THE ABIOTIC ACTION OF ULTRA-VIOLET LIGHT.

By H. S. NEWCOMER, M.D.

(*From the Laboratory of the Henry Phipps Institute of the University of Pennsylvania, Philadelphia.*)

PLATE 66.

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INTRODUCTION.

The following data as to the bactericidal action of ultra-violet light on typhoid bacilli were obtained by a method different from that heretofore used and the results give additional evidence toward settling certain disputed phases of the subject.

In 1910[1] and again in 1912[2] Victor Henri and his associates published articles on this subject in which they give evidence to show that the bactericidal effect of ultra-violet light first becomes appreciable at 3000 Ångström units, and increases progressively from there downward toward 2150 Ångström units. The amount of this progressive increase they did not make clear. The evidence tended to show that there was no maximum in this region. The organisms were exposed directly to the ultra-violet light source, so arranged as to limit to a certain extent its spectral range. This limitation was effected by choosing three sources of ultra-violet light such that their intensities in various regions were quite different, and to emphasize further this difference the spectral distribution of their radiation was cut down by the interposition of one of six light-screens. It is difficult from the tables of data to follow them to their conclusions. The upper limit for the action of ultra-violet light they fixed by screening out the lower wave-lengths with glass and determining the amount of screening necessary to prevent the effect.

Browning and Russ[3] state that they prepared plates by making a thin spread of bacteria on glass-supported films of nutrient material. These were exposed in

[1] Cernovodeanu, P., and Henri, V., *Compt. rend. Acad.*, 1910, cl, 52, 549.

[2] Henri and Henri, *Compt. rend. Acad.*, 1912, clv, 315.

[3] Browning and Russ read a paper on this subject recently before the Royal Society which has not yet appeared in print. Their reading is abstracted, however, in the *Brit. Med. J.*, 1917, i, 656.

As this proof goes to print the paper of Browning and Russ has appeared (Browning, C. H., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917, xc, the manner of a photographic plate at the back of a quartz spectrograph. The sterilizing action of the light gave a photograph of the active portion of the spectrum. They fixed 2960 Ångström units as the upper limit of effective light.

Lymann[4] has shown that the abiotic effect of ultra-violet light increases rapidly below 2000 Ångström units to become almost instantaneous at 1750. The lower limit of sensitivity has not been fixed. I have shown in a previous paper[5] that typhoid bacilli are slightly sensitive in the x-ray region (1 Ångström unit). There may, however, be one or more minima between 1 and 1750 Ångström units.

Method.

My experiments differ from previous ones in that water is used as a medium for the organisms. Experiments with the x-rays and with chemicals seem to show that nutrient media often protect the organisms from the action of abiotic agents. My experiments further differ from those of Henri and his associates in that the light incident on the organisms is confined in each case by the use of a quartz spectrograph to a very narrow region of the spectrum.

In the following experiments I have used a fresh water suspension of typhoid bacilli. A uniform quantity of the suspension was taken up on a platinum loop and used to fill a quartz capillary by capillary action. The quartz capillary was then fixed in an appropriate graduated rack in the focal plane of a quartz spectrograph and so

33). They found the cessation of abiotic action to be sharply at 2960 Ångström units for *Staphylococcus aureus*, and slightly higher, at 3000 Ångström units, for *B. typhosus*. An excellent plate for *Staphylococcus aureus* accompanying the article would indicate that the fall in abiotic action becomes perceptible at 2900 Ångström units and comes to zero at 2960 Ångström units. My quantitative data, graphically illustrated in Text-fig. 1, show that this fall to 2960 Ångström units is not so sharp as their plate would lead one to suppose. The probability that long exposures to sunlight have a killing effect would indicate that the curve of the figure approaches the horizontal axis asymptotically, that the curve is concave to the right as shown, rather than convex to the right, as an abrupt fall to zero would imply. My actual numerical data further imply this concavity. Browning and Russ required much longer exposures to kill the organisms than I did. My spectrograph is the same as theirs and my iron spark is probably not brighter than the tungsten arc which they used. The differences in exposure required by us would therefore seem to offer interesting corroborative evidence as to the protective effect of a nutrient medium for bacteria.

