THE EFFECTS OF ULTRAVIOLET RADIATION ON SPORES OF THE FUNGUS ASPERGILLUS NIGER

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In the course of a series of studies now in progress concerning the effects of low velocity electrons upon the spores of highly standardized cultures of the Ascomycete fungus Aspergillus niger, it became of interest to undertake comparative work with ultraviolet light upon the same material. A large amount of work has been done upon the comparative killing effects of various wave lengths of ultraviolet upon unicellular organisms. Thus Ehrismann and Noethling (1932) and Oster (1934) have undertaken careful studies of the effects of equal intensities of ultraviolet of various wave lengths on the growth of yeast cells. Lacassagne (1930) and Wyckoff and Luyet (1931) have made extensive surveys of the types of cell injury which may supervene on the ultraviolet irradiation of yeast. Fulton and Coblentz (1929) have tested the validity of the Bunsen-Roscoe relationship for intermittent ultraviolet irradiation of Penicillium digitatum. Nadson and Philippov (1928) and Hutchinson and Newton (1930) have reported stimulation in yeast under low dosages of ultraviolet, and Chavarria and Clark (1924) report similar stimulation for growth rate in cultures of Montonyella. Schulze (1909) has reported a series of observations on the morphology of the mycelia of Mucor stolonifer irradiated with sublethal dosages of ultraviolet at 2900 Å. The production of giant cells has been observed by Elfving (1890), Reinhard (1923), and Lacassagne (1930) and Holweck (1932). Ramsey and Bailey (1930) have observed that conidia may be produced in a strain of Fusarium when exposed to ultraviolet which normally does not show such structures. The inhibitive effect of long exposures to ultraviolet upon spore production, and the apparently stimulative effect of short ones on Mucor were reported by Purvis and Warwick

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(1907), while similar stimulative effects have been reported by Stevens (1928), Dillon-Weston (1932), Hutchinson and Ashton (1930), Ramsey and Bailey (1930), Bailey (1932), and Smith (1935). Bovie (1914, 1916) has reported the destructive action on germination with ultraviolet ranging from the longest wave lengths to less than 1700 Å. Smith (1935) has studied the effect of temperatures in conjunction with ultraviolet killing. Stevens (1928) has reported mutation under ultraviolet irradiation, in Glomerella cingulata, as has Dickson (1932) for Chaetomium cochliodes.

For the most part, the effects to be described are similar to those reported by one or more of these authors, but for several reasons the work is considered worthy of report at this time. The radiation sources available made it possible to obtain high intensities of practically monochromatic radiation. It is of interest to compare these ultraviolet results with those obtained with low voltage cathode rays, using the same cultures of material and the same culturing methods. Furthermore, certain unique qualitative results have been observed.

Source and Character of Radiation

The source of radiation was a low pressure, hot cathode, mercury vapor discharge lamp in a glass having a high transmission for ultraviolet radiation. This lamp operates at a current of 0.25 ampere at 60 volts. Its output is practically monochromatic radiation of wave length 2537 Å. About 95 per cent of the energy radiated in the ultraviolet region lies in the 2537 Å line. At a distance of 1 meter from the lamp the intensity of this radiation is approximately 25 microwatts/cm².

The intensity of radiation was measured by means of a sodium cathode phototube having a thin window of high transmission glass. Since this phototube has a long wave limit in the neighborhood of 4000 Å its readings could be used as a measure of the 2537 Å radiation. The tube was calibrated by Dr. B. T. Barnes of the Nela Park Laboratory of the General Electric Company.

Material and Methods

The material used was exclusively the asexual spores of the Ascomycete fungus Aspergillus niger, the black bread-mold. These spores were obtained from cul-
tures of the fungus which have been bred in pure strain and highly standardized, and cultured on a standard potato-maltose agar for a number of years (1934). The spores are uniformly spherical, with a nucleus which, at the ages rayed, is spherical and centrally placed in the spores. The spores vary in diameter from 3–5 μ.

Spores of between 3 and 12 days of age were brushed from the culture dish onto cellophane strips, a fine camel’s hair brush being used for this purpose. The cellophane strips, with the spore surface toward the light source, were then exposed to the radiation under the conditions described for each experiment. Within 6 hours after exposure they were “printed” as a monolayer of sparsely distributed spores, onto the moist standard potato-agar medium, and were cultured at constant temperatures for from 6 to 12 hours. At this time the agar surface was examined under the microscope and the living and dead spores were counted. Numerous objective fields were counted so as to get an accurate appraisal of killing effect for each printed area.

