THE DESTRUCTION OF BACTERIA THROUGH THE ACTION OF LIGHT.

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It is generally assumed that artificial light is more harmful to the eyes than daylight. This has been ascribed by many to the quality of the artificial light rather than to the faulty distributions and intensities which seem quite as likely to be the causes of the observed effects. That this harmfulness may be due to ultra-violet radiation in the light seems, at first sight, plausible, particularly in view of the known facts concerning the bactericidal action of these radiations. As the opening step in a series of investigations on the true cause of the difference in the physiological action of natural and artificial illumination, it seemed desirable to determine definitely the wave-lengths which accomplish the destruction of bacteria. Previous investigators generally have made use of full sources of light, for example, the carbon arc, iron arc or mercury arc, without any analysis of the radiation. The use of a quartz spectroscope and accessories naturally suggests itself as a means of getting more exact information.

The objects of this work may be specifically stated as follows: To determine precisely the wave-lengths most destructive of bacteria; to find out whether or not simultaneous irradiation with rays of greater penetration is essential to the bactericidal action; and to investigate the effect of dissolved food materials, or other material, on the resistance of bacteria.

A large amount of work has been done on the destructive effects of light beginning with Downes and Blunt (77) and including DuClaux (85), Roux (87), Arloing (85), Buchner (92), Marshall Ward (93), Ledoux Lebard (93), Richardson (93), Dieudonne (94), Finsen and his pupils (99–07), Jodlbauer and his pupils (05–09), and Cernovdianu and Henri (10). It is not necessary to give abstracts of all of the papers above referred to. To sum up the entire series, the conclusion finally reached is that light containing radiations of a length less than 2,800 Angstrom units will, after a sufficient time, kill bacteria. No investigator whose works I have been able to locate has analyzed the radiation used with any kind of spectrometer and in only one case (Henri) have filtering screens been used. The work of the Finsen school is particularly interesting because of its application to the treatment of cutaneous bacterial diseases as lupus vulgaris and lupus erethymatosus (due to the tubercle bacillus). The light used for this purpose is the so-called Finsen Ray (Violet Ray, or Actinic Ray). To pro-

duce curative effects, the entire radiation of a carbon arc must be used, the ultra-violet alone being ineffective, according to Finsen. Further, the light must be concentrated by condensing lenses. In these lamps, the light-path includes a system of quartz lenses and, in addition, ten or twelve inches of distilled water which must absorb all of the far ultra-violet and infra-red. The bactericidal effect must therefore be ascribed to the intermediate waves. The exposures made are for a minimum of seventy minutes. There can be no question of the fact that cases of lupus have been cured by the repeated use of this light.

Within the last three years the Cooper-Hewitt Company, under the direction of Doctors Henri, Hebronner and Recklinhausen, have produced a water sterilizer making use of a quartz mercury arc—a source particularly rich in ultra-violet rays. The published advertisements claim that with an expenditure of $\frac{3}{4}$ of a kilowatt, 5,000 gallons per hour can be sterilized completely. These sterilizers are in use on a large scale in the city water works of Luneville (France). In this apparatus the water flows over a partition in a thin sheet at a distance of a few centimeters from a quartz lamp of the same type as one used in this investigation. The effects are ascribed to the ultra-violet rays in the light. No screens of any kind are used.

Any study of the destruction of bacteria must consider the following factors:

(1) Character of the medium containing the organisms at the beginning of the test, whether solid or liquid, nutrient or non-nutrient, absorbing or non-absorbent, oxygenous, or not—also the changes which may be produced in the medium due to the action of the light.

(2) The presence or absence of protective layers due to the medium or to superficial layers of bacteria.

(3) The character of the radiation (a) wave length, (b) intensity, (c) heat content.

(4) Time of radiation.

In this investigation these factors were taken care of severally as follows: (1) As a solid medium agar-agar was used; as liquid media both normal salt solution (non-nutrient) and water containing dissolved food materials. No investigation of the effect of the radiation on the medium was made. Agar is highly absorbent and the only effect to be anticipated in the water is through the production of hydrogen peroxide and the amount of this has been determined by other investigators to be negligible. (2) On agar the presence of a protective layer of the medium was avoided by using surface colony bacteria only. The probability of the occurrence of superficial growths serving to protect more deeply buried bacteria was minimized by exposing the plates immediately after inoculation. (3) The character of the radiation was altered in intensity by using various current

strengths with an iron arc and by using a mercury arc on parallel tests. For each source different distances were used. The radiation was analyzed with a two-prism quartz spectroscope and the wave-lengths exactly determined by comparison with known lines in the mercury arc spectrum. Glass screens of different thicknesses were employed when the work was done at short distances. The direct heating effects were avoided, where necessary, by water cooling the exposed plates. (4) Various times of exposure were made ranging from ten seconds to three hours according to circumstances.

