

preparations, and the fact that they occur in fibers other than those carrying the centrifugal motor volley, point to the synchronously active muscle as the causal agent. The suggestion may be advanced that the action potential of the excited muscle is capable of stimulating intramuscular motor and sensory nerve endings.

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**Bactericidal Effect of Ultraviolet Rays on Non-Spore Forming Bacteria and Mold Spores.**

F. W. TANNER AND J. W. APPLING.

*From the Department of Bacteriology, University of Illinois, Urbana.*

Fellers<sup>1</sup> and Garret and Arnold<sup>2</sup> reported effective sterilization of drinking glasses by ultraviolet light produced by the "Sterilamp". Owing to the ease with which such objects can be exposed, this means of sterilization has many advantages. The "Sterilamp" was employed in this investigation as the source of ultraviolet light. In one series of experiments exposures of drinking glasses were made in the Steril-Ray Cabinet, in which 4 Sterilamps were used. For the remainder of the experiments, results of which are reported in this investigation, a laboratory cabinet, fitted with 2 Sterilamps, was used. The inside measurements of this cabinet were 14 x 14 x 10.5 inches and it was lined with polished aluminum foil. The shelf was made of one-half inch mesh screen and placed about 8 inches from the lower surface of the Sterilamps.

The first experiments were carried out with suspensions of bacteria and mold-spores in Petri dishes. Films of nutrient broth cultures were made on the bottoms of sterile Petri dishes as suggested by Ross.<sup>3</sup> Agar medium in Petri dishes was also sprayed with an atomizer. Another method of inoculation was to rinse the containers with a suitable suspension of the bacteria. In order to study the lethal effect of ultraviolet light on non-sporulating bacteria and on mold-spores, one ml portions of the suspensions were placed in sterile Petri dishes, exposed definite times to the light, and finally melted agar was added to the exposed suspensions. The most satisfactory method for inoculation consisted of rinsing the drinking

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<sup>1</sup> Fellers, R. V., *The American City*, August, 1938.

<sup>2</sup> Garret, O. F., and Arnold, R. B., *Milk Plant Monthly*, August, 1938.

<sup>3</sup> Ross, Virginia L., M.S. Thesis, University of Michigan, 1938.

glasses with the suspension of bacteria and draining for about one minute after which the receptacles were shaken to remove the greater part of the liquid. Glasses were kept covered with parts of sterile Petri dishes except when being exposed to the ultraviolet light.

The following non-sporulating bacteria were used in the preliminary work: *Escherichia coli* (Frank strain), *Escherichia coli* (American Type Culture Collection No. 4348), *Staphylococcus aureus*, and *Serratia marcescens*. The results of a semi-quantitative study using various dilutions of *Escherichia coli* (Frank strain), which were exposed in the laboratory cabinet, are given in Table I. These results indicate clearly the relation of the initial concentration to the time required to kill all of the organisms. Too little importance has been given to this factor. However, if the initial numbers are made comparable, then the relative resistance of different bacteria and mold-spores to exposure may be compared. In this way it was found that order of decreasing resistance to ultraviolet light for the bacteria studied was: *Staphylococcus aureus*, *Escherichia coli* (Frank strain), *Escherichia coli* (A. T. C. No. 4348), and *Serratia marcescens*.

TABLE I.  
Effect of Concentration of *Escherichia coli* (Frank strain) to Exposure to Ultraviolet Light. (Laboratory cabinet.)

Approx. initial conc. of <i>Escherichia coli</i>	Growth after exposures of: (min.)						
	0	0.5	1	2	3	4	5
12 x 10 <sup>6</sup>	++++	+++	+	+	+	30	3*
12 x 10 <sup>4</sup>	+++	++	0	1	0	0*	4*
12 x 10 <sup>3</sup>	++	40	1	0	0	0*	30
12 x 10 <sup>2</sup>	+	0	0	0	0	0	1

\*Colonies unlike those of *Escherichia coli* were present.

