A method of investigating the action of ultraviolet rays on bacteria.

By

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(Received Jan. 11, 1919)

The influence of light rays especially of ultraviolet rays on bacteria has long since been studied by many investigators. Their bactericidal action[1], the inhibitory effect on the growth of bacteria[2], the metamorphosing action[3] and the destructive action on the virulency of pathogenic germs[4] have been established.

In order to study these effects more closely, monochromatic lights should be used as the source of light, and its effects should be examined separately for every light of different wave length[5]. Recently Hertel[6] determined the bactericidal limits of wave lengths of light, by

1 A. Downes & Th. P. Blunt, Proc. R. Soc., London, **26**, 488 (1877).
E. Duclaux, C.R., **100**, 184 (1885).
H. Buchner, Arch. f, Hygiene, **17**, 179 (1893).
M. Ward. Proc. R. Soc., Londen, **52**, 393 (1892-3).
H. Thiele & K. Wolf, Arch. f. Hygiene, **57**, 29 (1906).
A. Keller, Centralbl f. Bakteriol., Ref., **40**, 186 (1907).
J. Malgat, Centralbl. f. Bakteriol., Ref., **44**, 122 (1909).
R. Lemoine, Centralbl. f. Bakteriol., Ref., **50**, 286 (1911).
W. Hausmann, Centralbl. f. Bakteriol., Ref., **51**, 618 (1912).
E. Jacobsthal &. F. Tamm, Münchener med. Wochenschr., 61 Jg., 2324 (1914).
H. S. Newcomer, J. Exp. Med., **26**, 841 (1917).
Miramond de Laroquette, Annales de l'institut Pasteur, **32**, 170 (1918).

2 S. Arloing, C. R., **100**, 378 (1885).

3 V. Henri, C. R., **158**, 1032 (1914),
Ibid., **159**, 340 (1914),
Mrs. V. Henri, C. R. **159**, 413 (1914).

4 G. Orsi, Centralbl. f. Bakteriol., Orig., **43**, 846 (1907).

5 H. Strebel, Deutsch. med. Wochenschr., **27** Jg., 69 & 87 (1901).
E. Hertel, Zs. f. allg. Physiol., **4**, 1 (1904).
E. Gill, Phil. Mag., **19**, 290 (1910).
V. Henri & his wife, C. R., **155**, 315 (1912).

6 E. Hertel, Zs. f. allg. Physiol., **5**, 95 (1905).

projecting various parts of the spectrum, upon the inoculated culture-medium separately. The determination of intensities of bactericidal power of every part of the spectrum was done by comparing the numbers of living bacteria in the physiological saline water suspension before and after its exposure. This method is, however, not adequate, because it is quite difficult to make exact count of the number of bacteria, and almost impossible to illuminate by a monochromatic light.

To avoid these disadvantages, a quartz spectrograph was employed in the present experiment, and the photographic plate was replaced by a thin culture-medium inoculated on a glass plate with a certain kind of bacteria, and different exposures were given on one plate one above the other. The part of the culture-medium, wherein the bacteria were killed, became transparent after incubation, while the remaining part got semitransparent by their growth, thus the image of spectrum lines being finely impressed on a culture-medium. From this the limits of wave lengths of light where the action ceases were determined for given exposures; and a curve showing the relation between bactericidal limits for different exposures was plotted. This curve we shall call the "bactericidal curve," and it somewhat resembles the absorption curves in spectroscopy.

This method of obtaining the "bactericidal curve" was already reported by the writer at the annual general meeting of the "Kyoto Medical Association" (Kyoto Igaku Kai) held in November, 1917, and a short abstract was given in its journal[1]. After the publication of this, the writer noticed a similar work by Browning and Russ[2], but he has not yet been able to obtain their original paper.

Method of Experiments.

A quartz spectrograph of size A made by Hilger was employed, and this was placed in such a position as to keep the plate holder horizontal. As the source of light, a carbon arc, iron arc and condensed sparks of iron and cadmium excited by the Blitzen transformer of 1/4 kw, were used.

The inoculated culture-medium of bacteria was prepared in the following manner. A glass plate of suitable size was held horizontally,

[1] T. Mashimo, Kyoto Igaku Kai Zassi, **15** (1918).

