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Comparison of Chlorine and Ozone as Virucidal Agents of Poliomyelitis Virus.*

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Levaditi, Kling and Lépine,[1] upon finding that 0.4 ppm of chlorine inactivated poliomyelitis in 24 hours, assumed that chlorination of water by the methods usually employed for purification of drinking water, was adequate for destruction of the virus.

As a result of numerous recent observations[2-7] in which poliomyelitis virus has been recovered from sewage, from stools of active cases and from stools of carriers, Kempf and Soule[8,9] reopened investigations on the subject of the virucidal effects of chlorine on this virus. They found that concentrations of 0.5 ppm of chlorine failed to inactivate the MV strain of poliomyelitis virus in 1:1650 dilution in 1½ hours at a temperature of 21-24°C and a pH of 8.5. Trask and Paul[10] likewise report that one strain with which they worked was resistant to chlorine.

Studies in this laboratory[11] on the comparative germicidal effects of chlorine and ozone on cysts of *Endamoeba histolytica* and on certain bacteria, showed ozone to be the more active agent. It was, therefore, decided to compare ozone and chlorine as virucidal agents of poliomyelitis virus.

Materials and Methods. Two strains of virus were used during the experiments. The MV, an extensively used experimental strain, was employed in the first experiment and Le, a strain recently isolated in Los Angeles, in the second and third experiments. At the time of the experiments, the pool of MV virus used produced symptoms in 100% of the animals inoculated at a titer of 10^{-1} while the pool of Le virus did the same at a titer of 10^{-3}.

The virus to be tested was diluted in saline solution, placed in test tubes and treated as follows:

1. *Chlorine.* In the chlorination tests each tube contained 15 cc of virus and chlorine consisting of 13½ cc of virus and 1½ cc of sodium hypochlorite or of gaseous chlorine solution of sufficient strength to give the desired terminal residual. Tubes were corked and agitated immediately upon mixing. They were shaken at 2-minute intervals during the period of the experiment. Each tube constituted a unit for each time period to be appraised, pH and residual values being determined at the end of each period. Tubes were kept in a constant temperature bath during the experiment.

2. *Ozone.* Ozonized air was bubbled through 50 cc of the mixture to be tested, a suitable diffusor carrying the ozone to the bottom of the tube. Amounts of virus mixture necessary to test pH and residual ozone and for inoculation into animals were removed at the required

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1 Levaditi, C., Kling, C., and Lépine, P., *Bull. Acad. de Méd.*, Paris, 1931, **105**, 190.

2 Paul, John R., Trask, J. P., and Culotta, C. S., *Science*, 1939, **90**, 258.

3 Trask, James D., Paul, John R., and Vignec, A. J., *J. Exp. Med.*, 1940, **71**, 751.

4 Howe, Howard A., and Bodian, David, *J. Infect. Dis.*, 1940, **66**, 198.

5 Kramer, S. D., Gilliam, A. G., and Mollner, J. G., *Pub. Health Rep.*, U. S. P. H. S., 1939, **54**, 1914.

6 Piszczek, E. A., Shaughnessy, H. J., Zichis, J., and Levinson, S. O., *J. A. M. A.*, 1941, **117**, 1962.

7 Kessel, John F., Moore, Frederick J., Stimpert, F. D., and Fisk, R. T., *J. Exp. Med.*, 1941, **74**, 601.

8 Kempf, J. Emerson, and Soule, Malcolm H., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 431.

9 Kempf, J. Emerson, Wilson, Martha F., Pierce, Marjorie E., and Soule, Malcolm H., *Am. J. Pub. Health*, 1942, **32**, 1366.

10 Trask, James D., and Paul, John R., *Am. J. Pub. Health*, 1941, **31**, 239.

11 Kessel, John F., Quiros, Maria, Allison, Donald K., and Kaime, M., manuscript.

TABLE I.
Summary of Inoculation Experiments. Initial pH 6.9. Temperature 20°C.

Exposure times	7½ min	15 min	30 min	60 min	120 min
Exp. No. 1, MV virus 1:10. Sept. 30, '42					
Ozone*	2/2†	2/0	1/2	—	—
Controls					8/8
Exp. No. 2, Le virus 1:1000. Nov. 18, '42					
Ozone*	0/2	0/2	0/2	0/2	0/2
Controls					2/2

Exp. No. 3, Le virus. Dec. 27, '42						
Exposure times	2 min	5 min	15 min	45 min	90 min	180 min.
Controls, virus 1:1000						
Without thiosulfate						3/3
With thiosulfate						3/3
Ozone—Residual 0.05—0.45 ppm						
Virus 1:10			3/3	1/3		
" 1:100			1/3	0/3		
" 1:1000	0/3	0/3	0/3	0/3		
Chlorine—Virus 1:1000						
Gaseous						
Residual 0.25-0.3 ppm			2/3			0/3
" 0.5-1.0 "		2/3	1/3	1/3	1/3	0/3
Sodium hypochlorite						
Residual 0.5-1.0 ppm			2/3	1/3	1/3	0/3

* Residual of ozone in ppm did not exceed 0.2 in any test.
† Numerator—Animals developing paralytic poliomyelitis. Denominator—No. of animals inoculated.

intervals, at which time pH and residual values were determined.

