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Action of Certain Germicides on Meningococcus.

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Susceptibility to death by chemical poisoning is a significant aspect of the biology of any bacterium and especially of a pathogen as important as meningococcus. This microörganism is generally supposed to be a delicate one, easily killed by antiseptic agents, but it is rarely included among the gram-negative organisms employed in the estimation of the germicidal activity of disinfectants, probably because it is somewhat exacting in its requirements for abundant growth on artificial media.

Methods. Most of the experiments were performed with a Type I strain of maximal virulence for mice, *i. e.*, less than 10 organisms sufficed to initiate a fatal infection by the mucin method.[1] In some experiments Type II organisms were also used, but in no experiment in which a comparison was made was any significant difference detected in the susceptibility of the two types to the germicides tested.

The meningococci were grown on the surface of a solid medium consisting of tryptic digest of egg-white, one percent glucose, buffer and agar,[2] in ordinary pint medicine bottles.

After 18 hours' growth the microörganisms were recovered in saline, centrifuged and resuspended in various dilutions of the chemical agent to be tested. Usually 10 cc volumes were used containing 5 to 10 billion microörganisms per cc. Care was taken to insure thorough mixing and dispersion of the bacteria. The suspensions were allowed to stand at room temperature for 15 to 30 minutes and then centrifuged. The sediment, carefully drained of supernatant, was washed by resuspending in 10 cc of saline and centrifuging again. A loopful of the washed sediment was then streaked onto the surface of a freshly poured blood agar plate, which was incubated and examined twice a day for 3 days because occasionally some colonies grew out slowly. Colonies of doubtful appearance were checked by microscopic examination of stained smears and subcultures.

1 Miller, C. P., and Castles, Ruth, *J. Inf. Dis.*, 1936, **58**, 263.

2 Miller, C. P., and Castles, Ruth, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

Unless this routine was followed, contradictory findings resulted. If, for instance, the bacterial growth was washed off the media with the germicide itself, the lethal action of the latter was much reduced by its contact with the agar and protein degradation products comprising the media, and even if the culture was washed off with saline and added directly to the germicide, enough ingredients of the media were carried along to diminish appreciably the effectiveness of the antiseptic.

Results and Comments. The tables show only the results of the cultures at and near the limit of each chemical's ability to kill all of the test organisms under the conditions described.

TABLE I.
Viability of Meningococci After Treatment with Germicides.

Chemical	Concentration		Growth
Salts of heavy metals			
Mercuric Chloride	.025%	.00092 *M*	0
	.012	.00046	0 or very few‡
	.006	.00023	++
Phenyl Mercuric Nitrate	.006%	.000184 *M*	0
	.003	.000092	0 or +‡
	.0015	.000046	0 or +‡
	.00075	.000023	++
Copper Sulphate	.012%	.000751 *M*	0
	.006	.000376	0 or very few‡
	.003	.000188	+
	.0015	.000094	++
Halogens			
Chlorine Water	1/4 saturated		0
	1/8		+
	1/16		++
Compound Solution Iodine, U.S.P.	2.5%		0
Lugol's Solution	0.62		+
	0.16		++
Sodium Fluoride	5.0%	1.19 *M*	+
	2.5	.595	+±
	1.0	.238	++
Sodium Azide, NaN_3	10.0%	1.535 *M*	0 to +‡
	5.0	.768	+±
	1.0	.1535	++
Potassium Tellurite	.2%	.00788	0
	.04	.001576	+
	.008	.000315	++
Organic Compounds			
Phenol	1.0%	.015 *M*	0
	0.25	.026	++
	0.06	.0063	++

Chemical	Concentration		Growth
Formaldehyde	0.4%	.132 *M*	0
	0.1	.033	0
	0.025	.008	+
Propylene Glycol	60.0%	7.88 *M*	0
	50.0	6.56	0 or +‡
	40.0	5.26	0 or +‡
	30.0	3.94	++
Alcohol	30.0%	6.51 *M*	0
	20.0	4.33	0 or ±‡
	10.0	2.16	+
*Quinone	.01%	.000925 *M*	0
	.001	.0000925	++
	.0001	.00000925	++
*Quinhydrone	.01%	.000458 *M*	0
	.001	.0000458	++
	.0001	.00000458	++
*Iodoacetic Acid	.1%	.00537 *M*	0
	.01	.000537	++
	.001	.0000537	++
Synthetic Detergents: anionic			
†Duponol L S Sodium Oleyl Sulphate	.05%		0
	.01		++
	.002		++
†Triton W-30 Sodium Salt of Alkyl Phenoxy Ethyl Sulphonate	.05%		0
	.01		+
	.002		++
Synthetic Detergents: cationic			
†Hydrocide Alkyl Hydroxy Benzyl Dimethyl Ammonium Phosphate	.25%		0
	.05		++
	.01		++
†Zephiran Alkyl Dimethyl Benzyl Ammonium Chloride	.025%		0
	.005		0 or +‡
	.001		++

*These compounds were very kindly supplied by Dr. E. S. G. Barron of this Department.

†These compounds were very kindly supplied by Dr. Zelma Baker of this Department.

