Effect of Alcohols on Lecithinase of *Clostridium perfringens.** (23941)

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Kushner has reported recently(1) that the presence of certain alcohols in the growth medium inhibited markedly the formation of lecithinase by cells of *Bacillus cereus* while having little effect on the growth of the organisms. The data reported revealed that the effect of the alcohols was on the synthesis of lecithinase rather than on the preformed enzyme.

Inasmuch as we have been concerned with a study of the influence of nutrition on synthesis of lecithinase by *Clostridium perfringens*(2), it appeared desirable to extend the experiments of Kushner to include this organism.

Materials and methods. The organism studied was *Cl. perfringens* strain 111-3 and was maintained by transfer every 3-4 weeks in nutrient broth containing chopped beef heart. The experimental medium contained 2 g of proteose peptone No. 3 (Difco), 1 g of sucrose, 0.83 g of K_2HPO_4, 0.16 g of KH_2PO_4, 20 mg of $MgSO_4 \cdot 7H_2O$, and 1 mg each of $FeSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 4H_2O$ and NaCl per 100 ml of deionized water and was dispensed in 10 ml amounts into 15 x 125 mm screw cap tubes. The pH prior to sterilization in the autoclave (121°C, 15 min.) was 7.2-7.4. The alcohols were added aseptically to the media at the time of inoculation.

The inoculum was prepared from a 16-20 hr culture grown in the experimental medium at 39°C in an atmosphere of H_2. All media were boiled routinely in a water bath for 10 min. and cooled quickly under running water prior to inoculation in order to expel dissolved O_2. The inoculum culture was centrifuged, the bacterial cells washed once with sterile medium, resuspended in 10 ml of the medium and 2 drops of this suspension used to inoculate each experimental tube. Growth was measured turbidimetrically after 4-5 hr incubation in a Coleman Junior Spectrophotometer at a wavelength of 650 mμ employing a medium blank.

Lecithinase (*alpha* toxin) was measured by the Nagler reaction. Lecithovitellin was prepared by mixing one egg yolk in 500 ml of 0.9% salt solution followed by adsorption with 20 g of Norite. This was accomplished by shaking the flask on a mechanical shaker for 30 min. followed by centrifugation to remove the Norite. The remaining solution was then passed through a Selas filter and stored in the refrigerator. Toxin titers were determined by mixing 3 ml each of lecithovitellin and saline and 2 ml of culture supernate obtained by centrifugation and incubating at 43°C for 1-2 hrs. The culture supernates were adjusted to pH 7 in order to minimize non-specific reactions. The resulting turbidities were measured in the Coleman at 650 mμ. Control tubes were identical except for the addition of polyvalent gas gangrene antitoxin (Lederle) to the culture supernate before it was mixed with the lecithovitellin.

Results. The data for two representative experiments are presented in Table I. Methanol and ethanol were approximately equivalent in activity both with regard to growth and toxin inhibition. At concentrations of 0.5 and 1.0% growth was reduced 10-34% (av 20%) while lecithinase activity was inhibited 35-71% (av 52%). At a concentration of 2% both methanol and ethanol caused an inhibition of lecithinase of 90% or greater with a concomitant decrease in growth approximating 50%. Propanol and butanol were decidedly more toxic to growth and toxin formation than the lower chain alcohols but even with these alcohols the effect on lecithinase activity was approximately twice the effect on growth especially at the lower concentrations.

The results cited do not permit a distinction between possible effects of the several alcohols on the synthesis of lecithinase or the stability of this toxin. It was therefore desirable to study the action of the alcohols on preformed

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toxin. This was accomplished by growing the organism in media free of alcohol, recovering the supernatant culture fluid after removal of the cells by centrifugation and then adding the alcohols individually to aliquots of the culture supernate. The results of such studies (Table II) revealed that the alcohols had no effect on stability of preformed toxin hence suggesting that their effect during growth is exercised by inhibiting toxin synthesis.

TABLE I. Effect of Alcohols on Growth and Lecithinase Production of *Clostridium perfringens*.

Additions, %		Growth, O.D.*	Toxin, O.D.†	Growth inhibition, %	Toxin inhibition, %
none		.68	.45		
methanol,	.5	.58	.24	14.7	46.6
	1.0	.50	.14	26.5	68.9
	2.0	.35	.03	48.5	93.4
ethanol,	.5	.56	.23	17.6	48.8
	1.0	.45	.13	33.8	71.1
	2.0	.35	.01	48.5	97.8
n-butanol,	.5	.40	.03	41.2	93.4
	1.0	.05	.00	92.6	100.0
none		.57	.57		
methanol,	.5	.51	.37	10.5	35.1
	1.0	.43	.22	24.5	61.4
	2.0	.25	.06	56.1	89.5
ethanol,	.5	.50	.37	12.3	35.1
	1.0	.43	.31	24.5	45.6
	2.0	.22	.05	61.4	91.2
iso-propanol	.5	.43	.28	24.5	50.9
	1.0	.30	.12	47.4	78.9
	2.0	.03	.00	94.7	100.0
n-butanol,	.5	.25	.03	56.1	94.7
	1.0	.17	.00	70.2	100.0

* Optical density at 650 mμ after 5 hr incubation at 39°C in an atmosphere of H_2.

† Optical density at 650 mμ of culture supernate after mixing with lecithovitellin and incubating at 43°C for 90 min.

Other details as described in text.

TABLE II. Effect of Alcohols on Preformed Lecithinase of *Clostridium perfringens*.

Additions	Lecithinase activity Exp. 1 O.D.*	Exp. 2 O.D.
none	.48	.29
methanol	.50	.28
ethanol	.43	.27
iso-propanol	.48	.26
n-butanol	.50	.29

* Optical density at 650 mμ of culture supernate after mixing with lecithovitellin and incubating at 43°C for 2 hr. Alcohols added to culture supernate in final conc. of 2%. Other details as described in text.

The results with *Cl. perfringens* were roughly parallel to those obtained with *B. cereus*(1) except that the several alcohols were decidedly more toxic for growth of the former organism than the latter. However, it is manifest that the inhibitory effect of the alcohols on toxin synthesis was far greater than the effect on growth suggesting a preferential effect on this function rather than a general metabolic inhibition.

Summary. A study of the effects of several alcohols on growth and lecithinase synthesis by *Cl. perfringens* revealed that although both functions were inhibited by appropriate concentrations, toxin synthesis was considerably more susceptible than growth. In general the inhibitory effects of the alcohols increased with increasing molecular weight.

1. Kushner, D. J., *Nature*, 1957, v179, 781.

2. Jayko, L. G., Lichstein, H. C., *Bact. Proc.*, 1957, p93.

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