TABLE IV—EFFECT OF U-1247 AND PARGYLINE ON TYRAMINE BLOOD PRESSURE RESPONSES

Treatment[a]	Tyramine Dose, mg./Kg.	ΔxBP, mm. Hg	±S.E.	No. Rats in Group
Control	Saline	12.0	2.16	6
Control	12.5	16.2	4.00	6
Control	50	38.5	5.35	6
Pargyline	12.5	37.0	11.05	6
U-1247	12.5	33.8	10.80	6

[a] Rats pretreated with U-1247 or pargyline (100 mg./Kg. p.o.) 72, 48, and 24 hr. prior to tyramine challenge (p.o.).

significant blood pressure reduction in both groups.

Effect of U-1247 and Pargyline on the Pressor Response to Tyramine—The mean blood pressure response to 0.01 mg./Kg. i.v. of tyramine in 7 rats was 7.7 (±2.5). After pretreatment with pargyline or U-1247, the mean response to tyramine in 5 rats was increased to 24.4 (±10.4) and 32.6 (±8.5), respectively. These differences from control were significant at the 0.1 level for pargyline and the 0.01 level for U-1247.

The effect of U-1247 and pargyline on perorally administered tyramine is summarized in Table IV.

In control rats there was a significant difference in the blood pressure response between 12.5 mg./Kg. and 50 mg./Kg. of tyramine. After pretreatment with either pargyline or U-1247, the response to 12.5 mg./Kg. of tyramine was increased, and no longer was significantly different from the response to the 50 mg./Kg. dose.

These data indicate that U-1247 and pargyline are capable of potentiating both intravenous and peroral tyramine.

DISCUSSION

This study indicates that within the pargyline series a triple bond is not essential for *in vitro* or *in vivo* MAOI activity. The *in vitro* data suggest that U-1247 is approximately 25 times less active than pargyline, while *in vivo* the two compounds are approximately equal in potency. The *in vivo* conversion of U-1247 to a more active form is one possible explanation of these results. The bioconversion of the allenic compounds to acetylenic derivatives, however, is unlikely as Swett (1) has indicated both the *N*-2 and *N*-3 butynyl-*N*-methylbenzylamine are inactive *in vivo*. The possibility of U-1247 and pargyline being converted to a common active form cannot be discounted from the data and is worthy of further study.

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Keyphrases

Monoamine oxidase inhibitors
Allenic amines—synthesis
LD_{50} value—allenic amines
Hypotensive, antihypertensive activity
Vapor-phase chromatography—identity
IR spectrophotometry—structure

Influence of Various Pretreatments (Carriers, Desiccation, and Relative Cleanliness) on the Destruction of *Bacillus subtilis* var. *niger* Spores with Gaseous Ethylene Oxide

By JOHN E. DOYLE and ROBERT R. ERNST

The ethylene oxide resistance of *Bacillus subtilis* var. *niger* spores inoculated on a variety of carriers was determined. After initial experiments indicated that cleanliness of the spores was important, desiccated and undesiccated spores of varying degrees of cleanliness on aluminum foil and chromatography paper were studied. Under the experimental conditions, desiccation of the spore population had no influence on susceptibility to ethylene oxide. The degree of cleanliness of the spore preparation was the significant factor in excessive resistance. The bacteriological controls used for testing ethylene oxide sterilization processes should be adequately cleaned so that they may accurately monitor the process.

GASEOUS ETHYLENE OXIDE is used for the sterilization of materials which cannot be sterilized by steam. As the utilization of heat- and moisture-sensitive materials increases, the importance of ethylene oxide sterilization increases for the hospital and pharmaceutical related fields.

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Essential to an ethylene oxide process is proper monitoring. The USP XVII, page 811 states: "The efficacy of any procedure for the sterilization of pharmaceutical products by ethylene oxide should be tested thoroughly by inclusion of items contaminated with spores of known microorganisms in representative locations in typical sterilizer loads and by subsequent demonstration that these microorganisms have not survived the treatment."

Since the evaluation of the efficiency of an ethylene oxide process depends a great deal on the bacteriological controls, the controls chosen should be uniform as regards to resistance to the sterilant. This paper deals with the effect of cleanliness and desiccation on the resistance of spores to ethylene oxide.

METHODS

Bacillus subtilis var. *niger* 356 S. C. No. 4 (1) was used in this study. The culturing methods have been described previously (2). Washing the spores was accomplished by centrifugation at 20,500 × g for 10 min., decanting the supernate, followed by resuspension in sterile distilled water. This procedure was repeated for further washings when indicated. Dilutions were made in sterile distilled water to produce the desired inoculum. Inoculums were 0.01 ml. per carrier materials.