As stated above two sources of radiation were used—the iron arc and the quartz mercury arc. The iron arc was a right-angled hand lamp with half-inch wrought iron electrodes turned to points—the positive pole at 30 degrees, the negative at 15 degrees. The lamp was run on a 110-volt direct current circuit under various current strengths ranging from three to twenty. The current strengths were adjusted by means of a series rheostat. The mercury lamp was a quartz Cooper-Hewitt arc and was operated on the same circuit, *i. e.*, 110-volt direct.

The lamps were supported before an adjustable slit which was kept 2.5 cm. by .05 cm. at all times. The light was collimated by a quartz lens 3.8 cm. in diameter and 25 cm. in focal length and passed through a system of two 60-degree quartz prisms 5 cm. in altitude (cut with their axes perpendicular to their bases). The dispersed light was focused by a second quartz lens similar to the collimating lens and gave a spectrum between (from the visible end of the red to the limit of the ultra-violet) 12.7 cm. and 15 cm. in length. This spectrum could be focused very sharply on a screen and, though curved and somewhat distorted because of the aberration of the lenses, gave excellent definition of closely adjacent lines. The spectral band was, of course, sharply curved in a horizontal plane due to the shorter focus for the ultra-violet radiations. A sheet of uranium glass was supported in an adjustable holder and used to explore and focus the ultra-violet. The ultra-violet lines showed up sharply and distinctly on this plate and could be located accurately. Similarly supported on a stand was a small (13 mm.) total reflection prism of quartz which, when placed in the converging light, served to reflect it downward to a focus on a small, adjustable, horizontal table on which the cultures were placed for exposure. The entire apparatus, with the exception of the lamp, was enclosed in a light-proof case provided with a head-cloth to make inspection and adjustment possible during runs.

After the apparatus was rigidly adjusted to minimum deviation of the sodium line, the spectrum of the mercury arc was explored with the uranium glass and compared with a photograph of the arc spectrum made in the Cooper-Hewitt laboratories. This photograph was marked in wave-lengths. All of the lines in the photograph were located and, in addition, 4 lines in the neighborhood of 1900–2000 not visible on the photograph.

The positions and wave-lengths of all of the lines were accurately noted on the top of the horizontal table as they were thrown down in succession by moving the reflecting prism. The iron arc spectrum was next calibrated by direct comparison with the mercury spectrum. The reflecting prism was placed, as is usual in such comparisons, so that it covered half of the slit and both spectra were thus thrown edge to edge on the uranium screen. In this way the wave lengths of eight lines in the ultra-violet of

TABLE NO. 1.

Material.	Thickness.	Limits.
Glass—		
plate—greenish	3.15 mm.	Cuts clean to 334—dims 334.
plate—greenish	2.0 mm.	Cuts clean to 334—
plate white	1.96 mm.	Dims 291, 303 and 313 a little.
	3.99 mm.	Dims 313 a little, shows 302 very faint.
cover glass	.15 mm.	Dims 302 and 291, no effect on 313.
cover glass	.12 mm.	Extinguishes all beyond 253.
		Cuts 265 and 253 to about $\frac{1}{5}$. Dims 291.
watch crystal	1.85 mm.	Nearly extinguishes 291.
		Dims 303 and 313.
Quartz—		
plates	3.0 cm.	No effect.
Distilled water		
in glass cells		
quartz ends	1 cm.	No effect.
	15 cm.	No effect.
Celluloid—		
transparent		
yellowish	1.63 mm.	Cuts to 334. 334 very dim. 365 dim.
Agar-agar—		
solid plate on quartz	1.5 mm.	Cuts sharp to 290—no effect above.
Grease—(beeswax)	.9 mm.	Cuts 236—dims 253 and 313 a little.
films on quartz		Transmits 275.
on glass (thin white)		Transmits 313.

the iron arc were accurately determined and others were subsequently located by comparison with the excellent chart of the iron spectrum prepared by Buisson and Fabry.

