following UV irradiation is also used to enhance survival of the organism (Gough, 1969).

The phenomenon of photoreactivation was reported by Kelner (1949) who found that visible light aided in the recovery of Streptomyces griseus conidia from damage caused by UV radiation. Photoreactivation, unlike dark repair, acts specifically on the bonds between the pyrimidines of the four carbon cyclobutane ring (Witkin, 1969A) and requires the application of light of wavelengths from 300-500 nm after irradiation with UV light. The particular wavelength required will vary with the organism used. Photoreactivation is believed to be the simplest repair mechanism as compared to dark repair, photoprotection, and MMR, and is theoretically the least likely to introduce errors in the DNA chain (Witkin, 1969B). Micrococcus radiodurans has been studied extensively due to its very efficient repair mechanism. This bacterium has been noted to be very resistant to UV radiation although dimerization of the DNA does occur.

Goodgal (1950) and Brown (1951) reported the existence of a photoreactivating mechanism in Neurospora crassa. The UV damage was found to be reversed by the use of wavelengths of light in the 400 nm region as compared with 365 nm effective for E. coli (Terry, Kilbey, and Howe, 1967). These workers found that all six strains of Neurospora tested were capable of photoreactivation. For simplicity, only the meth-7 (4894)A strain was investigated further. A decrease in colony forming ability of the strain grown on a 1% sorbose supplemented medium was used as an index of damage.

Photoreactivation in Neurospora, as in E. coli, was found to be temperature dependent. Terry, Kilbey, and Howe (1967) found that if the irradiated conidia are kept at 37 C, the cells lose their ability to photoreactivate. Gampel and Toha C. (1969) found that the same degree of photoreactivation could be applied to old as for young macroconidia of N. crassa indicating no loss in photorepair capacity due to age.

The preparation of a crude extract of a photoreactivating enzyme from Neurospora crassa has been described by Terry, Kilbey, and Howe (1967) and Terry and Setlow (1967). The activity of this extract was judged by its ability to split thymine dimers and thereby increase the transforming ability of UV irradiated Hemophilus influenzae DNA. Since the action spectrum of the enzyme isolated from E. coli differed from the one isolated from Neurospora, the two enzymes are thought to be different.

Various wavelengths of light have been found to stimulate or repress the growth of organisms. Short-red radiation (600-700 nm) has been found to stimulate phytochrome which activated germination of Lactuca sativa seeds (Rollin, 1966). Rollin found that far-red radiation (700-800 nm) inhibited this phytochrome response. The phytochrome system has also been found in rye seedling shoots (Holdgate and Goodwin, 1965). Interference in growth by near UV (365 nm) and green-yellow light (546-579 nm) of Ginkgo pollen and

HeLa cells was noted by Klein and Edsall (1967). The yellow-green light inhibition could be negated by red radiation (800 nm) but the near UV light damage was insensitive to photorestoration.

The UV and blue regions of the spectrum seem to be the most effective for stimulation of sporulation in many strain of fungi (Leach, 1962A, 1962B). Sexual and asexual reproduction are enhanced most efficiently in Pleospora herbarum by the exposure of two wavelengths, 237.8 or 313.3 nm (Leach, 1963). This effect was found to be dependent on the time of exposure, the wavelength, and the intensity of light.

Mutagenic and lethal effects of UV and visible light on cells with no added sensitizers have been reported by many investigators (Jagger, 1967). Webb and Lorenz (1970) noted no oxygen enhancement of inactivation at 254 nm and 313 nm but a strong enhancement was identified at 365 nm and 390-750 nm. These results indicated that oxygen dependent damage induced by near UV can be partially repaired by the excision repair system in E. coli. Oxygen has long been known to sensitize an organism to the effects of ionizing radiation as stated in the book by Grosch (1965). This appears to be due to the formation of free radicals in oxygenated water. The oxygen in combination with a free radical can form hydrogen peroxide, or the peroxy radical (Smith and Hanawalt, 1969). The peroxy radical is known to have three times the oxidizing capacity of the hydroxyl

radical. This would explain the sensitizing effect of oxygen on the cell which is irradiated with ionizing radiation.

Although peroxide reactions are believed to be produced much more readily in an environment treated with ionizing radiation than one treated with UV light, these reactions have been found to occur due to UV. Wilbur et al. (1957) found that UV irradiation produced lipid peroxides in Claetopterus pergamentaceous eggs. In addition, UV irradiated methyl linoleate was found to produce effects in normal cells similar to those of UV irradiation. Wilbur and his associates felt that part of the damage by UV was due to lipid peroxides. Alper (1954) found that the Shigella phage S13 and coli phage T3 were inactivated by ascorbic acid and by hydrogen peroxide acting as a reductant; but he concluded that peroxide must decompose under irradiation, into active radicals which attack phage. This reaction occurs to a greater extent under ionizing than UV radiation. The effects of hydrogen peroxide on phage suspensions were enhanced when oxygen was removed by passage of nitrogen through the suspension. It is theorized by Alper that the reducing agents interfer with the ability of the phage to absorb to the bacterial host.

Fuerst and Stephens (1970) published an extensive investigation concerning the effects of oxygen, carbon dioxide, Genetrons, Freons, and their analogous hydrocarbons

on non-irradiated and Co-60 irradiated strains of Neurospora. Effects on sexual and asexual growth were noted in this report. Westergaard's medium was used for the sexual crosses. Fuerst and Stephens found that carbon dioxide completely inhibited perithecia formation when Em5297a is crossed to St. Lawrence 74A while Genetron 23 seemed to have no effect on this cross. Methane was found to slightly inhibit perithecia formation. No attempt was made to count perithecia formed in this study. Only an estimate of the difference in number of perithecia produced in response to a particular gas as compared to the control was reported. In a later publication, Stephens, DeSha, and Fuerst (1971) confirmed their original theory that the boiling point and molecular size of the gas tested was correlated with the enhancing or protective effect of the gas atmosphere on Co-60 gamma irradiation induced damage.

The investigation reported here consists of an extension of this earlier work making use of an improved method for gassing and newly developed techniques of studying effects of these treatments on perithecia formation and development. In addition, a monochromator was designed for these experiments, so that it could be ascertained if Em5297a possessed a photoreactivating enzyme and determine the effect of other wavelengths of light on mycelial growth as well as perithecia formation.

MATERIALS AND METHODS

1. Strains of microorganisms employed.

Neurospora crassa Em5297a (FGSC# 352) and St. Lawrence 74A (FGSC# 262) used in these studies were obtained from the Fungal Genetic Stock Center, Dartmouth College, Hanover, New Hampshire. (Recently this fungal collection has been moved to Humboldt State College, Arcata, California.) Both strains were maintained on *Neurospora* complete medium agar slants and stored at 4 C. All cultures were tested on CoLab's Oxoid cornmeal agar slants prior to experimentation to ascertain that the cultures were genetically pure for mating type.

2. Growth media used.

The *Neurospora* minimal medium of Ryan et al. (1953) was later modified by Somers et al. (1957). This modification consisted of 5 g potassium sodium tartrate, 0.33 g ammonium chloride, 1 g ammonium nitrate, 0.5 g magnesium sulfate, 1 g potassium dihydrogen phosphate, 0.1 g sodium chloride, 0.1 g calcium chloride, 20.0 g sucrose, 2.5×10^{-6} g biotin, 2 ml of trace elements, and 1,000 ml distilled water. The final pH of the medium was adjusted to 5.4. The trace element solution consisted of 88 g sodium tetraborate, 64 mg ammonium molybdate tetrahydrate, 950 mg ferrous sulfate heptahydrate,

2,480 mg zinc sulfate heptahydrate, 270 mg cupric chloride dihydrate, 72 mg manganous chloride tetrahydrate, and 1,000 ml of distilled water.

The Neurospora crassa complete medium (Wagner et al., 1950) consisted of 2.5 g yeast extract, 2.5 g malt extract, 2.0 ml casein hydrolysate, 2.0 g peptone, and 1,000 ml of minimal medium with 20 ml of glycerol instead of the usual sucrose. The final pH of the medium was adjusted to 6.0.

CoLab's Oxoid cornmeal agar was used for the sexual crosses between "A" and "a" Neurospora strains in preference to either Baltimore Biological or Difco cornmeal agar since the perithecia formed on the Oxoid cornmeal agar were darker and more numerous. Westergaard's medium (Westergaard and Mitchell, 1947) is the preferred medium for experiments involving mating of Neurospora. For this reason, it was used initially in the research leading up to the work reported in this dissertation. Its use was discontinued since the conidiation on Westergaard's medium was more intense than than produced on cornmeal agar. This heavy conidiation made it harder to count perithecia and was a potential source of contamination when the petri plates were opened.

3. The source of the gases employed.

The empirical formulae and molecular weights of the gases used in these studies are shown in Table 1. The gases were obtained from Matheson Gas Products, La Porte, Texas, and East Rutherford, New Jersey.

Table 1.--Gases tested for the induction of perithecia production, conidia formation, and mutagenicity in *Neurospora crassa**.

Name of Gas**	Formula	Molecular weight
Oxygen	O_2	32.00
Freon C-318; octafluorocyclobutane	C_4F_8	200.04
Perfluorobutene-2; perfluoro-2-butene	C_4F_8	200.04
Genetron-23; fluoroform	CHF_3	70.02
Methane	CH_4	16.04
Butene-1	C_4H_8	56.10

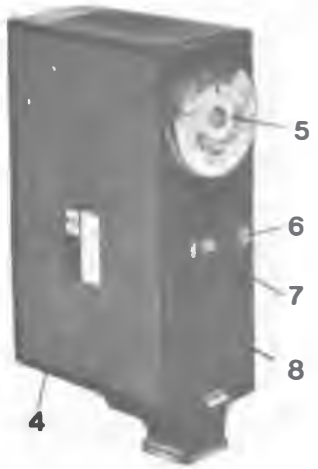
*The *Neurospora crassa* crosses tested were Em5297a X St. Lawrence 74A.

**Descriptive data for these gases were published by Matheson Gas Products (1969), East Rutherford, New Jersey.

4. Monochromator for photoreactivation studies.

A monochromator was needed for these studies so that the effect of different wavelengths of light on Em5297a could be investigated. A Bausch and Lomb Spectrophotometer 20 was used for the main body of the instrument. The tungsten filament lamp normally incorporated in the spectrophotometer as a light source was replaced for the purpose of these experiments with an electronic flash unit. The electronic photoflash was considered superior to the tungsten lamp for photoreactivation experiments since an intense amount of light could be reflected in 1 msec; so the effect of heat on the conidial sample would be negligible. The flash was originally a part of a Kalimar portable flash unit shown in Figure 1. The flash head and its reflector were removed from the gun and inserted into the space the tungsten lamp occupied. Longer lengths of lead wire were soldered to the flash head and triggering unit so that the flash could be triggered independently of the main spectrophotometer. The duration of this flash was approximately 1 msec and the lamp had a 4-5 sec recycling time. The slit was removed from the monochromator since 5 X 50 mm capillary tubes were twice the width of the original slit opening. A styrofoam stand was cut so that the capillary tube would stand upright while the light was being flashed. The diffraction grating used in the Bausch and Lomb Spectrophotometer to separate the wavelengths of light was maintained in the monochromator

Figure 1.--Portable flash unit substituted for the tungsten filament lamp in the Bausch and Lomb Spectrophotometer. The flash head (3) was removed from the gun and rewired so that the flash head itself was the only part of the gun actually inside the spectrophotometer.



1. Open Flash Button
2. Synchro Cord
3. Flash Head
4. AC Inlet
5. Exposure Calculator Dial
6. Neon Glow Lamp
7. AC-DC Switch
8. Battery Compartment Cover
9. AC Cord

so the wavelength could be selected. Since color of a light beam can be used to indicate wavelength, this criterion was used in these experiments to make certain that the proper wavelength within ± 50 nm was focused on the tube. A schematic of the monochromator designed for this study is shown in Figure 2. The flash unit is in the position normally occupied by the tungsten filament lamp. Although the photomultiplier tube was left in the monochromator, the styrofoam holder blocked the light path so no measurement of the intensity of the beam could be made.

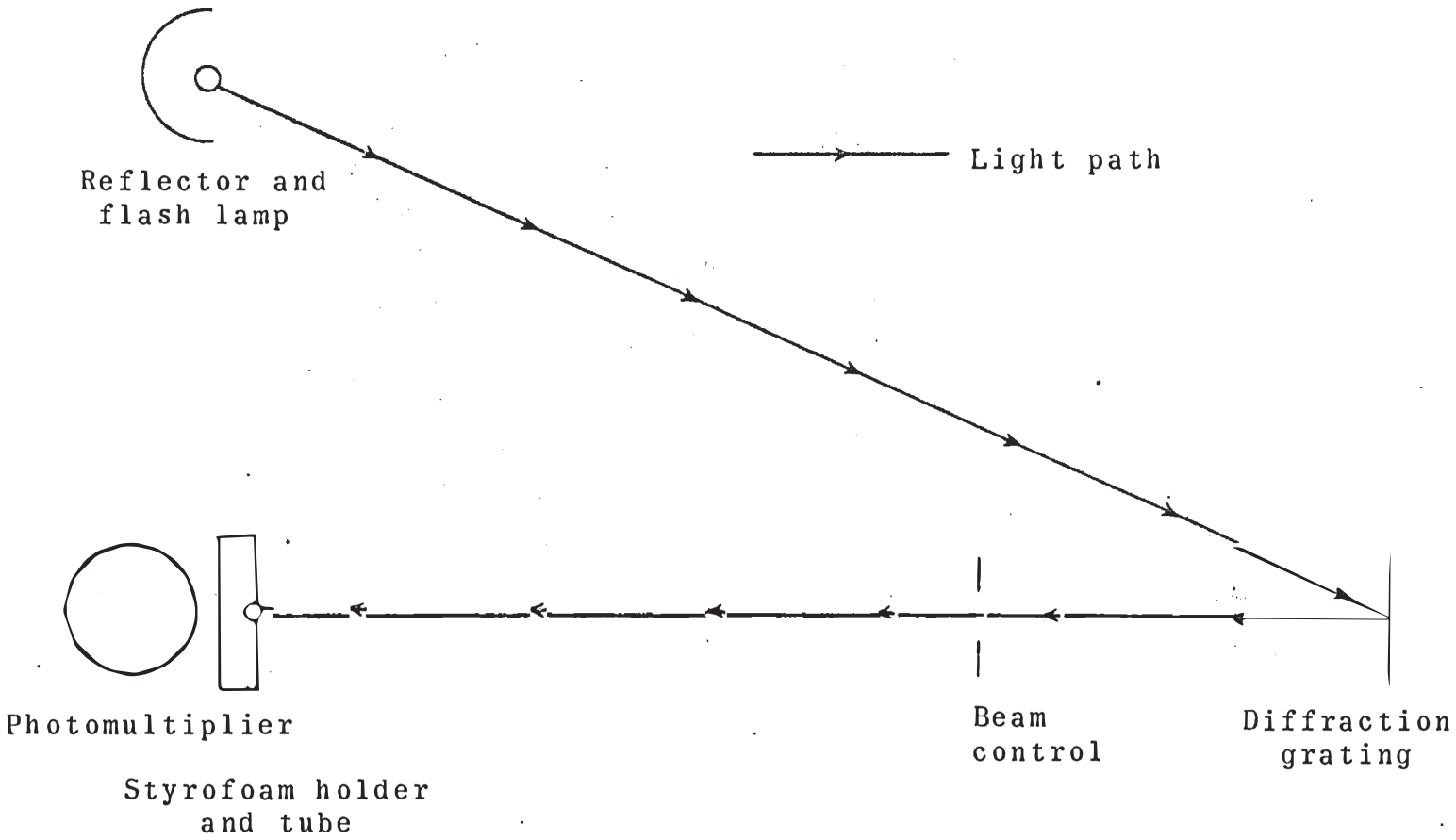
5. Neurospora suspension for gassing and irradiation.