The procedures similar to those used by Ernst and Shull (3) were used in this study for determination of ethylene oxide resistance.

RESULTS

Initially in the investigation, it was of interest to determine the resistance of *Bacillus subtilis* var. *niger* on a variety of carrier materials. However, a spore suspension was used that had been washed only two times. Table I describes the variety of resistances obtained. There appeared to be little effect due to absorbency or nonabsorbency of the carrier. No consistent difference of resistance between hard and porous surfaces was observed

TABLE I—RESISTANCE OF *Bacillus Subtilis* VAR. *Niger* SPORES[a] ON VARIOUS CARRIERS

Carrier Material	Total Survival, min.	Partial Survival, min.	Total Kill, min.
Acetate fabric, 100%	5	45	60
Acetate-rayon fabric	45	60	90
Aluminum foil	5	7	10
Bibulous paper	3	4	5
Chromatography paper	3	4	5
Fluorohalocarbon film	5	10	15
Heavy paper	20	45	60
Nylon fabric	5	10	15
Nylon film	5	7	10
Polypropylene film	5	7	10
Polyvinyl chloride film	4	25	25
Rubber glove film	5	10	15
Wax paper	5	10	15

[a] Spore suspension (10^8 spores/carrier) washed two times in sterile distilled water. Ethylene oxide concentration, 1,200 mg./L.; relative humidity, 40%; temperature, 130°F.

TABLE II—RESISTANCE OF *Bacillus subtilis* VAR. *Niger* SPORES[a] ON VARIOUS CARRIERS

Carrier Material	Total Survival, min.	Partial Survival, min.	Total Kill, min.
Acetate fabric, 100%	1	3	5
Acetate-rayon fabric	5	7	10
Aluminum foil	5	10	15
Balsa wood	5	7	10
Chromatography paper	5	10	15
Cotton fabric, 100%	5	10	15
Glass tubing	3	7	10
Polyethylene film	3	7	10
Rayon fabric, 100%	5	10	15
Reinforced cellulose	5	10	15
Rick-rack	3	7	15
Rubber glove film	3	10	20
Wax paper	3	7	10
Yellow sponge ("Ivalon")	5	10	15

[a] Spore suspension (10^8 spores/carrier) washed eight times in distilled water. Ethylene oxide concentration, 1,200 mg./L.; relative humidity, 40%; temperature, 130°F.

However, a large zone of partial survivors and high resistance was observed. This may have been due to the fact the spore suspension was only washed twice. A synergistic effect of the carrier material and the residual media may have produced high ethylene oxide resistance.

Therefore, a new suspension of *Bacillus subtilis* var. *niger* was prepared as before. However, this suspension was washed eight times. As shown in Table II, once again there appeared to be little effect on the resistance regardless of whether the carrier was absorbent or nonabsorbent. High resistance due to any carrier effect was not observed. The high degree of cleanliness appeared to reduce the relative resistance of the spores.

Since the cleanliness of a spore suspension appeared to be an important aspect of its relative resistance, the effect of the relative degree of cleanliness on the resistance of spores to ethylene oxide with respect to both nonabsorbent and porous carriers was investigated. Since hard surfaces have been reported to produce a greater resistance than porous surfaces, an aluminum foil carrier was compared with a porous paper carrier. Also because desiccation (4, 5) has been reported to increase the resistance of spores, this aspect was investigated as well.

A new suspension of *Bacillus subtilis* var. *niger* was prepared as before. Equal aliquots of the spore culture broth were separated into five groups. Washing was accomplished as described previously. One suspension was not cleaned, one was cleaned once, *etc.* Then the aluminum foil and chromatography paper strips were inoculated with the five suspensions. A portion of each was allowed to dry and equilibrate at room temperature. The other portion was placed in a desiccator and dried over calcium sulfate desiccant at room temperature in excess of 1 month. Hence there were "normal" dried and desiccant dried aluminum foil and paper carriers of varying degrees of cleanliness as shown by the number of resuspensions in distilled water.