[2] C. H. Browning & S. Russ, Proc. R. Soc., London, **90**, B, 107 (1918).
Cf. Nature, **101**, 112 (1918), cf. H. S. Newcomer, J. Exp. Med., **26**, 841 (1917).

and a metal frame of similar shape but of somewhat smaller dimensions was placed just on it. As the culture-medium agar agar was used. After being melted by heating gently, a certain amount of it was poured on the glass plate until the medium got a thickness of about three mm. After fixing it, the bacteria-suspension in physiological saline solution was smeared on its surface and then it was brought to a suitable degree of moisture, sufficient to prevent the dislocation of bacteria by their own motion, but keeping them alive. The metal frame was then taken off, and the plate thus prepared was put in the plate-holder horizontally. The bacteria used were as follows :—Bacillus coli communis, B. subtilis, B. mycoides, B. pyocyaneus, Sarcina, Micrococus pyogenes, Vibrio.

After exposures being given, the preparation was put in an incubator and it was kept there 12–24 hours long to favour the growth of bacteria. The transparent image of spectral lines then appeared clearly on a somewhat white background, and this was next put in a formalin-vapour to fix it.

As an illustration one of reproductions corresponding to the table 3 is shown in Fig. 1, and the bactericidal curve determined from it is given in Fig. 2.

Results of Experiments.

The results of the experiments are given in the following tables.

A. Bactericidal curves.

TABLE 1.

Germ : Bacillus coli communis.
Culture-medium : Agar agar.
Source of light : Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
1	2418 ÅU	2749 ÅU
3	2327 „	2749 „
6	2067 „	2749 „
12	2067 „	2784 „
15	1913 „	2841 „
20	1895 „	2878 „
40	1895 „	2878 „
80	1877 „	2948 „

TABLE 2.

Germ: Bacillus subtilis.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
1	2382 ÅU	2749 ÅU
2	2382 „	2749 „
4	2348 „	2749 „
6	2348 „	2749 „
8	2327 „	2784 „
10	2250 „	2841 „
15	2085 „	2841 „
20	2067 „	2841 „
41	2067 „	2874 „
80	2000 „	2874 „

TABLE 3.

Germ: Bacillus mycoides.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
$\frac{1}{4}$	2749 ÅU	2749 ÅU
$\frac{1}{2}$	2749 „	2413 „
1	2749 „	2327 „
2	2841 „	2250 „
3	2841 „	2067 „
4	2841 „	1990 „
5	2841 „	1913 „
6	2841 „	1895 „
8	2874 „	1895 „
10	2874 „	1887 „
20	2948 „	1874 „
40	2986 „	1859 „

TABLE 4.

Germ: Bacillus pyocyaneus.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
$\frac{1}{4}$	2749 ÅU	2495 ÅU
$\frac{1}{2}$	2749 „	2327 „
1	2749 „	2327 „
2	2749 „	2067 „
3	2749 „	1996 „
4	2841 „	1895 „
5	2841 „	1895 „
6	2841 „	1895 „
8	2874 „	1887 „
10	2874 „	1887 „
20	2948 „	1869 „
40	2948 „	1859 „

TABLE 5.

Germ: Sarcina.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
$\frac{1}{4}$	2749 ÅU	2632 ÅU
$\frac{1}{2}$	2749 „	2360 „
1	2749 „	2327 „
2	2749 „	2250 „
3	2749 „	2224 „
4	2749 „	2110 „
5	2749 „	2110 „
6	2841 „	2085 „
8	2841 „	2067 „
10	2841 „	2000 „
20	2841 „	1944 „
40	2784 „	1895 „

TABLE 6.

Germ: Micrococcus pyogenes.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
1	2749 ÅU	2632 ÅU
2	2749 „	2551 „
4	2749 „	2348 „
6	2749 „	2348 „
8	2749 „	2327 „
10	2749 „	2327 „
15	2841 „	2250 „
20	2841 „	2250 „
40	2948 „	2085 „
80	2948 „	2067 „

TABLE 7.

Germ: Vibrio (Intestine Vibrio).
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
$\frac{1}{4}$		
$\frac{1}{2}$	2749 ÅU	2418 ÅU
1	2749 „	2360 „
2	2749 „	2327 „
3	2749 „	2224 „
4	2749 „	2110 „
5	2749 „	2067 „
6	2841 „	2067 „
7	2841 „	2000 „
10	2841 „	2000 „
20	2874 „	1913 „
40	2948 „	1895 „

TABLE 8.

Germ: Water-Vibrio (Takasu Vibrio).
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
1	2749 ÅU	2327 ÅU
2	2841 „	2085 „
3	2841 „	2000 „
4	2841 „	2000 „
5	2841 „	2000 „
6	2841 „	1944 „
8	2841 „	1895 „
10	2874 „	1895 „
20	2874 „	1887 „
40	2948 „	1877 „

TABLE 9.

