"A STUDY OF THE GERMICIDAL VALUE OF ULTRA VIOLET LIGHT PARTICULARLY THAT PRODUCED BY THE STERILAMP"

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Thesis

Submitted to the Faculty of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of Master of Science

Virginia Lucille Ross

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INTRODUCTION

The fact that light, particularly that portion of the spectrum known as ultra violet, exercises a definite germicidal action has been known for many years, and much experimentation has been carried on for the purpose of determining the exact nature and extent of this action, as well as to discover any possible practical applications. Recently the possibilities inherent in ultra violet radiation have been brought particularly to the notice of both the bacteriologist and the general public by the appearance of the "Sterilamp", an ultra violet ray tube manufactured by the Westinghouse Lamp Company.

Broad claims have been made concerning the abilities of the Sterilamp with particular reference to its use commercially as a sterilizing agent, suggested uses ranging from the purification of air to the preservation of food. The object of this study was the determination of the actual bactericidal value of the Sterilamp and the formation of a theoretical foundation upon which to base a conception of its practical value.

Historical

The term "ultra-violet" includes a fairly wide band of the spectrum; so, since the announcement of Downes and Blunt (18) in 1877 that ultra-violet would kill the bacteria present in decaying vegetable matter, investigators have attempted in the interest of a higher level of efficiency to narrow this range to certain specific wave lengths as proving most bactericidal. The first, and simplest, conception was that of Hertel (17) (1905) concurred with by Coblentz and Fulton (9) (1924) and by Bayne-Jones (3) (1923), that the bactericidal power of ultra-violet rays increased progressively as the wave length decreased down to 1860 Angstrom units, with the stipulation that the penetrating power of the wave lengths below 1600 Angstrum units is so slight that the rays may not pass into the bacteria far enough to have much effect.

However, the results obtained by other investigators do not support this theory. In 1917, H. S. Newcomer (23), working the typhoid fever organism found that the wave band range between 2435 and 2640 Angstrom units was the most effective. Browning and Russ (16) (1917-18), using agar surface cultures, a tungsten lamp, and a quartz spectrometer, give a maximum of 2800 to 2540 Anstrom units. Cernovodeanu and Henri (8) (1910), using a Cooper-Hewitt lamp and bacterial emulsions, found a maximum at about 2800 Anstrom units. Mashimo (21) using agar surface cultures and a quartz spectrometer, found a maximum at 2750 Angstrom units. Band (21), working with bactericidal rays in the spectrum of the carbon arc lamp, showed that there were

two maxima: the first from 3600 to 3400 Angstrom units; and the second from 3000 to 2000 Angstrom units, with the greatest point of destruction of the two resting at 2500 Angstrom units. Gates (15) (1930), using measured monochromatic energy found a striking maximum between 2600 and 2700 Angstrom units. Duggar and Hollaender (11) in 1934 and later Dreyfer and Campbell-Renton (10) in 1936 confirmed the maximum found by Gates. Duggar and Hollaender (11) used various wave lengths between the limits of 2537 and 6120 Angstrom units and found that in general the maximum lethal effect was at 2652 Angstrom units when using Bacillus megatherium, Bacillus subtilis, and Serratia marcescens as test organisms, although the spores of Bacillus megatherium exhibited a greater sensitivity to a wave length of 2894 Angstrom units. In the experiment of Dreyfer and Campbell-Renton (10) organisms were again exposed to varying wave lengths of light. The bactericidal effect varied with the organism exposed, but in all cases the line 2655 Angstroms was most effective, followed in order by the lines 2536, 2804, 2483, and 2700 Angstroms.

Other factors concerned in the bactericidal effectiveness of ultra-violet radiation have been the subjects of investigation. Dreyfer and Campbell-Renton (10) showed through reducing the intensity of light by screening that the time required for a given bactericidal effect was inversely proportional to the intensity of the light. Bachem and Dushkin (1) (1933) exposed Serratia marcescens to ultra-violet rays of varying intensity and for varying lengths of time and obtained results confirming these, as long as the sensitivity of the bacteria to ultra-violet re-

mained constant. However, they found that this sensitivity increased rapidly for the first two hours after plating, then after reaching a broad maximum, decreased sharply up to ten hours. Henri and Cernovodeanu (8) (1910) observed that the germicidal action of the ultra-violet rays deceased more rapidly than the inverse square of the distance from the source.

Henri (8) also noted that different microbes varied in their sensitiveness. Duggar and Hollaender (11) found that the level of energy required to kill bacteria was far below that necessary for any effect on the virus of tobacco mosaic; while the level of energy required to give any particular survivor value was greater for spores than for vegetative cells, and the resistance of the spores of Bacillus megatherium was greater than that of Bacillus subtitlis.

There has been much conflicting data presented as to the action of ultra-violet radiation on yeasts. Presenting data on one side of the question are Feuer and Tanner (13) (1920), who found that yeast cells could live only a few seconds to a few minutes longer than bacteria when a very thin layer of a water suspension was exposed at a distance of twenty-five cm from the source of ultra-violet; Buchta (7) (1914), who found that Saccharomyces cerevisiae and Saccharomyces ludwigii could not survive more than three minutes exposure to ultra-violet; Maurain and Warcollier (20) (1909), who found that ultra-violet would completely arrest fermentation in cider which was exposed in a very thin layer; Schnitzler and Henri (26) (1910), who obtained an inhibition of the acetic acid fermentation of wine by ultra-violet

radiation; and Lanzillotta (18) (1920), who found that an exposure of twenty minutes killed the cells. On the other hand De Fazi (12) (1921) claimed that in his experiments he exposed brewer's yeast for twelve hours to the ultra-violet rays from a 1200 candle power lamp at a distance of twenty cm, and that not only was the yeast not injured by this treatment, but that its fermentative activity was actually increased. All of the bacteria in the yeast were destroyed after a brief exposure. Lindner (21) (1923) found that the exposure of yeasts under fermentation conditions favored activity of the yeasts, but exposure of cells in a shallow layer of liquid was fatal, and herein may lie the explanation.

Reports about molds are also conflicting. Some workers state that they are killed by exposure to ultra-violet radiation without much difficulty. Read (25) (1934) destroyed heavy infections of Aspergillus, Penicillium, Rhizopus, and Mucor on the surface of loaves of bread in forty-five seconds by means of lethal rays from "C" carbons at a distance of eight inches. Read found that the open Carbon lamps emitted a higher intensity of the shorter fungicidal wave lengths than quartz-mercury lamps.

Experimental

A. The bactericidal and fungicidal powers of the Sterilamp.

The Sterilamp is an argon, neon and mercury vapor filled tube producing rays of which approximately ninety-three percent fall in the ultra-violet range while ninety-one percent are 2537 Angstrom units. It will be noted that this latter value coincides very closely with the values found by a majority of investigators to be highly bactericidal, and by several, to produce the maximum lethal effect. The lamp is the invention of Dr. Harvey C. Rentschler, Director of research of the Westinghouse Lamp Company, and Dr. Robert F. James of the same company. Drs. Rentschler and James have reported that cultures of Eberthella typhi, exposed to the emanations of the Sterilamp at a distance of four inches, were killed in eight seconds, cultures of Staphlococci, in twenty-four seconds, and cultures of Aspergillus, in nineteen minutes. These reported results, however, are not commensurate with the results and suggested applications released in newspaper reports, which give the lamp as killing bacteria in less than one second, instantly sterilizing breaths and instantly killing the bacteria on any article exposed to its emanations. The Sterilamp was reported as having been tried and found effective in restaurants, bakeries for keeping food sterile, manufacturing plants for both food and personal articles, hen houses, dairies, and hogpens. Also used to keep down the danger of infection in toilets. The lamp has been used in the operating rooms of three hospitals, Duke Hospital at Durham, N.C., Mayo Clinic at Rochester, Minn., and the New York Medical Center. Dr. Deryl

Hart, surgeon-in-chief of Duke Hospital at a meeting of the American Institute gave the results of 800 operations in air purified by the ultra-violet light. He said the death rate in chest operations was cut from five and five-tenths per cent to two and nine-tenths per cent. Infections after hernia operations were cut from three and three-tenths per cent to zero.

The concentration of light at the wave length of 2537 Angstrom units was reported to have produced a lamp which might be used without danger to the eyes. Therefore, in the gathering of data for this paper, at first no protective device was used. It was found, however, by several workers with the Sterilamp that exposure of the eyes for more than a very few minutes to the emanations of the lamp results in very badly inflammed eyes; so for the completion of the work, dark goggles were used which were so constructed as to completely shield the eyes.

In these studies the tubes and transformers were furnished by the Westinghouse Lamp Company and set up as they recommended.

In order to obtain as uniform an inoculum as possible throughout the complete study the following steps were taken:

I. A twenty-four hour culture of each organism grown in nutrient broth was used.

2. This culture was uniformly diluted by placing one loopful with a platinum wire loop four mm in diameter in ten cc. of nutrient broth.