[4] Lymann, T., Spectroscopy of the extreme ultra-violet, London, 1914, 103.

[5] Newcomer, H. S., *J. Exp. Med.*, 1917, xxvi, 657.

exposed to a narrow region of the spectrum. After exposure the contents of the capillary were washed out and rinsed by suction in liquid agar and plated, the plates incubated, and counts made. The quartz spectrograph was a Hilger size C, giving a spectrum from 2100 to 8000 Ångström units 19 cm. long. The focal plane of the spectrograph formed an acute angle of about 30° with the light path. For most of the work the condensed iron spark was used as an ultra-violet source. It was placed 10 cm. from the entrance slit of the spectrograph. It was operated by a 10 kilowatt closed circuit transformer using 110 volts alternating current on the primary. The secondary voltage was about 10,000. The condensers had a capacity of 0.05 microfarad. The entrance slit of the spectrograph was opened to 1.5 mm., a width such that the individual lines of the iron spark were spread out into bands of the same width in projection as the quartz capillary. This produced an overlapping of the lines, giving the effect of a continuous spectrum. As a result any setting involved an exposure to a wave-length interval 15 to 40 Ångström units long. Fig. 1 reproduces a photograph of the iron spark spectrum, the upper band being the spectrum with a narrow slit, and the lower, the spectrum as used with a wide slit. The figure gives some information as to the relative intensity of light at different wave-lengths.

My data give the approximate wave-length range of the light falling on the quartz capillary for each setting. These wave-lengths were determined by measurements with a wave-length scale on photographs of the spark spectrum, the rack holding the capillaries being graduated to correspond with the wave-length scale. No attempt is made to give wave-lengths closer than to within 5 Ångström units. The data are computed on a basis of controls having counts of 100 colonies per sq. cm. The actual controls varied from 10 to 800 per sq. cm., usually about 150. When the controls gave more than 1,000 per sq. cm. the suspension contained so many organisms that some of them were protected from the light by being in the shadow of others.

OBSERVATIONS.

Table I gives the data for 10 and 5 minute exposures to the iron spark.

TABLE I.

Exposures to the Iron Spark of 10 and 5 Minutes' Duration.

Wave-length.	10 min. exposures.	5 min. exposures.	Wave-length.	10 min. exposures.	5 min. exposures.
Å. u.			*Å. u.*		
1990–2005	41*	73	2530–2560		0
1995–2010	50	62	2540–2570	0	3
2195–2215	8		2550–2580	0	
2205–2225	11		2560–2590	0, 0	½
2235–2255	25		2580–2610	0	0
2240–2260	28	50	2600–2630	0	¼
2250–2270	65		2610–2640	0	0
2270–2290	30		2645–2675	1	9
2280–2300	26		2650–2680	½	1
2290–2310	2,22	51	2655–2685	1½, 1½	3½
2325–2350	2½	4	2680–2715	0, ½	3, 3
2330–2355	1	14	2700–2735	0	1
2335–2360	2½	3	2760–2795	0	
2350–2375	3½	11	2780–2815	0	9,10
2365–2390	⅕	1½	2785–2825	1	
2385–2410	0, ½	0, 1½, 3	2790–2830	2	5
2405–2430	$\frac{1}{10}$	1¼	2795–2835	1½	4½
2435–2460	0	2	2845–2885	7	
2455–2480	1	2½	2850–2890		16, 17, 20
2485–2510	0, 0	0, 1, 1	2855–2895	3, 2½	5, 7½, 7½
2495–2525	0	1, 1, 2	2875–2915	7	
2500–2530	0	0	2945–2985	40, 45, 50	
2525–2555	0	¼			

* In the tables the data represent typhoid organisms per hundred remaining alive after the given exposure.