Germ: Water-Vibrio (Kôbe Vibrio)
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
$\frac{1}{4}$	2749 ÅU	2551 ÅU
$\frac{1}{2}$	2749 „	2445 „
1	2749 „	2360 „
2	2749 „	2348 „
3	2749 „	2327 „
4	2749 „	2327 „
5	2784 „	2110 „
6	2784 „	2110 „
8	2841 „	2067 „
10	2874 „	1913 „
20	2874 „	1877 „

B. The determination of the limits of wave lengths of light having the bactericidal action. (Long exposure)

TABLE 10.

Germ: Bacillus coli communis.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron and cadmium.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
150	2948 ÅU	1856 ÅU

TABLE 11.

Germ: Bacillus coli communis.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron and cadmium.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
300	2986 ÅU	1856 ÅU

C. The effect of rays upon media sensitized by methylene blue.

TABLE 12.

Germ: Bacillus subtilis.
Culture-medium: Agar agar containing methylene blue (0·01%).
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
1	2632 ÅU	2551 ÅU
2	2749 „	2360 „
4	2749 „	2348 „
6	2749 „	2327 „
8	2749 „	2327 „
10	2749 „	2250 „
15	2749 „	2250 „
20	2841 „	2144 „
40	2841 „	2085 „
80	2948 „	1895 „

TABLE 13.

Germ: Bacillus coli communis. Before smearing methylene blue (0·01%) added to bacteria-suspension.
Culture-medium: Agar agar.
Source of light: Condensed spark of iron.

Time of exposure in minutes	Upper limit of wave length having bactericidal action	Lower limit of wave length having bactericidal action
80	2841 $\overset{c}{A}U$	2250 $\overset{o}{A}U$

From the data given above, it may be seen that the bactericidal effect of the light begins suddenly at about $\lambda 295\,\mu\mu$, reaches the maximum at about $275\,\mu\mu$, and then decreases slowly. The lower limit of the bactericidal action at the extreme ultraviolet is about $186\,\mu\mu$, this number agreeing well with that given by Strebel[1]. It is important to notice that this effect of the rays is independent of the nature of the bacteria and the culture-media employed.

The bactericidal power of methylene blue under the exposure of light was next studied, but the effect could not be decidedly observed, so far as the present experiment is concerned. This is not in harmony with that of Keller[2].

From the results mentioned above, it may be considered that the most part of the sunlight which has a bactericidal action lies in the ultraviolet region beyond $\lambda 295\,\mu\mu$. To confirm this, the writer prepared three inoculated culture-media, the first was covered by a glass plate of ten mm. thickness to cut off the rays beyond $295\,\mu\mu$, the second by a glass plate of one mm. in thickness which is sufficient to prevent drying but transmits the rays, while the third was kept uncovered. Each of these culture-media were exposed to the sunlight for thirty minutes in August. After the incubation it was found that the bacteria in the second and third media were sterilized, but not in the first.

Discussion.

Cernovodeanu and Henri[3] observed that the microscopic figures of bacteria sterilized by ultraviolet rays were the same as those killed by heat-coagulation, and consequently they considered that the bactericidal action of ultraviolet rays was to cause the coagulation of the protoplasm of bacteria. They also considered that the hydrogen peroxide produced in the culture-medium by exposing it to the ultraviolet rays was not

1 Loc. cit.

2 A. Keller, Centralbl. f. Bakteriol., Ref., **40**, 186 (1907).

3 P. Cernovodeanu & V. Henri, C. R., **150**, 52 & 549 (1910).

the cause of the bactericidal action. Besides this, there are many other opinions[1] on this subject, but they are not concordant.

In our case the image of the spectral lines impressed on the inoculated culture-medium seems to be caused perhaps by the sterilization of bacteria by the light rays, but not by its inhibitory action on their growth, for even with the very long incubation of 48 hours or more the transparency of the image of the spectral lines is not altered.

The ultraviolet rays which sterilize the bacteria seem not to produce any toxical substances for bacteria in the culture-medium, because in that part where the image of the spectral lines was impressed, newly inoculated germs grew just as well as on an untreated medium.

Now, ultraviolet rays behave inhibitorily toward fermentations, which are most important factors in the metabolism of organism. In order to see whether this action of the rays has a certain relation to their bactericidal effect, the writer examined the action of the rays on ferments by the method described above.

The starch (Kuzu in Japan) paste, coagulated by steam was used as a culture-medium, and a small quantity of Taka-diastase was smeared on its surface. This preparation was put in a special plate holder of brass having water cooling. Even with four hours exposure to the light, no trace of the effect could be detected.

Again, egg-albumin coagulated by steam, was smeared with a small quantity of pancreatin, or pepsin containing dilute hydrochloric acid of 0.2 per cent. With four hours exposure, the result was found to be also negative.