A three day old culture of Neurospora crassa Em5297a grown on a complete medium agar slant was washed with 5 ml of sterile distilled water and filtered through 2.5 cm of sterile cotton in a disposable 10 ml syringe in order to remove hyphal fragments from the conidial suspension. Sterile water was used to dilute this suspension until a desired absorbance reading, according to the experimental procedure used, was reached. A Bausch and Lomb Spectrophotometer 20, set at 500 nm, was used for all the experiments.

6. Measurement of mycelial growth.

Treated Neurospora crassa Em5297a microconidia, absorbance 0.05, were diluted 1:100 with distilled water. The dilution procedure involved adding 1 ml of the conidial

Figure 2.--Schematic of the monochromator designed for the photoreactivation experiments. The flash head removed from the electronic gun, replaced the tungsten lamp in the monochromator. The Pyrex tube which is supported by the styrofoam holder is in the path of the light beam. The wavelength was determined by a diffraction grating control knob (not shown).


Reflector and
flash lamp
Light path
Photomultiplier
Styrofoam holder
and tube
Beam
control
Diffraction
grating

suspension to 99 ml of sterile water. Aliquots in the amount of 0.05 ml were pipetted into sterile 125 ml Erlenmeyer flasks containing 25 ml of Neurospora minimal medium. Since the inoculum was so dilute, the strain required 5 days instead of the normal 65 to 72 hr to produce 60 to 70 mg mycelial pads. The flasks were kept in the dark at 25 C during the entire period. Pads were harvested and dried at 100 C for 2 hr and weighed on a Roller-Smith Balance to the nearest mg.

7. Measurement of perithecia production.

Experimentally treated Neurospora crassa Em5297a microconidia, absorbance 0.05, was diluted 1:100 as described before. Sterile Brewer plates with metal tops holding absorbent discs were filled with 15 ml of sterile CoLab's Oxoid cornmeal agar. After the plates were cool, 0.05 ml aliquots of the diluted Em5297a suspension were delivered to the center of the plates using a Schwarz BioResearch Autopipettor. The disposable tips for the pipettor were sterilized in a petri plate 24 hr before use with several drops of ethylene oxide. The automatic pipettor allowed for the fast, accurate dispension of the conidial suspension onto the plates. Inoculated cornmeal agar plates were left in the absence of light at 25 C for 24 hr, after which 0.05 ml of St. Lawrence 74A, absorbance 0.05, was pipetted onto the surface of the agar in the inoculated plate. This time the conidial suspension was spread over the surface of

the agar with the use of a sterile 5.0 X 7.5 cm rectangular spreader. The plates were kept in the dark at 25 C. Dark perithecia, sexual spore bodies, were produced in one week. The spreading technique allowed for even distribution of perithecia on the surface of the agar, so that the perithecia could be more easily counted with the aid of a bacterial colony counter. A photograph of such a plate is shown in Figure 3. Before counting, however, the plates were washed with 2% Clorox to inactivate the conidiaspores which had formed. A stronger concentration of Clorox or the same concentration of other products such as Purex was found to bleach the perithecia and was thought that this might possibly have an effect on the ascospores inside the perithecia.

8. Gassing and irradiation in the quartz top flask.

The quartz top flask, which had a capacity of 175 ml, had Pyrex tubing in the Pyrex bottom for the introduction of a gas. Five ml of the Em5297a suspension, absorbance 0.05, was used in this flask. Gas was applied either prior to or following irradiation and was bubbled into the conidial suspension. The gas was dispensed into the flask from a lecture bottle through copper tubing to the Matheson manual flowmeter. In later studies, the Hastings Mass Flowmeter was connected to the manual flowmeter for more accurate delivery of the gas into the suspension. The gas flow from the lecture bottle was difficult to control

Figure 3.--Photograph of an Oxoid cornmeal agar plate with approximately 250 perithecia distributed over the surface. Perithecia formed are the product of untreated Em5297a crossed to St. Lawrence 74A.

with the aid of the outlet valve on the cylinder; therefore, it was necessary to use a manual flowmeter control between the gas cylinder and the mass flowmeter to more easily control the rate of flow of the gas. Readings on the mass flowmeter are equivalent to specific amounts of a gas, as measured in ml water displacement/min. The calibration curves which were drawn using water displacement values and assuming a negligible solubility will vary to an extent depending on the gases used. These calibration curves are illustrated in Figure 4. A Sylvania germacidal lamp was used for the irradiation of the microconidial Em5297a suspension which was gas treated or untreated. UV irradiation was applied to the sample at the rate of 270 ergs/mm^2/sec as measured with the aid of a Blak-Ray UV monitor. The arrangement for irradiation of the Neurospora spores is shown in Figure 5.

9. Gassing and irradiation in the quartz tube.

The quartz tube, which had a capacity of 5 ml, had a pyrex top allowing for the entry and exit of the gas. Four ml of the Em5297a microconidial suspension, absorbance 0.05, was used in this flask. Gas was applied either prior to or following irradiation and was bubbled directly into the conidial suspension as described previously. The amounts of the gases used for the experiments as calculated in ml/min water displacement, were calculated from the calibration curves in Figure 4. Two germacidal Sylvania lamps were

Figure 4.--Calibration curves of various gases experimentally determined and used in the investigations as measured on the Hastings Mass Flowmeter in standard cc/min.

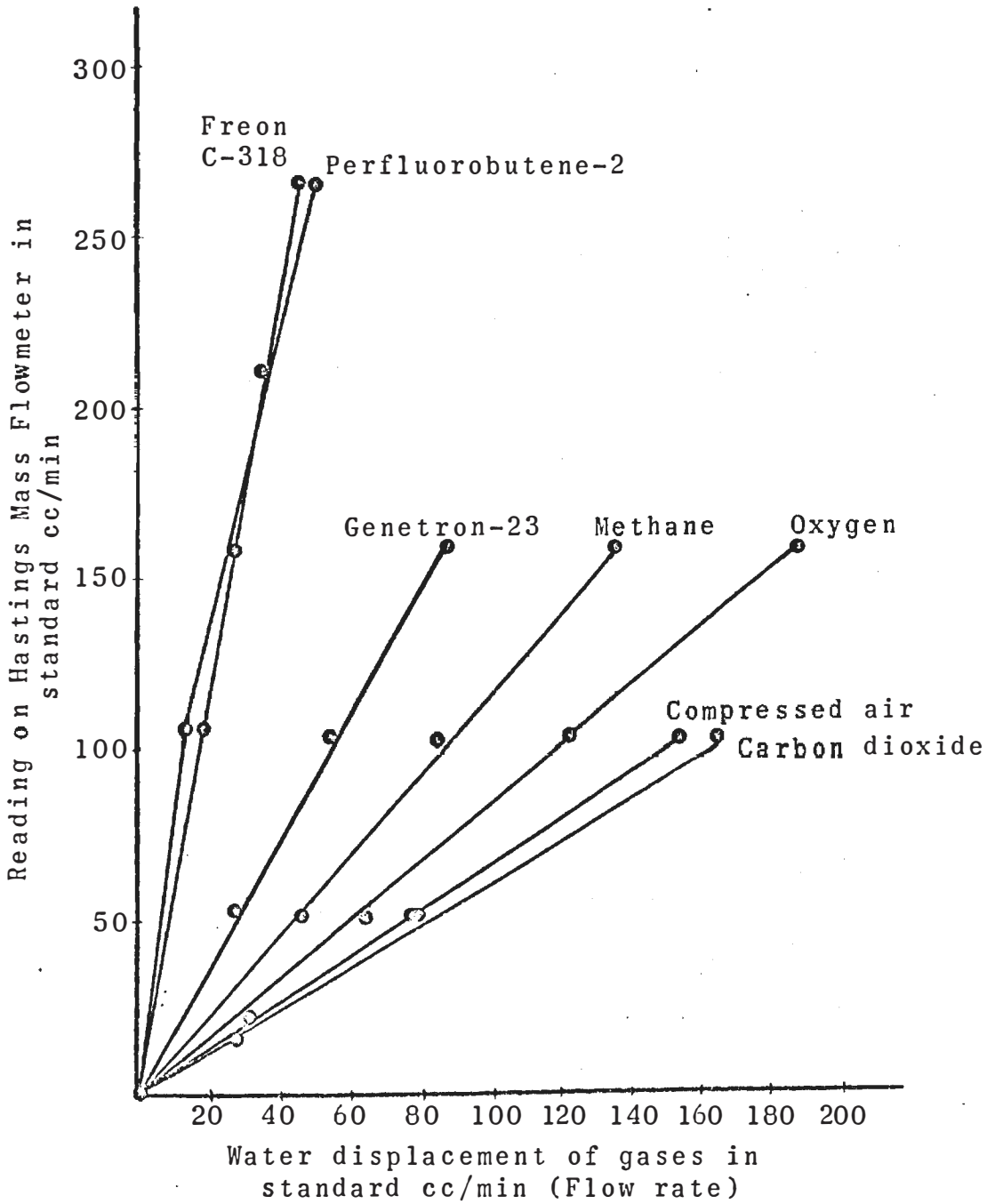

300
250
200
150
100
50
Reading on Hastings Mass Flowmeter in standard cc/min
Freon C-318
Perfluorobutene-2
Genetron-23
Methane
Oxygen
Compressed air
Carbon dioxide
20 40 60 80 100 120 140 160 180 200
Water displacement of gases in standard cc/min (Flow rate)

Figure 5.--Photograph of a quartz top flask situated under the 36" germacidal UV lamp in position for irradiation.

placed 18.5 cm on each side of the quartz tube for irradiation of the conidial suspensions in the tubes. The UV light was delivered at the rate of 135 ergs/mm^2/sec from each lamp as measured at this distance with the aid of a Blak-Ray UV monitor. The total dose, then coming from both lamps, was 270 ergs/mm^2/sec. A photograph of the flask and lamps in position for irradiation is shown in Figure 6.

10. Photoreactivation with the use of fluorescent lights.

A conidial suspension of Neurospora crassa Em5297a, absorbance 0.25, was irradiated in the quartz top flask at the rate 320 ergs/mm^2/sec for 30 min using a 36" Sylvania germacidal lamp. Aliquots of the treated suspensions were pipetted into 25 ml of minimal medium for the mycelial studies and onto 15 ml of cornmeal agar for the perithecia studies. The inoculated plates and flasks were placed under two 18" fluorescent lights mounted in a desk lamp, and irradiated for 30 min in an effort to photoreactivate the irradiated conidia. Survival of the conidia as measured by dry mycelial weight and perithecia formation was performed by methods already described.

11. Monochromatic light photoreactivation.

Four ml of a Neurospora crassa Em5297a microconidial suspension, 0.15 absorbance, was irradiated for 10 min in the quartz tube flask with a total irradiation dose of 270 ergs/mm^2/sec. The conidial suspension was then kept in

Figure 6.--Photograph of a quartz tube situated between the four 18" germacidal UV lamps in position for irradiation.

the dark at 25 C for 30 min. Prior to photoreactivation, Pyrex tubes (50mm X 5mm) were filled with 0.6 ml of the conidial suspension and received three light flashes from the electronic flash. The flashes were timed 10 sec apart with shaking between flashes to insure full photoreactivation. An aliquot of the treated suspension, 0.5 ml, was mixed with 0.5 ml of sterile water in a two ml vial. A 0.05 ml inoculum was added to each 125 ml Erlenmeyer flask containing 25 ml of minimal medium, for the mycelial growth studies, and onto each petri plate containing 15 ml of Oxoid cornmeal agar, for the studies of perithecia formation. A 0.05 ml microconidial sample of the Neurospora crassa strain, St. Lawrence 74A, which had an absorbance of 0.05 was pipetted onto each plate 24 hr after the initial plating of the Em5297a strain. Both inoculated plates and flasks were left in the dark at 25 C. Harvesting of the mycelial pad was performed after 5 days growth and counting of the perithecia was done after 10 days using the methods described previously.

12. Catalase testing for peroxides.

Catalase from Worthington Biochemical Corporation in the amount of 0.01 ml which contained 474 units of the enzyme was added to known dilutions (30% to 0.002%) of hydrogen peroxide. The first well in the Autotray by Astec, Inc. contained 0.2 ml of 30% hydrogen peroxide while the other 14 wells contained 0.1 ml of distilled water. One-tenth

ml of 30% hydrogen peroxide from the first well was added to the second well and mixed, and 0.1 ml of this solution in the second well was added to the third. This was repeated until the final dilution at the 15th well was made. The extra 0.1 ml was discarded from the 15th well. The controls as well as the irradiated samples were tested for amount of bubbling in the clear Autotrays. Four ml of sterile water and 4 ml of an Em5297a conidial suspension, absorbance 0.05, were irradiated at 270 ergs/mm^2/sec in two of the quartz tubes for 20 min to ascertain if any detectable peroxides were formed due to the UV light. The irradiated samples were compared to the controls on the basis of bubbles produced for an estimate of peroxides present.