Exposures of mold-spores in suspensions showed the same general results as were obtained with the bacteria tested. Four different molds were used, namely, *Aspergillus glaucus*, *Aspergillus niger*, a Mucor, and a Penicillium. Some difficulty was experienced at first in the preparations of suspensions free from clumps of spores and from vegetative cells; however, the following procedure gave excellent results. Dextrose-agar-slant culture (8 to 16 days old) was flooded with salt solution, shaken, and, in most cases, the growth gently rubbed with the tip of a sterile wire. This suspension was poured into a sterile tube and the process repeated using, in all, 10 ml of salt solution. The suspension was mixed thoroughly by shaking vigorously and then filtered through 12 thicknesses of

sterile cheesecloth. It was found necessary to wet the filter with about 5 ml of salt solution before pouring the spore-suspension on it in order not to lose most of the suspension on the filter. The suspension was refiltered through the same filter (2 or 3 times) until examination with the microscope showed it to be free from clumps and vegetative cells. After counting the spores (Neubauer chamber) the suspension was diluted sufficiently to give readily countable plates on the controls. One ml portions were exposed in each Petri dish. The results obtained in one series of exposures are given in Table II. It will be noticed that the effect of irradiation was either partial or complete depending upon the initial concentration of spores. As the concentration decreased, the effectiveness of the irradiation as a sterilizing agent increased.

TABLE II.  
Effect of Ultraviolet Light upon Mold-Spores in Suspensions. (Laboratory cabinet used)

Mold used	Growth estimates after 48-hr incubation, room temperature.								
	Exposure time in min.								
	0	1	2	3	4	5	6	7	8
<i>Aspergillus glaucus</i>	++++								
Dilution 1:100	(10,000)*	+++	++	150	200	200	100	100	
1:10,000	100	90	5	3	2	3	0	0	
<i>Aspergillus niger</i>	++++								
Dilution 1:100	(40,000)*	+++	+++	++	+	200	200	130	
1:10,000	400	130	15	2	0	1	1	1	
<i>Mucor</i>									
Dilution 1:100	++++	+++	30	10	8	3	4	5	
1:10,000	+++	+++	30	2	4	1	2	0	
<i>Penicillium</i>	++++								
Dilution 1:10	(330,000)*	+++	++	+	+	300		300	200
1:1,000	(3,300)*	40	5	6	1	1		0	0
1:10,000	330	4	0	0	0	0		0	0

\*Figures obtained from count at 1:10,000 dilution.

In another series of exposures it was found that when the spores had germinated before exposure, a much greater resistance was evident. Examination with the microscope of the inoculated agar surface after 18 hours of incubation at room temperature revealed no mycelia in the cases of *Aspergillus glaucus* and the *Penicillium*; however, in the experiment with *Aspergillus niger* and the *Mucor* mycelia were present. From these observations the penetration of the hyphæ into the agar seems to have afforded a considerable degree of protection.

Experiments were also carried out to determine the lethal effect of ultraviolet rays on mold-spores present on paper board. Paper

and paper board have been suggested as the cause of mold spoilage of certain foods packaged in these materials. Increasing use of these materials has made it desirable to determine whether the contaminating microorganisms can be eliminated by irradiation with ultraviolet rays. Strips of cardboard (12 x 65 mm), such as is used for food containers, were sterilized in an autoclave. Each strip was inoculated separately by immersion for approximately 2 minutes in a suspension of mold-spores. They were then removed with sterile forceps, allowed to drain, and kept a short time in a sterile tube. The suspension of spores, practically free of clumps and vegetative cells, was prepared as previously described. By the method of inoculation which was employed it seems probable that many spores were introduced into the fibers.

