RESULTS OF IRRADIATING SACCHAROMYCES WITH MONOCHROMATIC ULTRA-VIOLET LIGHT

II. THE INFLUENCE OF MODIFYING FACTORS

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It has been shown previously (1, 2) that the action of monochromatic ultra-violet radiation on the yeast Saccharomyces cerevisiae is not an all or none effect, but is a graded result varying from induction of simple inability to form normal sized colonies to “death” of the cell, through different degrees of damage, and involving in some stages the formation of giant cells. The mean “survival” curves for irradiated 24 hour cultures (2) resemble the curve for a first order process, but on this assumption divergences indicate the presence of modifying factors.

The present paper deals with several factors which may modify the absorption of energy and the cell processes resulting from irradiation of yeast with ultra-violet light.

Age of the Cells

Similar to the effects observed with other organisms (3, 4), the age of the yeast culture has a marked influence on the relative resistance of the cells to lethal irradiation, as measured by the energy required to suppress budding (2). This is illustrated by a comparison of the results obtained on irradiating two cultures of widely different periods of incubation, (A) a 24 hour culture and (B) a 15 day culture of yeast, both incubated at 25°C. and irradiated in a large quartz monochromator at the wave-length 2535 Å. u. as previously described (2) after inoculation on malt agar contained in small Petri plates. As shown in Fig. 1, from 20 to 50 per cent more incident energy to produce a given effect is required for the 15 day culture than for the 24 hour cultures. This is best explained on the basis that the greater
resistance of the 15 day culture is due to the greater proportion of cells in the resting stage.

The integral curve of reproduction of yeast, under the conditions observed in these experiments (2), shows that in a 24 hour culture, cell division is still going on at a fairly rapid rate so that the cells of a given inoculated plate will be in various phases of the reproductive cycle at the time of irradiation. The results obtained by Wyckoff and Luyet (1) using 15 day cultures of yeast, in which they found the survival curve followed a multiple hit to kill relation, indicate that the reproductive state of the cell is an important factor in its resistance to changes produced by ultra-violet irradiation.

In contrast to the results obtained with ultra-violet irradiation, yeast cells exposed to X-radiation (1, 5) show a large number of two-cell groups even after long exposures. Lacassagne and Holweck (5) have referred to this ability of the cell to form one bud and no more as a case of “deferred death.” They also state that cells undergoing rapid division were more resistant than the older resting cells. These
results, along with those of Strangeways and Hopwood (6), that tissue cells in the phase just preceding the prophase are especially susceptible to X-radiation, suggest that ultra-violet energy is absorbed by the nucleus with a slight but significant difference from the way in which X-rays, cathode rays, and alpha particles are absorbed. The reasons for this difference have recently been treated quantitatively by Holweck (7).

![Image of survival curves](image)

**FIG. 2.** Survival curves of yeast cells exposed to monochromatic ultra-violet radiation of wave-length 2535 Å.u. at four different temperatures. A at 29.5°C., B at 24.0°C., C at 16.0°C., D at 8.0°C. The points indicated by clear circles are mean values, at 10 per cent intervals, obtained from the smoothed curves (cf. Fig. 1) secured in several experiments at each temperature.

**Temperature in Relation to the Lethal Effect**

All of the data given in previous papers (2) dealing with the inhibitory and lethal effects of ultra-violet energy on yeast were obtained on cells exposed at the temperature of the laboratory, between 22° and 25°C. Within this range no evident effects on the inhibitory process could be observed, indicating a low temperature coefficient.

Determination of the temperature coefficient of the lethal action was made from data obtained by a series of observations on cells irradiated at 2535 Å. u. at four temperatures (8°, 16°, 24°, and 29.5°C.).
REACTION OF YEAST TO ULTRA-VIOLET LIGHT. II

The tests were performed as before (2), but with the inoculated agar plate and glass slide exposed in a water-jacketed brass cell with the aperture on the exposed side covered by a crystal quartz plate and that on the back with a glass plate for locating the agar plate in the path of the light beam. Three tubes provided for the water inlet and outlet and for the insertion of an accurate thermometer into the water chamber. With a steady stream of water from a storage vessel it was found that the temperature could be controlled to within plus or minus one-half of one degree for the duration of a given series of exposures. Before each plate was exposed it was allowed to remain in the chamber 10 minutes in order to come to the observed temperature. The mean values for several experiments at each temperature are plotted in Fig. 2. The temperature coefficient was obtained by taking the reciprocal of the energy ratio for a 10°C change (Table I).

Using the outer limits (29.5°C and 8°C) an average value of 1.10 is obtained. Using the range between 8°C and 24°C, mean values for the 8°C series give an average value of 1.08. When Experiment 91 is used, at 8°C, in which the temperature could not be observed to change over the entire course of the exposures, a lower value of 1.058 is obtained for the range 8–24°C. This is in good agreement with the low value 1.06 obtained for lethal action on bacteria (8).