‡Designates concentrations of germicide at which the results were variable: *i.e.*, growth in some experiments, no growth in others.

Compounds containing a heavy metal were the most effective germicides and of these phenyl mercuric nitrate was the best. This

is in accord with the findings of Weed and Ecker,[3] who found it the most potent of a number of mercurials studied. Next in order were the two inhibitors of respiratory enzymes: quinone and quinhydrone, which were lethal in concentrations of 0.01% (= 0.0009 and 0.00045 *M* respectively). Iodoacetic acid was somewhat less active. These results were to be expected from the observations of Barron.[4, 5]

The next most active germicide was the cationic detergent Zephiran, which killed regularly at 0.025% and usually at 0.005%. The other cationic detergent, Hydrocide, was much less effective, failing to kill at 0.05%. The two anionic detergents, Duponol and Triton W.30, were more effective than Hydrocide and less active than Zephiran. The activity of the anionic compounds is surprising in view of the work of Baker, Harrison and Miller,[6, 7, 8] who studied the effect of a number of detergents on the metabolism of 6 gram-negative organisms and on the viability of three, and found the gram-negatives resistant to the anionic compounds. Similar observations are reported by Dubos.[9]

Among the common antiseptics, alcohol was more active than was to be expected, killing most of the test organisms at 20% concentration. Formaldehyde was regularly lethal in concentrations of 0.1%. But phenol, chlorine water and Lugol's solution (Compound Solution of Iodine, U.S.P.) required much higher concentrations than had been anticipated. Phenol was ineffective in less than one percent concentration, chlorine water in less than one-fourth saturation and Lugol's solution in less than 2.5%. The latter was found by Nye[10] to be the most effective of the germicides tested with *Staphylococcus aureus.*

The action of propylene glycol on meningococcus was similar to that found by Robertson[11] on pneumococcus.

Potassium tellurite was lethal at a concentration of 0.2% but not at 0.04%. This was first used by Conradi and Troch[12] and later by Fleming,[13] who found most gram-negative organisms including

3 Weed, L. A., and Ecker, E. E., *J. Inf. Dis.*, 1933, **52**, 354.

4 Barron, E. S. G., *J. Biol. Chem.*, 1936, **113**, 695.

5 Barron, E. S. G., and Friedemann, T. E., *J. Biol. Chem.*, 1941, **137**, 593.

6 Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **73**, 249.

7 Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **74**, 611.

8 Baker, Z., Harrison, R. W., and Miller, B. F., *J. Exp. Med.*, 1941, **74**, 621.

9 Dubos, Rene J., University of Pennsylvania Bicentennial Conference, 1941.

10 Nye, Robert N., *J. A. M. A.*, 1937, **108**, 280.

11 Robertson, O. H., personal communication to the author.

12 Conradi, H., and Troch, P., *Munch. Med. Woch.*, 1912, **59**, 1652.

13 Fleming, Alexander, *J. Path. and Bact.*, 1932, **35**, 831.

gonococcus sensitive to it, although meningococcus seems not to have been tested in his experiments. Rose and Georgi[14] found potassium tellurite in 0.03% concentration to inhibit the growth of gram-negatives.

Quite unexpected were the results with sodium fluoride and sodium azide, which failed to kill meningococci under the conditions of these experiments in concentrations of 5%. The former is a well known inhibitor of glucose fermentation by cells and cell-free extracts. Snyder and Lichstein[15] report that 0.01% sodium azide was sufficient to restrain the growth of gram-negative bacteria on blood agar and so have Mallman, Botwright and Churchill.[16] A second paper by Lichstein and Snyder[17] recommends the addition of sodium azide to media as the most satisfactory method of preventing the spread of Proteus and of exerting a general bacteriostatic action against all gram-negative organisms.

It seems clear from the experiments herein described that meningococcus is unlike other gram-negative bacteria in being resistant to certain chemicals which are toxic to the members of this group usually subjected to germicidal tests. Smith[18] suggested that the Neisseriae might be exceptional among the gram-negatives in their susceptibility to the bacteriostatic action of various dyes, but in most of his experiments he used only *N. catarrhalis* as representative of the whole genus because of the difficulty of growing meningococcus and gonococcus on his standard media.

Summary. Meningococci were subjected to the action of a number of germicides by suspending aliquot quantities in various concentrations of each chemical, removing them by centrifugation, washing and culturing the washed sediment on blood agar plates. Several of the chemicals usually regarded as potent germicides failed to kill all or most of the meningococci.

Conclusions. Meningococci are more resistant to the lethal action of certain chemicals than the gram-negative organisms usually employed in germicidal tests.

[14] Rose, Kenneth D., and Georgi, Carl E., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 344.

[15] Snyder, M. L., and Lichstein, Herman C., *J. Inf. Dis.*, 1940, **67**, 113.

[16] Mallman, W. L., Botwright, W. E., and Churchill, Elbert S., *J. Inf. Dis.*, 1941, **69**, 215.

[17] Lichstein, H. C., and Snyder, M. L., *J. Bact.*, 1941, **42**, 653.

[18] Smith, Homer W., *Am. J. Hyg.*, 1922, **21**, 607.