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Ethylene Oxide Penetration of the Silicone Coating Used as a Lubricant on Disposable Syringe Rubber Plunger Tips and Hypodermic Needles

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Abstract □ The ability of 100% ethylene oxide to penetrate the silicone coating used on disposable hypodermic needles and syringe rubber plunger tips has been determined. It was shown that ethylene oxide has the ability to penetrate through the silicone coating and thus kill the spores of *Bacillus subtilis* var. *niger*, ATCC 9372, which were introduced underneath the silicone coating.

Keyphrases □ Ethylene oxide penetration—silicone lubricant □ Silicone lubricant effect—ethylene oxide bactericidal action □ *Bacillus subtilis*—ethylene oxide penetration determination

With the introduction of medical devices made from comparatively low melting point plastics, the ability to sterilize these instruments by autoclaving was no longer feasible; subsequently, ethylene oxide has been utilized and found effective (without high temperature) as a chemical sterilant.

Recent usage of silicone as a lubricant for disposable hypodermic needles and syringe rubber plunger tips raised the question of whether the ethylene oxide gas can penetrate the silicone coating and sterilize the surface beneath. It was, therefore, the purpose of this study to test the effectiveness of 100% ethylene oxide [4-hr. cycle, 48.8° (120° F.)] as a penetrating sterilant through a silicone coating. A number of preliminary tests were necessary to establish procedures and suitable materials. In the selection of materials it was necessary to obtain a nonbacteriostatic solvent capable of dissolving silicone (to expose the organisms which were covered by it), due to its immiscibility with the water present in the culture medium. Initial tests indicated *n*-hexane (1), purified (Curtin Co.), was an effective solvent. In turn, methanol (Baker analyzed reagent) was added to increase the miscibility of the *n*-hexane. The bacteriostatic properties of *n*-hexane had to be determined to assure that there was no killing factor other than ethylene oxide.

MATERIALS AND METHODS

Bacillus subtilis var. *niger*, ATCC 9372, was selected as the test organism since its spores are known to be highly resistant to ethylene oxide gas [Beeby and Whitehouse (2); Ernst and Shull (3, 4); Kelsey (5); and Phillips (6)]. Sterile trypticase soy broth (TSB) was used as the culture medium (20 ml. per culture tube), and disposable hypodermic syringes (2.5 ml.) and needles [16 gauge × 3.81 cm. (1.5 in.)] were used as the test samples. Commercial silicone fluid M360 (Dow Corning Co.), a colorless, highly water-repelling, nontoxic, nonvolatile, low-surface tension, and chemically thermally inert substance (1) was used as the coating agent.

Spore Strips Treated with *n*-Hexane—To determine whether *n*-hexane had a bacteriostatic effect on *B. subtilis* var. *niger* spores, three different concentrations (10^3, 10^5, and 10^7) of spore strips (American Sterilizer Co.) were immersed in *n*-hexane for 1 hr. These were then divided into two groups. The first group was cultured in TSB; the other was mixed with 1 ml. of 90% methanol (shaken for 20 min. on an automatic shaker) and then cultured in TSB for 48 hr. at 32°.

Vegetative Cells Treated with *n*-Hexane—To determine the bacteriostatic properties of *n*-hexane on vegetative cells of *B. subtilis* var. *niger*, four separate amounts (0.1, 0.2, 0.5, and 1.0 ml.) of *n*-hexane were mixed with 0.5 ml. of 90% methanol and 0.06 ml.

Table I—Effect of *n*-Hexane on Spore Strips of *B. subtilis* var. *niger*

Concentration of Spores	Total No. of Samples	24-hr. Growth	48-hr. Growth
10^3	5 Without methanol	1 Light growth 4 No growth	1 Heavy growth 4 No growth
10^3	5 With methanol	4 Light growth 1 No growth	4 Heavy growth 1 No growth
10^5	5 Without methanol	5 Medium growth	5 Heavy growth
10^5	5 With methanol	5 Heavy growth	5 Heavy growth
10^7	5 Without methanol	5 Heavy growth	5 Heavy growth
10^7	5 With methanol	5 Medium growth	5 Heavy growth

Table II—Effect of *n*-Hexane on Vegetative Cells of *B. subtilis* var. *niger*

Amount of *n*-Hexane, ml.	Total No. of Samples	Amount of Organisms, ml.	24-hr. Growth
0.1	10	0.06	All heavy growth
0.1	10	0.005	All heavy growth
0.2	10	0.06	All heavy growth
0.2	10	0.005	All heavy growth
0.5	10	0.06	All heavy growth
0.5	10	0.005	All heavy growth
1.0	10	0.06	All heavy growth
1.0	10	0.005	All heavy growth

Table III—Effect of *n*-Hexane on Spores Suspended in Methanol

Amout of *n*-Hexane, ml.	Total No. of Samples	24-hr. Growth	48-hr. Growth
0.1	10	10 Light growth	10 Heavy growth
0.2	10	8 Heavy growth 2 No growth	8 Heavy growth 2 No growth
0.5	10	8 Heavy growth 2 No growth	10 Heavy growth
1.0	10	8 Heavy growth 2 No growth	10 Heavy growth

of a heavy growth culture of *B. subtilis* var. *niger* and were shaken for 1 hr. on an automatic shaker (Group 1). Following the same method, a second group was mixed with a small amount of the heavy growth culture (0.005 ml.). Both groups were then cultured in TSB for 24 hr. at 32°.