Figure 1 compares the relative resistance of spores with relative degrees of cleanliness when placed upon aluminum foil carriers. If the suspension is not washed at all, a high resistance (over 48 hr.) to sterilization is obtained, probably due to the occlusion of the spores in the media. With one resuspension a broad partial survival region was observed,

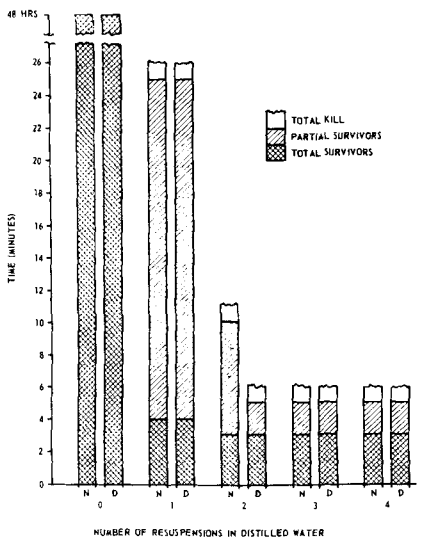

Fig. 1—Effect of relative cleanliness and desiccation on the ethylene oxide resistance of Bacillus subtilis *var.* niger *spores (10^6 spores/carrier) on aluminum foil. N = not desiccated, D = desiccated. Ethylene oxide concentration, 1,200 mg./L.; relative humidity, 40%; temperature, 130° F.*

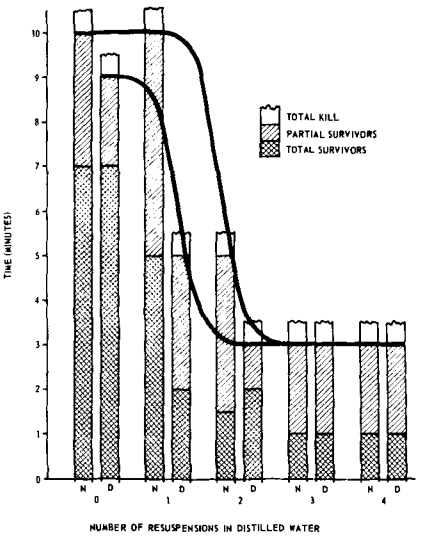

Fig. 2—Effect of relative cleanliness and desiccation on the ethylene oxide resistance of Bacillus subtilis *var.* niger *spores (10^6 spores/carrier) on porous paper. N = not desiccated, D = desiccated. Ethylene oxide concentration, 1,200 mg./L.; relative humidity, 40%; temperature, 130° F.*

indicating that some spores were being randomly protected. However, sterility was obtained in 25 min. With a second resuspension there was still some effect due to the relative uncleanliness of the suspension, except in the case of the desiccated carriers. After the third resuspension, the resistance dropped to 3 min. total survival, requiring only 5 min. for total kill. The partial survival region is approximately 2 min. Desiccation appeared to have little effect on the resistance of the spores.

Figure 2 compares the resistance of spores of relative degrees of cleanliness when placed upon a porous paper carrier. Complete resistance to ethylene oxide with the porous paper was never obtained under these conditions, even when the suspension was not washed at all. It appeared that the desiccated carriers were less resistant than the undesiccated carriers when the spore inoculum was washed less than three times. Upon further washing, undesiccated and desiccated strips had equal resistances.

Some experiments were performed with the spore preparation which was not washed, at 10^7 spores per paper carrier, and an even greater partial survival region was observed. One would expect more resistance with carriers contaminated with a greater number of spores. However, the larger region of partial survival shows the greater likelihood of protection by occlusion due to the larger amount of media as well as the larger number of spores.

When one compares the degrees of cleanliness of spores on the aluminum foil and paper carriers, it is evident that the cleanliness of the spore suspension used determines great differences in relative susceptibility. In every degree of cleanliness the aluminum foil carrier resulted in a greater resistance than the porous paper with a comparative difference in resistance becoming quite small with three washings.

The small difference of greater resistance imparted by a hard surface over a porous one is easy to understand, since diffusion is impossible or hindered through a hard surface carrier material, whereas the gases can diffuse in all directions through a porous one. Also the spores would tend to protect each other for they tend to ball up on a hard surface material whereas the spores become somewhat separated when inoculated on a porous paper or any porous material.

Desiccation of spores did not appear to play a large role in the experimental conditions. Even at 10% RH, 130°F., and 1,200 mg./L. ethylene oxide, little differences in resistance between clean desiccated and undesiccated spores of *Bacillus subtilis* var. *niger* were observed. The sterilization time for both, however, was extended to 1 hr.