EXPERIMENTAL RESULTS

Jagger (1967) published a thorough review of the inhibiting effects of ultraviolet light on large molecules, viruses, bacteria, and other cells. The presence of dark repair enzymes in micro and macro organisms were noted to increase their survival when compared to cells not possessing this enzyme system. Jagger (1967) noted that perhaps the most important modification of damage caused by far UV irradiation is photoreactivation. Visible light reactivation has been found, according to this review, in all taxonomic orders in which it has been sought, although individual strains were found which did not possess this enzyme. From this review, it seems that there exists a definite need to study the effects and modifications of UV light induced damage on specific systems in organisms, for example, sexual spore formation in fungi. Studies were performed in our laboratory with Neurospora crassa Em5297a. The action of UV was investigated on the survival of the organism by measuring impairment of sexual spore formation after crosses to a non-irradiated strain. In order to measure inhibition of perithecia formation, a quantitative method had to be devised for counting the fruiting bodies.

In preliminary studies, microconidia of Neurospora crassa Em5297a were irradiated in either a quartz top flask or a quartz tube with a total irradiation dose of 270 ergs/mm^2/sec in order to determine the LD/70 dose for the strain. UV effects on mycelial growth were determined after 5 days incubation in the dark at 25 C. A graph was plotted of the average dry mycelial weight of the strain after irradiation in the two flasks as shown in Figure 7. It can be seen that the quartz tube is a more efficient system for UV irradiation than the quartz top flask since the LD/70 is reached in 11 min in the tube and in 21 min in the flask. Aliquots of the same microconidial suspensions were tested for loss of perithecia forming ability. The irradiated conidia of Em5297a were mated 24 hr after the initial plating on the cornmeal agar plate with the St. Lawrence 74A strain. Perithecia were counted one week later. Data obtained, showing UV effects on perithecia formation in the two types of flasks used are shown in Figure 8. There was little effect produced due to UV, on the total number of perithecia formed from conidia irradiated in the quartz top flask; however, UV exposure is apparently much more detrimental when applied to the conidial suspension in the quartz tube. An increase in the number of perithecia was noted after 20 min of UV irradiation in some experiments not reported here, but the results were not always reproducible.

Figure 7.--Effect of UV exposure for various time intervals on mycelial growth of Neurospora crassa Em5297a conidia as tested in two types of irradiating vessels.

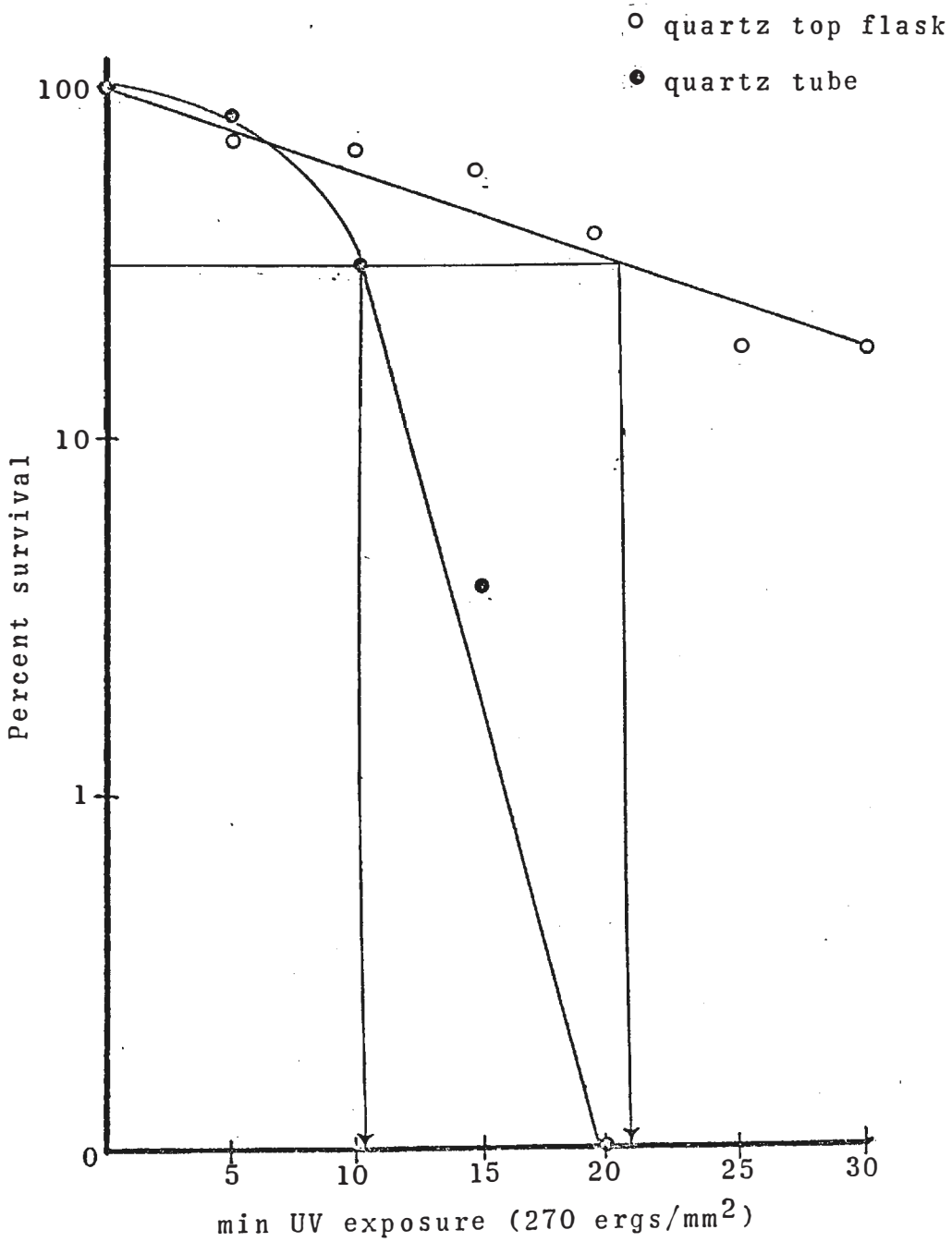
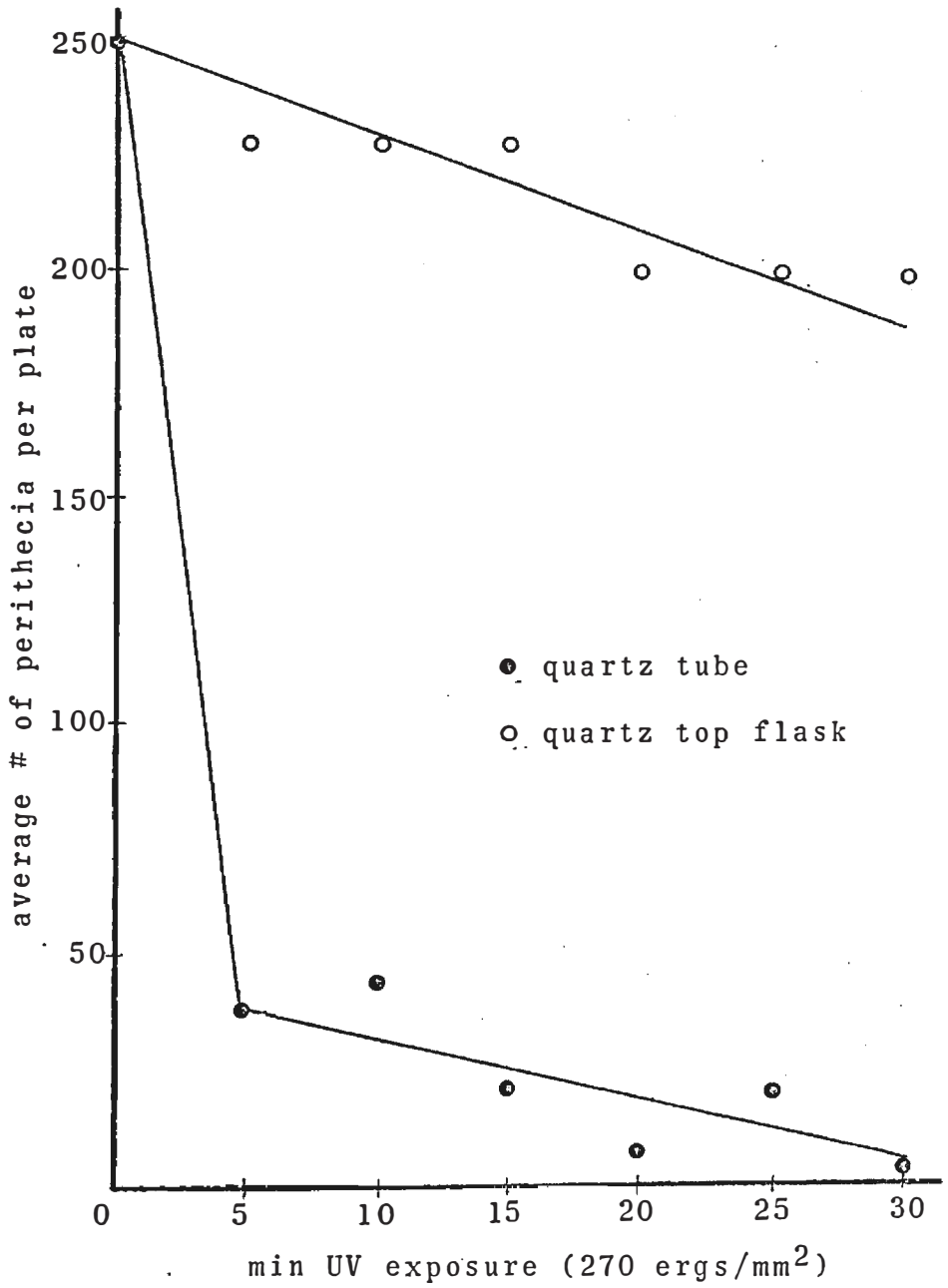

quartz top flask
quartz tube
100
10
1
0
Percent survival
5
10
15
20
25
30
min UV exposure (270 ergs/mm2)

Figure 8.--Effect of UV exposure at various time intervals on perithecia formation of Neurospora crassa Em5297a irradiated in two different vessels and then mated to the St. Lawrence 74A strain.

The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.


250
200
150
100
50
0
5
10
15
20
25
30
average # of perithecia per plate
quartz tube
quartz top flask
min UV exposure (270 ergs/mm2)

An experiment was devised to test for the presence of peroxides in irradiated conidial suspensions. Catalase, which is an enzyme that splits peroxide into water and oxygen, was added to known concentrations of hydrogen peroxide and the amount of bubbling was noted. Two tenths of a ml of 30% hydrogen peroxide was placed in one well of an Autoclear tray. A 0.1 ml aliquot of the solution was withdrawn from this and added to 0.1 ml of sterile distilled water in the following well. The solutions were mixed by drawing some of the liquid in and out of the pipette repeatedly. The procedure was repeated until a concentration of 0.002% hydrogen peroxide was reached. The results of this test, shown in Table 2, were then compared to two 0.1 ml samples of water, with and without the Neurospora suspension, which were irradiated at 270 ergs/mm^2/sec for 20 min in the quartz tube. These UV irradiated samples were tested in the same manner indicated for the dilution of hydrogen peroxide. This test was performed in order to ascertain if peroxides were being produced by the UV light which might effect the conidial cells. The quartz tube was used since it had proven to be a more efficient system based on the LD/70 data and therefore probably allowed more UV penetration. Peroxides were not detected with this test in the experimental samples, but since the controls contained 0.004% peroxide and above, for purposes of this study it was concluded that no peroxides were formed during the UV irradiation.

Table 2.--Detection of peroxides by observing amount of bubbling when 474 units of catalase were added to known amounts of peroxide.

Percent of peroxide	Irradiated samples*	Oxygen production**
30.0		++++
15.0		++++
7.5		+++
3.75		+++
1.88		+++
0.94		+++
0.47		++
0.24		++
0.12		++
0.06		++
0.03		++
0.015		++
0.008		+
0.004		+
0.002		-
	H_2O alone	-
	H_2O + Em5297a conidia	

*Water and conidial samples in water were irradiated at 270 ergs/mm^2/sec for 20 min in quartz tubes.

**The amount of bubbling was observed in response to the addition of catalase to identical aliquots of previously diluted samples of peroxide.

++++ extensive bubbling
+++ moderate bubbling
++ light bubbling
+ 10-20 bubbles
- no visible reaction

According to Jagger (1967) oxygen has little or no effect on enhancement of damage produced due to far UV irradiation on bacteria, fungi, or algae. The following experiments were undertaken to compare oxygen and compressed air effects on mycelial growth and sexual spore (perithecia) formation in Neurospora, using the gases alone and gas treatments in conjunction with UV irradiation.

An experiment was performed to determine if there was any effect of compressed air or carbon dioxide as compared to atmospheric air on the irradiated samples. The quartz tubes were irradiated at 270 ergs/mm^2/sec for 15 min. Treatment with the compressed air or carbon dioxide was done either prior to or following irradiation. There was a 30 min delay period after gassing and before irradiation or plating. The results in Table 3 indicate that compressed air given before irradiation can stimulate recovery of Em5297a. Daylight, in absence of UV radiation, is stimulatory for the organism. It can be noted that there is no photoreactivation of the strain when daylight treatment is given to the UV irradiated cells. Compressed air and carbon dioxide given to non-UV treated cells was slightly inhibitory but carbon dioxide did not aid in the recovery of UV irradiated conidia. Table 4 contains data showing recovery of UV induced damage to perithecia formation of Em5297a crosses in response to atmospheric air and compressed air. All of the perithecia forming plates were incubated in the

Table 3.--Comparison of growth of Neurospora crassa Em5297a, measured as percent dry mycelial weight compared to the atmospheric air control grown in daylight.

Treatments	Compressed air*		Carbon dioxide*		Atmospheric air*	
	Grown in total darkness %	Grown in daylight %	Grown in total darkness %	Grown in daylight %	Grown in total darkness %	Grown in daylight %
Control (no irradiation)	72	92	72	83	80	100
Irradiation prior to gas	0	0	0	0	0	0
Irradiation following gas	9	41	0	0	0	0

*Atmospheric air refers to the air normally present in the laboratory. Gases were delivered to the conidial suspension at the rate of 30 ml/min for 10 min.

**UV irradiation was delivered to the suspensions for 15 min at 270 ergs/mm^2/sec in the quartz tube.

Table 4.--Comparison of growth of Neurospora crassa Em5297a, as measured by % survival of perithecia formation in response to irradiation*** and gas treatments* after which this strain was mated to St. Lawrence 74A.

Treatments	Compressed air**		Carbon dioxide*		Atmospheric air*	
	% survival	Average # of perithecia**	% survival	Average # of perithecia**	% survival	Average # of perithecia**
Control (no irradiation)	100	200	100	180	100	190
Irradiation prior to gas	0	0	12	32	0	0
Irradiation following gas	50	180	0	0	0	0

*Atmospheric air refers to the air normally present in the laboratory. Gas was delivered to the conidial suspension at the rate of 30 ml/min for 10 min.