Lastly, catalase in the juice of leaves of clover (Triforium repens, L) and oxydase in apple and in egg-plant (Solanum melongena, L) were exposed to the light of a quartz mercury lamp, but the result was also negative, even after thirty minutes exposure.

Considering these facts, we see that the inhibitory action of the ultraviolet rays on fermentation has no relation to their bactericidal action.

As to the nature of the bactericidal action of the ultraviolet rays, no definite conclusion could be drawn. It seems, however, to me that

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1 V, Henri & his wife C. R. **159**, 413 (1914).
Cf. P. Achalme, Électronique et biologie, p, 334 (1913).
W. E. Burge, Amer. J. Physiol., **43**, 429 (1917).

the bactericidal curves obtained above may be useful for further study in the mechanism of this phenomenon.

In the present investigation, non-pathogenic germs were used, and a further investigation on pathogenic germs will be carried out in the near future.

The writer's cordial thanks are due to Profs. T. Mizuno and M. Kimura and to Assistant Prof. U. Yoshida for their kind suggestions and advice, and also to Assistant Prof. T. Toyoshima for his kind supply of the purely isolated germs of bacteria.

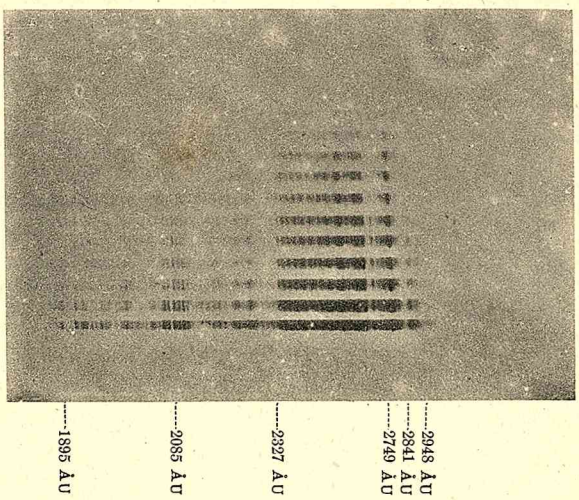
1895 ÅU
2085 ÅU
2927 ÅU
2749 ÅU
2841 ÅU
2948 ÅU

Fig. 1.

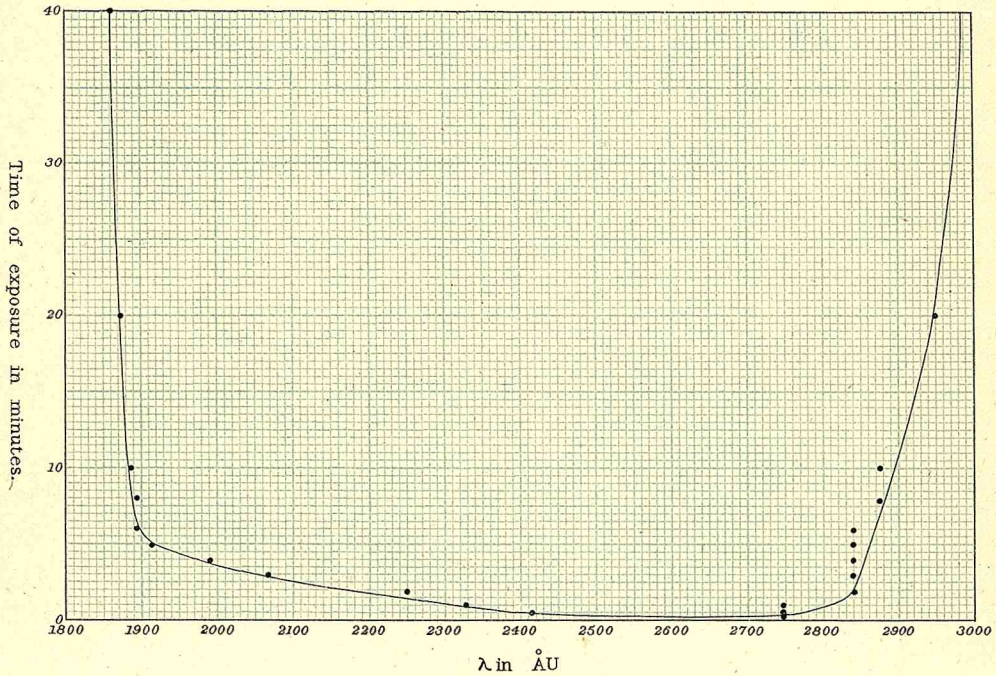
Time of exposure in minutes.
40
30
20
10
0
1800
1900
2000
2100
2200
2300
2400
2500
2600
2700
2800
2900
3000
λ in ÅU

Fig. 2.