THE EFFECT OF ULTRAVIOLET AND WHITE LIGHT ON GROWTH RATE, LYSIS, AND PHAGE PRODUCTION OF BACILLUS MEGATHERIUM

BY JOHN H. NORTHROP

WITH THE TECHNICAL ASSISTANCE OF MARIE KING

(From the Laboratory of The Rockefeller Institute for Medical Research, Department of Bacteriology, University of California, Berkeley)

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SUMMARY

Cultures of *megatherium* 899a, growing under different conditions, were exposed to ultraviolet or white light.

1. Cultures exposed to ultraviolet light and then to white light continue to grow at the normal rate. Cultures exposed to ultraviolet light and then placed in the dark grow at the normal rate for varying lengths of time, depending on conditions, and then lyse with the liberation of from 5 to 1000 phage particles per cell, depending on the culture medium.

2. Increasing the time of exposure to ultraviolet light results in an increase in the fraction of cells which lyse in the dark. The lysis time decreases at first, remains constant over a wide range of exposure, and then increases. The lysis can be prevented by visible light after short exposure, but not after long exposures.

3. The time required for lysis is independent of the cell concentration.

4. Effect of temperature. After exposure to ultraviolet light, the cell concentration increases about 4 times at 20 °, 30 °, or 35 °C., but only 1.5 to 2.0 times at 40-45 °C. This is due to the fact that the growth rate of the culture reaches a maximum at 38 °, while the lysis rate increases steadily up to 45 °.

5. Terramycin decreases the growth rate and lysis rate in proportion.

6. At pH 5.1, the cultures continue to grow slowly in the dark after exposure to ultraviolet light.

7. *Megatherium* sensitive cells infected with T phage lye more rapidly than ultraviolet-treated 899a, and visible light does not affect the lysis time.

The results agree with the assumption that exposure to ultraviolet results in the production of a toxic (mutagenic) substance inside the bacterial cell. This substance is inactivated by white light.

**The Effect of Ultraviolet and White Light on Growth Rate, Lysis, and Phage Production of B. Megatherium**

Exposure of organisms to ultraviolet light results in an increase in the proportion of mutants and eventually in the death of the organism. Exposure of...
organisms to ultraviolet is much more effective, if the organisms are kept
subsequently in the dark, than if they are exposed to intense white light
(The subject has been critically reviewed by Muller, 1954.)

![Image of growth curves showing effects of ultraviolet and white light on Bacillus megatherium.](image)

**Fig. 1.** Effect of ultraviolet and white light on the growth of *megatherium 899a*. 10 ml. of a suspension of *megatherium 899a* in logarithmic growth in 5 per cent YEP (yeast extract peptone) in quartz test tubes were exposed to a General Electric germicidal lamp for 2 minutes at a distance of 1 cm. 1 tube was then placed in a water bath at 30°, 2 cm. from the lens of an American Optical Co. microscope lamp (model 370). A second tube was put in the same water bath, but kept dark. A third tube was not exposed to ultraviolet. All tubes were stirred by bubbling air.

The white light may be assumed to reanimate cells which have been killed by the ultraviolet light ("photoreactivation"), or to destroy some toxic compound formed by the ultraviolet light. Novick and Szilard (1949) have shown that this latter assumption explains the facts very well, and hence, it is unnecessary to suppose that the visible light reanimates cells killed by the ultraviolet.

If lysogenic cultures are exposed to ultraviolet, they grow for some time
and then lyse (Lwoff, Siminovitch, and Kjeldgaard, 1950). This process may be prevented by visible light (Jacob, 1950). In the case of lysogenic cultures, therefore, the number of living and growing cells may be determined by direct observation, since the dead cells disintegrate. The results of an experiment carried out in this way are shown in Fig. 1. The figure shows that both cultures exposed to ultraviolet grow at very nearly the same rate as the control for 2 hours, whether they are in white light or not. At the end of this time, the cells in the dark tube lyse with the liberation of about 1000 phage particles per cell. The culture placed in the light shows only about 10 per cent lysis.

It is evident that the colony counts from these cultures are entirely misleading. The colony count of the dark culture, for instance, drops to less than 10 per cent of the original value immediately after the ultraviolet treatment, and yet the culture from which the sample was taken continues to grow at the normal rate. The colony count of the culture in the light is also only about half that of the control; yet this culture continues to grow indefinitely. These paradoxical results are due to the fact that the colony counts of the cultures are incorrectly placed on the time axis. The colony count immediately after ultraviolet exposure, for instance, represents those cells which were able to survive 2 minutes of ultraviolet and also 8 to 10 hours in the dark on the agar plate. It does not represent the number of viable cells immediately after the ultraviolet exposure, and, in fact, it is impossible to place the colony count on the time axis without further information. The difficulty cannot be avoided by defining viable cells as those capable of forming colonies, since it is still not possible to say when the ability to form colonies is lost.