3. The diluted culture was smeared over the surface of an area of four square inches of an agar plate previously poured and allowed

to harden. This smear was made with a sterile cotton swab.

4. The smear was allowed to stand one-half hour at 37°C before exposure to the Sterilamp so as to permit excess moisture to evaporate and remove any possibility of the exercising of a protective influence by the broth in which the organisms were suspended.

By removal of the petri dish cover the cultures so prepared were exposed to the emanations of a single Sterilamp at a distance of six cm (± 2mm) from the tube. (The fact that the cultures were not exposed to any bactericidal action of the emanations until the cover was removed is shown in table I) A group of identically prepared plates were opened for equal periods of time without exposure to the emanations from the Sterilamp, and these plates were used as controls. All plates were incubated for twenty-four hours at 37°C. As the colonies were largely confluent, it was impossible to make exact counts, so the growth was recorded as ranging from negative to four plus. Four plus growth was that typical of the control plates, two plus growth was approximately one-half as dense; while one plus was defined as from ten to one hundred discrete colonies and plus-minus, as less than ten.

Bactericidal

I. Unshielded lamp.

a. Non-spore-forming bacteria.

A series of non-spore-forming bacteria of common occurrence and representative nature were chosen as test organisms. These were Staphylococcus aureus, Eberthella typhi, Escherichia coli, Mycobacterium phlei, Pseudomonas aeruginosa and Aerobacter aerogenes.

The strains used were obtained from the stock cultures.

The time of exposure varied from five seconds to one minute. There was a marked bactericidal effect from five seconds exposure of all of these organisms, while fifteen seconds exposure reduced the average count of each, with the exception of Mycobacterium phlei, to less than ten. Mycobacterium phlei appears slightly more resistant. The differences in the average count of these six bacteria at the end of fifteen seconds exposure cannot be considered as particularly significant, as it is practically impossible to obtain exactly the same number of organisms on each smear.

b. Spore-forming bacteria.

(1) Bacillus subtilis and Bacillus mycoides were selected as test organisms, as well as an unidentified spore-former (xl), isolated from a plate previously exposed to the light. There was no discernible bactericidal effect on any of the three organisms from five seconds exposure. From fifteen seconds exposure there was an appreciable lethal effect on Bacillus subtiles; while at the end of thirty seconds exposure the plates showed only two plus growth. The plates of Bacillus mycoides and (xl) were nearly sterilized by thirty seconds exposure.

(2) To further demonstrate the effect of the presence of spores on the bactericidal power of emanations from a Sterilamp, an eight hour and a two months culture of Bacillus mycoides were exposed to the unshielded Sterilamp for intervals of five, ten and fifteen seconds. The cultures were examined microscopically prior to inoculation, and the eight hour culture was found to be free from spores, while the two month culture contained 100 per cent spores. The

results indicate a greater resistance in the spore containing culture, but show a marked bactericidal effect on this culture at the end of fifteen seconds exposure. The reduction obtained was from the four plus growth of the control plates to two plus growth, or a reduction of nearly one half in the number of spores which germinated.

II. Shielded lamp.

a. In a second series a variation was made in the technique of exposure by the use of a device for the control of the amount of light emitted by the Sterilamp. The purpose of this device was to make possible a more equitable comparison of lamps of varying size and consisted of a metal shield in which there was a narrow opening (3mm wide and 35mm long) through which passed the rays from the lamp. Control plates exposed under the shielded portion of the lamp showed no diminution in growth, demonstrating the fact that the metal was impermeable to the bactericidal rays and acted as an effective shield. The distance from the lamp to the plate was fifteen cm, and the periods of exposure were increased to from one to seven minutes. Under these conditions an exposure of two minutes resulted in approximately the same degree of bactericidal action as was effected by an exposure of five seconds to the unshielded light. Eberthella typhi again appeared slightly less resistant to the rays and Bacillus subtilis decidedly more resistant than Escherichia coli while Mycobacterium phlei did not show as great a relative resistance.

III. Dry plates.

To test the effectiveness of the Sterilamp on the bacteria

when exposed on a dry glass surface, the same technique for smearing was used as in I and II with the exception that the smears were made and dried directly on the inner glass surface of a sterile petri dish. The smears were then exposed to the emanations of a shielded Sterilamp for intervals of from one to seven minutes, nutrient agar was poured over the cultures, and they were incubated at 37°C for twenty-four hours. The results showed no pronounced difference from those obtained in II. Staphyloccoccus aureus, Eberthella typhi, and Bacillus subtilus appeared slightly more resistant when exposed on the dry glass. Escherichia coli showed about the same degree of resistance as when exposed on agar.

Fungicidal

I. Yeasts.

Feuer and Tanner found pigmented yeasts to be more resistant to the action of ultra-violet than White (13). Of the three yeasts selected, two Saccharomyces ellipsoideus and Saccharomyces cerevisiae, are unpigmented, and the third, Torula rosea, produces a rose pigment. The technique for exposing the yeasts was that described on page seven with the exception of the replacement of nutrient agar by dextrose agar, and the periods of exposure to the emanations of the unshielded Sterilamp were five, ten and fifteen seconds. The plates exposed for five and ten seconds showed a marked diminution in growth; those exposed fifteen seconds a decrease of approximately one half as compared to the control plate; and nearly all of the organisms were destroyed when Saccharomyces cerevisiae was exposed for thirty seconds. These results are comparable with those obtained for the spore producing bacteria and there is no appreciable differ-

ence between the effect on the unpigmented true yeasts, and the pigmented false yeast.

II. Molds

The molds selected as being diversified types and typical of the most common offenders in food spoilage were Aspergillus niger, Rhizopus nigricans, Penicillium italicum, Oospora lactis, and two cultures isolated from eggs and known as X3 and X6. The procedure was changed slightly to conform with the difference in the nature of the test organism.

(1) A three day old culture grown on dextrose agar was used so that the culture would be fully matured and contain both vegetative cells and fruiting bodies.

(2) The growth from the slant culture was suspended in sterile saline. This suspension was smeared with a sterile cotton swab over an area of four square inches on the inner surface of a sterile petri dish, and allowed to dry at room temperature for one-half hour before exposure to the rays of the Sterilamp.

(3) After the molds were exposed to the shielded Sterilamp, dextrose agar was poured over the smears, and the plates were allowed to incubate for twenty-four hours at room temperature.

As it was impossible to obtain satisfactory growth of Oospora lactis by this technique, this organism was spread on the surface of an agar plate rather than on the dry glass surface and exposed to the emanations from the unshielded Sterilamp. Table VI shows that culture X3 and culture X6 were not effected by five minutes exposure, and that thirty minutes exposure had only a slight effect

on Aspergillus niger and Rhizopus nigricans and none on Penicillium italicum. Cultures of these organisms kept continuously irradiated over a period of eleven hours showed no signs of growth during or after this period of irradiation. The medium was moistened with sterile saline to compensate for the loss of moisture during the period, and half of the plates were reinoculated with the molds. The newly inoculated plates grew well, demonstrating that the medium was still suitable for growth.

Oospora lactis, which possesses a different type of fruiting body than the above molds, was shown to be less resistant to the ultra-violet irradiation. Some effect was noted after fifteen seconds exposure to the unshielded light; thirty seconds exposure nearly sterilized the plates while sixty seconds exposure did completely sterilize them. These results would show Oospora lactis to possess about the same degree of resistance to the Sterilamp as the spore-forming bacteria.