The higher value (1.10) obtained when the 29.5°C series is used may

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<th>Killed</th>
<th>Energy required</th>
<th>Reciprocal of energy ratio for 10°C change</th>
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<tr>
<td></td>
<td>At 24°C.</td>
<td>At 8°C.</td>
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<td></td>
<td>Mean</td>
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<tr>
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<td>80</td>
<td>751</td>
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TABLE I
Data Used in the Determination of the Temperature Coefficient for the Lethal Action of Ultra-Violet Radiation of Wave-Length 2535 Å. u. on Yeast
be due in part to the influence of another reaction, since it has been demonstrated that 30°C. is a critical temperature for this strain of yeast (9). The values obtained suggest that the effects produced by the radiation are physical or direct rather than chemical in nature.

If the rate of "killing" is plotted as the logarithm of the energy required to "kill" a given percentage (50 per cent) against the reciprocal of the absolute temperature, according to the equation of Arrhenius, the temperature characteristic can be determined for the process (Fig. 3). This is found to have a mean value of about 640, a value much lower than any found for ordinary chemical processes (10).

Effects of Ultra-Violet Energy upon the Nutrient Medium

To determine whether the irradiation of the malt agar medium was a factor in the inhibitory and lethal effects (11), plates of the medium
were exposed for periods ranging from 5 minutes to 2 hours; the yeast was then seeded on these surfaces by a fine mist from a vaporizer, the cells being allowed to settle on the agar with as little excess moisture as possible. No effect on the subsequent growth of the cells could be detected. As the droplets of water containing the yeast were so small as to show no visible moistening of the agar surface, it is unlikely that any toxic substances formed by the action of the radiation upon the medium could have been diluted to an appreciable extent.

Test of Toxicity

To test the possibility that toxic substances formed in the cells killed by the irradiation may diffuse out subsequently and aid in the formation of the abnormal and inhibited cell groups observed, the following tests were made.

Four malt agar plates were inoculated with a yeast suspension of standard turbidity and allowed to drain. In the meantime a heavy suspension of yeast from the same culture, filling a large Petri plate to a depth of 8 mm., was exposed at a distance of 20 cm. from a horizontal quartz mercury vapor arc operated at 67 volts and 5.5 amperes. During irradiation the suspension was stirred at frequent intervals and samples were removed at the end of 1, 5, 10, and 60 minutes exposure. Small drops of these samples were immediately pipetted on to marked areas of the plates previously seeded with non-irradiated cells. After 36 hours incubation at 25°C. the plates were examined and the following observations made; (a) all of the areas covered by drops of the exposed suspensions showed the same number of normal colonies as the adjacent control areas, with the exception of the 1 minute exposure. The latter areas showed approximately three times the normal number of colonies, with 3 per cent of the colonies showing some giant cells. (b) In the 5 minute areas the normal sized colonies were surrounded by numerous small colonies and one- and two-cell stages. These small colonies are evidently survivors from the irradiated suspension. (c) In the 10 minute areas the normal sized colonies were surrounded by many one- to three-cell stages, many of which contained giant cells. (d) The 60 minute areas contained hundreds of single cells surrounding the normal colonies or
clumped at the edge of the area. The mean diameter of the normal colonies in the various areas was the same as that of colonies in the control regions.

From these observations we may conclude that cells killed or damaged by ultra-violet radiation, under the conditions here described, do not liberate toxic substances capable of affecting the subsequent growth of adjacent normal yeast cells.

The Bunsen-Roscoe Reciprocity Law

No extended series of experiments was made to test the validity of the Bunsen-Roscoe reciprocity law. However, it was necessary to test the effects produced by the irradiation for variations of intensity of the same or somewhat greater degree than those commonly present in the actual experiments. In two different experiments the intensity of the incident light at 2804 Å. u. was decreased by 30 per cent by increasing the distance of the test object from the exit slit. This difference in intensity is considerably greater than any actually experienced at any given wave-length during the course of the investigation (2).

On plotting the percentage survival against the energy incident upon the receiving surface for the two experiments (at 10 ergs per mm.² per sec. and 7 ergs per mm.² per sec.) it was found that the deviation between the two curves was considerably less than the maximum deviation between curves plotted from data obtained at constant intensity. During the course of the complete tests the actual variation of intensity of the incident light was never found to exceed 20 per cent, over all wave-lengths, so that the Bunsen-Roscoe law has been considered to be valid within the narrow limits of intensity variation permitted. For wide variations in intensity, however, it is probable that the law would not hold (8) and that the Schwartzchild exponent $q$ in the equation $I^qL = K$, would be greater than one.

CONCLUSION

Possible variation in the probability that absorbed quanta of ultra-violet energy will produce observable inhibitory and lethal effects in the yeast cell, due to non-uniformity in sensitivity of the different regions of the cell, may be further modified by the reproductive stage
REACTION OF YEAST TO ULTRA-VIOLET LIGHT. II

of the cell at the time of irradiation. Tests of the survival of yeast cells of 15 day and 24 hour cultures indicate that the older resting cells are more resistant to ultra-violet irradiation effects than cells undergoing rapid cell division.

The effects of temperature changes within the range of normal growth are evidently small, as judged from the temperature coefficient (1.10).

Possible inhibitory effects due to the action of ultra-violet radiation on the malt agar medium and to toxic substances diffused from cells killed by irradiation were not found under the conditions of the experiments.

Tests of the validity of the Bunsen-Roscoe reciprocity law for variation in the intensity of the incident ultra-violet radiation up to 30 per cent indicate that for this range the rate of absorption of quanta by the cell does not produce any marked change in the lethal effects observed.

CITATIONS