The chlorinated and ozonized virus mixtures to be injected into monkeys were treated with identical amounts of sodium thiosulfate immediately upon removal from the test mixtures. One cc of the treated virus was injected intracerebrally into each test animal (*Macaca mulatta*) within 10 minutes after neutralization.

The ozone used was produced by a laboratory model of the Sterozone ozonizer, designed by one of the authors, D.K.A. Ozone produced by this method contains negligible amounts of nitrogen oxide as determined by the diphenyl benzidine test. The orthotoluidine method was used to determine the residual in ppm, both for ozone and chlorine. The pH determinations were made electrometrically.

Conclusions and Summary. 1. Gaseous chlorine and hypochlorite in residual amounts of 0.5 ppm failed to inactivate a 1:1000 dilution of Le strain of poliomyelitis virus after an exposure of 90 minutes but inactivated the virus by 180 minutes. These results compare favorably with those of Kempf and Soule in working with the MV strain of virus. 2. Ozone in residual amounts not exceeding 0.45 ppm, inactivated a 1:1000 dilution of Le virus in 2 minutes and a 1:100 dilution of the same virus in 45 minutes. A 1:10 dilution of MV virus was not inactivated in 30 minutes and a 1:10 dilution of Le virus was not inactivated in 45 minutes. The extra amount of brain material in the lower dilutions constitutes an excess of organic matter which probably accounts for the longer time necessary for inactivation of virus at these dilutions.

It is observed from these results that an identical dilution of the same strain and pool of poliomyelitis virus when exposed to chlorine in residual amounts ranging from 0.5-1.0 ppm and to ozone in residual amounts between 0.05-0.45 ppm under the same controlled experimental conditions was inactivated almost immediately by ozone, *i.e.*, within 2 minutes, while an interval ranging between 1½ hours and 3 hours was required for inactivation by chlorine.

Thompson[12] in a recent review of the significance of the findings concerning the occurrence of poliomyelitis virus in intestinal con-

[12] Thompson, Rudolph E., *Water and Sewage*, 1943, **81**, 24.

tents and sewage and of its inactivation by current water purification procedures suggests either "superchlorination" or that "ozone treatment might be effective."

The germicidal effects of ozone have been noted in the past but recent quantitative determinations have not been reported and its effect on viruses has not been observed. The current results indicate that ozone acts as a strong virucidal agent and warrant additional experimentation.

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Studies on the Nutrition and Metabolism of *Pasteurella pestis.*

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Although *Pasteurella pestis* has been the subject of a great deal of medical and immunological research, very little work has been done on the physiology of the organism itself. During recent years, attempts have been made to apply technics used in the study of bacterial metabolism to certain pathogenic organisms. The experiments reported in this paper were designed to establish the basic growth requirements of the plague bacillus, and to investigate to some extent the nature of its dissimilatory processes.

Rao[1] claims that 3 amino acids, cystine, phenylalanine, and proline, are essential for the development of *P. pestis,* and that haematin and riboflavin may be considered as accessory growth factors. However, he does not give evidence that the organism can grow with a mixture of these compounds alone, and provides neither a carbon nor a nitrogen source other than amino acids in most of his media. In an excellent study dealing with the nutrition of the members of the genus *Pasteurella,* Berkman[2] could find no effect of 12 nutrilites on the development of their strains of *P. pestis* in a medium containing glucose as well as a mixture of amino acids.

Experiments were carried out in this laboratory and at the Hooper Foundation for Medical Research with a non-pathogenic strain (1122) of *P. pestis,* and with 3 selected pathogenic strains: 337 (from India), and "Yreka" and "Shasta" from California. Since glucose was found to be the best carbon source and ammonium salts an excellent nitrogen source for all organisms tested, the basic medium was prepared with 0.2% glucose, 0.1% NH_4Cl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.005% $FeCl_3$, .001% $CaCl_2$, and M/30 Sorensen KH_2PO_4-Na_2HPO_4 buffer at pH 7.0. Five ml amounts of the basic medium were dispensed in 17 mm test tubes to insure a fair degree of aeration without agitation. All cultures were incubated at 29°C. On the first transfer from blood agar slants, none of the strains tested could grow in the above medium unless small amounts (0.002%) of cystine and phenylalanine were added. Initially large inocula had to be used, and visible turbidity was slow to appear, evidence pointing to a selection of the cells most capable of reproducing in this environment.[3] With subculturing in the same medium, growth became rapid. The addition of 0.002% proline hastened the development of strains 337 and "Shasta" on first transfer, but had no appreciable effect on their subsequent growth. The other strains tested were unaffected by proline. No nutrilite function of haematin, biotin, pantothenic acid, *p*-aminobenzoic acid, riboflavin, nicotinic acid, thiamin, or pyridoxine could be detected for any of the strains.

More detailed studies carried out with strain 1122 showed that:

1. Cystine could be replaced with thiosulfate, sulfite, thioglycollate, or homocystine, but not with methionine.

[1] Rao, M. S., *Indian J. M. Research,* 1939, **27**, 75.

[2] Berkman, S., *J. Infect. Dis.,* 1942, **71**, 201.

[3] Doudoroff, M., *J. Bact.,* 1942, **44**, 451.