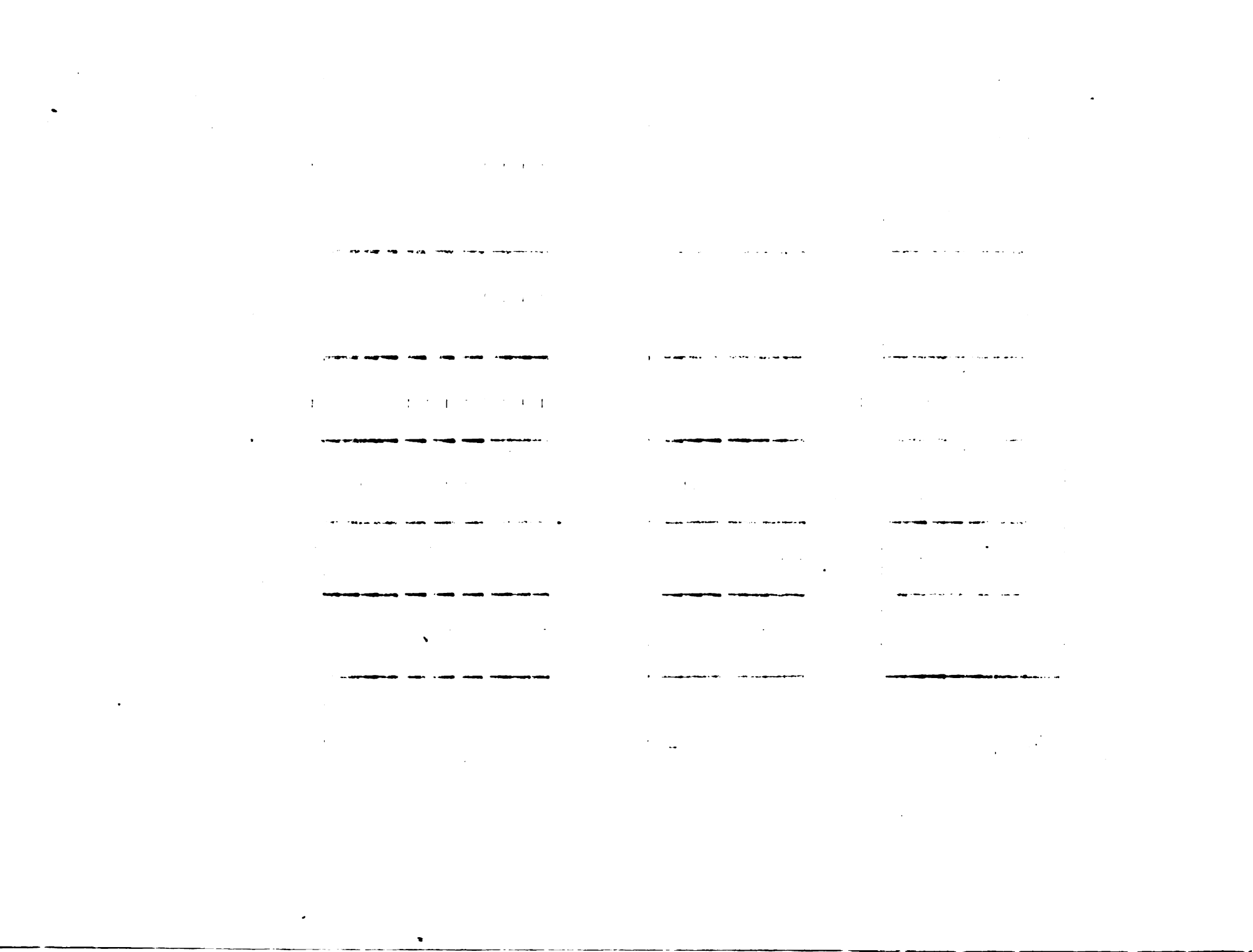
Table I. Bactericidal power of emanations of a Sterilamp on organisms protected by a petri dish cover.

Trial	Unexposed	Exposed 10 min. with cover on
1	4+	4+
2	4+	4+
3	4+	4+
4	4+	4+
Average	4+	4+

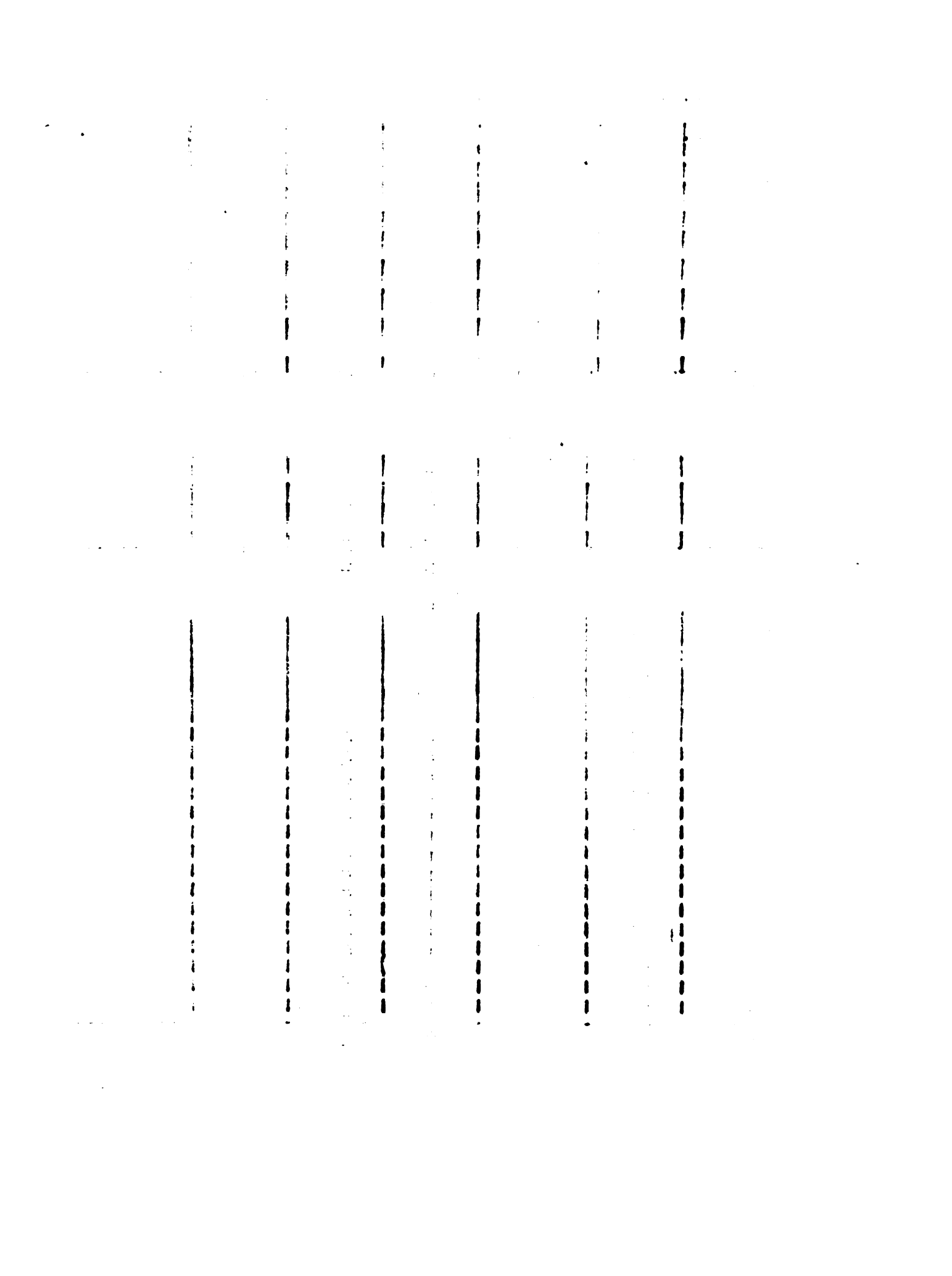
Table II Bactericidal Power of Emanations from a Sterilamp on organisms smeared on agar plates.

Trial	Time of exposure in Seconds					
	0	5	10	15	30	1 min
		Staph. aureus				
1	4+	2+	+	+		
2	4+	+	± (2)[x]	+ (11)		
3	4+	+	±	± (3)		
4	4+	2+	+	± (9)		
5	4+	2+	+	± (9)		
Average	4+	2+	+	±		
		E. Typhi				
1	4+	+	+	± (4)		
2	4+	+	+	±		
3	4+	+	± (6)	± (1)		
4	4+	+	±	± (1)		
5	4+	+	+	± (10)		
6	4+	+	± (3)	± (1)		
7	4+	+	-	± (1)		
8	4+	+	± (2)	± (1)		
Average	4+	+	±	±		
		Esch. Coli				
1	4+			-	+	-
2	4+			-	-	-
3	4+			-	-	-
4	4+			-	-	-
5	4+	3+	-	-		
6	4+	2+	-	-		
7	4+	2+	+	-		
8	4+	+	-	-		
9	4+	2+	+			
10	4+	2+	± (10)			
11	4+	2+	± (10)			
12	4+	2+	±			
Average	4+	2+	±	-	-	-

[x] Number of colonies on plate.



