10475

Action of Ketene on Gonococcus and Meningococcus.*

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The toxicity inherent in the cells of gonococcus and meningococcus—commonly ascribed to "endotoxins"—probably handicaps them as effective antigens and restricts their usefulness as immunizing agents. We have made numerous attempts to diminish the toxicity of these organisms without impairing their antigenic properties.

Tamara and Boyd[1] reported detoxification of *B. dysenteriæ* Shiga by acetylation without alteration of its antigenic activity though the extent of this change is not apparent from the data presented in their preliminary communication. Acetylation was accomplished by means of ketene gas generated by the method of Herriott.[2] By the same technic Pappenheimer[3] was able to reduce very greatly the lethal action of diphtheria toxin without destroying its ability to combine and flocculate with antitoxin. Short exposure of toxic filtrates to ketene gas gave Goldie[4] similar results. Hyman[5] reports that ketene reduces the toxicity, but also the antigenicity of diphtheria toxin.

Ketene $\overset{H}{\underset{H}{}}>C=C=O$ is a gas which liquefies at −56°C. It reacts with amino acids, alcohols, amids and other compounds by acetylation.[3, 6, 7, 8]

Methods. Ketene was generated in an apparatus similar to that described by Herriott.[2] Vaporized acetone was passed over a hot platinum filament and then through a chamber cooled by solid carbon dioxide where polimers and unchanged acetone were removed by condensation. The ketene gas was bubbled slowly (about 1 bubble per second) through the bacterial suspension containing phenol red. Its reaction was maintained at pH 7.6-7.8 by frequent additions of 10% sodium hydroxide to neutralize the acetic acid as it formed.

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1 Tamura, J. T., and Boyd, M. J., *Science*, 1936, **83**, 61.

2 Herriott, Roger M., *J. Gen. Physiol.*, 1934, **18**, 69.

3 Pappenheimer, Alwin M., Jr., *J. Biol. Chem.*, 1938, **125**, 201.

4 Goldie, H., *Compt. Rend. Soc. Biol.*, 1937, **126**, 1, 4.

5 Hyman, L. W., *J. Bact.*, 1939, **37**, 228.

6 Staudinger, H., *Die Ketene*, Stuttgart, F. Euke, 1912.

7 Herriott, R. M., and Northrup, J. H. *J. Gen. Physiol.*, 1934, **18**, 35.

8 Stern, K. G., and White, A., *J. Biol. Chem.*, 1937, **122**, 371.

The duration of treatment with ketene varied in different experiments from 20 to 60 minutes.

The microörganisms were cultivated in pint medicine bottles on solid media consisting of tryptic digest of egg white, 1% dextrose buffer and agar. They were removed with saline, washed once and resuspended in distilled water, about 0.3 g moist, packed organisms in 5.0 cc suspension.

Cultures of the bacterial suspensions showed them to be sterile after 5 minutes' exposure to the gas. Control suspensions were, therefore, killed by heating at 70°C for 15 minutes. Acetic acid was added, then neutralized with sodium hydroxide in the same manner and quantity as the ketene-treated sample.

Toxicity of the bacterial suspensions was measured by their lethal action on white mice. Immediately after completion of the ketene treatment, series of 20 g mice were injected intraperitoneally with graded doses. Death usually occurred within 36 hours, but the mice were observed for 6 days. Preliminary experiments made clear the necessity of grading the dosage by small increments and of injecting enough mice with each dose to be statistically significant.

Results. Comparison of the mortality rates of the ketene-treated and control suspensions over the whole series of each titration showed that some degree of detoxification occurred in almost every one of a considerable number of experiments. At best it was never very great. An *average* result (neither maximal nor minimal) is exemplified in Table I.

Meningococcus behaved similarly to gonococcus.

Duration of detoxification. The chemical reaction responsible for detoxification is in whole or in part a reversible one. This fact is demonstrated by the experiment shown in Table II.

The dose of acetylated gonococci which killed only 2 of 6 mice was repeated after one week's storage in the ice box and it then killed all of 6 mice.

TABLE I.

Mortality of White Mice Injected Intraperitoneally with Acetylated (for 40 min.) or Control Suspensions of Gonococcus.

Dose, cc	Acetylated suspensions	Control suspension
1.4	5/8*	5/5
1.0	4/8	7/8
.75	2/8	4/8
.5	2/8	5/8
.3	0/8	3/8
.2	0/8	2/8

*Numerator denotes number of deaths; denominator denotes number of mice injected.

TABLE II.
Reversibility of Detoxification by Acetylation.

	Dose, cc	Acetylated* suspension	Control suspension (not acetylated)
Gonococcus suspension injected immediately after ketenization	0.5	2/6	6/6
Same gonococcus suspension injected 6 days after ketene treatment	0.5	6/6	

* Numerators indicate the number of mice killed by the injection. Denominators show the total number of mice injected in this experiment.

Effect of acetylation on the antigenic properties of gonococci. Three rabbits were immunized by a series of intravenous injections with suspensions of gonococci treated with ketene immediately before each injection. These rabbits survived weekly intravenous injections, increasing from 0.5 cc to 4.0 cc of the concentrations previously mentioned, without mortality. At the end of 6 weeks antiserum showed about the same antibody content by precipitin test and the same degree of specificity as that made by injections of unacetylated organisms or gonococcus protein.[9]

Conclusions. The toxicity of gonococcus and meningococcus cells is appreciably but temporarily reduced by acetylation with ketene.

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Experimental Intersexuality: Masculinization of Female Rats by Postpartum Treatment with Anterior-Pituitary-Like Hormone.*

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Papanicolaou and Falk[1] reported a masculinization of female guinea pigs due to treatment with anterior-pituitary-like hormone. This masculinization consisted of growth and enlargement of the clitoris. They also noted a "masculinizing effect" on the skeletal musculature due to the same treatment.[2] These "masculinizing" effects were associated with a marked development of the interstitial

[9] Boor, A. K., and Miller, C. P., *J. Exp. Med.*, 1934, **59**, 63.

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[1] Papanicolaou, G., and Falk, E. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **21**, 750.

[2] Papanicolaou, G., and Falk, E. A., *Science*, 1938, **87**, 238.