**Percent survival refers to the amount of plates out of the total number which showed perithecia formation. Average # given is average # per plate for surviving plates only.

***UV irradiation was delivered to the suspensions for 15 min at 270 ergs/mm^2/sec in the quartz tube.

The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.

dark since metal tops were used on the plates to avoid condensation of water. There is no real inhibition of perithecia formation due to carbon dioxide or compressed air as there was with the mycelial data; however, carbon dioxide given after, and compressed air applied before, irradiation appeared to stimulate the recovery of the perithecia forming capacity.

In another experiment, microconidia of Em5297a were irradiated in the quartz top flasks at 270 ergs/mm^2/sec for varying periods of time. After irradiation in atmospheric air, 30 ml/min of oxygen was dispensed for 10 min into the flasks. This amount, a total of 300 ml, displaced all air in the flask. The conidial suspensions in the flasks were left in the dark, in the oxygen atmosphere 30 min prior to plating. Aliquots of the treated suspensions were diluted, and pipetted into 125 ml Erlenmeyer flasks containing 25 ml of minimal medium. The inoculum was grown for 5 days at 25 C in the dark. The mycelial pads were harvested, dried, and weighed to the nearest mg. The results of this experiment are shown in Table 5. It can be noted that oxygen gives a protective effect to the UV irradiated cells which peaks after 20 min of UV exposure. The same experiment was duplicated using the quartz tube to study effects of oxygen using this system. Oxygen was bubbled at 30 ml/min for 10 min which was more time than was required to displace all of the air in this flask since the total water volume

Table 5.--Survival of microconidia of Neurospora crassa Em5297a after UV irradiation followed by gas treatments for 30 min in quartz top flasks.

270 ergs/mm^2/sec* min	Survival after treatment measured as dry mycelial weight**					
	Atmospheric air		Oxygen***		Compressed air***	
	mg	%	mg	%	mg	%
0	66 ± 5	100	55 ± 8	83	63 ± 4	95
5	41 ± 6	62	30 ± 2	45		
10	40 ± 3	61	30 ± 4	45		
15	37 ± 4	56	40 ± 5	61		
20	26 ± 4	39	49 ± 2	74		
25	15 ± 8	23	20 ± 5	30		
30	13 ± 6	20	13 ± 3	19		

*The UV dosage was measured 7.5 cm from the Blak-Ray UV monitor.

**The strain was grown on liquid medium for 5 days at 25 C in the dark.

***Oxygen and compressed air were administered at a rate of 30 ml/min for 10 min giving a total of 300 ml. The gas atmosphere was not disturbed in the flask for 30 min before samples were taken.

The data presented constitutes means of 8 replicas ± standard error.

of the tube was 5 ml. The actual bubbling time was considered important in this experiment. Em5297a was grown in the minimal medium as before, after dilution, at 25 C for 5 days in the dark in order to measure the inhibition of mycelial growth. Table 6 indicates the results of this study. The protection noted due to oxygen on irradiated cells in the quartz top flask was even more striking when the same treatment was applied in the quartz tube. In both flasks, oxygen alone, with no irradiation, tended to inhibit growth. Figure 9 shows the effect of oxygen on irradiated Em5297a conidial suspensions in the two flasks. The lethality curves for both suspensions in the two vessels are quite similar with the recovery peak appearing 5 min earlier for the cells irradiated in the quartz tube than in the quartz top flask.

Experiments involving UV and oxygen effects on perithecia formation were performed simultaneously with the mycelial growth studies. Oxygen was applied to both vessels after irradiation. As in mycelial studies, aliquots were pipetted onto petri plates containing cornmeal agar and the Em5297a strain was mated 24 hr later with St. Lawrence 74A. The data in Figure 10 indicates that in the quartz top flask, the perithecia forming ability of the Em5297a strain was not effected by UV to any significant degree; however, UV in conjunction with oxygen proves to be detrimental to this system. In the quartz tubes, however, UV exposure is much

Table 6.--Survival of microconidia of *Neurospora crassa* Em5297a after UV irradiation followed by gas treatment in quartz tubes.

270 ergs/mm^2/sec* min	Survival after treatment measured as dry mycelial weight** Atmospheric air mg	%	Oxygen*** mg	%
0	69 ± 6	100	51 ± 4	74
5	55 ± 6	79	41 ± 2	59
10	24 ± 9	35	46 ± 8	67
15	3 ± 8	4	54 ± 5	78
20	0	0	32 ± 4	46
25	0	0	0	0
30	0	0	0	0

*The UV dosage was measured 7.5 cm from the Blak-Ray UV monitor.

**The strain was grown on liquid medium for 5 days at 25 C in the dark.

***Oxygen was administered at a rate of 30 ml/min for 10 min giving a total of 300 ml. The gas atmosphere was not disturbed in the flask for 30 min before samples were taken.
Both nitrogen and compressed air were applied at the rate of 30 ml/min for 10 min. With no irradiation, the mean for compressed air gassed conidia was 65 ± 5 and 69 ± 4 for nitrogen. An average of 41 ± 5 was obtained for conidia irradiated 10 min and gassed with nitrogen, indicating recovery had occurred.
The data presented constitutes means of 8 replicas ± standard error.

Figure 9.--Comparison of survival of Neurospora crassa Em5297a conidia, UV irradiated at different time intervals followed by oxygen treatment at 30 ml/min for 30 min in the quartz top flask and quartz tube.

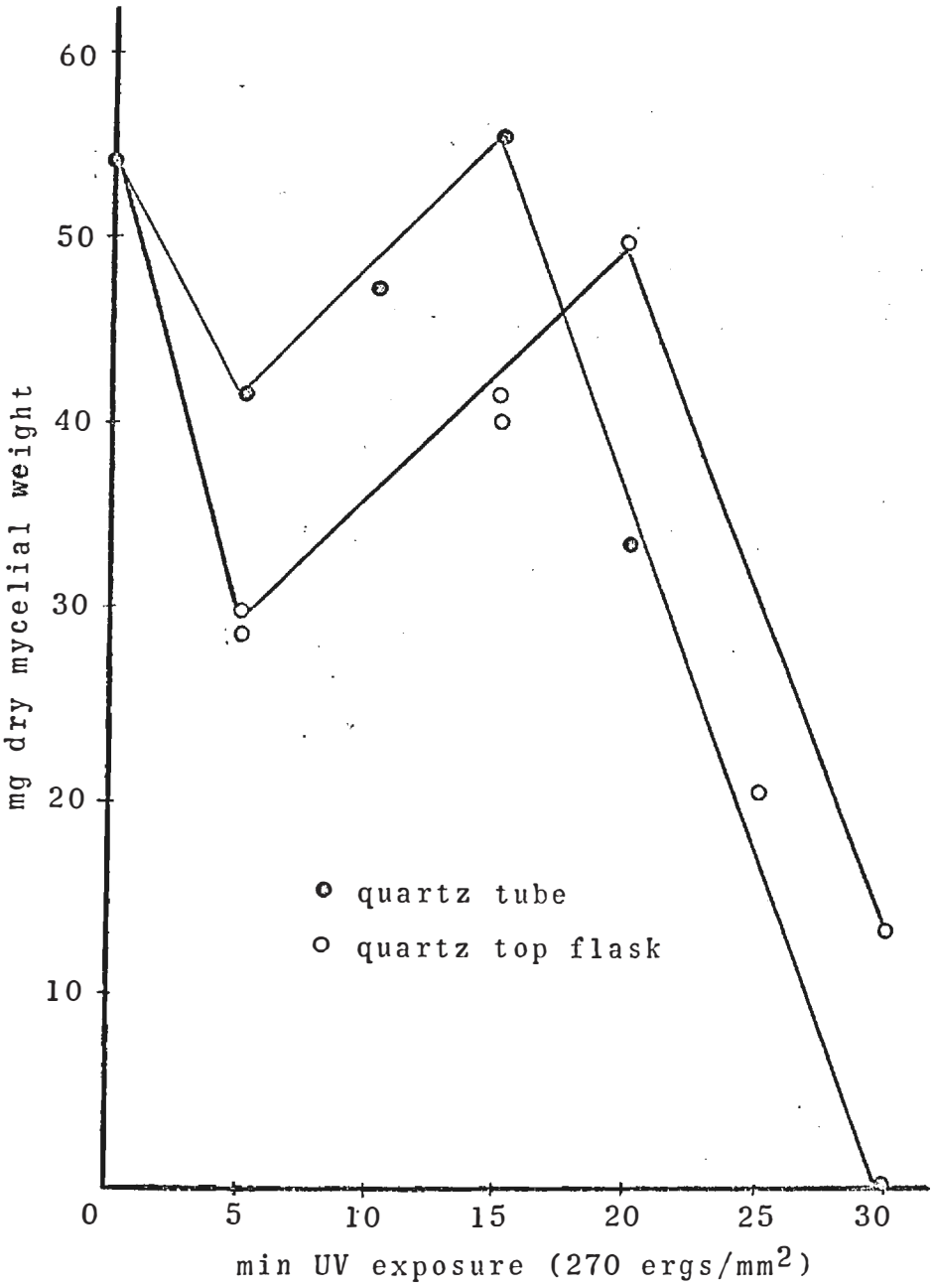
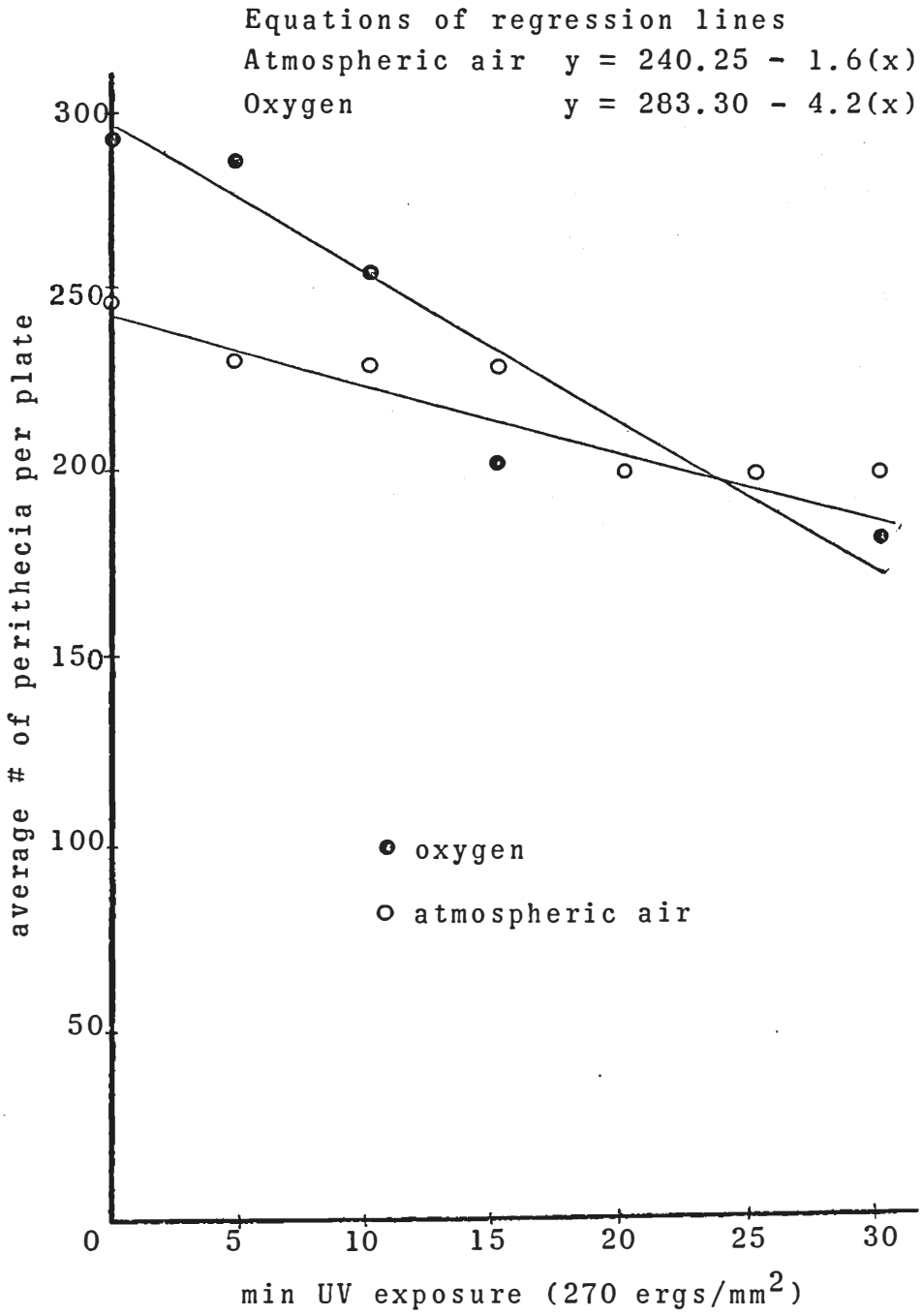

60
50
40
30
20
10
mg dry mycelial weight
quartz tube
quartz top flask
0
5
10
15
20
25
30
min UV exposure (270 ergs/mm2)

Figure 10.--Perithecia formation of Neurospora crassa Em5297a crossed to St. Lawrence 74A after UV irradiation followed by gas treatment in the quartz top flask.

The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.


Equations of regression lines
Atmospheric air y = 240.25 - 1.6(x)
Oxygen y = 283.30 - 4.2(x)
300
250
200
150
100
50
0
5
10
15
20
25
30
average # of perithecia per plate
oxygen
atmospheric air
min UV exposure (270 ergs/mm2)

more detrimental to the capacity of perithecia formation of the strain in atmospheric air, as indicated by the data in Table 7. There was recovery of perithecia forming ability as indicated by an increase in perithecia resulting from exposure of 20 min in air. This protective effect was also noted in the mycelial studies with oxygen. t appears, though, that oxygen given after UV treatment, in the quartz tube, is even more damaging than when applied in the quartz top flask.

Effects of fluorinated hydrocarbons and their analogues were compared to those of oxygen in the next set of experiments. The UV irradiation was applied to the conidial suspension in quartz top flasks for a period of 30 min at 270 ergs/mm^2/sec. Results obtained as shown in previously described Figure 7 indicate only a 20 to 30% survival of conidia as measured by mycelial growth. In this experiment, the conidial suspensions were gassed prior to irradiation. Thirty min after gassing, the flasks were allowed to aerate. The gas treatment consisted of a 10 min period in which gas bubbled into the suspension. The treated and untreated suspensions were pipetted into each of 125 ml Erlenmeyer flasks containing 25 ml of minimal medium as described for the mycelial growth studies. Pads were harvested after 5 days, dried, and weighed to the nearest mg. Results are tabulated in Figure 11. After 30 min of UV irradiation, 24 mg of dry mycelial weight indicates a 34% survival as

Table 7.--Perithecia formation of treated *Neurospora crassa* Em5297a crossed to St. Lawrence 74A after UV irradiation followed by gas treatment* in quartz tubes.

