GERMICIDAL ACTION OF DAYLIGHT ON MENINGOCOCCI IN
THE DRIED STATE

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Received for publication September 11, 1943

INTRODUCTION

The study reported in the preceding communication (1943) showed that meningococci are much more resistant to dehydration than is generally supposed and that in films dried on glass, wood, or cotton fabric, they can survive for a week or more in the dark. The question of their susceptibility to the germicidal action of direct and diffuse sunlight seemed also to invite reexamination. The observations bearing on this question are few and contradictory and obviously were made in passing by workers concerned primarily with other aspects of the biology of the meningococcus.

Of the investigators who dried meningococci in films on glass or other objects, Bettencourt and Franca (1904) and von Lingelsheim (1905) reported them to die as quickly in the dark as in diffuse daylight. The time required for this to occur was 3 to 6 hours according to Bettencourt and Franca who worked in Lisbon and less than 12 hours in von Lingelsheim's experiments which were made in Upper Silesia. On the other hand, Flügge (1905) reported some observations made in his laboratory in Breslau by Kache in which dried meningococci died in 10 hours in diffuse daylight and 24 hours in the dark.

Additional evidence that diffuse daylight is injurious to meningococci has been contributed by experiments of a different sort. Several workers have noted that stock cultures live longer in the dark than in diffuse daylight at the same temperature, which has usually been room temperature. For example: Elser and Huntoon (1909) found that strains which lived 3 or 4 weeks at room temperature in the dark died within one week when exposed to diffuse daylight.

On the deleterious effect of direct sunlight there was agreement among all workers who investigated that point, although they did differ on the rapidity of its lethal action. It is interesting to compare the results reported from different places even though the experiments themselves were not identical. Bettencourt and Franca (1904) working in Lisbon found that exposure to direct sunlight for 2 hours was sufficient to kill meningococci although they were protected against overheating by running water. The date of his experiments was not given. In experiments made by Lingelsheim (1905) in Upper Silesia in June, 4 to 6 hours of direct sunlight was lethal. Kucher (1906) found 8 to 10 hours of direct sunlight necessary to kill meningococci in March in Berlin. Elser and Huntoon (1909) reported 8 to 9 hours in August in New York City.

1 This investigation was aided through the Commission on Meningococcal Meningitis, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, United States Army.
METHODS

The methods employed were those described in the preceding paper (Miller and Schad, 1943). Young cultures of meningococci were deposited on glass beads, pieces of wood, or pieces of cotton cloth, dried in a desiccator over sulphuric acid or calcium chloride and placed in new, unscratched Petri dishes which, like those in the preceding study were left unsealed. They were then exposed to different intensities of natural illumination by placing them in different parts of the laboratory; a room approximately 17 ft. x 16 ft. lighted by two south windows, and another room with a single north window. At the north window they were always placed far enough away from the window to protect them from direct sunshine in the early morning and late afternoon. The Petri dishes containing the pieces of wood and cotton fabric were turned over in the middle of each day to insure uniform exposure to light. Controls were run in each experiment by placing similar objects in a dark cupboard in the same room or in light-proof boxes along side those exposed to light.

Notes were kept on the weather and the appearance of the sky throughout each day. Most of the experiments were made in the winter and early spring. The laboratory in which they were conducted is located on the campus of the University of Chicago, about a mile and a half west of Lake Michigan. The prevailing winds are from the West and reach us after blowing over miles of urban settlement, some of which is highly industrialized.

RESULTS

Exposure to direct sunlight which had passed through ordinary window pane, killed the microorganisms within a few hours. This result was not entirely due to heating, because the period was not prolonged very much when the Petri dish was kept cool by means of ice. Even during cloudy weather when the sky was overcast throughout the day, the microorganisms died off much more rapidly close to the window than they did a distance of 12 feet from the nearest window. A typical example is given in table 1, which demonstrates the bactericidal action of daylight on meningococci dried on the surface of glass beads, contained within a Petri dish made of pyrex glass, in a room illuminated during the day by light passing through an ordinary window pane.

The germicidal effect of diffuse daylight on the meningococci dried on wood (ordinary tongue depressors) and different kinds of cotton cloth is shown in table 2. It will be seen that on each of these materials the meningococci survived a shorter time in daylight than they did in the dark. The difference was less on wood than on the cotton fabrics, as one might expect, because the latter transmitted a certain amount of light. As noted in the preceding paper (1943), the number of living meningococci recovered from these materials each day did not diminish with uniform regularity because of the physical impossibility of making complete contact with the culture medium as was possible in the case of the glass beads.

