ACKNOWLEDGMENTS AND ADDRESSES

Received August 14, 1968, from the *Divisions of Pharmacology and Medicinal Chemistry, Pharmacy Research Institute, University of Connecticut, Storrs, CT 06268*

Accepted for publication October 25, 1968

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

This work was supported in part by University of Connecticut Research Foundation grant 400-5-178.

Effect of Polyethylene Glycol 300 on the Viability of Bacterial Spores

ROBERT L. ROBISON and MELVIN H. WEINSWIG

Abstract □ In an earlier study polyethylene glycol 300 (PEG 300) reduced the dry-heat sterilization time for the spores of two species of aerobic bacilli. Further investigation indicates that PEG 300 also affects the viability of the spores without heat.

Keyphrases □ Polyethylene glycol 300 (PEG 300) effect—bacterial spores □ Bacterial spore germination—PEG 300 effect □ Oil, bacterial growth—PEG 300 effect

The authors recently reported that polyethylene glycol 300 (PEG 300) significantly reduced the dry-heat sterilization time for the spores of two species of aerobic bacilli (1). Since this was unexpected and contrary to an earlier report that polyethylene glycol (PEG) did not affect sterilization time (2), additional studies were conducted to confirm the sterilizing activity of PEG 300 with heat and to determine its effect without heat. The results of experiments conducted without heat follow.

Several low-molecular weight glycols are effective in low concentrations as air sterilizers (3, 4). Glycerol and various polyols inhibited bacteria through an osmotic effect (5). A specific inhibitory effect has been suggested for propylene glycol (6).

Although PEG compounds have been used in pharmaceutical preparations for many years, only recently has any antibacterial activity been attributed to these compounds. Cox (7) reported in 1965 that PEG affected the survival of bacteria as aerosols. Subsequently (8) he reported the toxic effect of solutions of various PEG compounds on vegetative bacterial cells. Sachs and Alderton (9) described a procedure for separating spores from vegetative cells using a two-phase aqueous polymer system containing PEG 4000 and potassium phosphate. They found that spores recovered in this manner showed no reduction in thermal resistance or viable count. The authors have not found any reports that any of the PEG compounds adversely affect the viability of bacterial spores.

EXPERIMENTAL

The growth of the spores of *Bacillus subtilis* ATCC 9372 and *Bacillus stearothermophilus* and the preparation of the spore test disks have been reported (1). The results reported here were obtained concurrently with those of the previous study, the test interval being one in which the spores were highly heat resistant in sesame oil and in air. The procedures and materials used were the same as those used in the heat study, the only difference being that the spore disks were held in the sealed oil or PEG 300 ampuls at room temperature for periods up to 3 weeks before transfer to the culture medium.

Analysis of PEG 300—The PEG 300 used in this study was analyzed to determine if some minor component other than the PEG 300 might be responsible for the results. Table I suggests that PEG 300 alone was involved.

RESULTS

B. subtilis—When PEG 300 was used as a control in the oil-sterilization study (1), the time from first exposure of the spores to PEG 300 until inoculation into the culture medium rarely exceeded 24 hr. Under those conditions PEG 300 did not affect the germination time of the unheated controls. When the *B. subtilis* spore disks were stored in PEG 300 for longer periods at room temperature, a different result was observed as shown in Table II. *B. subtilis* spores did not survive after storage in PEG 300 for 1 week, whereas those stored in the unpreserved sesame oil and in the phenol and benzyl alcohol-preserved sesame oil survived up to 2 weeks. No viable counts were made, thus it is impossible to determine what the viability loss rate was or the time required to kill all cells. Although these tests were much more limited than those in which heat was used, there is good correlation between the data obtained under the two conditions, *i.e.*, decreased survival times in the presence of PEG 300 compared to those in sesame oil.

B. stearothermophilus—Control disks of *B. stearothermophilus* produced visible growth within 12 hr. after transfer to the culture medium. Germination time was not altered by storage in either the

Table I—Analysis of PEG 300

Moisture[a]	0.12%
Ethylene glycol[b]	0.18%
Diethylene glycol[b]	0.24%
Aldehyde[c]	14 p.p.m.
Ethylene oxide[d]	Negative

[a] Karl Fischer method. [b] GLC, flame detector. [c] Aldehyde (as formaldehyde) spectrophotometric determination. [d] Spectrophotometric method.

Table II—Survival of *B. subtilis* ATCC 9372 in Anhydrous Vehicles—110,000 Spores per Disk

Vehicle	Initial	Storage at Room Temperature (Weeks) 1	2	3
PEG 300	2/2[a]	0/2[b]	0/2	—[c]
Sesame oil	2/2	2/2	2/2	1/2
Sesame oil + 2% benzyl alcohol	2/2	2/2	2/2	2/2
Sesame oil + 0.5% phenol	2/2	2/2	2/2	0/2

[a] 2/2 = Number of tubes with growth/number of tubes inoculated with disks. [b] A second test using another batch of PEG 300 also caused loss of viability. [c] Test not run. Fluid thioglycollate medium incubated 14 days at 30–32°.