Spores in Methanol Treated with *n*-Hexane—To determine a possible bacteriostatic effect of *n*-hexane on spores in the methanol suspension, spores (15×10^6 per ml.) were diluted with 90% methanol to approximately 1000 spores per ml. Spore samples (1 ml. each) were thoroughly mixed for 30 min. (on automatic shaker) with different amounts (0.1, 0.2, 0.5, and 1.0 ml.) of *n*-hexane and then cultured in TSB for 48 hr. at 32°.

Samples Inoculated with Spores and Coated with Silicone—To determine whether silicone fluid inhibits the germination and/or growth of spores, 20 rubber plunger tips were dipped into a spore suspension (15×10^6 per ml.) and then allowed to air dry for 6 hr. at 25°. A measurement (using Sahli-type hemoglobin pipet to pick up the spore solution adhering to the plunger tip) was made which was 20 λ and contained approximately 300,000 spores adhering to the rubber surface.

In addition, 20 needles were immersed into the 15×10^6 spore solution for 4 hr. and then allowed to air dry for 6 hr. at 25°. The pour plate counting method determined that approximately 32,000 spores were under the coating per needle (number of spores under coating = number of spores rinsed off before coating − number of spores rinsed off after coating). Experimental needles and rubber plunger tips were then coated with silicone (the thickness of the coating and technique were as normally used by the manufacturer, which is a maximum of 1 mg./cm.^{2} of exposed surface area of rubber tip and 1 mg. per needle). Controls were not coated with silicone. All the samples were then cultured in TSB for 24 hr. at 32°.

Inoculated Samples Sterilized by Ethylene Oxide—To determine the ability of 100% ethylene oxide gas to penetrate and sterilize through the silicone coating, the rubber tips and needles were inoculated with spores, coated with silicone (same method as mentioned previously), and assembled as a syringe. The syringes were then sterilized with 100% ethylene oxide with a concentration of approximately 2628 mg./l. for 4 hr. at 48.8° (120°F.) and 4 p.s.i. pressure. Then the experimental group had the silicone coating removed with *n*-hexane, and a control group was left coated. Both groups were cultured in TSB at 32° for 10 days.

Table IV—Effect of Silicone Coating on the Inoculated Samples—Not Sterilized

Treatments	Samples	24-hr. Growth
With coating	10 Plungers	All heavy growth
With coating	10 Needles	All heavy growth
Without coating	10 Plungers	All heavy growth
Without coating	10 Needles	All heavy growth

Table V—Effect of Ethylene Oxide Sterilization on Inoculated Samples

Treatment	Samples	10-Day Cultivation
Coating removed	10 Plungers	All no growth
Coating removed	10 Needles	All no growth
Coating not removed	10 Plungers	All no growth
Coating not removed	10 Needles	All no growth

RESULTS

The results are shown in Tables I through V.

DISCUSSION AND CONCLUSION

The results of Table I show that a concentration of 10^3 spores on paper strips, after immersion in *n*-hexane, was not successfully cultured. The *n*-hexane, being nonsoluble in water, formed a layer in the liquid growth medium; however, when mixed with 90% methanol, *n*-hexane solubility was increased, thereby releasing the spores for germination and growth. There is no indication that *n*-hexane acts as a bacteriostatic agent to both the spores and vegetative forms of *B. subtilis* var. *niger;* therefore, *n*-hexane was used as a solvent for the silicone fluid.

The number of microorganisms involved did not prove to be an influencing factor for the bacteriostatic character of *n*-hexane. The results of Table II indicate that a small number of microorganisms was not inhibited by any of the levels of *n*-hexane.

Results of Table IV indicate that the silicone coating (which was coated by the spray method) did not cover a great number of the inoculated spores (10^6); therefore, the silicone coating was not considered a complete masking factor.

Results of this study indicated that 100% ethylene oxide does penetrate the silicone fluid used as a coating agent and does kill the test organisms present on the surface and beneath the coating.

REFERENCES

(1) Medical Materials Data, Dow Corning, Bulletin 14-003.

(2) M. M. Beeby and C. E. Whitehouse, *J. Appl. Bacteriol.*, **28** 349(1965).

(3) R. R. Ernst and J. J. Shull, *Appl. Microbiol.*, **10,** 337(1962).

(4) *Ibid.*, **10,** 342(1962).

(5) J. C. Kelsey, *J. Clin. Pathol.*, **14,** 59(1961).

(6) C. R. Phillips, *Amer. J. Hyg.*, **50,** 280(1949).

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