Trial	0	5	10	15	30	1 min.
	Myco. phlei					
1	4+	2+	+	+		
2	4+	2+	+	+		
3	4+	2+	+	+		
4	4+	2+	+	+		
5	4+	2+	+	+		
6	4+	2+	3+	+		
7	4+	2+	+	+		
8	4+	3+	2+	2+		
9	4+	3+	2+	2+		
10	4+	3+	2+	2+		
Average	4+	2+	2+	+		
	Pseud. aeruginosa					
1	4+	+	±(4)	±(2)		
2	4+	+	±(4)	±(1)		
3	4+	+	±(3)	±(1)		
4	4+	+	±(3)	±(2)		
5	4+	±	-	-		
Average	4+	+	±	±		
	Aerobacter aerogenes					
1	4+	4+	2+	+		
2	4+	4+	2+	2+		
3	4+	4+	2+	2+		
4	4+	4+	2+	+		
5	4+	4+	2+	+		
6	4+	3+	2+	+		
7	4+	2+	+	±(10)		
8	4+	2+	+	±(3)		
9	4+	2+	+	±(4)		
10	4+	+	+	+		
11	4+	+	+	±(4)		
12	4+	+	+	±(1)		
13	4+	+	+	-		
14	4+	+	+	-		
15	4+	+	+	-		
16	4+	2+	+	+		
17	4+	2+	+	±(10)		
18	4+	2+	+	+		
19	4+	2+	±(13	±(1)		
20	4+	+	-	±(1)		
21	4+	2+	±(2)	±(1)		
Average	4+	2+	+	±		



B. subtilis

Trial	0	5	10	15	30	1 min.
1	4+	4+	4+	4+		
2	4+	4+	4+	4+		
3	4+	4+	4+	4+		
4	4+	4+	4+	4+		
5	4+	4+	4+	4+		
6	4+	4+		2+	2+	
7	4+	4+		2+	2+	
8	4+	4+		2+	2+	
Average	4+	4+	4+	3+	2+	

B. mycoides

1	4+	4+			±(3)	± (1)
2	4+	4+			±(4)	±
3	4+	4+			±(6)	-
Average	4+	4+			±	-

Freshly isolated unidentified spore forms

1	4+	4+		+	±(6)	
2	4+	4+		+	±(3)	
3	4+	4+		+	-	
Average	4+	4+		+	±	

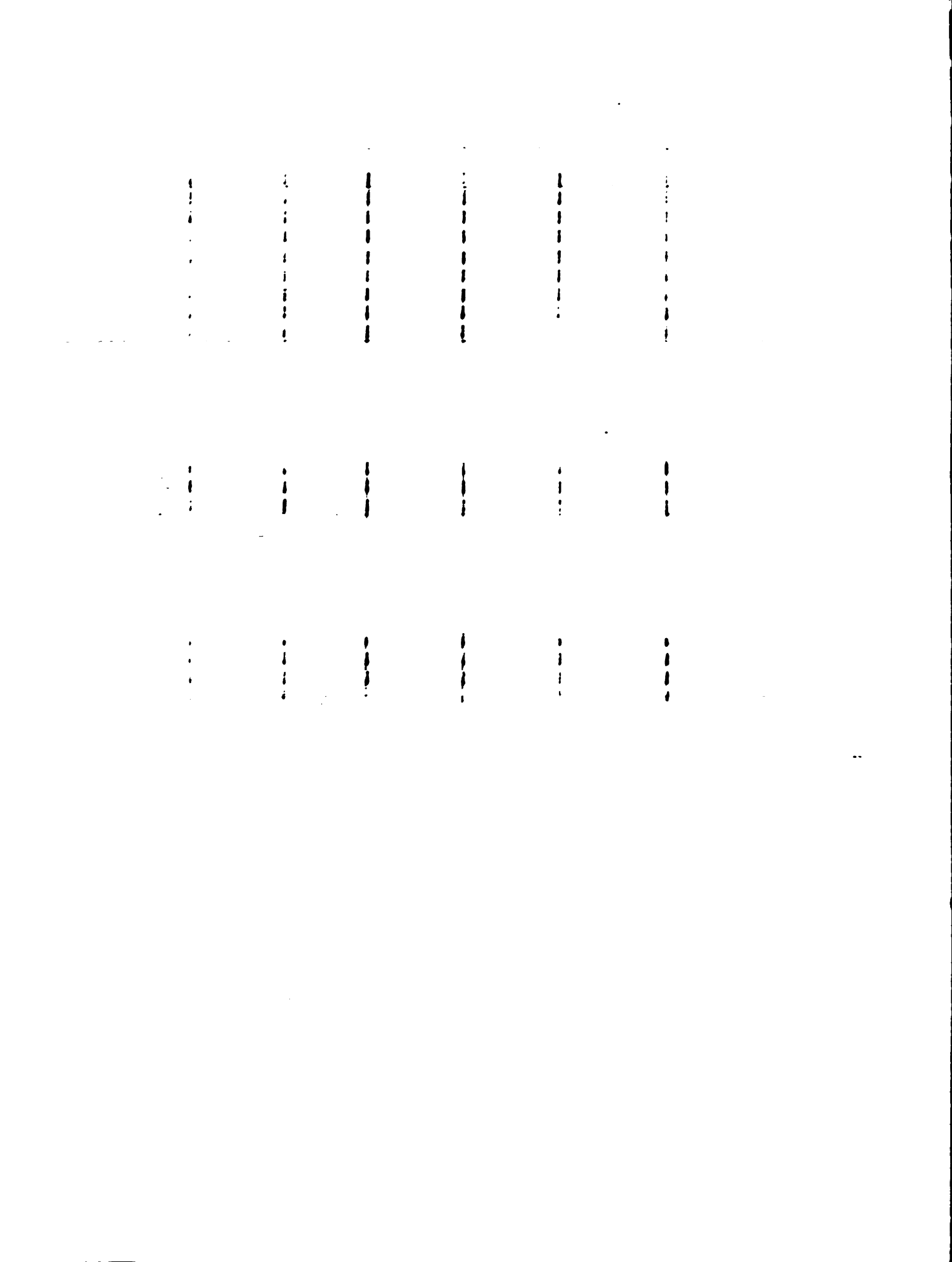


Table III Effect of presence of spores on the bactericidal power of emanations from a Sterilamp.

Trial	Time of Exposure in Seconds				
	0	5	10	15	
		B. mycoides			
1	4+	4+	2+	+	8 hr. culture
2	4+	3+	2+	+	
3	4+	3+	2+	+	
Average	4+	3+	2+	+	
1	4+	4+	3+	2+	2 mo. culture
2	4+	4+	3+	2+	
3	4+	4+	3+	2+	
Average	4+	4+	3+	2+	

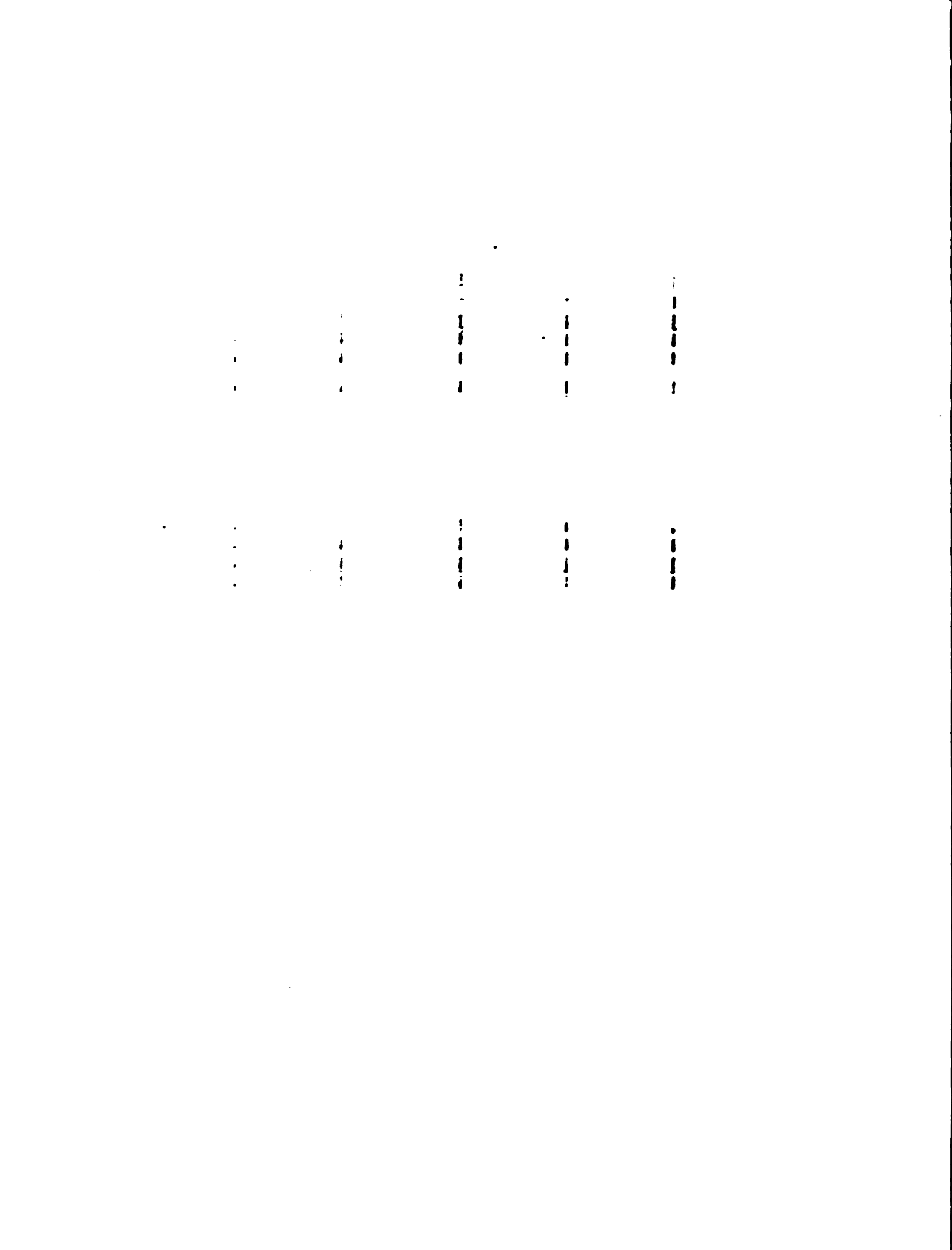


Table IV Bactericidal Power of Emanation from a Sterilamp with a Controlled amount of light obtained by passing the rays through a narrow opening on organism smeared on surface of Agar Plates.