If, on the other hand, the injury is due to some toxic (mutagenic) substance formed in the cell by the ultraviolet and inactivated by light, then the results are to be expected. This mechanism also explains the effect of temperature on the reaction. Cultures exposed to ultraviolet and then kept in the dark at 15° retain the ability to grow, when exposed to light, about four times as long as those kept at 35°. The inactivation reaction, therefore, has a high temperature coefficient. This is not true of photochemical reactions (Novick and Szilard, 1949; Giese et al., 1956).

Effect of Temperature, Light, pH, Terramycin Concentration, and Cell Concentration on Growth and Lysis of Megatherium 899a after Exposure to Ultraviolet. Table I

Time of Exposure to Ultraviolet.—Increasing the time of exposure to ultraviolet increases the lysis until it becomes complete. The lysis time remains constant, at first, and then increases on long exposure. Lysis after long exposure cannot be prevented by visible light. This result indicates the existence of two reactions. The toxic product formed in the cell by the ultraviolet alters some essential cell constituent (probably DNA) resulting in a mutation. Very
little toxic agent is sufficient to carry out this reaction, and an excess does no more damage. Hence the percentage of lysis and the lysis time remain constant

for a wide range of ultraviolet exposure. (This is the usual effect of ultraviolet on the mutation rate.) Further exposure injures the metabolism of the cell and eventually kills it, possibly through direct action of the ultraviolet light, since this effect is not reversible by visible light. This explanation is partly sub-

### Table I

<table>
<thead>
<tr>
<th>Culture media and megatherium strain</th>
<th>Temp.</th>
<th>pH</th>
<th>$B_o$</th>
<th>Growth rate/hr</th>
<th>Lysis time hrs.</th>
<th>$B_{max}/B_o$</th>
<th>Phage particles per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEP</td>
<td>0</td>
<td>5.1</td>
<td>0.1-1.0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Megatherium 899a</td>
<td>1 min.</td>
<td>35</td>
<td>$6.8 \times 10^7$</td>
<td>1.8</td>
<td>50</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>$1 \times 10^7$</td>
<td>1.7</td>
<td>100</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>5/10</td>
<td>35</td>
<td>$1 \times 10^7$</td>
<td>1.1</td>
<td>100</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>35</td>
<td>$1 \times 10^7$</td>
<td>0.1</td>
<td>100</td>
<td>80</td>
<td>2.2</td>
</tr>
<tr>
<td>0.5 γ terramycin/ml.</td>
<td>2</td>
<td>35</td>
<td>$1 \times 10^7$</td>
<td>0.7</td>
<td>100</td>
<td>2.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Asparatic acid–arginine

<table>
<thead>
<tr>
<th>Temp.</th>
<th>$10^{-2}$ M Mg++</th>
<th>$10^{-4}$ M Mg++</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 sec.</td>
<td>35</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.3</td>
</tr>
<tr>
<td>YEP</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>Megatherium sensitive</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Megatherium sensitive + T phage</td>
<td>0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Diluted with 0.5 N HCl for a wide range of ultraviolet exposure. (This is the usual effect of ultraviolet on the mutation rate.) Further exposure injures the metabolism of the cell and eventually kills it, possibly through direct action of the ultraviolet light, since this effect is not reversible by visible light. This explanation is partly sub-
stantiated by the fact that the sensitive strain of *B. megatherium* does not lyse after short exposure, but is killed after long exposure. The failure of such strains to lyse is due to the fact that ultraviolet and mutagenic agents, in general, merely increase the proportion of mutants already present, rather than cause new ones to appear (Muller, 1954). There are no lysogenic mutants in cultures of the sensitive strain, and, hence, exposure to ultraviolet cannot increase them.

*Effect of Time of Exposure to White Light.*—Under the conditions used in these experiments, 20 minute exposure to white light decreased lysis to about half and 40 minutes prevented practically all lysis.

*Effect of Cell Concentration.*—The percentage of lysis and the time required for lysis after 2 minutes of exposure to ultraviolet is practically independent of the cell concentration. If anything, the dilute suspension lysed first. These experiments were done by exposing a suspension of $1 \times 10^8$ B/ml. to ultraviolet for 2 minutes and then diluting to $1 \times 10^4$ and $1 \times 10^5$ B/ml. at once. The suspensions were all shaken at $35^\circ$ and plated for phage at 15 minute intervals. No damage had been done to the cells at the time the dilution was made, since they grew normally if placed in the light. If the toxic product formed by the light were present in the culture medium, then it would be expected that the more concentrated suspension would lyse first. Since lysis occurs at about the same time in the concentrated suspension as in the one which had been diluted $1/10^6$, the toxic substance is probably in the cell (Roberts and Aldous, 1949). This may explain why the preventive effect of light does not appear in molecular reactions, since in these the concentration of reactants is very low, while inside a cell it may be very high. Effects similar to those ob-
served with cells might be expected with very concentrated (>30 per cent) solutions of nucleic acids, but not with dilute ones. It may be for this reason that light has no effect on ultraviolet-inactivated phage particles.