At this stage the extent and intensity of the ultra-violet portions of the mercury and iron arcs were compared directly and it was unmistakable that, at ampereages above twelve, the iron arc gave shorter ultra-violet radiations (running down to 1850, the limit set by the absorption of the quartz) than does the 110-volt mercury arc and, in spite of the closely set

lines nearly uniformly distributed, an intensity at all points comparable with that of the isolated lines of the mercury arc. The iron arc ought, therefore, to be, excepting for its unsteadiness, better suited to work in the ultra-violet than is the quartz mercury lamp. As might be expected, the intensity of the ultra-violet radiations from the iron arc increases with the ampereage used. The relative intensities were in all cases judged by the energy of fluorescence on the uranium glass.

The transmission limits of certain thicknesses of glass, of layers of water and of other substances were measured in order that plates of these materials might be used as screens to cut off the shorter wave lengths when this was desirable. The transmission was determined by the use of the uranium glass and the mercury arc. The results of the tests are shown in Table No. 1.

The purpose was to start with certain typical bacteria both pathogenic and non-pathogenic forms. The ones selected were B. prodigeosus, B. pseudomonas pyocyaneus, B. coli communis, and B. typhosus. In most of the work following cultures were made of these types in nutrient and non-nutrient water and exposed in definite wave-lengths of radiation for a certain known period. The exposed emulsions were then sown into agar plates in Petri dishes and incubated, with control cultures, for 24 hours at 37.5 degrees centigrade. Examination with a hand lens over a counting plate made possible the determination of the effect of the radiation. Some determinations of the effects of the radiations on motility were made using hanging drop preparations of the liquid cultures and (as noted subsequently) a number of series were run through with surface colonies made by direct inoculation of agar plates with a platinum needle.

In order to check the work of others done with the full radiation of the sources, the lamps were set up with proper diaphragms and supports and experiments run through as noted in Table No. 2.

These plates were incubated for 24 hours and the presence of the living bacteria demonstrated by the development of the green pigment. The tests, as actually made, were in the reverse order of the numbers here given. The conclusion is unavoidable—that pyocyaneus, when in surface colonies on agar, is not killed by the full radiation of the mercury lamp even at very short distances and long times. The glass screen used on cultures 1 and 13 cut off all radiation shorter than 365 units. (See Table No. 3.)

In making these tests the arcs were turned vertically and the emulsions exposed in quartz-bottomed cells. An electric fan placed on a level with the cell, at one side, was an essential part of this arrangement. The fan kept the cell cool and, in the use of the iron arc, prevented the condensation of the smoke of the arc on the quartz plate. One cubic centimeter from each sample was sown into the agar plates and, where any growth developed, the colonies were counted. The controls developed above 50,000 bacteria per cc.

TABLE NO. 2.

B. PSEUDOMONAS PYOCYANEUS—FRESH SURFACE CULTURES ON AGAR.

FULL RADIATION OF THE MERCURY ARC.

No.	Distance.	Time.	Results.	Remarks.
1	5 cm.	1 hr.	+	Under glass—water-cooled
2	5 cm.	1 hr.	+	Under quartz—water-cooled
3	8 cm.	1 min.	0	Under quartz—plate very hot
4	8 cm.	1 min.	+	Under quartz
5	8 cm.	15 min.	+	Under quartz—water-cooled
6	10 cm.	1 min.	+	Under quartz
7	10 cm.	1 min.	+	
8	22 cm.	1 min.	+	Under quartz
9	22 cm.	5 min.	+	Under quartz
10	22 cm.	5 min.	+	Under quartz
11	22 cm.	5 min.	+	Under quartz
12	22 cm.	5 min.	+	Under quartz
13	22 cm.	7 min.	+	Under glass
14	22 cm.	7 min.	+	Under quartz
Controls				

+ = normal growth. 0 = no growth.

TABLE NO. 3.

PYOCYANEUS IN WATER. FULL RADIATION.

A.—Mercury lamp.

No.	Distance.	Time.	Results.	Remarks.
1	8 cm.	1 min.	0	3 cc. of emulsion
2	8 cm.	1 min.	0	1 cc. of emulsion
3	Control		+	

B.—Iron arc. 12 amperes.

1	8 cm.	30 min.	0	3 cc. emulsion
2	8 cm.	20 min.	0	3 cc. emulsion
3	Control		+	
4	8 cm.	1 min.	0	3 cc. emulsion

These results show that pyocyaneus, when in emulsion in water, is killed with ease and certainty by very short exposures—on agar it is not at all affected. This is remarkable.

The following tests were made with B. typhosus.

TABLE NO. 4.