Trial	Time of Exposure in Minutes							
	0	1	2	3	4	5	6	7
	Staph. Aureus							
1	4+		2+	+	+			
2	4+			2+	+	+		
3	4+			±	±	±		
4	4+				-			
5	4+		2+	1+				
6	4+		2+	1+				
Average	4+		2+	+	±	±		
	Eberth. typhi							
1	4+			+	±(1)[x]			
2	4+		-		+	±(1)		
3	4+				-		±(1)	
4	4+				+	+	±(3)	
5	4+			-	-			
6	4+				±(1)	-	±(2)	
Average	4+		-	±	±	±	±	

[x] Number of colonies on plate.

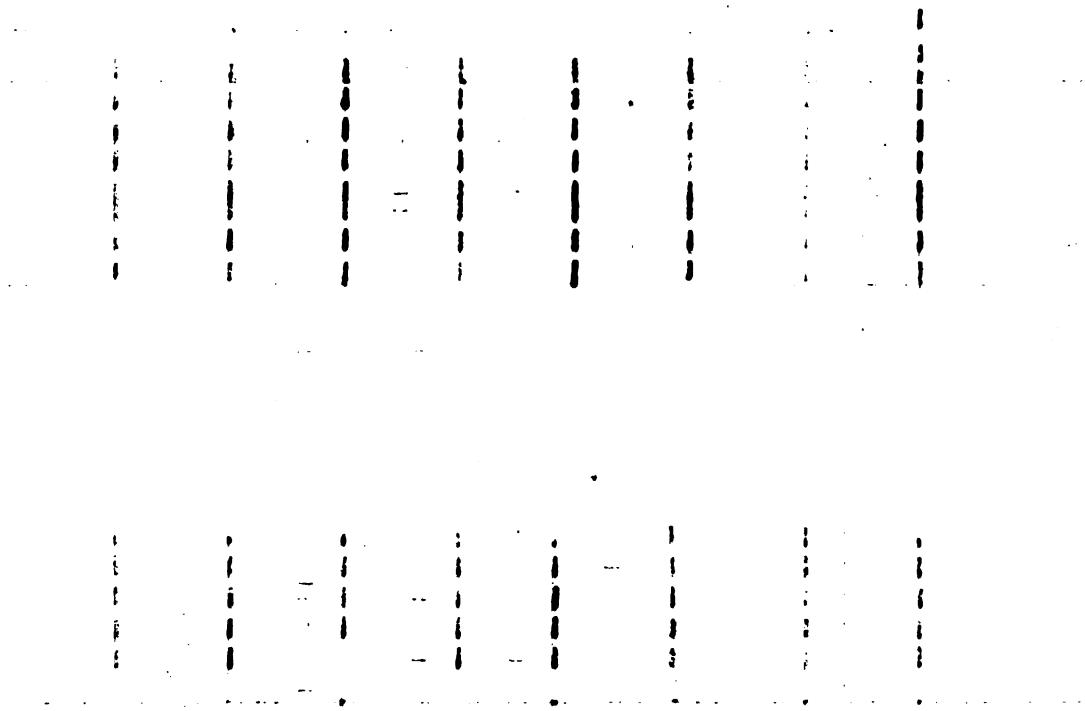


Table IV Cont. Bactericidal Power of Emanation from a Sterilamp with a controlled amount of light obtained by passing the rays through a narrow opening on organisms smeared on surface of agar plates.

Trial	Time of Exposure in Minutes							
	0	1	2	3	4	5	6	7
				Escherichia Coli				
1	4+		4+	3+	3+			
2								
3	4+				3+	+	+(42)[x]	
4	4+				+	+	-	
5								
6	4+							+(27)
7	4+							±(1)
8	4+	4+	3+	3+		2+		
9	4+	4+	3+	2+		+		
10	4+	4+	3+	2+		+		
11	4+	4+	3+	2+		+		
12	4+					-		
13	4+							-
Average	4+	4+	3+	2+	2+	+	±	±
				Myco. phlei				
1	4+			+	±(10)	±(5)		
2	4+				±(9)	±(6)	±(1)	
3	4+				±(1)	-	-	
4	4+					-		
5	4+					-		
Average	4+			+	±	-	-	
				B. subtilis (spores present) (24 hr. culture)				
1	4+		4+	3+	2+			
2	4+			±(4)	0	+(1)		
3	4+					3+		
4	4+			3+	2+			
5	4+			3+	2+			
6	4+					+		+
7	4+					+		
Average	4+		4+	2+	+	+		+

[x]Number of colonies on plate.

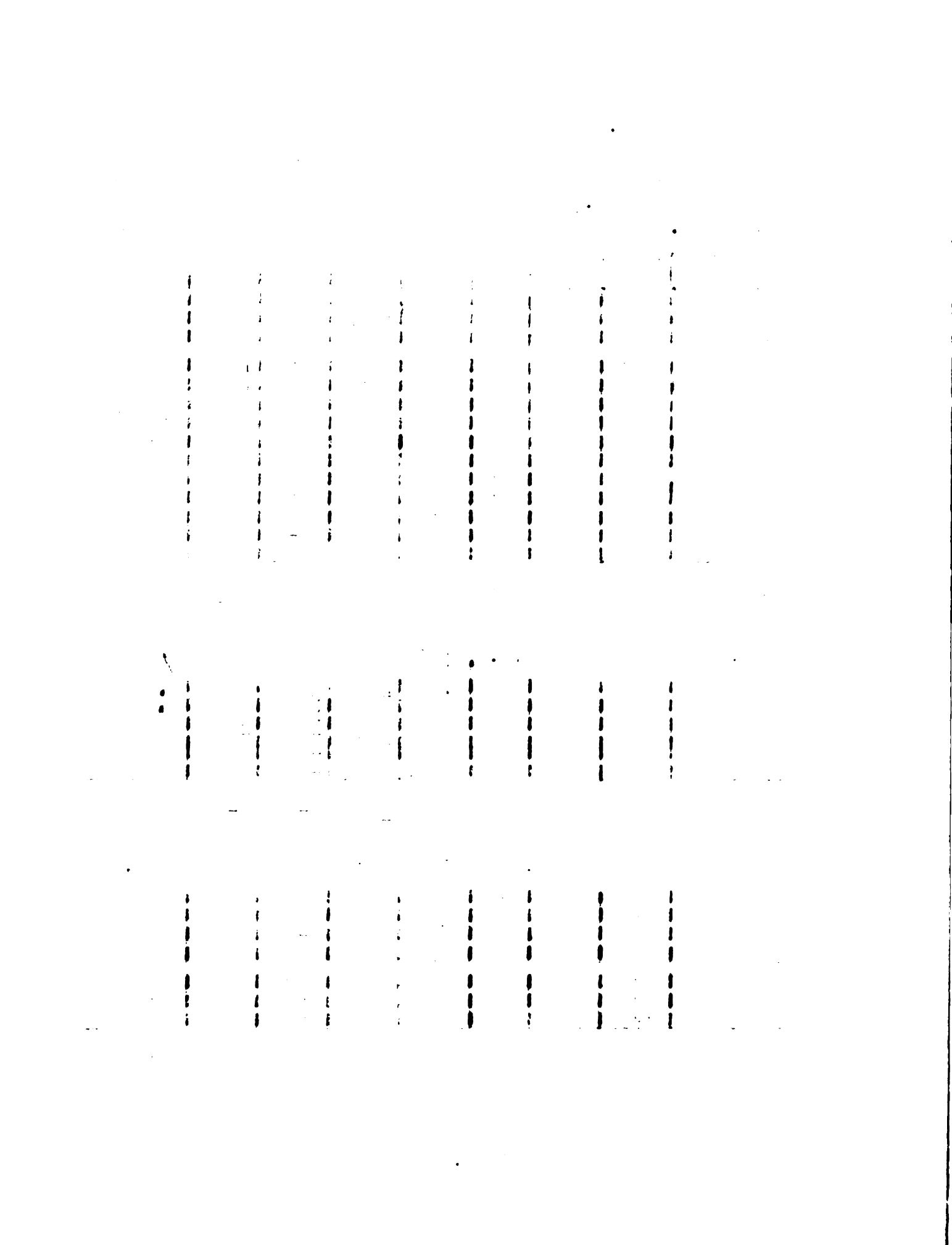


Table V Relative bactericidal power of emanations from unprotected Sterilamp and of a controlled amount of light obtained by passing the rays through a narrow opening on Esch. Coli.