Table III—Survival of *B. stearothermophilus* in Anhydrous Vehicles—100,000 Spores per Disk

Vehicle	Initial	Storage at Room Temperature (Weeks) 1	2	3[a]
PEG 300	2/2[b]	2/2[c]	2/2[c]	—
Sesame oil	2/2	2/2	2/2	—
Sesame oil + 2% benzyl alcohol	2/2	2/2	2/2	—
Sesame oil + 0.5% phenol	2/2	2/2	2/2	—

[a] No tests run at 3 weeks. [b] 2/2 = Number of tubes with growth/number of tubes inoculated with disks. [c] Onset of growth was delayed. Fluid thioglycollate medium plus 0.1% soluble starch incubated 4 days at 60°.

preserved or unpreserved sesame oil up to 2 weeks at room temperature. At the sublethal but somewhat damaging temperatures used in the sterilization study (1) visible growth frequently was delayed 48 hr. or more. When the *B. stearothermophilus* spores were held in PEG 300 for one day or less there was no noticeable reduction in germination time. Spores held in PEG 300 for 1 week did not show growth until 18–24 hr. after culturing, and those stored 2 weeks showed slight additional delay in onset of growth. Although Table III does not indicate sporicidal activity, the delay in growth suggested that PEG 300 exerts an inhibitory action not caused by the oils.

Delay of Growth in Culture Medium Containing PEG 300—*B. subtilis* spore disks were inoculated into fluid thioglycollate containing concentrations of PEG 300 ranging from 1 to 20%. Growth occurred in all culture tubes but concentrations above 7.5% caused delayed onset of growth. Visible growth in the cultures containing up to 7.5% PEG 300 occurred within 24 hr. after inoculation. Media containing 20% PEG 300 did not show growth until approximately 48 hr. after inoculation and intermediate concentrations gave corresponding delays in visible growth.

DISCUSSION

The present experiments only permit speculation as to how PEG 300 affects the viability of spores. Inhibition of bacteria by related types of compounds has been attributed to osmotic effects (5) or to a specific inhibitory effect (6). The polyethylene glycols are good solubilizers and possibly they solubilize certain of the involved spore components. Since spores are at least peripherally penetrated by relatively lipid-soluble molecules such as the ethylene glycols (10), it seems likely that both physical and physicochemical phenomena might be involved in the loss of viability. It has been suggested for vegetative cells, that the PEG compounds may be involved in phase separation of groups which would normally be in solution and attached to the cell membranes, thus causing a detrimental change in their structure (8).

The degree to which PEG adversely affects a variety of sporulating organisms is not known. Cox found that 50% of *B. subtilis* var. *niger* spores were inactivated in 10 min. in PEG 200 at 37° (11). This strain is presumably the same as that used in this work and [illegible] does not lend to any generalization regarding a spectrum of [illegible]vity. The lack of substantial activity against *B. stearothermophilus* without heat is not unexpected since the spores of this species are more highly resistant to destruction than mesophilic spores (12).

REFERENCES

(1) R. L. Robison and M. H. Weinswig, *J. Pharm. Sci.*, **56**, 1418(1967).

(2) J. H. Brewer, in "Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization," 2nd. ed., G. F. Reddish, Ed., Lea & Febiger, Philadelphia, Pa., 1957, p. 158.

(3) T. T. Puck, *J. Exptl. Med.*, **85**, 729(1947).

(4) G. Sykes, in "Disinfection and Sterilization," 2nd ed., E. & F. N. Spon Ltd., London, England, 1965, p. 270.

(5) M. Barr and L. F. Tice, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 221(1957).

(6) *Ibid.*, **46**, 217(1957).

(7) C. S. Cox, "Protecting Agents and Their Mode of Action," *1st Intern. Symp. Aerobiology*, Naval Biological Laboratory, Naval Supply Center, Oakland, Calif., 1965, p. 345.

(8) C. S. Cox, *J. Gen. Microbiol.*, **45**, 275(1966).

(9) L. E. Sachs and G. Alderton, *J. Bacteriol*, **82.**, 331(1961).

(10) P. Gerhardt and S. H. Black, in "Spores II," H. O. Halvorson, Ed., Burgess, Minneapolis, Minn., 1961, p. 218.

(11) C. S. Cox, personal communication.

(12) A. Briggs, *J. Appl. Bacteriol.*, **29**, 490(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 25, 1968, from the *College of Pharmacy, Butler University, Indianapolis, IN 46202* (M.H.W.) and the *Lilly Research Laboratories, Indianapolis, IN 46206* (R.L.R.)

Accepted for publication November 5, 1968.

Abstracted from a thesis prepared for the Graduate School o Butler University by Robert L. Robison in partial fulfillment of Master of Science degree requirements.

The authors are indebted to the Lilly Research Laboratories for generously supplying materials and facilities for this investigation.

The authors wish to thank Mr. Jerry Hale, Mrs. Bonnie Rutherford, and Dr. J. L. Spahr, Eli Lilly and Co., for the analytical data on polyethylene glycol.