AGAR PLATES INOCULATED WITH TYPHOSUS AND EXPOSED TO THE FULL RADIATION OF THE MERCURY LAMP.

No.	Distance.	Time.	Results.	Remarks.
1	5 cm.	1 min.	+	Under glass—3.15 mm.
2	5 cm.	1 min.	Inhibited	Under quartz
3	10 cm.	1 min.	Inhibited	
4	10 cm.	5 min.	Inhibited	
5	10 cm.	10 min.	0	Water-cooled under quartz
6	10 cm.	10 min.	+	Water-cooled under glass 3.15 mm.
7	Control		+	
8	Control	10 min.	+	Water-cooled under glass .13 mm.

The greater susceptibility of this bacterium (as compared with pyocyaneus) is here evident. The growth is seriously interfered with even for exposures so short as one minute and, in spite of the heavy inoculation with bacterial scum, none survive after 10 minutes at 10 cm. It is to be observed that the destructive effects are completely eliminated by a screen of glass .13 mm. thick which cuts out radiations shorter than 250 and impedes all shorter than 313. Unless care be taken in work of this kind destruction of the bacteria is likely to result from overheating—a temperature of 60 degrees centigrade being fatal. As above noted, the exposed plates were water-cooled and thermometers were supported over them to make certain that the temperature never rose above 40 degrees.

An emulsion was now made of typhosus in normal salt solution. Portions of this emulsion were treated in glass cells under quartz covers to the full radiation of the mercury lamp and were then sown into agar plates and incubated. The results are tabulated below. The numbers given show the bacteria alive per cc. after exposure.

Analysis of this set of results justifies the conclusion that at a distance of 5 cm. exposures of longer than 30 seconds will kill all of the typhoid bacteria in emulsion in water if the layer does not exceed .5 cm. in depth. It is to be observed that in deeper layers the action is less effective and that

TABLE NO. 5.

TYPHOSUS IN NORMAL SALT SOLUTION—FULL RADIATION OF MERCURY ARC.

Nos.	Distance.	Time.	Depth— 5 mm.	1 cm.	1 mm.	Under Glass 5 mm.
1–2–3	5 cm.	5 sec.	23	2500		500
4–5–6	5 cm.	10 sec.	15	90		250
7–8–9	5 cm.	30 sec.	0	22		720
10–11–12	10 cm.	30 sec.	21	20	210	
13–14–15	10 cm.	1 min.	2	28	2	
16–17–18	10 cm.	3 min.	12	4	contaminated	

4 controls—21 cc.—too many to count.
2.1 cc.—30,000 and 27,000 per cc.

through glass (watch crystal transmitting nothing below 290) the development of this particular bacterium is inhibited. This last result is not consistent with other tests. The water worked with contained about 30,000 bacteria per cc. which is a number considerably higher than would ordinarily occur in contaminated drinking water. If the results in the last tabulation are expressed in percents they seem more striking.

Bacillus coli communis was subjected to the following tests under full radiation (mercury arc).

TABLE NO. 6.

No. 1. Freshly inoculated agar plate 12 cm. from the lamp for 15 min. (under quartz). Growth—normal.

No. 2. Emulsion in distilled water—3 mm. deep—exposed at 12 cm. for 8 min. Agar plate inoculated from the emulsion by use of a platinum needle. No growth.

No. 3. Like No. 2—for 1 min. No growth.

The single surface colony determination (1) can hardly be depended on as it stands alone. Here, however, as elsewhere the bactericidal action of the radiation is seen to be much more rapid and effective on bacteria in water than on surface colonies in any type.

Emulsions were now made of coli in distilled water, in normal salt solution and in ordinary tap water and were exposed in glass cells under quartz to the full radiation of the mercury arc. One cc. from each sample was sown into agar and incubated as in the earlier work. The results follow:

TABLE NO. 7.

No.	Distance.	Time.	Depth.	Result.	Remarks.
	5 cm.	1 min.	5 mm.	0	In distilled water
2	5 cm.	1 min.	2 mm.	0	In salt water
3	5 cm.	1 min.	5 mm.	0	In tap water
4	5 cm.	5 min.	5 mm.	0	In distilled water
5	10 cm.	1 min.	5 min.	3/cc.	In distilled water
6	10 cm.	1¼ min.	5 mm.	+	In salt water
7	10 cm.	5 min.	5 mm.	1/cc.	In distilled water
8	10 cm.	5 min.	5 mm.	0	In salt water
9	10 cm.	5 min.	5 mm.	0	In tap water
10	10 cm.	5 min.	2½ mm.	-	Lost
11	10 cm.	15 min.	5 mm.	0	In distilled water

Two controls—1200/cc.—normal.