Trial	Unexposed	Exposed 5 sec. without slit	Exposed 2 min with slit
1	4+	2+	2+
2	4+	2+	2+
3	4+	2+	2+
Average	4+	2+	2+

Table VI. Bactericidal Power of Emanation from a Sterilamp with a controlled amount of light obtained by passing the rays through a narrow opening on organisms dried on glass surface of petri dishes.

Trial	Time of exposure in minutes							
	0	1	2	3	4	5	6	7
		Staph. Aureus						
1	4+					+	+	
2	4+					+	3+	
3	4+					±(3)*	±(1)*	
4	4+					+	±(7)*	
5	4+					+		
6	4+					+		
Average	4+					+	+	
		Eberth. typhi						
1	4+					+		
2	4+					+		
Average	4+					+		
		Esch. coli						
1	4+	4+				+(1)*		±(1)*
2	4+	2+				-		+(87)*
Average	4+	3+				±		±
		B. sub-tilis						
1	4+					3+	+	
2	4+					3+	2+	
3	4+					3+		
4	4+					3+		
Average	4+					3+	2+	

* Number of colonies on plates.

Table VII. Bactericidal Power of Emanations from a Sterilamp on Yeasts Smeared on Agar Plates.

Trial	Time in seconds					
	0	5	10	15	30	60
	Torula rosea					
1	4+	3+	2+	2+		
2	4+	3+	2+	2+		
3	4+	3+	2+	2+		
Average	4+	3+	2+	2+		
	Sacch. ellipsoideus					
1	4+	3+	3+	2+		
2	4+	3+	3+	2+		
3	4+	3+	3+	2+		
Average	4+	3+	3+	2+		
	Sacch. cerevisiae					
1	4+	3+	3+	2+		
2	4+	3+	3+	2+		
3	4+	3+	3+	2+		
4	4+			2+	-	-
5	4+			2+	±(4)*	-
6	4+			2+	±(4)*	±(1)*
Average	4+	3+	3+	2+	±	-

*Number of colonies on plates

Table VIII. Bactericidal Power of Emanations from a Sterilamp with a controlled amount of light on molds dried on a glass surface.

Trial	Time in minutes		
	0	½	30
	Culture X6		
1	4+	4+	
2	4+	4+	
Average	4+	4+	
	Culture X3		
1	4+	4+	
2	4+	4+	
Average	4+	4+	
	Aspergillus niger		
1	4+		3+
2	4+		3+
Average	4+		3+
	Rhizopus nigricans		
1	4+		3+
2	4+		3+
Average	4+		3+
	Penicillium italicum		
1	4+		4+
2	4+		4+
Average	4+		4+

Table IX. Bactericidal Power of Emanations from a Sterilamp on Oospora lactis spread on surface of agar plates.

Trial	Time in seconds							
	0	5	10	15	30	60	120	180
1	4+	4+	4+	3+	±	-	-	-
2	4+	4+	4+	3+	-	-	-	-
3	4+	4+	4+	3+	±(12)[x]	-	-	-
Average	4+	4+	4+	3+	±	-	-	-

[x] Number of colonies on plate.

B. Action of the Sterilamp on media.

Browning and Russ (6) (1917) found that agar which has been irradiated by ultra-violet with a tungsten arc as source was not inferior as a medium for the growth of Staphylococus aureus to agar which had not been so irradiated. However, Coblentz and Fulton (9) (1924) found that irradiation of agar by ultra-violet inhibited the growth of Escherichia coli on this medium and Belford (2) (1927) corroborated this finding. Proks (2) (1933) also reported that the irradiation of media makes it less suitable for growth. Blank and Arnold (5) (1930) reported that the exposure of any one of twenty carbohydrates and three carbohydrate derivatives to rays of the wave length 2537 Angstrom units for periods from one to five hours inhibited the growth of spores of Bacillus subtilis when that carbohydrate was incorporated in the medium. They also obtained similar results for agar and for agar-water gels. Baumgartner (2) (1936) found that the irradiation of agar gels or of sucrose solution inhibited the growth of Bacillus subtilis and of Escherichia coli if these substances were incorporated in the medium. Baumgartner reported that the irradiation was accompanied by a marked production of acid, approximately one-half of which was formic. When ten ml. of sterile nutrient agar were exposed to ultra-violet at a distance of five cm. from the source for four hours, the pH dropped from 7.2 to 4.6. Neutralization of this acid restored the ability of the culture to support growth. Pratt (24) (1936) irradiated agar with a Sterilamp at a distance of four cm. for three to six hours and observed that three

hours irradiation had little effect on the growth of Bacillus subtilis spores on this agar, but that six hours irradiation almost entirely inhibited their growth. A mixture of spores and vegetative cells would still grow on agar irradiated for eight hours, and Escherichia coli, on agar irradiated for seven hours. Irradiation of one per cent glucose solutions irradiated nine hours by the Sterilamp dropped in pH from 5.8 to less than 5.

It was necessary, therefore, to prove that the action of the emanations was a direct one and was not due to changes produced in the medium on which the organisms were placed. The effect obtained from the exposure of organisms on glass surfaces served as a partial control, but aside from this two types of control experiments were run.

I. In the first of these, nutrient agar plates were exposed to the emanations of a Sterilamp at a distance of six cm. for five minutes and then inoculated in the usual way with Escherichia coli or with Staphylococcus aureus. The same amount of growth was obtained as was obtained on plates which had not been exposed.

2. Secondly, experiments were run to determine the effect of exposure on the pH of the medium exposed. A series of plates, each containing fifteen cc. of nutrient agar, pH 6.7, were exposed at a distance of six cm. At the end of five minutes and at the end of thirty minutes plates were removed and the pH tested colorimetrically. No change in pH was found.

Dextrose broth (initial pH 6.8) was exposed (in 200 cc. amounts) at a distance of six cm. and the pH tested at intervals. There was no change in pH up to eight and one-half hours, but there was a drop in pH to 6.65 at the end of thirty hours.

Table X. Bactericidal effect of agar exposed to Sterilamp for five minutes.

Trial	Unexposed	Agar inoculated after exposure	Agar inoculated before exposure
	Staph. aureus		
1	4+	4+	+
2	4+	4+	±(1)*
3	4+	4+	±
Average	4+	4+	±
	Esch. coli.		
1	4+	4+	-
2	4+	4+	-
3	4+	4+	-
4	4+	4+	-
Average	4+	4+	-

* Number of colonies on plate.

Table XL. Effect of emanations of a Sterilamp upon pH of agar.

Trial	Time of Exposure		
	0	5 min.	30 min.
1	6.7	6.7	6.7
2	6.7	6.7	6.7

Table XII. Effect upon pH of dextrose broth of irradiation by Sterilamp.

Time of irradiation	pH
0	6.8
2½ hours	6.8
8½ hours	6.8
30 hours*	6.65

* Experiment stopped at 30 hours due to marked growth of bacteria in the broth.

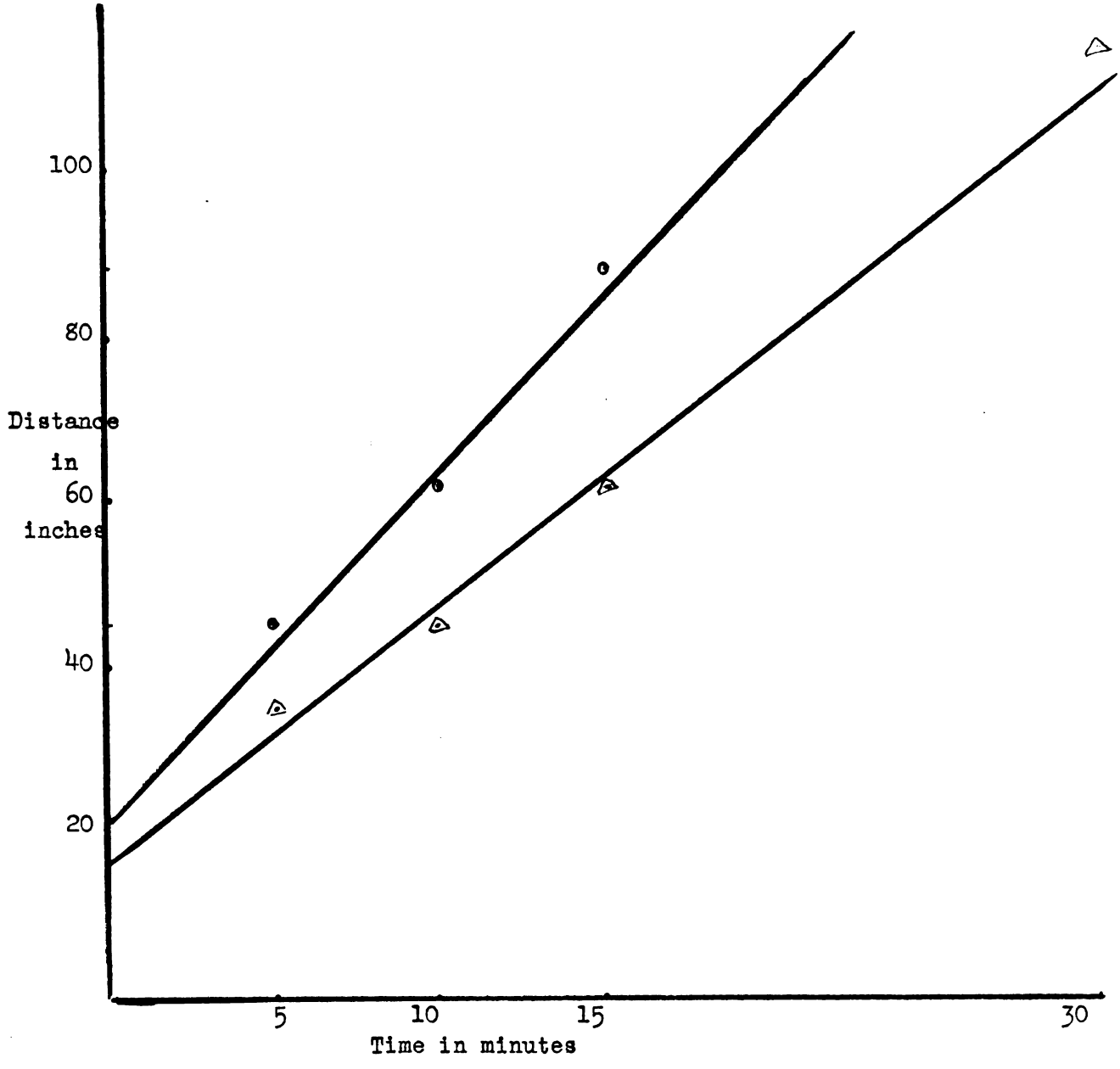
C. Practical limits of time and distance on the bactericidal power of emanations from a Sterilamp.