It is to be noted that the abiotic effect is low from 2100 to 2300. This corresponds to a portion of the iron spark spectrum of relatively weak intensity. In order to determine the nature of the effect in this region exposures were made to the copper and zinc spark, sources with lines of about equal intensity in this and higher regions of the spectrum. Table II gives the results of these exposures and shows that the effect is about the same in this region as elsewhere.

TABLE II.

Exposures to the Copper and Zinc Sparks.

Copper spark.		Zinc spark.		
Wave-length.	10 min. exposures.	Wave-length.	10 min. exposures.	5 min. exposures.
Å. u.		*Å. u.*		
2130–2140	57, 22	1990–2105	1	14
2180–2195	30, 23	2130–2145	5	21
2205–2225	30	2190–2210	37	
2235–2250	32	2250–2270	37	61
2265–2290	47	2490–2530*		1
2295–2320	79, 81	2545–2575*		0
2360–2385	24	2800–2840*	2	1½
2590–2620	47, 63			
2750–2785	49			

* Very strong lines.

The relative intensity of the light in the various portions of the spectrum could only be measured photographically, and comparisons were further made difficult by the fact that the plate gradually decreased in sensitivity in the far ultra-violet. Between 2100 and 2800 the exposures of Table I showing a slight relative decrease in abiotic effect correspond well with weak regions in the spectrum. Above 2800 the abiotic activity of the light begins gradually to fall off. This can be seen well in Table I and in Table III for 2 minute exposures.

TABLE III.

Exposures to the Iron Spark of 2 Minutes' Duration.

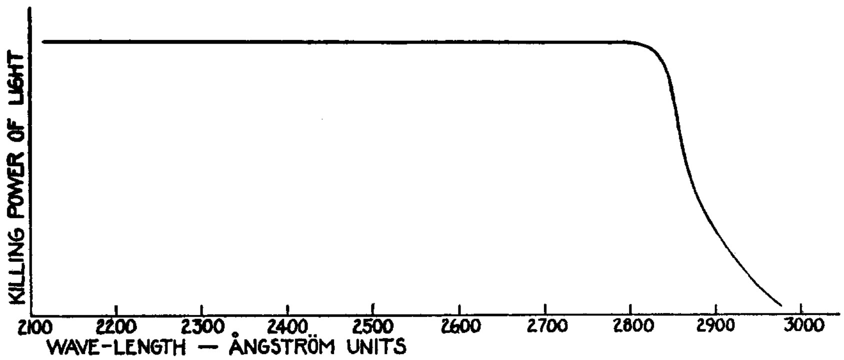
Wave-length.	2 min. exposure.	Wave-length.	2 min. exposure.
Å. u.		*Å. u.*	
2365–2390	13, 12, 13	2580–2610	1, 0, 1, 0
2385–2410	16, 20	2680–2715	22, 20, 21
2435–2460	13, 12½, 12	2850–2890	45, 52, 45
2530–2560	4, 2, 2, 1½, 1		

From Table III the decrease would appear to begin at 2700, but at this point the iron spark spectrum is rather weak. At 2750 the spectrum is very strong and here exposures corresponded with those made at 2600, also a relatively strong place in the spectrum. The iron spark spectrum is slightly weaker below 2500 than above that point. Below 2320 it is very weak. Above 2780 it weakens perceptibly. About 2870 corresponds to a region of the spectrum equal in intensity to the 2700 region. At 2870 the tables show a decrease in abiotic activity. This decrease when corrected for the weakness of the spectrum amounts to a fall of one-half. The last appreciable abiotic effect occurs somewhere between the moderately strong 2950 and 2990 lines. In this region the intensity is about the same as at 2700 and 2870. At 2945 to 2985 exposures for 10 minutes averaged 55 per cent killed, for 20 minutes 81 per cent killed. An average of eight exposures at 2990 to 3030 for 20 minutes gave no mortality. These exposures at 2945 to 2985 and 2990 to 3030 were controlled for evaporation and for scattered light. Longer exposures were impracticable because of the increasing influence of both these factors, particularly the former. Complete evaporation of the contents of the capillary occurring in about 40 minutes will completely or almost completely kill the typhoid organisms.