To get some notion of the portion of the spectrum responsible for this effect,
each of a series of Petri dishes containing beads coated with meningococci was wrapped with a double thickness of colored or uncolored cellophane. One con-

TABLE 1

Viability of meningococci dried on glass and exposed to different degrees of natural light

<table>
<thead>
<tr>
<th>TIME</th>
<th>SOUTH WINDOW IN DIRECT SUNSHINE</th>
<th>SOUTH WINDOW OUT OF DIRECT SUNSHINE</th>
<th>12 FT. FROM WINDOW</th>
<th>DARK CUPBOARD</th>
<th>NORTH WINDOW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23 hours</td>
<td>0</td>
<td>±</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>29 hours</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>++++</td>
<td>0</td>
</tr>
<tr>
<td>48 hours</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+++</td>
<td>0</td>
</tr>
</tbody>
</table>

Weather:
March 9, 1942—Morning cloudy with occasional brief sunshine. Afternoon bright and clear except for occasional clouds.
March 10, 1943—Bright most of the day. Occasional clouds.
* All in same room measuring approximately 17 x 16 ft. with 2 south windows.
† Another room 16 x 9 ft. with 1 north window.

+++ Confluent growth
+++ Innumerable colonies
++ 100-400 colonies
+ 25-100 colonies
± fewer than 25 colonies
0 no growth

TABLE 2

Viability of meningococci dried on wood and cotton in the dark and in diffuse daylight

<table>
<thead>
<tr>
<th>TIME</th>
<th>WOOD</th>
<th>COTTON GAUZE</th>
<th>COTTON TOWELING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Daylight</td>
<td>Dark</td>
</tr>
<tr>
<td>1 day</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>2 days</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>3 days</td>
<td>++++</td>
<td>++</td>
<td>2 col.</td>
</tr>
<tr>
<td>4 days</td>
<td>++</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 days</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>7 days</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Weather:
May 5, 1942—Cloudy all day (1st day).
6, 1942—Cloudy all day.
7, 1942—Cloudy except for about 2 hours of sunshine in the middle of the day.
8, 1942—Bright sunshine all day.
9, 1942—Cloudy with rain.
10, 1942—Sunshine all day.
11, 1942—Cloudy with rain.

Control was placed in a light-proof paper box such as is used for photographic materials and another was left unwrapped. All of the Petri dishes, including the dark

* Dupont Cellulose Film non-moistureproof, purchased from the Dennison Manufacturing Company.
TABLE 3
Viability of meningococci dried on beads, exposed to diffuse daylight filtered through glass and cellophane of different colors

<table>
<thead>
<tr>
<th>TIME</th>
<th>CONTROL (DARK)</th>
<th>RED</th>
<th>ORANGE</th>
<th>YELLOW</th>
<th>GREEN</th>
<th>BLUE</th>
<th>PURPLE</th>
<th>COLOR-LESS</th>
<th>CONTROL, NO CELLOPHANE</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 hours</td>
<td>++++</td>
<td>+++</td>
<td>+++±</td>
<td>+++±</td>
<td>1 col.</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>44 hours</td>
<td>++++</td>
<td>+++</td>
<td>+++±</td>
<td>+++±</td>
<td>1 col.</td>
<td>++</td>
<td>5 col.</td>
<td>3 col.</td>
<td>0</td>
</tr>
<tr>
<td>58 hours</td>
<td>++++</td>
<td>+++</td>
<td>+++±</td>
<td>+++±</td>
<td>+</td>
<td>++</td>
<td>5 col.</td>
<td>3 col.</td>
<td>0</td>
</tr>
<tr>
<td>4 days</td>
<td>++</td>
<td>++</td>
<td>±±</td>
<td>±±</td>
<td>1 col.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 days</td>
<td>+</td>
<td>+</td>
<td>±±</td>
<td>±±</td>
<td>2 col.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 days</td>
<td>+</td>
<td>±</td>
<td>±±</td>
<td>±±</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8 days</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9 days</td>
<td>±</td>
<td>±</td>
<td>±±</td>
<td>±±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 days</td>
<td>±</td>
<td>±</td>
<td>±±</td>
<td>±±</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 days</td>
<td>1 col.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 days</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Dates and Weather:
March 24, 1942—1st day bright sunshine.
25, 1942—Hazy until mid-morning, sunshine until late afternoon.
26, 1942—Cloudy.
27, 1942—Cloudy with rain and snow, clearing in late afternoon to hazy sky.
28, 1942—Overcast in the morning, cloudy and snow in afternoon.
29, 1942—Cloudy except for short intervals of sunshine in afternoon.
30, 1942—Hazy sunshine in morning, cloudy with snow in the afternoon.
31, 1942—Cloudy except for about 1 hour of bright sunshine in early afternoon.
April 1, 1942—Hazy until mid-morning, bright sunshine thereafter.
2, 1942—Hazy until mid-morning, bright until late afternoon.
3, 1942—Bright sunshine all day.
4, 1942—Sunshine until mid-afternoon, becoming cloudy.