It is evident from this tabulation that coli communis is very sensitive to the action of this light. Exposures of one minute at five centimeters destroy them completely. The action is unaffected by the small quantities of dissolved and suspended matter in the tap water.

A series of parallel tests were run with this organism using the iron arc. The emulsions were placed in quartz bottomed brass cells which, from their construction, were very hard to sterilize so that several of the emulsions were contaminated as noted. (See Table No. 8.)

The comparisons of the mercury and iron spectra referred to earlier in this paper indicated that the iron arc should be at least as effective as the mercury arc in treating bacteria. The results in this last table together with those previously stated for pyocyaneus, show that they are, in fact, of the same order of effectiveness.

The conclusion of this work up to this point is that bacteria of the coli type (including typhosus) are, when in emulsion in water, readily killed by short exposures to lights containing amongst others radiations shorter than 2,500 units in length. Pyogenic bacteria—at any rate pyocyaneus—are somewhat more resistant. This is practically the same conclusion as that reached by Henri. We will now pass on to a study of the effect on these bacteria of isolated ultra-violet waves.

The reflecting prism of the quartz spectroscope described early in this paper was adjusted to throw down a certain line of the mercury arc or a certain narrow group of lines of the iron spectrum. In this way an illuminated area, 2 cm. by 2 or 3 mm., was obtained on a certain mark on the horizontal table. The colony or emulsion was placed over this mark and properly screened from stray light by means of a slotted diaphragm.

TABLE NO. 8.

COLI IN EMULSION—FULL RADIATION OF THE IRON ARC.

No.	Distance.	Time.	Depth.	Result.	Remarks.
1	8 cm.	1 min.	4 mm.	contaminated	
2	8 cm.	5 min.	4 mm.	1/cc.	
3	8 cm.	15 min.	4 mm.	contaminated	
4	8 cm.	30 min.	4 mm.	1/cc.	
5	8 cm.	5 min.	2 mm.	0	
6	8 cm.	1 min.	2 mm.	0	
7	25 cm.	10 min.	4 mm.	contaminated	

Two controls—normal—about 30,000 per cc.

The first experiments were made with surface colonies of pyocyaneus on agar-agar. A pencil mark, 1 cm. or 1.5 cm. in length, was made on the bottom of the dish near the center. The plate was then inoculated from a culture tube by drawing a platinum needle along the mark. It had been found (see Table 2) that the full radiation at short distance and long time had no effect whatever on pyocyaneus on agar so that it is not surprising to find the analyzed radiation equally ineffective. Table 9 is a condensed report of the experiments.

Similar experiments on pyocyaneus on agar, using the mercury arc, gave the results shown in Table 10.

In using these isolated lines a distance was taken such that the radiation certainly completely covered the area of inoculation. These tables show that isolated ultra-violet radiation of the intensity here used, as short as 240 $\mu\mu$, will not kill pyocyaneus in surface colonies on agar. It must be remembered, however, that the full radiation did not affect surface colonies of this organism. (See Table 2.) The same organism in emulsion in water was readily killed by full radiation. (Seee Table 3.) Because of the suspicion that some of the bacteria were protected by a film of agar (the high absorbing power of which has been stated), no further experiments were made using this method.

A glass plate was cut and a slot ground in it with an opening 2 mm. by 10 mm. and 5 mm. deep. This plate was sterilized by boiling in distilled water for 40 minutes, the slot filled with an emulsion of the bacteria by means of a sterile bulb pipette, the slot then covered with a thin, sterile quartz cover and exposed on the horizontal table as before. The emulsion was then sown into agar plates and incubated. In this way the effect of the ultra-violet radiation of a limited range of wave-lengths (2 $\mu\mu$ approxi-

TABLE NO. 9.

PYOCYANEUS ON AGAR—DISPERSED RADIATION OF THE IRON ARC.