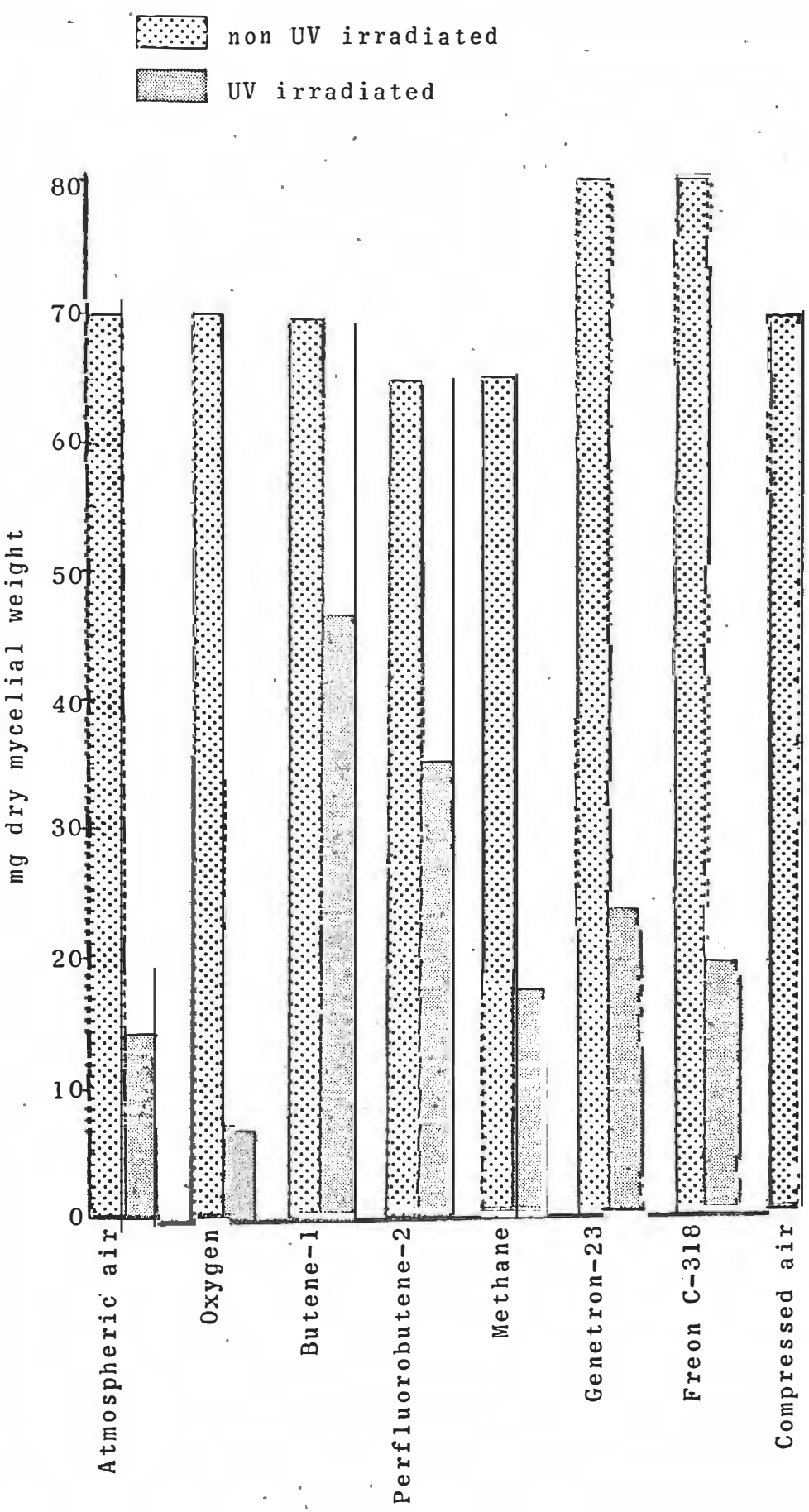
270 ergs/mm^2/sec min	After dilution average # of perithecia per plate**		
	Atmospheric air	Oxygen	Compressed air
0	230	270	230
5	35	16	
10	40	48	
15	22	50	
20	10	65	
25	20	25	
30	10	13	

CoLab's Oxoid cornmeal agar was used as the medium for the crosses.

*Gases, oxygen and compressed air, were delivered to each flask for 10 min at 30 ml/min. Atmospheric air refers to air present in the laboratory.

**The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.

Figure 11.--Survival microconidia of Neurospora crassa Em5297a irradiated with 270 ergs/mm^2/sec of ultraviolet light after gas treatment, 30 ml/min for 10 min, in quartz top flask. The gas was allowed to remain in the flask for 30 min before aeration. Em5297a was grown for 5 days in liquid minimal medium at 25 C in the dark.

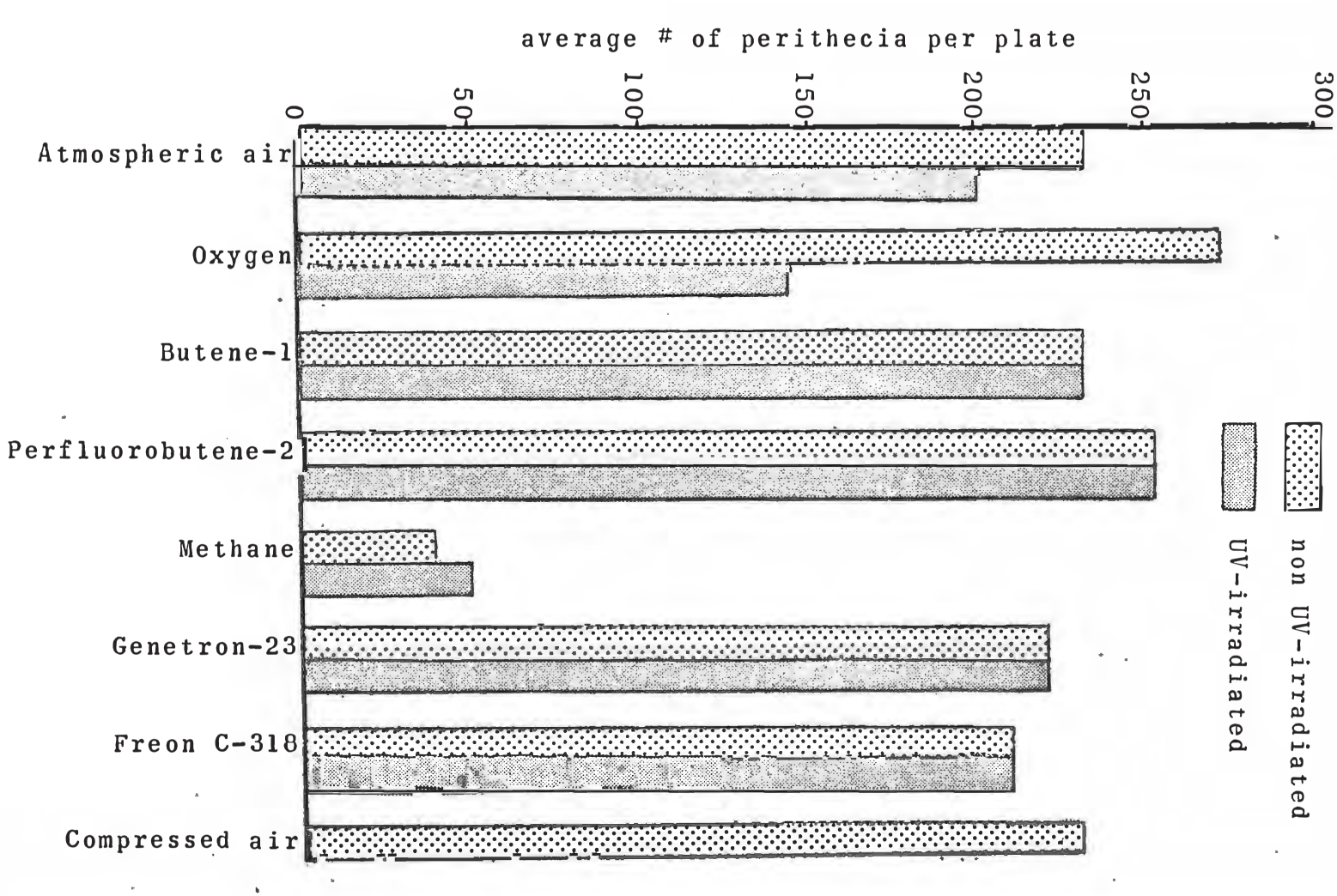

non UV irradiated
UV irradiated
mg dry mycelial weight
0
10
20
30
40
50
60
70
80
Atmospheric air
Oxygen
Butene-1
Perfluorobutene-2
Methane
Genetron-23
Freon C-318
Compressed air

compared to the control. Oxygen and methane enhanced the detrimental effect of UV as seen by the depression of mycelial growth. Although there is not a great deal of difference between the mycelial weights of the control and the Genetron-23 and Freon C318 treated samples, experiments in our laboratory have shown that these gases alone, without irradiation treatment, stimulate mycelial growth. In conjunction with UV radiation, butene-1, perfluorobutene-2, and Genetron-23 offered varying degrees of protection. In comparison, oxygen proves itself to be the most detrimental to mycelial growth when applied prior to irradiation.

Effects of fluorinated hydrocarbons and their analogues on Em5297a were compared to the effects rendered by oxygen in conjunction with UV as measured by perithecia forming ability of the strain. As before, the suspensions in the quartz top flasks received a UV dosage of 270 ergs/mm^2/sec and gassing was done prior to irradiation. The data are shown in Figure 12. Since the control data calculations indicated a standard deviation of ±15, there was no significant difference in the effect of any of the gases on Em5297a in the absence of UV irradiation treatment. Oxygen or methane applied prior to irradiation seems to have a detrimental effect on subsequent perithecia formation as well as on mycelial growth. Other data reported in this dissertation indicated that oxygen applied after, instead of before irradiation, also inhibited perithecia formation.

Figure 12.--Perithecia formation of Neurospora crassa Em5297a crossed to the St. Lawrence 74A strain after prior treatment to the gas followed by the UV irradiation (270 ergs/mm^2/sec) in quartz top flask. The gas was dispensed for 10 min at the rate of 30 ml/min. The gas was allowed to remain in the flask for 30 min before aeration. The two strains were crossed on Oxoid corn-meal agar.

The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.


average # of perithecia per plate
0
50
100
150
200
250
300
Atmospheric air
Oxygen
Butene-1
Perfluorobutene-2
Methane
Genetron-23
Freon C-318
Compressed air
non UV-irradiated
UV-irradiated

The previous experiments indicated that in using the fluorinated gases and their analogues, the quantity of gas and the actual exposure time as measured in ml/min of gas bubbled in the flask were important considerations in the effects observed on the conidia, both in perithecia formation and mycelial growth. The suspensions in the quartz top flask were irradiated at 270 ergs/mm^2/sec. Three gases were used: Freon C318, Genetron-23, and methane. All gases were introduced into the flasks using the Hastings Mass Flowmeter so that 30 ml/min was dispensed for each of the three gases used. The gases were bubbled into the Em5297a microconidial suspensions prior to irradiation for 10 min, 20 min, and 30 min, so that a total of 300, 600, and 900 ml of each gas was applied. The flasks were aerated 30 min after gassing just prior to irradiation. The effects of the gases were tested on mycelial growth of the Em5297a strain. The diluted, treated samples were pipetted into minimal medium and the mycelium was grown for 5 days in the dark at 25 C. The mycelial pads were harvested, dried, and weighed to the nearest mg. Table 8 shows the results of this experiment. Both Freon C318 and Genetron-23 at a concentration of 900 ml showed marked stimulation of mycelial growth and the higher concentrations, 600 and 900 ml, of methane inhibition was noted. The 14 mg reported for the irradiated control represents 20% survival of the conidia. UV irradiation in conjunction with the gas treatment is more difficult to analyze. A depression is noted when Freon C318

Table 8.--Survival of microconidia of Neurospora crassa Em5297a after gassing microconidia with varying amounts of gasses and then irradiating for 30 min with 270 ergs/mm^2/ sec of ultraviolet light in the quartz top flask.

Treatment**		Mean of dry mycelial weight*		
Gas	Concentration ml	Gas treatment mg	UV treatment after gas & aeration mg	UV treatment in air only mg
Air	atmospheric	70 ± 4		
Compressed air	300	69 ± 4		
UV (in air)	atmospheric			14 ± 4
Freon C-318	300	68 ± 4	22 ± 3	
Freon C-318	600	63 ± 3	3 ± 3	
Freon C-318	900	95 ± 4	15 ± 4	
Methane	300	64 ± 4	17 ± 4	
Methane	600	54 ± 2	12 ± 5	
Methane	900	46 ± 2	17 ± 2	
Genetron-23	300	81 ± 9	14 ± 9	
Genetron-23	600	80 ± 9	26 ± 9	
Genetron-23	900	94 ± 8	8 ± 5	

*Neurospora was grown at 25 C for 5 days in 25 ml minimal medium in the dark.
**All gases were bubbled through the microconidial suspensions for 10, 20, and 30 min at the rate of 30 ml/min and were left in the gassing vessel for 30 min prior to plating and irradiation.
The data presented constitutes means of 8 replicas ± standard error.

is applied to the irradiated cells indicating that 30 ml/min of the gas for 20 min is more detrimental to the organism than gassing only for 10 or 30 min. The 30 min time interval may give the cell time to recover from the gassing and irradiation damage. A slight protective effect was noted for 600 ml of Genetron-23 while an inhibitory effect results when 900 ml of the gas was applied.

Aliquots of the same conidial suspension used for mycelial studies were tested for effects on perithecia formation of Em5297a. Freon C-318, Genetron-23, and methane were bubbled for 10, 20, and 30 min at a rate of 30 ml/min into the Em5297a microconidial suspensions which were then irradiated at 270 ergs/mm^2/sec. The diluted, treated aliquots were pipetted into Petri plates of Oxoid cornmeal agar. The opposite mating type, St. Lawrence 74A, was applied 24 hr later. The suspensions on the plates were allowed to grow for one week at 25 C in the absence of light. The perithecia were counted and the results are recorded in Table 9. A high concentration of 900 ml of methane, both with and without subsequently applied irradiation, seems to be less detrimental to perithecia formation than lower concentrations. This is in direct opposition to the results reported for methane in the mycelial studies. None of the gases tested were found to stimulate perithecia formation. A depression is noted for perithecia formation as was found for mycelial growth in the data for conidia treated with UV

Table 9.--Perithecia formation of Neurospora crassa Em5297a crossed to the St. Lawrence 74A strain* after gassing microconidia with varying amounts of gases and then irradiating for 30 min with 270 ergs/mm^2/sec of ultraviolet light in the quartz top flask.

