LOCUS OF ACTION OF STREPTOMYCIN IN THE DEVELOPMENT OF CLOSTRIDIA FROM SPORE INOCULA[1]

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Received for publication May 12, 1952

Quantitative data are unavailable on the effect of streptomycin on the germination of spores of anaerobic bacteria. Spore inocula were employed by Curran and Evans (1946), but these authors merely concluded that 100 units per ml did not prevent the spoilage of milk by *Clostridium botulinum* or putrefactive anaerobe no. 3679. It is the purpose of this investigation to report studies on the locus of action of streptomycin in the development of three *Clostridium* species from spore inocula.

MATERIALS AND METHODS

Organisms employed were *Clostridium perfringens*, *Clostridium chauvei*, and putrefactive anaerobe no. 3679, all obtained from the Department of Bacteriology, University of Texas, Austin, Texas. Spore suspensions were prepared according to a previously reported procedure (Wynne and Foster, 1948). Streptomycin was used as the calcium chloride complex (Merck) or the sulfate (Heyden Chemical Corporation). Media employed were fluid thioglycolate medium (Difco) and Brewer thioglycolate medium (Difco). The general method employed for germination studies has been described in previous papers (Wynne and Foster, 1948; Wynne and Harrell, 1951). Dilutions for spore counts in modified Yesair's pork infusion agar were such that the amount of streptomycin added per counting tube had no effect on counts.

RESULTS

Preliminary experiments with all 3 species at a level of approximately 500 spores per ml showed that 1,000 μg per ml were the lowest concentration consistently preventing appearance of turbidity in fluid thioglycolate medium. The streptomycin may have acted by (1) a sporicidal action, (2) an inhibition of germination, (3) an effect on vegetative cells, or (4) a combination of these factors.

Germination was apparently unaffected by 1,000 μg per ml of streptomycin (table 1). However, no turbidity was observed in the presence of streptomycin while all controls were turbid. With putrefactive anaerobe no. 3679, only a moderate degree of germination was obtained. With longer incubation times, countable

[1] This investigation was supported in part by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, and an institutional grant from the American Cancer Society.

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spores of this organism increased in controls without streptomycin, possibly due to resporulation.

It is admittedly conceivable that streptomycin may have exerted a sporicidal effect which corresponded to maximum germination obtained in controls. However, kinetic studies indicated that the rate of germination of putrefactive anaerobe no. 3679 was apparently not influenced by streptomycin. It would seem unlikely that a postulated sporicidal action would occur at the same rate as the germination process. Furthermore, streptomycin had no effect on counts when spores of the 3 species were incubated in the germination media under relatively aerobic conditions designed to inhibit germination. Apparently slow germination occurred with *C. chauvei* and *C. perfringens*, but this germination was unaffected by the antibiotic. A similar apparent lack of sporicidal action of streptomycin occurred in the germination experiments under conditions far more favorable for

TABLE 1

Germination of spores in the presence of streptomycin (in Brewer thioglycolate medium)

SPECIES	INCUBATION	STREPTOMYCIN PER ML	RESIDUAL SPORES	GERMINATED SPORES	PER CENT GERMINATION
	hr	*μg*			
Clostridium chauvei	0	0	880,000	—	—
	26	0	19,000	861,000	98
	26	1,000	13,000	867,000	99
Clostridium perfringens	0	0	820,000	—	—
	19	0	30,000	790,000	96
	19	1,000	13,000	807,000	98
Putrefactive anaerobe no. 3679	0	0	65,000	—	—
	25	0	26,200	38,800	60
	25	1,000	25,000	40,000	62

germination. It would seem unlikely that a sporicidal action just equivalent to germination would appear with two apparent rates of germination differing widely.

By the spore-free technique of Wynne and Harrell (1951), inhibition of vegetative cells by streptomycin was shown to correlate with the prevention of turbidity from spores in preliminary experiments and with effects of streptomycin in the counting medium. Therefore, it appears valid to conclude that streptomycin at a concentration of 1,000 μg per ml had no significant effect on spore germination.

Incubation of spores of putrefactive anaerobe no. 3679 and *C. perfringens* in the presence of 1,000 μg of streptomycin per ml in distilled water resulted in a marked decrease in counts. Comparable results were obtained at 4 C and 37 C. Since a sporicidal action was unlikely, it was thought that the antibiotic might be adsorbed on the spores and carried over into the counting medium, thus exerting an effect on resulting vegetative cells out of proportion to its apparent

concentration. Attempts to inactivate adsorbed streptomycin with hydroxylamine or competitively adsorb the antibiotic with acid-treated charcoal (Waksman, 1951) were unsuccessful. However, the presence of 0.05 g per ml of NaCl prevented the appearance of the streptomycin effect.

DISCUSSION

The observed lack of effect of streptomycin on spore germination might be due to impermeability of the heat resistant spore to the antibiotic. Alternatively, it may be postulated that the process of spore germination is fundamentally different from vegetative development (Wynne and Harrell, 1951; Wynne, 1952). The mechanism of action of streptomycin has been reported to involve inhibition of dephosporylation of mononucleotides and depolymerization of nucleic acids (Macheboeuf, 1948). In view of the insensitivity of germination to streptomycin, it is tempting to speculate that the process of germination in these species may not involve these reactions. Streptomycin also has been reported to inhibit the condensation reaction of the tricarboxylic acid cycle (Oginsky *et al.*, 1949). Although studies reported here and elsewhere (Wynne and Harrell, 1951; Wynne, 1952) indicate that germination of *Clostridium* spores may occur under relatively aerobic conditions, the lack of effect of streptomycin would indicate that germination may not involve the tricarboxylic acid cycle.

Prevention of the effect of streptomycin in distilled water by the presence of NaCl is compatible with the view that most of the streptomycin taken up by bacteria is adsorbed at the cell surface (Berkman *et al.*, 1948) and that inorganic cations compete with this antibiotic for adsorption centers in a manner similar to the reported competition between hydrogen ions and streptomycin (Abraham and Duthie, 1946). Since part of the adsorbed streptomycin was readily desorbed in water, it was concluded by Berkman *et al.* (1948) that slow desorption of the antibiotic *in vivo* may not be a factor in its observed therapeutic action after a detectable blood level is no longer present. However, it would appear that the dilutions made by these workers subsequent to streptomycin treatment of cells were such that the final levels of the antibiotic in the counting medium were at least 10,000-fold lower than the minimum inhibitory level. In the present studies a ready desorption of streptomycin did not occur, but it should be pointed out that dilutions used were such that final concentrations of streptomycin in the counting medium were less than 10-fold lower than the activity threshold. Therefore, it is possible that though the antibiotic concentration in blood or tissue fluid is below a detectable value, it may still be high enough for the concentration of adsorbed streptomycin at the cell surface to be effective.

SUMMARY

Under conditions employed in these investigations, the only locus of action of streptomycin demonstrated was on vegetative cell multiplication. No effect on germination was noted, nor could a sporicidal action be shown. An apparent adsorption of streptomycin to spores in distilled water was prevented by the presence of 5 per cent NaCl. The implications of these findings are discussed.

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