Aspergillus spores after ultraviolet irradiation do not germinate at a completely uniform rate. It was therefore necessary to set up an arbitrary criterion of survival. At the end of 6 hours of culturing some spores have swollen and sprouted, others do not achieve this condition until between 9 and 12 hours after printing. It was found that after about 10 hours all the spores which had at this time swollen in a characteristic manner would ultimately develop mycelia and give all other evidences of normal growth. Any that had not at this time swollen were found not to swell or germinate at any later period. They were arbitrarily construed as being dead. Therefore, spores were counted after 10 hours of culturing and were thus categorically classified as living or dead. Survival ratios on the basis of such counts were computed as the ratio between the percentage survival of the experimental spores and the percentage surviving in the controls (which accompanied each experiment).

RESULTS

1. Killing.—The survival ratios of Aspergillus spores exposed to monochromatic ultraviolet radiation of wave length 2537 Å as a function of total incident energy are presented in Fig. 1. The radiation intensity at the surface of the material was 88 microwatts/cm.2 or 880 ergs/cm.2/sec. In Fig. 1 are shown the results of three experiments, in which the total energy to which the spores were exposed was varied by exposing for various lengths of time between 3 and 51 minutes. The curves are of the sigmoid form usually observed. It is interesting to compare these results with those reported by Fulton and Coblentz (1929). They report 13.3 and 0.5 per cent survival after exposures of 1 and 4 minutes respectively to a quartz burner.
operating at 320 watts. From available data it is possible to make a rough estimate of the incident energy used in their experiments. The 1 minute exposure corresponds to about 846,000 ergs/cm.² of 2537 Å radiation. In their experiments, however, there must have been considerable additional energy of both shorter and longer wave lengths.

2. Effect of Relative Humidity upon Killing.—It was especially desired to determine whether the relative humidity of the atmosphere in which the spores were placed at the time of their irradiation was a factor in their resistance to ultraviolet radiation. Such a question is of considerable interest in connection with practical problems of ultraviolet fungicidal action. Three experimental groups of spores were therefore set up to be irradiated under identical conditions. One of these groups was treated under ordinary atmospheric conditions (relative humidity during irradiation being 67 per cent). A second group was kept for varying periods of time before irradiation in a closed moisture-saturated chamber with a cellophane top. This group was irradiated without removing the cellophane top. The transmission of the cellophane was measured by means of a phototube and the distance from the source adjusted so that the energy incident on the spores was the same as in the previous experiments. The results are presented in the curves of Fig. 2. The data as presented in Fig. 2 are in terms of survival ratios, and in this form show a striking similarity to the normal survival curves of Fig. 1. The absolute survival data (as distinguished from the ratio) of the desiccated spores, however, are about 20 per cent lower than those for the spores irradiated at atmospheric humidity. The absolute survival data of the “saturated” spores are approximately the same as those for the atmospheric spores, possibly a little lower, but not significantly so. The important observation, however, is that in terms of survival ratio the data from these aforementioned experiments fit into the same band as those from the atmospheric spores (cf. Figs. 1 and 2), indicating that relative humidity is not of great importance in determining the fungicidal efficacy of 2537 Å irradiation.

3. Killing As a Function of Ultraviolet Wave Length.—It is a matter of considerable interest, both theoretically and practically, to determine the relative efficiencies in killing of equal energies of ultraviolet of different wave lengths. Experiments were therefore under-
taken with four wave lengths of 3650 Å, 3129 Å, 3022 Å, and 2537 Å respectively, obtained by means of a monochromater. The results are plotted in Fig. 3 where survival ratios are shown as a function of

![Fig. 1. Survival ratio of *Aspergillus* spores exposed to 2537 Å radiation. Intensity 880 ergs/cm²/sec.—88 microwatts/cm² Atmospheric conditions.](image)

![Fig. 2. Survival ratio of *Aspergillus* spores exposed to 2537 Å radiation. Intensity 880 ergs/cm³/sec.—88 microwatts/cm³.](image)

total incident energy for different wave lengths. The curve for wave length 2537 Å is repeated from Fig. 1. These results show that the killing power falls off markedly with the increasing wave length and is negligible for wave length 3650 Å.
EFFECTS OF ULTRAVIOLET RADIATION ON SPORES

Fig. 3. Survival ratio of Aspergillus spores exposed to radiation of various wave lengths.