Treatment**		Average # of perithecia/plate***		
Gas	Concentration ml	Gas treatment	UV treatment after gas & aeration	UV treatment in air only
Air	atmospheric	225		
Compressed air	300	230		
UV (in air)	atmospheric			175
Freon C-318	300	120	130	
Freon C-318	600	150	70	
Freon C-318	900	175	180	
Methane	300	39	25	
Methane	600	85	50	
Methane	900	100	150	
Genetron-23	300	130	120	
Genetron-23	600	225	140	
Genetron-23	900	200	140	

*The two strains were mated on CoLab Oxoid cornmeal agar for one week at 25 C in the dark.

**The gases were dispensed for 10, 20, and 30 min at the rate of 30 ml/min and were left in the gassing vessel for 30 min prior to plating and irradiation.

***The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.

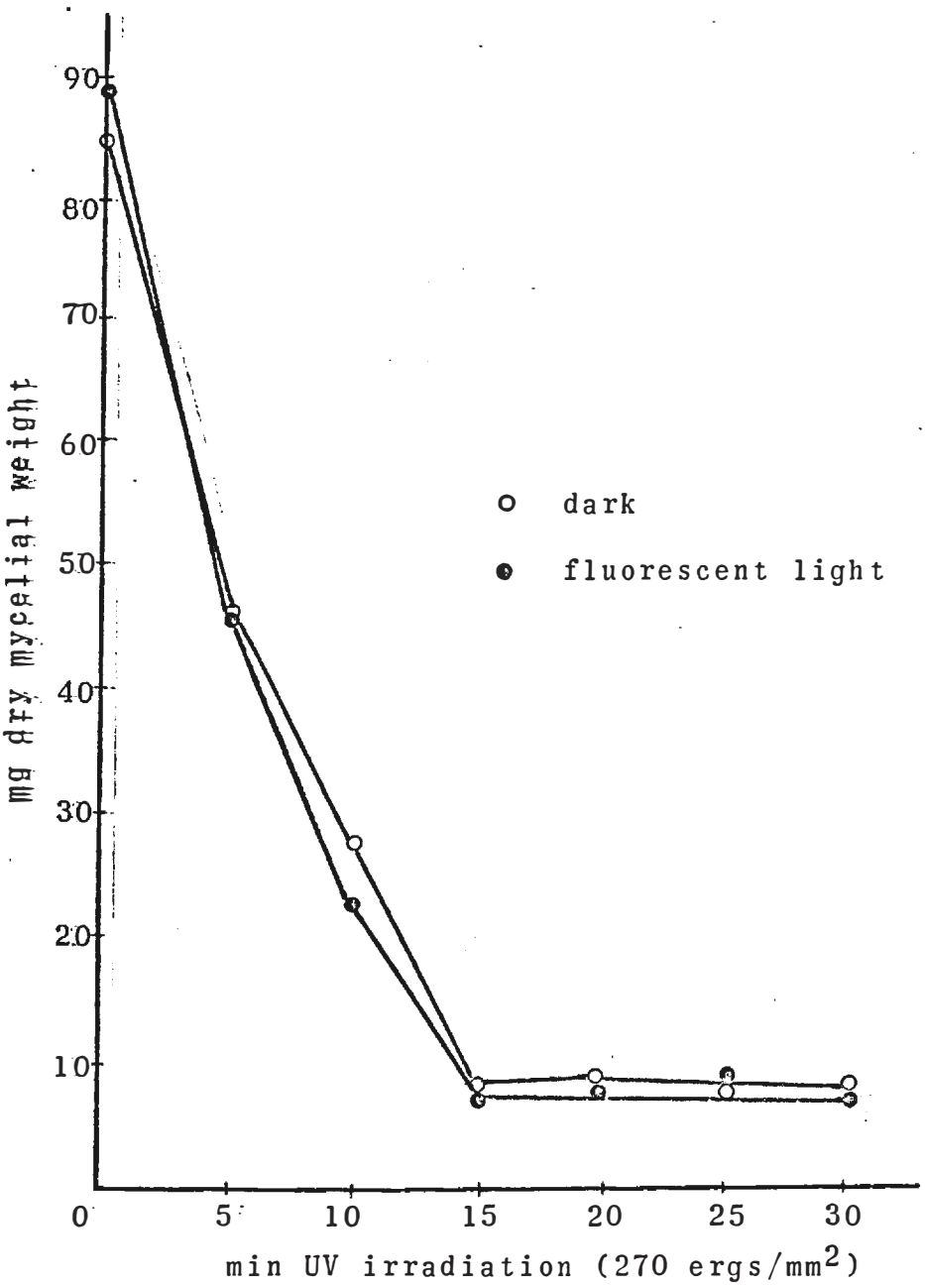
and Freon C-318. These studies indicate that the time interval for gas treatment of the cells appears to be very critical.

Terry, Kilbey, and Howe (1967) found that the Neurospora strains they used possessed a photoreactivation system. Experiments were designed to determine if Neurospora strain Em5297a had the enzymes to carry out these reactions as determined by an increase in survival of the organism in response to light. Early studies were accomplished using fluorescent lights as the photoreactivation source. A spore suspension of Em5297a with an optical density of 0.25 was irradiated in the quartz top flasks at 320 ergs/mm^2/sec for 30 min. Aliquots in the amount of 0.05 ml were pipetted into 25 ml of minimal medium. These Erlenmeyer flasks containing the conidial suspensions were left under the fluorescent lights for 30 min and the cells were allowed to grow in the absence of light at 25 C for 65 hr. The pads were harvested, dried, and weighed with the results shown in Figure 13. There was essentially no difference between the dark repair and photoreactivated samples as indicated by mycelial growth. From this experiment, it was concluded that at least using this method, the strain was not reactivated by light stimulation.

The effects of fluorescent light on the perithecia forming mechanism of UV irradiated Em5297a conidia were studied at the same time the mycelial studies were performed.

Figure 13.--Mycelial growth of Neurospora crassa Em5297a conidia left in the dark or treated with fluorescent light after UV irradiation in the quartz top flask and quartz tube.

This figure shows clearly that fluorescent light does not stimulate the photoreactivating enzyme in Neurospora crassa Em5297a.

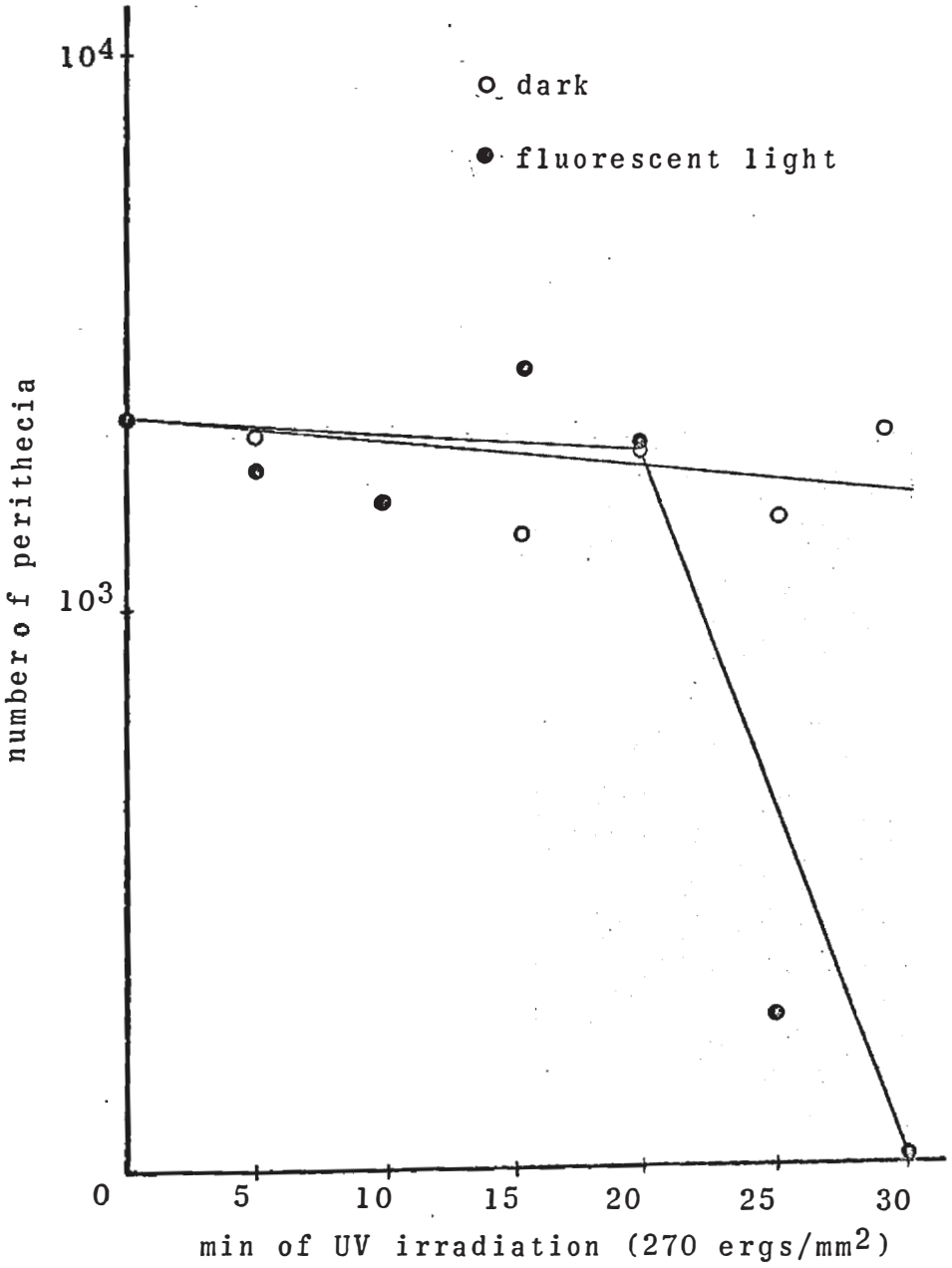

90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
mg dry mycelial weight
dark
fluorescent light
min UV irradiation (270 ergs/mm2)

The same dosage of UV light was used. Samples of the treated conidia were added to each cornmeal agar plate and these plates were left under the fluorescent lights for 30 min. The mating with the St. Lawrence 74A strain was done 24 hr later. The plates were kept in the dark until the perithecia were counted one week after Em5297a was crossed to the opposite mating type. The results are tabulated in Figure 14. The UV radiation slightly depresses perithecia formation, and the fluorescent light enhances the damaging UV effect even to a greater extent at 25 and 30 min.

It was concluded from these preceding studies that one waveband of visible or near visible light might be inhibiting growth so that the photoreactivating effect, if present, was negated. Another possibility is that the wavelength of light, responsible for stimulating the photoreactivating enzyme, was not intense enough in the light sources used. It was then decided that a monochromator would be necessary for identification of the inhibiting or reactivating wavelength, whichever was found to be the case. A monochromator was designed for this use from a Bausch and Lomb Spectrophotometer 20 and an electronic flash unit. The electronic flash was used instead of the tungsten filament lamp, since more recent experiments (Harm and Rupert, 1968; Harm, Harm, and Rupert, 1968; Harm, 1969A; Harm, 1969B) on photoreactivation in E. coli indicated that this light source was intense and yet fast enough so that the effect of heat on

Figure 14.--Perithecia formation of Neurospora crassa Em5297a conidia left in the dark, or treated with fluorescent light, after UV irradiation in the quartz top flask and quartz tube.

The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.


10^4
10^3
0
number of perithecia
dark
fluorescent light
5
10
15
20
25
30
min of UV irradiation (270 ergs/mm2)

the sample would be minimal. In the studies reported, only one light flash which had a duration of 1 msec was necessary to photoreactivate a sample of UV irradiated E. coli. The diffraction grating on the spectrophotometer was used to separate wavelengths of light for photoreactivation of UV irradiated Neurospora crassa Em5297a conidia. This strain was irradiated for 15 min at 270 ergs/mm^2/sec in the quartz tubes. Aliquots of the irradiated cells and duplicate samples of non-irradiated conidia were pipetted into Pyrex tubes to test the effect of various wavelengths of light on the Neurospora conidia. Three flashes were delivered at 10 sec intervals with shaking between the flashes to insure full photoreactivation. Diluted aliquots were pipetted into the minimal medium and grown at 25 C in the dark for mycelial growth experiments. Pads harvested at 72 and 120 hr were dried and weighed. The data for the selected wavelengths of light on the nonirradiated cells are shown in Table 10. There was no significant difference in survival of the conidia after a 72 hr incubation period. However, after 120 hr there was a 23 mg or 27% increase in growth of conidia at 500 nm. There was no significant effect of any of the other wavelengths of light on the nonirradiated cells grown for 120 hr as measured by dry mycelial weight. Table 11 contains the data for the effects of the wavelengths of light on mycelial growth of the UV irradiated conidia measured again at two different time intervals. After 72 hr there

Table 10.--Mycelial growth of Neurospora crassa Em5297a in response to selected wavelengths of light.

Wavelength used	Mycelial growth after 72 hr*	Mycelial growth after 120 hr*
nm	mg	mg
0	19 ± 1	86 ± 1
350	20 ± 1	81 ± 4
400	19 ± 2	86 ± 9
500	17 ± 5	109 ± 9
600	18 ± 2	78 ± 9
700	17 ± 3	82 ± 5
800	17 ± 1	82 ± 5
900	19 ± 1	88 ± 9

*The conidial samples were grown in the dark in 25 ml of minimal medium.
The data presented constitutes means of 8 replicas ± standard error.

Table 11.--Mycelial growth of UV-irradiated** *Neurospora crassa* Em5297a in response to selected wavelengths of light.

Wavelength used after irradiation	Mycelial growth after 72 hr*	Mycelial growth after 120 hr*
nm	mg	mg
0	trace	2 ± 3
350	trace	5 ± 3
400	trace	29 ± 10
500	trace	25 ± 11
600	trace	5 ± 3
700	trace	11 ± 3
800	trace	9 ± 2
900	trace	13 ± 5

*The conidial samples were grown in the dark at 25 C in 25 ml of minimal medium.
**Conidia were irradiated at 270 ergs/mm^2/sec for 15 min in quartz tubes.
The data presented constitutes means of 8 replicas ± standard error.