TABLE 4
Percentage transmissions by colored cellophane of lights of various wave lengths
Readings made and data calculated by Theodore T. Puck, Ph.D., Research Associate in Medicine, Department of Medicine of the University of Chicago.

<table>
<thead>
<tr>
<th>COLOR OF CELLOPHANE</th>
<th>WAVE LENGTH OF STANDARD FILTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>420 mu</td>
</tr>
<tr>
<td>None</td>
<td>100%</td>
</tr>
<tr>
<td>Red</td>
<td>0.8</td>
</tr>
<tr>
<td>Yellow</td>
<td>0.8</td>
</tr>
<tr>
<td>Green</td>
<td>1.8</td>
</tr>
<tr>
<td>Orange</td>
<td>0.8</td>
</tr>
<tr>
<td>Blue</td>
<td>11.0</td>
</tr>
<tr>
<td>Purple</td>
<td>15.0</td>
</tr>
</tbody>
</table>

The percentage transmitted in this table were obtained using an Evelyn photoelectric colorimeter and a set of standard light filters manufactured by the Rubicon Company.
control, were placed in front of a north window, care being taken to prevent any
direct sunlight from reaching them in the early morning or late afternoon. The
beads were withdrawn and cultured as described in the preceding paper.

The results of a typical experiment are presented in table 3 which shows that
meningococci covered with red cellophane survived as long as those completely
protected from light. Orange afforded the next most effective protection. The
other colors, yellow, green and blue, all permitted rather rapid death of the
microorganisms, the order of relative effectiveness among them differing in differ-
ent experiments. No effect of the colorless cellophane was detectable. The
spectral composition of the light transmitted by the cellophanes used is presented
in a table, which has been very kindly supplied by Dr. Theodore T. Puck.

The data show that the intensity of illumination transmitted by the red filter
is greater than that transmitted by the blue; that is, more red light passed
through the red filter than did blue light through the blue filter. Nevertheless,
the red light was practically without effect on the viability of the organisms. The
blue light which was weaker in intensity was highly bactericidal. The activity
of the light transmitted by the remaining filters seemed to be roughly propor-
tional to the amount of blue light which was transmitted.

**DISCUSSION**

It is clear from these experiments that meningococci dried in films on such
materials as glass, wood and cotton cloth are killed by exposure to daylight pass-
ing through two layers of glass: that of ordinary window pane and the pyrex
glass of which Petri dishes are made. Sunshine killed them very quickly—within
a few hours—even when they were protected against overheating. This finding
coincides with those cited from the literature in the introduction.

Exposure to diffuse daylight, that is the ordinary degree of natural illumination
present in front of a north window, also killed them rapidly, especially those dried
on glass beads which were transparent and on the cotton gauze and towelling
which were at least translucent. The germicidal action of diffuse daylight was
less rapid on the microorganisms dried on pieces of wood.

These findings agree with the observation of Kache, reported by Flügge (1905)
but are more convincing as he was unable to recover meningococci after 24 hours
in the dark. Our results are in complete accord with those of Buchbinder and
his associates (1941) who have made careful quantitative determinations of the
lethal effect of sunlight and daylight on streptococci. They found that diffuse
daylight passing through two thicknesses of glass was bactericidal and that this
property was approximately proportional to its intensity.

It is clear from the experiments with colored cellophane that the rays at the red
end of the visible spectrum are not the ones which kill meningococci in the dried
state. The colors of the cellophane are not to be regarded as pure colors which
can serve as accurate filters. For this reason the location and limits of the
germicidal portion of the spectrum cannot be defined. Experiments designed to
do so have been interrupted by circumstances beyond our control. The interest-
ing suggestion has been made by T. T. Puck that one would predict that any lethal radiation within the visible spectrum would be located among the shorter rather than the longer wave lengths, because of the color of meningococcus cells which is a light tan with a faint suggestion of pink.

SUMMARY

Meningococci dried in films on the surface of glass, wood, and cotton fabric were exposed to different intensities of natural illumination and cultured at intervals to determine the duration of viability. They were killed by direct sunlight within a very few hours even when protected against overheating. On glass beads and cotton gauze they were killed by diffuse daylight passing through two layers of glass—ordinary window pane and pyrex Petri dish, within 30 hours: and on cotton towelling and on wood, they died a little more slowly.

Light passing through cellophane of different colors did not kill them at the same rate. In red light they survived almost as long as in the dark.

CONCLUSIONS

Diffuse daylight as it exists in Chicago in the winter and spring is definitely germicidal to meningococci.

REFERENCES


PUCK, THEODORE, T. Personal communication to the author.