Since calcium sulfate desiccation had no effect on ethylene oxide resistance, spores were dried in the following manner: (*a*) Acetone drying of spores prior to inoculation on chromatography paper. (*b*) Vacuum drying of cleaned spores on chromatography paper at 100μ for 24 hr. (*c*) Chromatography paper carriers were inoculated with clean spores at 10^7 spores/carrier. Then the carriers were exposed to 100°C. in an oven for 12 hr., reducing the spore population per carrier to 10^6. These remaining viable spores should have been extremely dry. None of these means of drying clean spore preparations produced any difference in resistance to ethylene oxide. The uncleanliness of a spore preparation seems to be the major reason for unusual resistance.

If the spore crop is clean, the region of partial survivors is narrow. However, if the spore crop is not thoroughly washed, the region of partial survivors is broad. Materials inoculated with the spore suspension are randomly protected by occlusion in crystals, encapsulation in organic debris, or both. The broader the region of partial survivors, the more unreliable the sterility monitor becomes.

One can artificially produce conditions in which microorganisms become extremely resistant to ethylene oxide. When *Staphylococcus aureus* or *Bacillus subtilis* var. *niger* were suspended in blood

and then inoculated on aluminum foil, they failed to be sterilized with ethylene oxide. Likewise, *Bacillus subtilis* var. *niger* spores suspended in peptone and dried on aluminum foil with a contamination level of only 10 spores/carrier were very difficult to sterilize with ethylene oxide. This would be similar to the protective effect found by previous investigators (6), *i.e.*, occlusion of the spores in crystals.

It is also possible to produce artificial conditions which would seem to make the organisms extremely susceptible to ethylene oxide. Spores suspended in 2% glycerin and then dried on polystyrene were less resistant to ethylene oxide than spores suspended in distilled water (7). *Bacillus subtilis* var. *niger* spores were suspended in 10% glycerin, inoculated on chromatography paper strips, and dried at room temperature. After drying at room conditions, the glycerin will contain a certain amount of water depending on the room relative humidity, for it will gain or lose moisture depending on its surroundings. At 130°F., 1,200 mg./L. ethylene oxide, these strips showed the same resistance (3 min. for inactivation) at 40% RH as at 10% RH even though the strips at 40% RH would contain a greater amount of moisture after equilibration. A similar situation would indicate to the unwary that no optimum RH was required. However, clean spores inoculated from distilled water required 1 hr. at 10% RH and 3 min. at 40% RH for inactivation.

SUMMARY

The evaluation of the efficiency of an ethylene oxide process depends a great deal on the type of bacteriological control used. It is therefore recommended that an evaluation be made of the type of load to be sterilized, so that the bacteriological controls chosen will accurately monitor the process. If one uses unwashed spores for preparation of sterility monitors when sterilizing clean materials, then he will likely obtain erratic indications of nonsterility rather than sterility. If, however, one does attempt to sterilize dirty materials, contaminated with dirt, blood, feces, *etc.*, he must realize that the process at its best will sterilize occasionally and should only be considered a decontamination procedure. The only dependable method of testing a sterilization process is to determine if it kills living microorganisms. The microbial control chosen should simulate actual conditions of the materials being sterilized.

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Keyphrases

Ethylene oxide sterilization
B. subtilis spores—ethylene oxide effect
Staph. aureus—ethylene oxide resistant
Sterilization, ethylene oxide—variables affecting

Preferential Aggregation and Coalescence in Heterodispersed Systems

By NORMAN F. H. HO and W. I. HIGUCHI

A theoretical study of preferential coalescence and aggregation of small particles in heterodispersed systems has been carried out where moderate electrical barriers exist between the particles. Equations based on the concepts of Derjaguin, Verwey, and Overbeek were employed. Computation over a wide range of conditions has shown that small particles may aggregate (or coalesce) with themselves or with larger particles at rates that are 10 to 50 orders of magnitude faster than particles 10 times larger. These findings may explain (*a*) the relatively narrow particle size distributions observed in certain emulsions and flocculated suspensions and (*b*) the limited flocculation and coalescence behavior observed in certain instances.

THERE ARE many situations involving suspensions and emulsions where with time the particles or droplets of the dispersed phase simultaneously increase in size and narrow in their relative size distributions, and then later became quite stable kinetically. If the dispersed phase is soluble (or miscible) enough in the solvent, then the phenomenon may be accounted for by molecular diffusion (1) or Ostwald ripening. However, there are many examples in the literature (2–5) where the changes appear to occur primarily through particle–particle aggregation or droplet–droplet coalescence.

The authors have recently observed that urea-

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