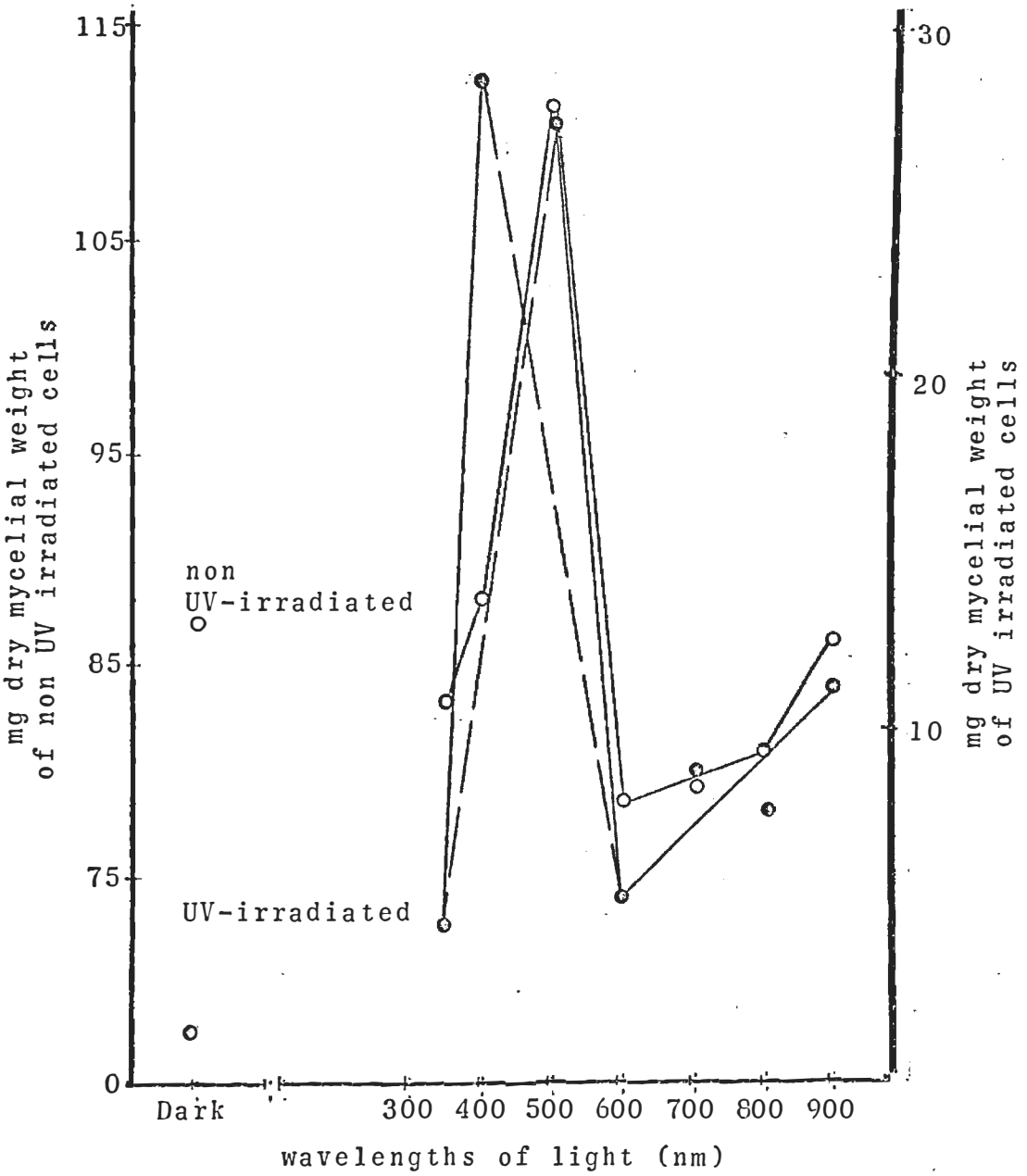
was no measurable growth in any of the flasks. However, after 120 hr of incubation, there was a marked increase of 27 mg at 400 nm and again 23 mg for 500 nm. There was, in addition, a slight increase due to the near infra-red and infra-red wavelengths which extend from 700 to 900 nm. This slight increase corresponds with the data for the non UV irradiated samples, although it is not a significant one.

Figure 15 shows the action spectrum of the irradiated and nonirradiated cells in response to the light flashes. It can be noted that the peak due to the wavelength of light at 500 nm for the irradiated cells was also obtained in the nonirradiated data. This wavelength of light seems to be stimulatory for this organism. The peak at 400 nm is probably due to photoreactivation of the UV damage incurred in these cells since, according to Terry, Kilbey, and Howe, 1967, photoreactivation in Neurospora occurs at 400 nm.

The effect of selected wavelengths of light was tested on UV irradiated Neurospora crassa Em5297a conidia from the aspect of damage to the perithecia forming mechanism in another series of experiments. The conidia were irradiated, as before, for 15 min in the quartz tubes. Aliquots of the irradiated and multiple samples of the non-irradiated conidia were pipetted into Pyrex tubes and the selected wavelengths were flashed 3 times each at 10 sec intervals to insure full photoreactivation. Diluted aliquots of the conidial suspension were pipetted onto cornmeal agar plates

Figure 15.--Comparison of the effect of selected wavelengths of light on the mycelial growth of UV irradiated and non-UV irradiated Neurospora crassa Em5297a. Conidia were irradiated at 270 ergs/mm^2/sec for 15 min in quartz tubes.

A scale which extended from 75 to 115 mg of dry mycelial weight was used for the non-irradiated samples and one from 0 to 30 mg was used for irradiated conidia.


115
105
95
85
75
0
30
20
10
mg dry mycelial weight
of non UV irradiated cells
mg dry mycelial weight
of UV irradiated cells
non
UV-irradiated
UV-irradiated
Dark
300 400 500 600 700 800 900
wavelengths of light (nm)

and the suspension was mated with the St. Lawrence 74A strain 24 hr later. The plates were always kept in the dark. Perithecia were counted one week later, and the data are shown in Table 12. There was a marked stimulation of perithecia formed in response to 700 and 800 nm of light for both UV and non-UV irradiated samples. It can be seen that there was a marked inhibition of perithecia formation when no light flashes were administered to the irradiated conidia. However, there was an increase in the number of perithecia produced when a light flash was given at 400 nm. This increase was probably due to photoreactivation of UV damage incurred as demonstrated with the mycelial growth studies. Figure 16 compares these peaks more graphically. The peak at 400 nm is not noted in the non-irradiated samples indicating a mechanism different than the stimulatory one noted at 700 and 800 nm. This stimulation of perithecia formation in response to wavelengths of light administered in the range of 700 and 800 nm probably peaks at 750 nm since both of the peaks for non-irradiated and irradiated samples can be extrapolated at a higher point on the graph as indicated by the dotted lines.

Table 12.--Perithecia formation of UV irradiated and non UV irradiated Neurospora crassa Em5297a in response to selected wavelengths of light.

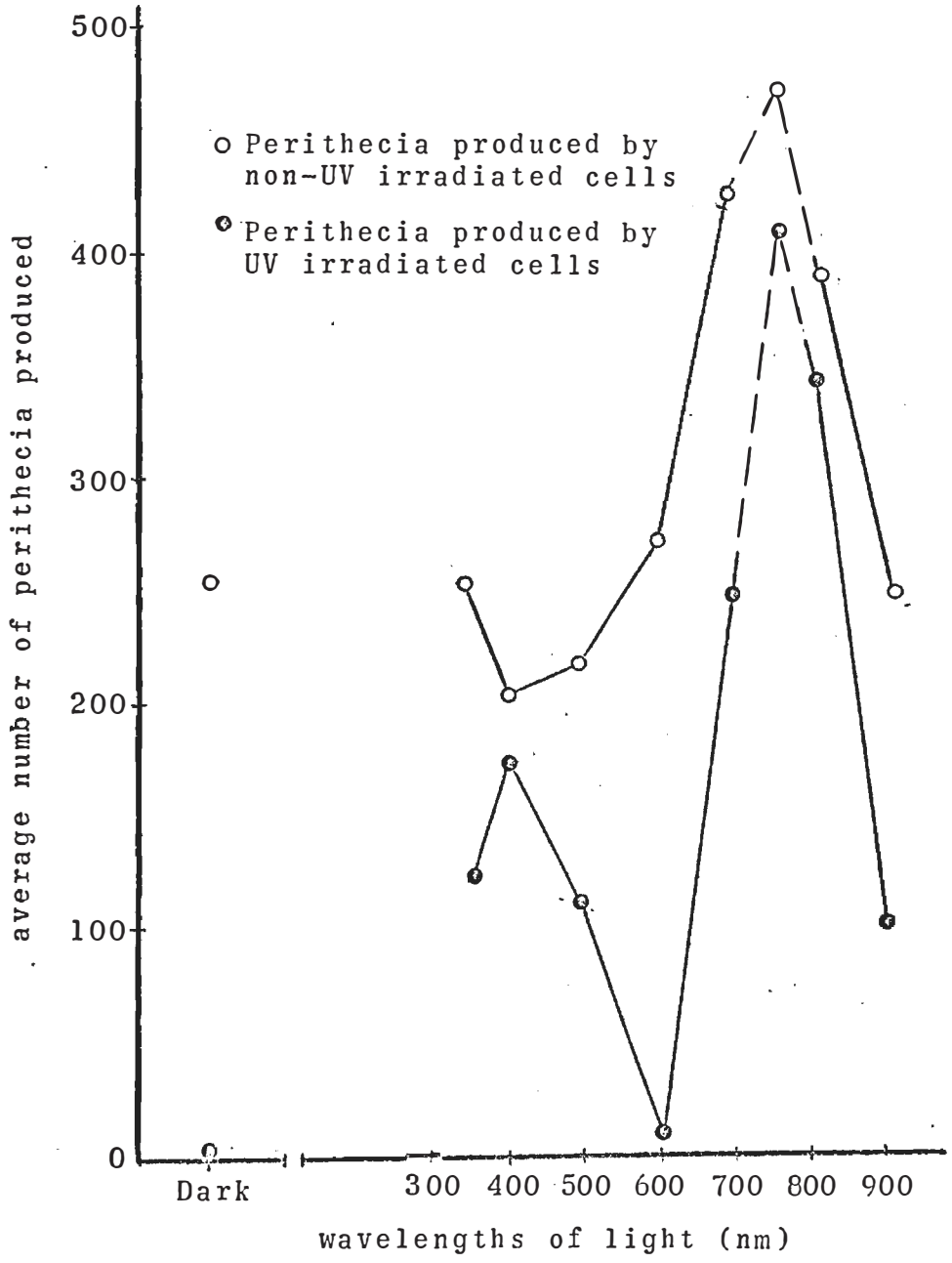
Wavelength used nm	Average # of perithecia formed/plate* No UV irradiation	UV irradiation
0	250	2
350	250	130
400	200	160
500	220	120
600	275	18
700	425	250
800	375	325
900	240	80

The UV dosage used was 270 ergs/mm^2/sec for 15 min.

*The suspension was plated on cornmeal agar plates and mated 24 hr later with St. Lawrence 74A. The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.

Figure 16.--Comparison of the effect of selected wavelengths of light on perithecia formation of UV irradiated and non-UV irradiated Neurospora crassa Em5297a. Conidia were irradiated at 270 ergs/mm^2/sec for 15 min in quartz tubes.

The rounding of figures was performed according to the committee on Standard Methods of the American Public Health Association recommendation.


500
400
300
200
100
0
average number of perithecia produced
Perithecia produced by non-UV irradiated cells
Perithecia produced by UV irradiated cells
Dark
300 400 500 600 700 800 900
wavelengths of light (nm)

DISCUSSION

Lethal effects induced by ultraviolet light and modifications of this response resulting from exposure to visible light, or due to various gas atmospheres, have been investigated and the data obtained are reported in this thesis. Neurospora crassa mutants have been found which lack dark repair (Stadler and Smith, 1968) and photoreactivation processes (Tuveson and Mangan, 1970); however, most strains of Neurospora that were investigated possess both of these systems. Terry, Kilbey, and Howe (1967) obtained six strains capable of photoreactivation: meth-7(4894)A; inos(37401)A; arg-5(27947)a; ad-3B(5-8-700)a; wild-type 74 Or-23-1a; and al-2, cot, pan-2A. Action spectra data indicated that the most effective wavelength for photoreactivation was in the 400 nm region as compared to 365 nm for E. coli and 440 nm for Streptomyces griseus. Tuveson and Mangan (1970) found a UV sensitive mutant of Neurospora isolated from FGSC #331 (cr, rg, pe, fl, multigroup strain consisting of four morphological mutants distributed on two chromosomes) deficient for photoreactivation when fluorescent lamps were used as the light source. In the experiments reported here, the survival of Neurospora crassa Em5297a was not found to be increased as the result of exposure to

fluorescent light for a 30 min time period. However, photoreactivation was found to occur at 400 nm, as based on mycelial growth and perithecia formation using a monochromator, with an electronic flash as the light source. These studies are the first ones reported in which quantitative measures of perithecia formation were used as an indication of survival of Neurospora crassa cells.

Two different vessels were used in the UV irradiation experiments. From the results obtained, the quartz tube type of vessel seemed to be a more efficient system since the LD/70 was reached in this flask in half the time required in the quartz top flask. Studies of perithecia formation indicated that a marked difference was apparent when the quartz top flask or the quartz tube were used in otherwise analogous experiments. Microconidia irradiated in the quartz top flask for the full 30 min time period exhibited little loss of perithecia forming ability; however, conidial cells irradiated in quartz tubes exhibited a pronounced drop in perithecia formation. In mycelial studies, no recovery was noted for irradiated conidia in quartz tubes, 20% or less survival was noted after 30 min exposure to UV. Perithecia formation as compared to mycelial growth from conidia irradiated in the quartz top flask seemed less effected by UV exposure, while in the quartz tube the perithecia forming system proved to be more sensitive.

Experiments with non-irradiated conidia grown in light and under dark conditions showed that mycelial growth was stimulated in the presence of light but no increase in perithecia was noted. Using the monochromator, light stimulation of conidia was found to occur at 500 nm for mycelial growth and at 700-800 nm for the perithecia forming capacity. This indicates that visible wavelengths such as 500 nm may be present in daylight in intense enough amounts to stimulate mycelial growth, while a 700-800 nm waveband would not be expected to occur in sufficient quantity to stimulate the perithecia forming capacity of the strain. In the light and dark studies, no light stimulation was noted for irradiated conidia, as was reported for the experiments with fluorescent light. These data indicated that perhaps the reason the cells do not react to fluorescent light or daylight is not merely a matter of lack of intensity of light but may involve a competition between photoreactivating and stimulatory mechanisms. In the monochromatic light investigations only one wavelength of light was given to one sample of either irradiated or non-irradiated conidial cells so that both enzyme systems would not be operating at the same time.