Region	1	2	3	4	5	6	
Wave-length	400	370	320	280	260	240	in $\mu\mu$

No.	Region.	Time.	Results.	Remarks.
1	1	15 min.	+	Lighter green than controls.
2	2	15 min.	+	Lighter green than controls.
3	3	15 min.	+	Lighter green than controls.
4	4	15 min.	+	Lighter green than controls.
5	5	15 min.	+	Lighter green than controls.
6	6	15 min.	+	Lighter green than controls.
7	1	30 min.	+	
8	2	30 min.	+	
9	3	20 min.	+	?
10	4	15 min.	+	
11	5	15 min.	+	
12	6	10 min.	+	
13	6	16 min.	+	
14	6	30 min.	+	
15	6	20 min.	+	
16	5	20 min.	+	
17	5	60 min.	+	Pale.
18	4	15 min.	+	
19	4	30 min.	+	
20	4	45 min.	+	
21	3	2 hr. 23 min.	+	

Controls—all normal.

mately) was determined with all other radiation excluded. It is to be noted that, in spite of the slight apparent intensity of the radiation here used compared to the full radiation of the source, we have, nevertheless, practically all of the radiation of that particular wave-length contained in the full radiation (or such of it as gets through the collimator slit)—for quartz and air in layers of the thickness here used are said to absorb waves of lengths above 200 $\mu\mu$ very little. Further, the volumes of the emulsion and the shape of the container were here so altered, in proportion to the solid angle subtended, that the total flux through the slit gave approximately the same total radiation per cubic millimeter of emulsion as was applied in the use of the full radiation. On account of the collimation and

TABLE NO. 10.

PYOCYANEUS ON AGAR—DISPERSED RADIATION OF MERCURY ARC.

No.	Line.	Time.	Results.	Remarks.
1	313 μμ	10 min.	+	
2	313	10 min.	+	
3	295	10 min.	+	
4	253	5 min.	+	Less green.
5	253	10 min.	+	
6	253	15 min.	+	Less green.
7	253	20 min.	+	
8	253	30 min.	+	
9	236	5 min.	+	
10	236	10 min.	+	
11	236	15 min.	+	

Controls—all normal.

focusing of the beam there was no decrease in the intensity due to the inverse square law and the conditions, as far as these lengths of ultra-violet radiation were concerned, were practically the same as if the emulsions were in the full radiation of the source at a distance of 25 centimeters, that is, the distance from the source to the collimating lens. The difference between the full radiation at the shorter distance and the dispersed radiation at the longer is threefold. In the first place, the full radiation may contain certain very short waves (under 185 μμ) which are absorbed by air and by thin quartz. Shuman found, however, that one millimeter of air absorbed completely all radiations under 170 μμ so that these waves are obviously an unimportant factor. In the second place, the ultra-violet in the full radiation is associated with longer waves of greater penetration which may render the organisms more susceptible. In the third place the intensity of the ultra-violet in the dispersed radiation is diminished through reflection from the prism faces and through absorption in the quartz prisms and lenses. The effects of these differenees will be studied in full later. The results of the experiments made by the method outlined above follow.

Study of these results especially (2, 3, 6 and 7) and comparison with the results obtained with the full radiation justified the conclusion that the isolated ultra-violet as here used is, for some reason, much less destructive of bacteria than is the full radiation. As this result was entirely unexpected a careful study was made of the differences in the two methods of irradiation. In the use of the isolated ultra-violet we have as above stated: (1)

TABLE NO. 11.

B. Coli in Slot Slide. +=Growth Normal.

No.	Line.	Time.	Results.	Remarks.
1	280 $\mu\mu$	2 hr.	+	Iron arc.
2	253	$2\frac{1}{3}$ hr.	+	Iron arc.
3	253	2 hr.	+	Iron arc.
4	265	2 hr.	+	Iron arc.
5	280	1 hr.	+	Iron arc.
6	250	$\frac{3}{4}$ hr.	+	Mercury arc.
7	236	1 hr.	+	Mercury arc.

Controls—all normal.

a greater distance from source to emulsion. This affects (a) the direct heating, (b) the intensity by inverse square effect, (c) the intensity through air absorption. (2) The radiation is transmitted through approximately four centimeters of quartz and, further, is weakened through two reflections from the prism faces. (3) The ultra-violet radiations are not associated with any longer waves of greater penetration.

In analyzing these differences it is to be observed that the emulsions were cooled by water or by fan and the temperatures kept low in both methods of exposing. Consequently no effect in either case can properly be ascribed to direct heating. Further, as stated on page 986, the collimation of the light prevented any decrease in intensity due to the inverse square law beyond the collimating lens. In these experiments the effective distance from the source was 25 centimeters—a distance at which the full radiation kills in fifteen minutes or less. In order to settle these points more definitely, and also to study the effect of air absorption, the following experiments with full radiation and long air path were made. In these experiments the distances used (See 1 and 4) are greater than the total path from source to emulsion in the spectroscope. The time required to destroy the bacteria is relatively greater than for short distances but destruction is complete in a maximum of 45 minutes.