Effect of Temperature on Growth Rate, Growth before Lysis, and Lysis Time

The effect of various temperatures on the growth rate, the growth before lysis occurs, and the lysis rate (1/t to cause lysis) is shown in Fig. 2. The lysis rate has a steady temperature coefficient of about 2 per 10 degrees from 20-45°C, while the growth rate, as usual, shows a maximum near 40°C. As a result, the ratio B at lysis/B at beginning decreases as the temperature increases. Lysis is not conditioned, therefore, by a certain number of cell divisions, but rather by a certain time limit.

The growth rate and lysis rate may be separated by growing the exposed cells in a very narrow range of pH near 5.0. Under this condition, very slow growth occurs, but no lysis (cf. Weatherwax, 1956).

If the growth is slowed by the addition of terramycin, however, the lysis time is also slowed, and the ratio B_{max}/B_{o} remains constant.

The time for lysis of ultraviolet-treated 899a cells is longer than that required for lysis of _Megatherium_ sensitive cells infected with T phage and the ratio B_{max}/B_{o} is higher in the lysogenic than in the infected system. White light has no effect on the lysis of infected cells (Fig. 3), nor does it affect the phage production in growing lysogenic cultures.

Lysis of infected cells requires about 0.6 hour at 35°C, while lysis of ultraviolet-treated lysogenic cells requires about 1.3 hours. It is probable that the lytic process itself is the same in both cells; if this is so, about 0.7 hour is required to start phage production in the lysogenic culture. The results in Fig. 3 also show that the lytic process itself is not affected by white light and hence, the light should not prevent lysis unless it is used at least half an hour before lysis would occur. This is approximately correct. Ultraviolet-treated cells will grow normally if exposed to light ½ hour after the ultraviolet (at 35°C) but not after 1 hour.

Effect of Culture Media.—In synthetic medium made up of aspartic acid, arginine, glucose, sodium and potassium phosphate, iron and magnesium sulfate, the culture is much more sensitive to ultraviolet light. This is due largely to the fact that this solution does not absorb ultraviolet as strongly as the peptone. The lysis time after exposure to ultraviolet is about the same as in peptone, but the growth and the phage yield per cell is less in 10^{-8} M MgSO_{4} and much less in 10^{-6} M MgSO_{4} than in peptone. The lower phage yield is no doubt due to the lower NA content of slow growing cells (Krueger and Mundell, 1938; Hedén, 1951; Northrop, 1953), since the cell NA is the precursor of phage NA (Hershey, 1953).

_Megatherium_ sensitive cells infected with C phage in very dilute suspensions, however, go through 2 or 3 cell divisions before lysis occurs (Northrop, 1953).
The increased phage yield in the presence of MgSO₄ (Northrop, 1951) is probably due to an increase in phage particles produced per cell rather than an increase in the number of phage-producing cells, as is the case when the culture is exposed to ultraviolet.

**Effect of Ultraviolet on the Mutation Rate of Terramycin-Resistant Mutants, and of Phage-Producing Cells**

The effect of ultraviolet and white light (or other mutagenic agents) on phage production of lysogenic cultures is similar in all respects to the effect on the mutation rate of various bacterial mutants. This suggests the probability that the change from a normal cell to a phage-producing cell is also a mutation.

This explanation is suggested also by the fact that the phage particle is a product of cell metabolism (Bordet, 1931; Northrop, 1938; Lwoff, 1954; Raet-
tig, 1955; Northrop and Murphy, 1956; Welsch, 1956) and its production is a
 genetic character of the bacterial cell, just as is the production of the trans-
 forming principle (Gratia, 1936; Fredericq, 1953; Lederberg and Lederberg,
 1953; Wollman, 1953). Exposure to ultraviolet light does, in fact, increase the
 mutation rate of terramycin-resistant mutants in B. megatherium cultures, but
 the increase is not so great as is the increase in phage-producing cells (unpub-
 lished experiments).

**Experimental Procedure**

Ultraviolet—General Electric germicidal lamp.
White light—American Optical Co. microscope lamp, model 370.
10 to 15 ml. cultures in logarithmic growth in 20 × 150 mm. quartz tubes were
placed in front of the ultraviolet lamp. The cultures were stirred by bubbling air
through them. The culture itself, or a diluted sample from it, was exposed to white
light by placing it in a glass water bath, directly in front of the lens of the microscope
lamp.

Cell concentration, colony counts, and phage plaque counts were made as previ-
ously described.

Yeast extract culture medium and aspartic acid–arginine culture medium were
also prepared as previously described (Northrop, 1957; 1951).
The samples for plaque assay were mixed with toluene and allowed to stand 2
to 3 hours before plating. Control experiments showed that no change in the phage
titer occurred after the toluene was added. The plaque count, therefore, unlike the
colony count, corresponds to the time at which the sample was taken.

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