If the Sterilamp is to be used in the practical fields which have been suggested as possibilities, there should be some conception of the distance over which it is an effective germicidal agent and the time required for its action at varying distances. Therefore plates were exposed at varying distances for five, ten, fifteen and thirty minutes. The test organism used was Escherichia coli, and the plates were made as described on page seven. The plates were arranged so as to be as nearly as possible in a position vertical to the emanations of the lamp, and the distances were measured from the center of the plate to the circumference of the tube. It was found that if any article intervened between the lamp and the plate in such a way as to cast a shadow over a portion of the inoculated area on the plate, the bactericidal action on this portion of plate was blocked out, and the shadow effect was evident in the growth on the plate. So, evidently the bactericidal action of the Sterilamp, either through direct action of the emanations or through a residual effect on the surrounding atmosphere possesses no ability to turn corners.

At the end of five minutes exposure two out of three plates were sterilized at a distance of nineteen inches; at the end of ten minutes not more than one colony remained on any plate exposed at a distance of twenty-seven inches; fifteen minutes exposure sterilized three out of four plates at thirty-five inches, and thirty minutes exposure sterilized four out of five plates at seventy-five inches. The one plus growth appeared to be most consistent, so the distance

values at which this degree of growth was obtained were plotted against the time necessary to obtain it.

In all probability, a battery of lamps would be necessary in commercial use of the Sterilamp. Therefore, these experiments were repeated using two lamps connected in series to determine the increase in the $\frac{\text{distance}}{\text{time}}$ ratios. Distances were measured from the center of the plate to a point midway between the two lamps. Not more than two colonies developed on any plate exposed for five minutes at a distance of thirty-five inches, and less than ten colonies developed on the plates exposed for ten minutes at forty-five inches and fifteen minutes at eighty-one inches. Results showed a marked increase in the distance over which a strong bactericidal effect was obtained in a given time, but the bactericidal effectiveness was not doubled by the use of two lamps.



EFFECT OF TIME AND DISTANCE ON BACTERICIDAL

POWER OF EMANATIONS OF THE STERILAMP

△ ONE LAMP

○ TWO LAMPS

Table XIII. Effect of time and distance on the bactericidal power of the emanations of one Sterilamp (as shown) on Escherichia coli.

Distance from lamp	Trial 1	2	3	4	5
	5 minutes exposure				
19 in.	-	±(1)*	-		
27 in.	±(10)*	+(15)*	±(8)*		
35 in.	+(50)*	+(25)*	±(5)*		
Unexposed	4+	4+	4+		
	10 minutes exposure				
19 in.	±(1)	-	-		
27 in.	±(1)	±(1)	-		
35 in.	±(2)	±(1)	±(1)		
45 in.	+(32)	+(30)	+(38)		
Unexposed	4+	4+	4+		
	15 minutes exposure				
19 in.	-	-	-	-	
35 in.	±(1)	-	-	-	
45 in.	±(3)	±(2)	±(6)		
62 in.	+	+	+		
Unexposed	4+	4+	4+	4+	
	30 minutes exposure				
5 ¼ in.	-	-	-	-	-
17 in.	-	-	-	-	-
33½ in.	-	-	-	±(1)	-
75 in.	-	-	-	+	-
93 in.	±(7)	±(2)	±(4)	±(10)	-
115 in.	+(37)	+(16)	+(20)	+(25)	+(34)
173 in.	2+	2+	2+	2+	2+
Unexposed	4+	4+	4+	4+	4+

* Number of colonies on plates

Table XIV Effect of time and distance on the bactericidal power of the emanations of two Sterilamps as shown on Escherichia coli.

Distance from lamp	Trial 1	2	3	4
	5 minutes exposure			
19 in.	-	-	-	
35 in.	±	-	± (2)*	
45 in.	±(15)	+(14)	+(29)	
Unexposed	4+	4+	4+	
	10 minutes exposure			
19 in.	±(1)	-	-	
27 in.	±(4)	± (2)	-	
35 in.	±(3)	±(1)	-	
45 in.	±(4)	±(6)	+(9)	
62 in.	±(28)	+(11)	+(34)	
unexposed	4+	4+	4+	
	15 minutes exposure			
27 in.	±(1)	-	-	
33 in.	±(1)	±(2)	-	±(1)
81 in.	+(4)	±(8)	±(4)	±(3)
88 in.	2+	+	+	
114 in.	2+	2+	2+	2+
unexposed	4+	4+	4+	4+

* Number of colonies on plate

D. Penetrability of various mediums to the emanations of the Sterilamp.

As ultra-violet irradiation has been a suggested means for the sterilization of both milk and water, whole milk and saline were two of the liquids selected for this study. The third, broth, was selected as a clear liquid containing organic matter. Escherichia coli was the test organism, and the cultures were prepared as on page seven in deep culture dishes. The cultures were covered with liquid to the desired depth and exposed for five minutes to the rays of the Sterilamp, which was placed six cm. above the level of the liquid. The liquid was carefully decanted, and the plates incubated for twenty-four hours at 37°C. Two sets of control plates were run for each series, one which was not exposed and one which was exposed without a liquid covering.

There appeared a marked bactericidal effect through a thin film of broth, and a noticeable one through a one mm. layer, but here penetration of the ultra-violet rays appeared to stop. Through whole milk there was found no evidence of any penetration. The rays penetrated well through saline, however, sterilizing two out of four plates which had been covered with saline to a depth of two cm.

Table XV Power of penetration of bactericidal emanations from a Sterilamp through various mediums with five minute exposure.

Trial	Depth of broth					
	Control	none	thin film	1mm	2mm	3mm
1	4+	+	+	2+	4+	4+
2	4+	-	+	3+	4+	4+
3	4+	-		3+	4+	4+
4	4+	-	4+	4+	4+	4+
5	4+	-	+	2+	4+	
6	4+	+(1)*	+	4+	4+	
7	4+			4+	4+	
8	4+			4+		
9	4+			4+		
10	4+			2+		
11	4+			2+		
12	4+			4+		
13	4+			4+		
14	4+			4+		
15	4+			3+		
Average	4+	-	+	3+	4+	4+

	Depth of milk					
1	4+	-	4+	4+		
2	4+	-	4+	4+		
3	4+	-	4+	4+		
Average	4+	-	4+	4+		

	Depth of saline						
		.5cm	1cm.	2cm	3cm	3.5cm	4cm
1	4+	-	-	-			
2	4+	-	-	-			
3	4+			±(2)	±(3)		
4	4+		-	+	±(1)	±(1)	+
5	4+					+	+
6	4+					+	-
Average	4+	-	-	±	±	+	-+

* Number of colonies on plate.