In regard to the effect of absorption in the quartz parts of the apparatus and the loss through reflection at the prism faces nothing very definite can be said *a priori*. The effect of the absorption of four centimeters of quartz on the shorter waves might be marked. As for reflection, the reflecting power of quartz in the ultra-violet has not been adequately investigated. Working near the absorption region for ultra-violet as we

TABLE NO. 12.

FULL RADIATION—THROUGH 3 MM. IN GLASS CELLS. (B. TYPHOSUS.)

No.	Distance.	Time.	Results.	Remarks.
1	55 cm.	45 min.	0 (1/cc.)	Mercury arc.
2	55 cm.	15 min.	Retarded	Slightly. Hg. arc.
3	25 cm.	20 min.	Lost	Slightly. Hg. arc.
4	55 cm.	35 min.	0 (1/cc.)	Iron arc.
5	25 cm.	10 min.	contaminated	Iron arc.

Controls—all normal—about 35,000 per cc.

are here we must, however, anticipate a large loss due to reflection at the oblique surfaces. An approximate calculation gave the loss at each surface to be of the order of 15 per cent. Special experiments were therefore necessary to determine definitely the effect of these factors on the destructive action of the full radiation.

EXPERIMENT NO. A.

The apparatus was set up as shown schematically in Fig. 1. The emulsion was contained in a small porcelain crucible (4 mm. diameter at the bottom) and was exposed to the full radiation of the mercury lamp at a distance of 20 mm. The radiation passed through 4.3 cm. of quartz obtained by superposing eleven quartz plates of various thicknesses. A

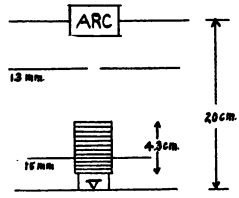

FIG. I

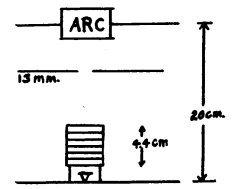

FIG. II

diaphragm under the arc cut the source of light to a circle of 13 mm. diameter. A second diaphragm with an opening 15 mm. in diameter was introduced into the pile of plates. The exposure was for 20 minutes and the destruction was complete. The control contained 30,000 typhosus per cc.—the exposed emulsion 0. Here we have 22 normal reflecting surfaces in the light path.

Experiment No. B.

This experiment was designed to check Experiment No. A. A single diaphragm (13 mm. opening) was placed as shown in Fig. 2 and the quartz path was made up of 6 plates aggregating 4.41 cm. Here we have, of course, twelve reflecting surfaces. The exposure was for 20 minutes as before. The destruction was practically complete—eight bacteria per cc. surviving. The control showed 35,000 per cc.

Experiment No. C.

In this experiment the effect produced by oblique reflections was investigated. Two quartz plates were set in the light path at angles equal to those offered by the prism faces in the spectroscope. In addition quartz plates were used giving a total quartz path of 4.5 cm. and an air path of 20 cm. (Fig. 3) The exposure was for 30 minutes. Destruction was complete—. the control showing 35,000 per cc.—the exposed emulsion 0.

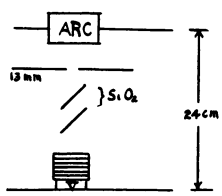

Fig III

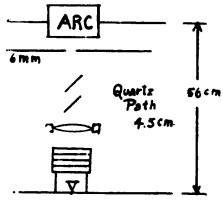

Fig. IV

Experiment No. D.

The effort was made here to reproduce the conditions in the spectroscope in regard to air-path, quartz-path, reflections and total irradiation. The apparatus was set up as shown in Fig. 4. The diaphragm opening was reduced to 6 mm. diameter. In the light-path were two oblique plates, one biconvex lens and four horizontal plates. The quartz path was 4.5 cm. —the air path 51.5 cm. We have here a longer air path than in the spectroscope—a longer quartz path, as many oblique reflections and, in addition, eight normal reflecting surfaces. The area over which the light that came through the diaphragm opening was diffused was measured and the ratio to the area of the crucible taken. This showed that 1-180 of the light that came through the hole fell on the emulsion. The area of the slit of the spectroscope was also measured and the area of diffusion in the plane of the collimating lens. This showed that 1-12 of the light that came through the slit fell on the lens. Calculations based on this data show that approximately twice as much ultra-violet radiation must fall, in any given time, on an emulsion on the table of the spectroscope as fell on the emulsion in the crucible in this last experiment. The exposure was

for one hour and the destruction was complete. The control developed 30,000 typhosus per cc.—the exposed emulsion 0 per cc.