Fig. 4. Effect of exposure time on germination of Aspergillus spores 2537 Å. 88 microwatts/cm.²
4. Delay in Germination Time.—Associated with the phenomenon of complete inactivation of irradiated spores by ultraviolet is a very marked delay of germination. This delay is a very definite function of the total incident energy to which the spores have been exposed. Experiments to test this were undertaken using 2537 Å radiation from the sources already described, and a constant energy input. Experiments were made with exposure times of 6, 12, 15, and 21 minutes, corresponding to total energies of 317,000, 634,000, 792,000, 1,110,000 ergs/cm.², and the percentage of spores showing mycelial beaks was recorded at various intervals between 7 and 18 hours after planting. The results are shown in the curves of Fig. 4. It will be seen that there is a marked increase in delay of total germination with exposure time. This effect is very sharply differentiated from radiation damage, inactivation, since even the very delayed spores, once germinated, thereafter developed morphologically normally, and for the most part grew at fairly normal rates.

5. The Bunsen-Roscoe Reciprocity Law.—The question as to whether constant dosages of radiant energy produce equivalent biological effects in living organisms, regardless of how applied, is one which has been given very considerable attention in the past. Experiments in this field were undertaken with ultraviolet. A constant energy input of 845,000 ergs/cm.² was given several cultures in five experiments. In the first case, the entire dose was given in 1 minute. In the second, the intensity was reduced to 1/2 and the time doubled. In the third, the intensity was quartered and the time increased fourfold and so on, the total variation in intensity being over a range of 16 to 1. The results are shown in Table I. It will be seen
that there is no systematic deviation in these values. Moreover, they fall within the calculated probable error for the number of spores counted. Within the accuracy of the measurements and the counts, therefore, it may be concluded that, in this experiment, the validity of the Bunsen-Roscoe relationship may be assumed for ultraviolet light of 2537 Å wave length, over a distribution of time and energy input per unit of 16-fold.

6. Morphological Changes.—No attempt was made to distinguish mutations, which have been reported a number of times with ultra-

![Diagram of Aspergillus spores](image)

**Fig. 5.** 1. Normal *Aspergillus* spore, 3–5 μ in diameter.
2. Normal swollen spore prior to sprouting.
3. Spores irradiated with 2537 Å ultraviolet while in the swollen condition just prior to sprouting (2). Instead of sprouting normally the spores undergo a continued swelling, reaching a diameter about three times normal before sprouting.
5. Spores irradiated during the early sprouting state (6). Continued growth results in the bead-like structure on the mycelium.

violet, and only incidental attention was paid to morphological changes in the irradiated organisms. However, one change, similar to that recorded by other workers, was met with sufficiently frequently and was sufficiently conspicuous to deserve mention. Undue swelling of spores before germination under fairly heavy dosages given after swelling had begun—a phenomenon frequently reported by other workers for yeast cells—was noticed with considerable frequency. Many times germination was completely inhibited under these conditions, and the cells reached giant size without ever showing a more advanced condition. With mycelia already produced, however, when
irradiated, the mycelia formed large swellings or beads at their extremities. These persisted, even when the mycelium had developed beyond the enlargement. The enlargement was thus left like a bead on a thread. Occasionally several of these beads developed, and persisted throughout the period of observation of the spore. The various types of abnormalities most frequently observed are shown in Fig. 5.

SUMMARY

The survival ratio of *Aspergillus* spores exposed to ultraviolet radiation has been measured as a function of total incident energy for wave lengths of 2537 Å, 3022 Å, 3129 Å, and 3650 Å.

The effect of humidity on killing of *Aspergillus* spores by ultraviolet radiation has been found to be negligible.

A delay in germination as a result of irradiation has been found.

The Bunsen-Roscoe reciprocity law has been found to hold within the limits of the radiation intensities studied.

Certain morphological changes have been observed.

REFERENCES


Elving, F., Studien über die Einwirkung des Lichtes auf die Pilze, Helsingfors, 1890.


EFFECTS OF ULTRAVIOLET RADIATION ON SPORES


Lacassagne, A., Différence de l'action biologique provoquée dans les levures par diverses radiations, Compt. rend. Acad. sc., 1930, 190, 524.