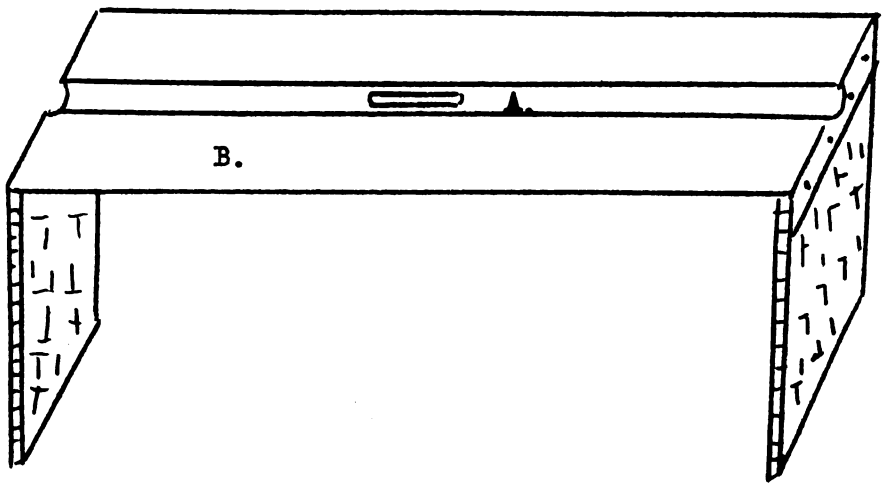
E. Comparison of the bactericidal power of the Sterilamp and that of a quartz tube.

In order that a fair comparison of the two types of lamp, which were very different in size, might be made, the amount of light to which the plates were exposed was controlled by passing the rays through the narrow opening in the metal shield described on page ten. Consequently the comparison is of the effectiveness of the emanation from the same surface area of each lamp, and not of each lamp as a complete unit. The organisms used were Escherichia coli, Staphlococcus aureus, Eberthella typhi, Mycobacterium phlei, and Bacillus subtilis, and upon three of these organisms there was shown a slightly greater bactericidal effect by the quartz lamp than by the Sterilamp. However, the Sterilamp demonstrated a greater inhibitative effect upon Mycobacterium phlei than did the quartz tube, and against Bacillus subtilis, the two lamps appeared to have about the same degree of effectiveness.

In the spectra of the two lamps there is slightly more dispersion about some lines in the spectrum of the Sterilamp than about the corresponding lines of the smaller tube, notibly about the most intense line, that at 2537 Angstrom units. There is a pronounced band in both spectra at 2485 Angstrom units and at 2655, and a series of lines between 2810 and 2840 Angstrom units which are a little more intense in the spectrum of the Sterilamp. The differences are so slight, however, that the spectra can be considered nearly identical. Apparently the chief advantage of the Sterilamp would lie in its much greater length.

The comparison of bactericidal effectiveness as carried out in this experiment suggests a method of standardization of measurement of the bactericidal power of ultra-violet ray producing tubes, using a standard tube as reference. The length of time required to obtain either complete sterilization or a certain degree of inhibition of growth by the emanations of a tube as compared to the time required by the reference tube can easily be determined. Accurate results can be obtained by preparing the plates carefully according to a standard technique such as that described on page seven. By so controlling the amount of light, the bactericidal effectiveness of the ultra-violet radiation can be determined without having as a variable the size and length of the tube.

Table XVI shows a comparison of three Sterilamps obtained in this way. It will be noted that there is a greater variation in results obtained for the same tube on different occasions than in those obtained for the three lamps on the same trial. This might be attributed to a difference in the density of the suspensions used on different dates.



Shield

A. Slit

B. Metal shield

C. Wooden support

Table XVI Comparative bactericidal power of emanations from a Sterilamp (1) and from a quartz tube (2) with a controlled amount of light obtained by passing the rays through a narrow opening.

Lamp	Trial	Time of exposure in Minutes					
		0	1	2	3	4	6
		Esch.	coli				
(1)	1	4+			2+		
	2	4+			2+		
	3	4+	4+	3+		3+	+
	4	4+	4+	3+		3+	+
	5	4+	4+	3+		+	+
	6	4+	4+	3+			
(2)	1	4+			+		
	2	4+			+		
	3	4+	2+	+		± (1)*	± (5)
	4	4+	2+	+		± (5)	± (5)
	5	4+	2+	+		±	± (8)
	6	4+	2+	± (10)		± (3)	±
Average (1)		4+	4+	3+	2+	2+	+
(2)		4+	2+	+	+	±	±

*Number of colonies on plate.

Table XVI Continued

Lamp	Trial	Time of exposure in minutes			
		0	1	2	3
		Staph. aureus			
(1)	1	4+			+
	2	4+			+
(2)	1	4+			-
	2	4+			-
Average (1)		4+			+
Average (2)		4+			-
		E. typhi			
(1)	1	4+			±
	2	4+			-
(2)	1	4+			-
	2	4+			-
Average (1)		4+			±
Average (2)		4+			-
		Myco. phlei			
(1)	1	4+			+
(2)	2	4+			2+
Average (1)		4+			+
Average (2)		4+			2+
		B. subtilis			
(1)	1	4+			3+
	2	4+			3+
(2)	1	4+			3+
	2	4+			3+
Average (1)		4+			3+
Average (2)		4+			3+

Table XVII Comparison of three Sterilamps with a controlled amount of light obtained by passing the rays through a narrow opening on organisms smeared on the surface of agar plates.

Tube	Trial	Time of exposure in minutes				
		0	3	4	5	6
843	1	4+	2+	+	+	
	2	4+		2+	+	+
	3	4+		±	±	∓
876	1	4+	2+	+		
		4+	2+	+		
	2	4+			+	
	3	4+		+		
937	1	4+			+	
	2	4+			+	+

Summary and Discussion

(A)

It was found that, in spite of claims to the contrary, exposure of the eyes to the emanations of the Sterilamp for more than a very few minutes caused a painful inflammation of the eyes. Therefore, it is not advised that the Sterilamp be used at any time unless the eyes are protected.

Of the non-spore-forming organisms exposed, no plate showed more than ten colonies after fifteen seconds exposure to the Sterilamp with the exception of Mycobacterium phlei, which appeared slightly more resistant. Staphylococcus aureus was killed in slightly less time and Eberthella typhi in a slightly greater than that reported by Drs. Rentschler and James. The instantaneous effects reported to the newspapers and popular magazines were conspicuously absent.

While fifteen seconds exposure had an appreciable lethal effect on spore-forming organisms, thirty seconds exposure was necessary to obtain the same degree of killing obtained by fifteen seconds exposure of non-spore-forming bacteria. A culture of Bacillus mycoides which contained spores was found to be more resistant than one which did not.

The Sterilamp when partially shielded gave the same relative results except with Mycobacterium phlei, which did not show as high a degree of resistance as formerly.

When the bacteria were exposed on dry plates, no marked difference in results was obtained. Staphylococcus aureus, E. typhi, and B. subtilis were slightly more resistant; the

results with E. coli were approximately the same.

The results obtained from the exposure of yeasts were comparable to those obtained with spore-forming-bacteria. There was a marked lethal effect at the end of ten seconds exposure, and nearly all of the organisms were killed by thirty seconds exposure. The pigmented yeast was not found to be more resistant than the unpigmented.

Thirty minutes exposure was necessary to produce any lethal effect on the molds exposed, with the exception of Oospora lactis. The extent of the lethal effect was very slight then although this is a much longer period than that reported by Rentschler and James. If the molds were irradiated continuously, mold growth was completely inhibited. Oospora lactis showed about the same degree of resistance as the spore-forming bacteria.

(B)

It was found that nutrient agar exposed to the unshielded Sterilamp for five minutes was not inferior to unexposed agar for the support of bacterial growth and that there was no change in pH after one half hour's exposure. The pH of dextrose broth was not affected by eight and one-half hour's exposure, but dropped slightly at the end of thirty hours. These time periods were much longer than any periods of exposure in this work; so the bactericidal effect must have been direct rather than because of any change in the media.

(C)

The intervention of any article between the plate and the lamp stopped bactericidal action; so there is apparently no

residual effect of the emanations on the air surrounding the plate, and any article to be sterilized should be in a direct line with emanations from the Sterilamp without intervening objects. When one Sterilamp was used, reduction to one plus growth was obtained at thirty-five inches on plates exposed five minutes, at forty-five inches after ten minutes, at sixty-two after fifteen minutes, and at one hundred and fifteen after thirty minutes. The bactericidal action obtained by the use of two lamps, connected in series, while much greater, was not correspondingly twice as great.

(D)

Although many of the uses which have been suggested to the public would presuppose some degree of penetration by the rays of the Sterilamp into opaque materials, this ability was not proven. Through whole milk there was no evidence of any penetration; through broth penetration stopped at one mm. Complete sterilization was obtained, however, through saline with a depth of two cm.

(E)

In comparing the germicidal effect of the emanations of the Sterilamp with those of a quartz tube, little difference was found. The emanations from the same surface area of the quartz tube as of the Sterilamp were more bactericidal for some organisms. The spectra were nearly identical.

A suggested method for controlling the amount of light to which plates are exposed and for comparing ultra-violet ray producing tubes is by means of a metal shield in which there is a small slit through which the rays pass. With this at a given distance from the plate, a standard method of preparing plates should be used. Much of the confusion because of varied results obtained by different workers

is due to the variations in the technique of exposure.

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