These experimental results seem to prove conclusively that the destructive action of the light ought not to be in any way diminished in passing through a spectroscopic apparatus of the type and size of the one employed in this investigation.

A final series of experiments was now made with isolated radiations. The mercury arc was used as before. On account of the ease and certainty of sterilization the small porcelain crucible was used in place of the slotted glass slide. Four or five drops of the emulsions were used and controls made up with the same amounts. The distances were so taken that the lines used, as located on the fluorescent glass, covered the entire emulsion. In order to make absolutely certain of this point the crucible was rotated in the line during exposure at the rate of one revolution in 45 minutes, by a small clinostat. The adjustments were very carefully made and checked repeatedly during runs. The results are summarized in Table No. 13.

The following facts seem evident from these results. Marked destructive effects can be produced by isolated ultra-violet rays 250 $\mu\mu$ or less provided sufficient time is taken. A three-hour exposure is necessary in order to produce a marked result with $\mu\mu$ 250. The destructive effects increase on moving into the shorter wave-lengths—a one-hour exposure in $\mu\mu$ 220 being equivalent to three hours in $\mu\mu$ 250. However even for these rays ($\mu\mu$ 220—the shortest given by the quartz mercury arc) a five-hour exposure does not accomplish complete destruction.

Comparison of these results with those obtained with the full radiation (Table No. 12, see page 988) forces us to the conclusion that the effectiveness of the mercury arc, and similar sources, in killing bacteria rapidly is due to some extent to the association of radiations of greater wave-length and greater penetrating power with the short ultra-violet. Ordinarily the whole effect is ascribed to the ultra-violet. It was thought possible that the comparative ineffectiveness of the isolated rays might be ascribed to absorption in the water of the emulsions but an experiment made with a quartz cell showed that one linear centimeter of normal salt solution plus coli had no apparent absorptive effect on any of the ultra-violet lines—220 or above. It is possible that the longer radiation may render the organisms more susceptible to the action of the shorter—possibly the cell wall absorbs the isolated short waves thus protecting the enclosed protoplasm but the longer radiations, when present, may, in a sense, carry the shorter waves through the cell wall and thus enable them to act destructively on the protoplasmic contents.

This hypothesis can be checked by subjecting emulsions simultaneously to ultra-violet radiation and to longer wave-lengths from a second source.

TABLE NO. 13.

ISOLATED RADIATION OF THE MERCURY ARC. (B. TYPHOSUS.)

Time.	Wave-length.					
	220 $\mu\mu$.		236 $\mu\mu$.		253 $\mu\mu$.	
1 hour	50,000 cut to 5,000	1 — 10				
2 hours			+ + +	1 — 1	+	1 — 1
3 hours	12,000 cut to 150	1 — 80	21,000 cut to 450	1 — 50	12,000 cut to 1,000	1 — 12
4 hours	21,000 cut to 160	1 — 130				
5 hours	12,000 cut to 60	1 — 200				

Ratio of test to control shown by the fractions.

A few experiments of this kind have been made but the number is not yet sufficient to furnish any definite conclusion. It is obvious also that experiments should be made using the radiations from the three ultra-violet groups recombined for, in the full radiation, these wave lengths act simultaneously.

The results of this work may be summarized as follows. The full radiation of certain sources containing waves below 250 $\mu\mu$ in length, will kill bacteria certainly and rapidly. Isolated ultra-violet of lengths 250–280 $\mu\mu$ will not affect bacteria in one drop of water in a four hour exposure. Isolated ultra-violet as short as 220–225 $\mu\mu$ will kill only after several hours exposure even when the isolated radiation is of the same intensity as that contained in the rapidly destructive full radiation. The destructive power increases rapidly with decrease in wave length. The final conclusion is that the destructive effect of ultra-violet light is in some way dependent on its association with longer radiations.

I wish to acknowledge my indebtedness to Dr. Arthur W. Goodspeed, Director of the Randal Morgan Laboratory of Physics, for assistance in carrying out this work. The examination of the exposed cultures—the counting of the colonies, etc. was done by Mr. Edward Pugh, a special student in Bacteriology, who has been associated with me from the first and whose assistance has been necessary and invaluable.

It is proposed to continue this work along the lines indicated in the paragraph at the top of this page.

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