The strips were exposed in pairs by inserting the ends of each strip into the wire mesh of the shelf in the laboratory cabinet. By this procedure the actual contact between strip and shelf was minimal. In addition, indirect exposure of the under side of the strip was more satisfactorily obtained. After a given exposure the strip was removed and placed in about 5 ml of melted dextrose-agar which had just been poured into a sterile Petri dish; following this another 5 ml of melted dextrose-agar was evenly distributed over the strip. As a result of this procedure, the strip was completely coated with agar. Incubation at room temperature for 48 hours was found to be satisfactory for making estimates of colony numbers. Check estimates made after 72 hours of incubation were difficult to evaluate. However, the results appeared to agree with findings obtained after 48 hours' growth. Table III gives the results of one such series of exposures and it shows clearly the ineffectiveness of ultraviolet light. Incomplete sterilization resulted in the case of *Aspergillus niger*. The fact that the under side of the strips frequently showed many more colonies than did the upper side indicated that the indirect exposure was much less effective than the direct one.

TABLE III.  
Effect of Sterilamps on Mold-spores on Cardboard Exposed in the Laboratory Cabinet.

Incubation of (hr)	Estimates of spores per strip after exposure of : (min.)															
	0		0.5		1		2		3		4		5		10	
Mold used	48	72	48	72	48	72	48	72	48	72	48	72	48	72	48	72
<i>Aspergillus glaucus</i>	55	85	43	*50	31	*34	16	*26*	6	*23*	7	*14*	3	* 3*	1	* 1*
<i>Aspergillus niger</i>	40	47	28	27	13	17	12	*15*	3	*15*	5	*11*	6	*13*	6	* 7*
Mucor	8	8	9	9	3	3	0	0	0	0	0	0	0	0	0	0
Penicillium	80	**	21	*27*	26	*36*	2	*10*	3	* 5*	3	* 4*	4	* 6*	0	1*

\*Most of colonies on under side of strip.

\*\*Too crowded to enumerate.

*Summary.* The results secured in these investigations show that the initial number of cells influences survival of microörganisms exposed to ultraviolet light. Dilute suspensions are destroyed more quickly than heavy ones. The order of decreasing resistance for the species used was: *Staphylococcus aureus*, *Escherichia coli* (Frank strain), *Escherichia coli* (American Type Collection No. 4348), and *Serratia marcescens*. For the mold-spores studied the order of decreasing resistance was: *Aspergillus glaucus*, *Aspergillus niger*, *Penicillium*, and *Mucor*.

Cardboard strips, which had been inoculated by immersion in a suspension of mold-spores, were not as thoroughly sterilized as were the suspensions themselves, when exposed to ultraviolet light in Petri dishes. On many of the strips it was possible to determine that the colonies originated from the under side of the strip more frequently than from the upper side. This was interpreted as indicating that indirect irradiation is much less effective than direct irradiation.

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#### **Bactericidal Effect of Ultraviolet Rays on Microorganisms on Restaurant Glass-Ware.**

J. W. APPLING AND F. W. TANNER.

*From the Department of Bacteriology, University of Illinois, Urbana.*

To study the bactericidal effect of irradiation with the Sterilamp on drinking glasses which had been used at soda fountains, it was necessary to select or devise a method of sampling the bacteriological contamination of the glasses. The multiple glass test proposed by the American Public Health Association<sup>1</sup> was used whenever a series of glasses from a soda fountain was examined. Other procedures were followed including the method used by Speck and Black.<sup>2</sup> In addition, a supplementary method was devised which permitted examination of the entire inner surface of the glass as well as the rims and proved to be simpler than that of Speck and Black. About 5 ml of a 25 ml water blank were poured into the drinking glass to be sampled and the entire inner surface of the glass was wetted and rubbed by means of a sterile cotton swab. The swab was then broken

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<sup>1</sup> Am. Pub. Health Assn., Yearbook, 1936-1937, p. 48.

<sup>2</sup> Speck, M. L., and Black, L. A., *Food Research*, 1937, **2**, 559.