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Residual Germicidal Action of Water and Plain Agar After Exposure to Ultraviolet Light.

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Downes and Blunt[1] stated that the most active rays on microorganisms were blue, violet and ultraviolet, the red and orange were not entirely inactive. Ward and Lodge,[2] using the electric arc, stated that ultraviolet light alone had a much more powerful bactericidal action. Parkinson[3] stated that action was most vigorous on first exposure and that the number of survivors did not bear a constant relation to the number originally present. Cernovodeanu and Henri[4] and de Voogt[5] stated that the rate of destruction was almost proportional to the square of the duration of exposure.

Materials which have been exposed to ultraviolet light, have been said to retain a bactericidal property. This paper presents the results of an investigation of that subject. Coblentz,[6] Bedford,[7] Norton,[8] and Walker and Pryer[9] reported what was called a residual germicidal action of water which had been exposed to ultraviolet light. Coblentz exposed nutrient agar plates to ultraviolet light and found that the exposed agar caused appreciable mortality of *Escherichia coli.* Bedford stated that radiation of media before inoculation inhibited the growth of microorganisms to a varying degree. Norton observed a lower count in radiated water but was never able to kill all of the bacteria, even when the count was as low as 100 per cc. He could observe no action after an hour. Walker and Pryer believed that a residual action was imparted to water by the light.

In our experiments 10 organisms were used: *Serratia marcescens, Escherichia coli, Escherichia acidi-lactici, Bacillus alcaligines, Micrococcus epidermis, Bacillus megatherium, Micrococcus roseus, Bacillus subtilis, Micrococcus varians,* and *Bacillus vulgatus.* To test the

[1] Downs, A., and Blunt, T. P., *Proc. Roy. Soc.*, 1877, **26**, 488.

[2] Ward, H. M., *Phil. Trans. Roy. Soc. London*, 1894, **185**, 961.

[3] Parkinson, N. F., *Ann. Rept. Prov. Bd. of Health*, Ontario, Can., 1914, **33**, 156.

[4] Cernovodeanu and Henri, *Comp. Rend.*, 1910, **149**, 365.

[5] Voogt, J. G. de, *Z. Hyg.*, **81**, 62.

[6] Coblentz, W. W., and Fulton, H. R., *U. S. Bur. of Stand. Sci. Papers*, 1924, **19**, 495.

[7] Bedford, T. H. B., *Brit. J. Exp. Path.*, **8**, 437.

[8] Norton, *A. J. P. H.*, 1928, 476.

[9] Walker and Pryer, *A. J. P. H.*, 1921, **2**, 703.

direct action of the light, agar plates were streaked with each of 10 organisms and exposed immediately to ultraviolet light for 6 minutes, half of the plate being covered with cardboard. For testing the residual action agar plates were poured, half of each plate radiated, and both halves of the plate streaked with the test organisms. Three sets of plates were made; one set was exposed for 6 minutes, another for 30 minutes, and the third for 60 minutes. All plates were incubated for 24 hours at 37°C., after inoculation.

Exposure of agar for 6 minutes to ultraviolet light did not impart residual germicidal property for the microorganisms used. Exposure for 30 minutes caused the agar to possess slight germicidal action for *Escherichia coli, Micrococcus epidermis,* and *Micrococcus roseus,* and a marked action for *Bacillus subtilis, Bacillus megatherium,* and *Bacillus alcaligines.* The other microorganisms seemed to be unaffected. Exposure of the agar for 60 minutes produced the same results, except that *Escherichia coli* was somewhat more inhibited by exposure for 60 minutes than for 30 minutes.

For studying the question with water, attempts were made to use a large and small number of bacteria. Fifty cc. of distilled and tap water were exposed in an 8-inch petri dish. An exposure of 6 minutes was found to be just as efficient as one of 30 minutes or longer. The water was at a distance of 25 cm. and varied in temperature between 36° and 42°C. The exposed water was transferred to sterile flasks by sterile pipettes. Nine cc. of the water were inoculated with 2 dilutions of the cultures and plates prepared at regular intervals. Control plates were made with water sterilized by passage through a Seitz filter. Some of the microorganisms died as quickly in the control water, which had not been treated with ultraviolet light, as in that which had been treated. This was the case with *Serratia marcescens, Escherichia coli, Escherichia acidi-lactici, Micrococcus varians,* and *Bacillus vulgaris. Bacillus subtilis* died rapidly in both waters, but showed a slightly higher death rate in the radiated water. The other species used showed a distinctly higher mortality in the radiated water.

Why the 5 species died so rapidly in the plain water was not studied experimentally. It may have been due to a toxic factor in the water or to some physical factor such as plasmoptysis.

An attempt was then made to study this question with yeasts. Several investigators have shown that ultraviolet light is toxic for yeasts. (Tanner and Feuer[10]; Lindner[11]; Nadson and Philippov.[12])

[10] Tanner and Feuer, *J. Ind. Eng. Chem.,* 1920, **12**, 740.

[11] Lindner, *Woch. Brau.,* 1922, **39**, 166.

[12] Nadson and Philippov, *Comp. Rend. Soc. Biol.,* 1928, **366.**

The last mentioned investigators showed that ultraviolet light when administered under the proper conditions stimulated growth and caused increased budding.

For studying the residual bactericidal action of radiated water for yeasts, 3 species were used: *Willia anomala, Saccharomyces ellipsiodeus* and *Pichia polymorpha.* They were handled in the same manner as described above for the bacteria. No residual germicidal property could be established for these 3 species. They died just as rapidly in the control water as in that which had been radiated.

Conclusions. If water and other materials exposed to ultraviolet light retain a bactericidal property, it varies greatly for different organisms. There is some doubt in the authors' minds as to whether the mortality of certain bacteria in radiated water is really due to a toxic factor contributed by the ultraviolet light.

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Is Ferratin Precipitinogenic?

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In 1893 Schmiedeberg[1] announced that it was possible to extract a protein from the liver by means of boiling water. The extract was filtered and the filtrate treated with tartaric acid for precipitation of the protein. Schmiedeberg concluded that this was a high iron containing protein but not a nucleoprotein. He named it ferratin. Subsequent investigators,[2] however, came to the conclusion that the protein extracted in this fashion was really a nucleoprotein, or derived from nucleoprotein.

Since by repeated extraction and precipitation it seemed probable that ferratin could be completely separated from the accompanying blood and lymph proteins, it seemed advisable to test its antigenic properties.

Our first experiment was unsuccessful and new ferratin was prepared with particular care to avoid its exposure to any undue hy-

[1] Schmiedeberg, *Arch. f. exp. Path. u. Pharm.*, 1893, **33**, 101.

[2] Beccari, cited from *Malys Jafresber*, 1902, **32**, 494. Wohlgemuth, *Z. f. Physiol. Chem.*, 1902, **37**, 474, and 1904, **42**, 519. Scaffidi, *Z. f. Physiol. Chem.*, 1907, **54**, 448. Salkowski, *Z. f. Physiol. Chem.*, 1908, **58**, 282.