Various wavelengths of light have been found to stimulate or repress the growth of organisms. Short red radiation (600-700 nm) has been found to stimulate phytochrome (P_R) which activates germination of Lactuca sativa seeds (Rollin, 1966). Rollin found that far-red radiation (700-800 nm)

inhibited this phytochrome response. The phytochrome system has also been found in rye seedling shoots (Holdgate and Goodwin, 1965). Interference in growth due to near UV (365 nm) and green-yellow light (546-579 nm) of Ginkgo pollen and HeLa cells was noted by Klein and Edsall (1967). The yellow-green light inhibition could be negated by red radiation (800 nm) but the near UV light damage was insensitive to photorestoration. It is supposed that the increase in perithecia produced by irradiated and non-irradiated conidia due to red radiation might be a result of a phytochrome process.

These studies indicate that a specific wavelength of light may stimulate non-irradiated and irradiated cells: 500 nm for mycelial growth and 700-800 nm for perithecia formation. If wavelength spectra are not done on irradiated and non-irradiated cells, an increase in amount of mycelia or number of perithecia due to two wavelengths might be reported as two photoreactive mechanisms, instead of photoreactivation at one wavelength and one light stimulation peak as in experiments reported in this dissertation.

Oxygen has been found to enhance damage due to ionizing radiation (Grosch, 1967) but little has been reported on the sensitizing effect of oxygen on UV irradiated cells. Webb and Lorenz (1970) noted oxygen dependent enhancement at 365 nm and 390-750 nm in E. coli cells which was not noted at 254 nm and 313 nm. These results indicated that

some UV effects can be enhanced by oxygen and this damage can be partially repaired by the dark repair system in E. coli. This sensitizing effect of oxygen to UV radiation may be the result of oxygen in combination with a free radical which can form hydrogen peroxide (H_2O_2) or the peroxy radical (HO_2) (Smith and Hanawalt, 1969). Peroxide reactions are believed to proceed much more readily in an environment of ionizing radiation than in UV light; however, peroxide effects have been noted due to UV light. Dendy, Smith, and Aebi (1967) found that peroxides inhibited DNA synthesis following irradiation with a UV microbeam. Wilbur, et al. (1957) found that UV irradiation produced lipid peroxides in Claetopterus pergamentaceous eggs. In addition, UV irradiated methyl linolenate was found to produce effects in normal cells similar to those resulting from UV irradiation. Wilbur, et al. (1957) felt that part of the damage induced by UV was due to lipid peroxides. In the studies reported in this thesis, no peroxides were detected in the external environment of the cell; however, this does not indicate that peroxides were not formed within the cell itself.

Protection of Shigella phage S13 and E. coli phage T3 due to the addition of oxygen to a phage solution irradiated with ionizing radiation was reported by Alper (1954). Alper found that phages, S13 and T3, were very sensitive to inactivation by reducing radicals produced in water by

x-radiation. The effects of hydrogen peroxide on phage suspensions are enhanced when oxygen is removed by the passage of nitrogen through the suspension. It was theorized by Alper (1954) that oxygen reacts with the reducing molecules, so that fewer reductants would be available for inhibition of the phage. The Neurospora mycelial data indicated that oxygen increases survival of UV irradiated conidia. Since oxygen applied to non-irradiated conidia slightly inhibits mycelial growth, recovery from the irradiation effect might be interpreted as a dose response phenomenon. An inhibition of cell division might occur so that fewer cells are in the radiation sensitive DNA-synthesis period and are, therefore, less liable to damage. In contrast oxygen alone was found to stimulate perithecia formation, and as may be expected from the earlier observation, perithecia formation was more sensitive to oxygen when UV light was applied.

The effects of compressed air administered at a rate of 30 ml/min for 10 min was compared to atmospheric air treatment in order to determine if detrimental or stimulatory effects were rendered to the Neurospora conidia. Prior gassing with compressed air was found to increase survival of the UV irradiated conidia in experiments where either mycelium or perithecia formation was measured. The amount of mycelia produced by conidia, gassed with compressed air, irradiated, and then grown in daylight was found to be 44% of the daylight compressed air control, compared to 10%

grown in the dark but treated otherwise in the same manner. Whether compressed air is enhancing the photoreactivating, the stimulatory, or some other system is not clear at the present time. The results obtained indicated that carbon dioxide was not responsible for this stimulation, although gassing with carbon dioxide after irradiation caused some recovery as indicated by an increase in the number of resultant perithecia. Compressed air was used in this study as a comparison to atmospheric air. The increase in mycelial growth due to prior treatment with compressed air indicates that some component or components in this air must be different than what is normally present in what is called atmospheric air. Data published by Bennett and Dossett (1970) substantiated this difference. Bennett and Dossett (1970) reported that because of the raised partial pressure of nitrogen, compressed air induced narcosis-like symptoms in cats which was also noted when so called inert gases of the noble gas series were tested, with the exception of helium and neon. There can be several theories advanced for this narcosis, but the most preferred one at this time seems to be the Meyer-Overton theory (Bennett and Dossett, 1970). This theory assumes that there is a preferential adsorption of the inert gas to the lipid constituent of cells. This effect would restrict the permeability of the membrane in a reversible manner as shown by data published by Bennett and Hayward (1967) indicating an

increase in Na^+ and Cl^- ions in the cells when inert gases were breathed by mammals, such as rats and man.

Effects of hydrocarbons and their fluorinated analogues were compared to oxygen in the data recorded in this dissertation. Of all the gases studied, oxygen was the most deleterious to Em5297a as indicated by resultant mycelial growth. Perhaps oxygen can more readily enter the system so that its action is more pronounced than that of other gases. Genetron-23 and Freon C-318, in absence of radiation, seemed to be stimulatory for Em5297a growth. The reason for this is not apparent. Besides this, exposure of conidia to methane was found to significantly lower the number of perithecia formed. Methane acts physiologically as an asphyxiant so that in high concentrations, it displaces the oxygen required for the organism to survive (Matheson Gas Data Book, 1966). Neurospora studies reported in this dissertation indicate that oxygen causes a slight inhibition of mycelial growth but a stimulation of perithecia formation. If oxygen is needed for perithecia to be formed, methane would be expected to inhibit this process. This inhibition effect is also substantiated by the findings of Fuerst and Stephens (1970). From sexual crosses like Em5297a X C83A (tryptophanless) and Em5297a X St. Lawrence 74A, gassed with methane after the strains were crossed, fewer perithecia resulted than from nongassed controls. It is interesting to note that Genetron-23, a methane analog, did not inhibit

perithecia formation. Perhaps the fluorinated analog can not be incorporated as readily as methane in Em5297a.

Fuerst and Stephens (1970) have shown that shorter chain molecules protect Neurospora against Co-60 induced radiation while the longer chain hydrocarbon analogues are more detrimental. This research indicates that these gases act in a different manner when applied to conidia prior to irradiation. Studies indicated that butene-1, a long chain hydrocarbon, stimulated mycelial growth of irradiated Em5297a to 65% of the value given for the non-irradiated control. Perfluorobutene-2 and Genetron-23 slightly enhanced growth of the strain. No protection by these gases against the effects of UV irradiation was noted for gassed and irradiated conidia examined for perithecia forming capacity. Both oxygen and methane were found to be detrimental to this system, either in the presence or absence of UV light. Bennett and Dossett (1970) reported that fluorinated compounds adsorb to lipid membranes, thus interfering with cell permeability, as noted in the previous discussion of compressed air induced narcosis. Neurospora possesses a tough chitinous cell wall, as compared to the relatively delicate membrane in animal cells, which were studied by Bennett and Dossett (1970). This might suggest why the fluorinated hydrocarbons are not as apt to render a detrimental effect as oxygen or methane in plant cells. Although the possibility can not be ignored that this interaction, gas

attachment with lipid, may be taking place, in vitro and in vivo studies involved with membrane interactions would be required to ascertain this reaction.

The possibilities for extension of this research are limitless. The fact that compressed air, butene-1, perfluorobutene-2, and Genetron-23 applied prior to UV irradiation stimulated the recovery of irradiated conidia suggest other investigations to see if the increased pressure of these gases is changing the permeability of conidial cells. Radioactive tracers could be used to ascertain if there is a lipid-gas interaction and if this causes a decrease in surface tension of the membrane. Changes in ion concentrations inside and outside the cell could then be calculated.

Oxygen or methane atmospheres applied without UV treatment, or supplied prior to UV irradiation, proved to be the most detrimental of all gases used. Tracer studies may establish where these gases are accumulated, which could lead to in vitro studies of gas effects on various enzyme systems.

Light stimulation of mycelial growth at 500 nm and of perithecia formation at 700-800 nm, which had not been previously described, was reported in this study. This finding could lead to a project involving the isolation of this enzyme system, after which studies of gaseous influence on the in vivo and in vitro system could be compared.

Em5297a was presumed to possess a photoreactivating enzyme, since light stimulation at 400 nm was found to enhance survival of UV irradiated conidia. Gassing effects on this enzyme could now be investigated to see if inhibition or stimulation of this process would be noted. The next step would be to isolate this repair enzyme and ascertain the effect of various gases on the in vitro system to see if the action is direct or indirect. Since photoreactivation, which has been thoroughly studied, is considered to be one of the most simple enzyme systems known (Witkin, 1969), it would provide an ideal mechanism for a study activities of these gases. Data obtained from gassing of the photoreactivating enzyme and other selected enzyme systems may help to better understand the action of gases on conidial cells as a whole.

SUMMARY

1. Various repair mechanisms, such as photoreactivation and dark repair, which are activated as a result of ultraviolet light damage, have been studied in Neurospora crassa by numerous investigators (Terry, Kilbey, and Howe, 1967; Stadler and Smith, 1968; Tuveson and Mangan, 1970). The lethality of UV radiation as a function of dosage was established for the conidia of Neurospora crassa Em5297a. Modifications of the response of the strain to these sublethal and lethal doses resulting from subsequent exposure to visible light, termed photoreactivation, or due to various gas atmospheres have been reported in this dissertation.
2. Inhibition of mycelial growth and perithecia formation were used as criteria for the extent of damage to the Em5297a strain as a result of UV irradiation. Minimal medium (Somers, et al., 1957) was used for growth of the mycelia and Oxoid cornmeal agar was used for the sexual crosses. Since perithecia formation required mating of the experimentally treated strain Em5297a, its' conidia were crossed to St. Lawrence 74A, the untreated parent.

3. A quantitative method was devised for counting of perithecia, so changes in numbers of the fruiting bodies, as the result of a specific treatment, could be tabulated. For this method, the treated conidia were diluted and pipetted to the surface of cornmeal agar. Twenty-four hours later, an aliquot of a conidial suspension of St. Lawrence 74A was spread on top of the previously inoculated plate. One week later, perithecia were visible. These fruiting bodies were evenly distributed over the surface of the agar so that they could be easily counted. Approximately 250 perithecia were produced for each plate in the control crosses.
4. Microconidia of Em5297a were found to exhibit 30% survival after 21 min of irradiation at 270 ergs/mm in the quartz top flask and 11 min of irradiation at 270 ergs/mm^2/sec in the quartz tube. The survival of the conidia was found to be increased when monochromatic light in the range of 400 nm was applied, indicating that Em5297a did possess a photoreactivating enzyme. Monochromatic light was obtained by using a diffraction grating in a Bausch and Lomb Spectrophotometer to separate the wavelengths of light, which were produced from a Kalimar electronic flash. Three flashes of the prescribed wavelength were given to each conidial sample. The flashes were of 1 msec duration and were spaced 10 sec apart. Shaking between flashes was accomplished to insure full photoreactivation.

5. Although daylight and monochromatic light of 500 nm was found to stimulate some process which tends to increase mycelial growth, visible light was not capable of stimulating photoreactivation. Possibly the intensity of the 400 nm wavelength was not strong enough to induce this process. Exposure of conidia to far red light, 700 to 800 nm, increased the number of perithecia produced by non UV-irradiated conidia. Thus, two stimulatory mechanisms were discovered for this strain, one which is responsible for stimulation of mycelial growth and another one for perithecia formation.
6. Since peroxides have been implicated in UV and ionizing radiation studies as a cause of damage to cells, a test was devised to ascertain if hydrogen peroxide was produced in the external environment of the conidia as a result of UV irradiation. Catalase, an enzyme which splits hydrogen peroxide into water and oxygen, was used in conjunction with known concentrations of hydrogen peroxide to indicate the amount of peroxide present in a UV irradiated sample of water and a conidial suspension. Peroxides were not formed as a result of UV treatment, at concentrations of hydrogen peroxide at 0.004% or greater, in the conidial suspension. Since no peroxides were detected in the external environment of the conidial cells, this was not considered to be a cause of UV damage to the cells.

7. Compressed air, applied before UV irradiation, was found to protect conidial cells against damage as indicated from studies of mycelial growth and perithecia formation. Cells treated with compressed air prior to UV irradiation and then grown in daylight showed 65% recovery as compared to 10% for cells treated in the same way but grown in the dark.

8. Oxygen applied to non-irradiated conidial cells increased the number of perithecia formed but inhibited mycelial growth, as compared to a control of conidial cells that received no oxygen treatment. An oxygen atmosphere, provided to the conidial suspension following UV light treatment, produced opposite results. The weights of the mycelia increased but the average number of perithecia decreased. In limited experiments, nitrogen was found to enhance recovery when the cells were exposed to it after UV irradiation.

9. Methane applied to non-irradiated conidial cells was found to significantly lower the number of perithecia formed. Since oxygen was noted to stimulate perithecia formation, methane, which is known to act as an asphyxiant, would be expected to inhibit this process. Genetron-23, a methane analog, exhibited no such inhibition. It is supposed that Genetron-23 may not enter the cellular system as does methane, so that no detrimental effect on Em5297a was noted.

10. Butene-1, perfluorobutene-2, Genetron-23, compressed air, oxygen, or carbon dioxide all produced some protection for Em5297a conidial cells against the effects of UV radiation. It is possible that the increased pressure of these particular gases changed the permeability of the conidial cells; however, it is not clear by what mechanism.

11. From the studies reported in this dissertation it is supposed that the photoreactivating enzyme is present in Neurospora and that the methods developed to study effects of gas treatment with and without UV exposure are dependable and were successfully applied to the findings reported here. The systems studied deserve further consideration and possible future investigations.

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