In order to limit further the upper end of the abiotic spectrum exposures were made directly before the iron spark but behind a piece of optical glass 3 mm. thick. The distance from the spark was 4 cm., the increase in energy due to lessened distances alone amounting to 256 times. Besides this, the whole area of the spark was effective rather than a portion corresponding to the slit area. This introduced another factor so that there was an increase of about one thousandfold. The glass transmitted no light below 3100; above 3300 it was almost transparent. These exposures of 5 and 10 minutes' length had no effect on the typhoid organism. The terrestrial sun's spectrum ends somewhere near 2970. Exposures to sunlight of 20 minutes' duration produced no effect.

I, therefore, conclude that the killing power of ultra-violet light begins to decrease at about 2800, at first very gradually, reaches one-half of its value at about 2870, one-tenth of its value at 2950, and less than one one-hundredth at 2990. Text-fig. 1 gives a sketch of the probable form of the abiotic curve. As far as I can judge equal intensities produced equal effects in the region 2100 to 2800. If there is a maximum in this region it is at the most only slight and would be in the neighborhood of 2600.

The relative effect of much shorter exposures is shown in Table IV. These exposures give at the same time further data for determining the relation between quantity of energy and per cent of organisms killed. This relation, most likely an integral of the probability law, must be known in order to derive from the tables the form of the abiotic energy curve of Text-fig. 1.



TEXT-FIG. 1. Graph representing the approximate amount of abiotic energy in ultra-violet light as referred to the typhoid bacillus.

TABLE IV.

Short Exposures to the Iron Spark.

Time.	Wave-length.	
	2385-2410 Å. u.	2580-2610 Å. u.
sec.		
60	14, 16, 14, 12	1, 5, 6
50		2
40		3, 3, 2
30	36, 24, 12	5, 4, 5
20		11, 7, 8, 4, 10
15	72, 50, 42	
10		12, 25, 11, 12, 17, 40

The Wratten and Wainwright panchromatic plate requires an exposure of about $\frac{1}{4}$ second under these conditions. At 2600 a 95 per cent sterilization of a *Bacillus typhosus* suspension is secured in about 200 times this length of time. The typhoid organism therefore compares very well with the slow silver papers in degree but not in range of sensitivity.

In similar but less extensive experiments I have found *Staphylococcus aureus* to behave exactly like *Bacillus typhosus*.

CONCLUSIONS.

Typhoid bacilli are about one two-hundredth as sensitive to ultraviolet light of wave-lengths 2100 to 2800 Ångström units as is the photographic plate. This sensitivity then falls off, decreasing rapidly to almost zero sensitivity at about 2970, the beginning of the sun's spectrum.

I wish to thank Dr. Paul Lewis for suggestions and advice in the arrangement of the experiments.

EXPLANATION OF PLATE 66.

FIG. 1. The iron spark spectrum between 2100 and 3200 Ångström units. The upper band is the spectrum with a narrow slit, the lower band, the spectrum with a wide slit as for bacterial exposures. Below 2320 Ångström units the faintness of the spectrum is real. Below 2200 the increasing faintness is due to the plate.

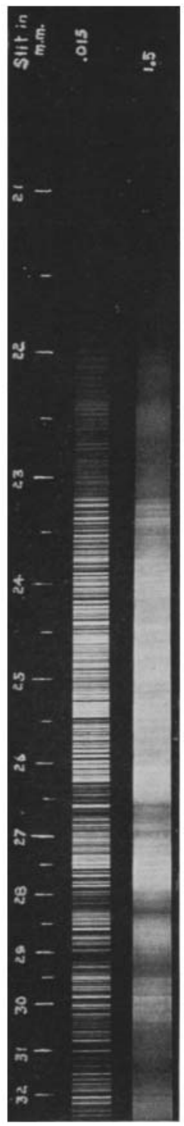


FIG. 1.

(Newcomer: Abiotic action of ultra-violet light.)