Biological-Chemical Indicator for Ethylene Oxide Sterilization

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The use of a biological-chemical indicator for ethylene oxide sterilization of disposable medical and pharmaceutical materials is reported. The indicator system affords an immediate visible indication of gas penetration into the materials during the sterilization cycle and also serves as a biological (spore) control. Preparation of the indicator is discussed in some detail, including the organisms and chemicals employed and the basic chemical reactions. The usefulness of the indicator for determination of gas penetration is discussed in terms of diffusion of the gas and permeability of the materials, including latex and plastic films.

A NUMBER of indicators have been suggested for use with ethylene oxide. For the most part these depend on a pH change or other type of chemical reaction, and some attempt has been made to have these indicate time as well as the presence of ethylene oxide. Since moisture content and other factors have a great deal to do with the efficiency of gas sterilization, the most satisfactory indicator of sterilization is the use of positive spore controls. These have the disadvantage that one must wait for the required incubation before reading the results.

In the sterilization of many of the disposable medical and pharmaceutical materials such as gloves, syringes, transfusion, and infusion sets, it is of the utmost importance to determine whether the gas is reaching the intricate spaces within these items and sterilizing them. As pointed out by Rubbo and Gardner (1), this is particularly true of such items as syringes with rubber plungers which might have two or more ribs in contact with the glass or plastic barrel.

If one depended on pressure alone to get the gas into these spaces, then this pressure would be too great to be reached by most gas-sterilization processes. One must also take into consideration the diffusion characteristics of the materials employed, and this makes a visual indicator quite valuable. Most of the chemical indicators are so sensitive that a very small amount of gas might cause a color change and yet not be sufficient to kill any bacterial spores present. For this reason the authors have sought an indicator system that combined both a chemical indicator, which did not depend on a pH change alone, and bacterial spores.

CHEMICAL INDICATOR

The compound 4-(4-nitrobenzyl) pyridine[1] has been described as a sensitive reagent for the determination of alkylating agents (2, 3). This reagent reacts with ethylene oxide to form a methine dye which is blue in an alkaline medium. (Scheme I.) The mechanism in the reaction of the reagent with an alkylating agent has been reported (4).

4-(4-Nitrobenzyl) pyridine has been used in this laboratory as an ethylene oxide indicator in solution, dried on filter paper, and adsorbed on silica gel. It has been found useful to indicate ethylene oxide permeability of latex and plastic films, ethylene oxide retention of plastics and elastomers, and as an exposure indicator for ethylene oxide sterilization.

The indicator paper is prepared by first dipping Whatman No. 1 filter paper in a 2% aqueous solution of sodium carbonate. After drying, the paper is dipped in a 2% solution of the indicator reagent in acetone. The indicator paper thus formed can then be cut into convenient-sized strips and inoculated with bacterial spores.

Indicator strips prepared as described will turn blue within a few seconds when exposed to the vapors of pure ethylene oxide. The same strips have been exposed to sterilization gas mixtures of ethylene oxide and carbon dioxide in ratios of 10:90 and 20:80, and it was observed that as long as the strip remained in the mixed-gas atmosphere the color did not change. Furthermore, if the strip was sealed in a permeable plastic container which would retain some residual gas mixture after removal from the sterilizer, no color change would occur. However, when the paper was removed to the air after exposure, the blue color would develop. This effect was explained by the fact that carbon dioxide prevented the reaction product from assuming the alkaline form until the CO_2 was diffused from the paper.

EXPERIMENTAL

In order to determine ethylene oxide penetration to the area between the plunger-stopper rings of both glass and plastic syringes, indicator strips were cut to size and inserted in this area. (Fig. 1.) The assembled syringes were placed in a glass desiccator; the desiccator was evacuated to approximately 25 in. of mercury and ethylene oxide admitted to a concentration of approximately 1000 mg./L. No color change of the strips in the syringes was noted until after about 1.5 hr. of exposure. After this time, a faint blue color was noted, which continued to darken. After 3 hr. of exposure, the syringes were removed from the desiccator. For the next 24 hr. the indicator strips continued to darken to a deep blue.

It appeared from the above experiment that dif-

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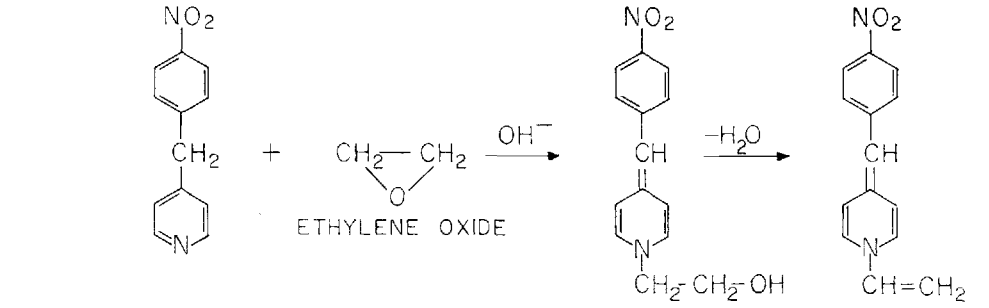
[1] The authors acknowledge the suggestion of Dr. David N. Kramer, Army Chemical Center, Md., that we investigate this compound as an indicator.

The cost of the preparation of the color picture which appears in this article has been borne by the authors.



Scheme I

fusion of ethylene oxide gas through to the area between the syringe stopper rings is slow; however, the gas will eventually penetrate to this area and in sufficient concentration to kill the control organisms. There was also an indication that ethylene oxide continues to diffuse from the stopper for a period of time after the syringes are removed from a sterilizer. To investigate these observations further, syringes were assembled with 5 stoppers and indicator strips in each barrel and exposed as in the previous experiment. When the syringes were removed from the desiccator after 3 hr. of exposure, the strips in the 2 outer stoppers were blue and the 3 inner stoppers were white. Twenty-four hours after the syringes were removed, the strips in the stoppers next to the outer stoppers had turned blue and the center strip remained white; 36 hr. after exposure, the center strip had turned blue. These are exaggerated laboratory tests, since no syringe has over 1 rubber plunger.

This might indicate that the gas remains in the rubber or within the syringe, tubing sets, or other items, and might possibly contaminate drugs being administered to the patient. To prove this is not the case, gas chromatographs are run to show that all the gas has diffused out within the 10-day quarantine period and is further demonstrated by the toxicity tests which are run on each lot.

BIOLOGICAL INDICATOR

As spore controls we have employed both *Bacillus stearothermophilus* and *Bacillus globigii*. For our

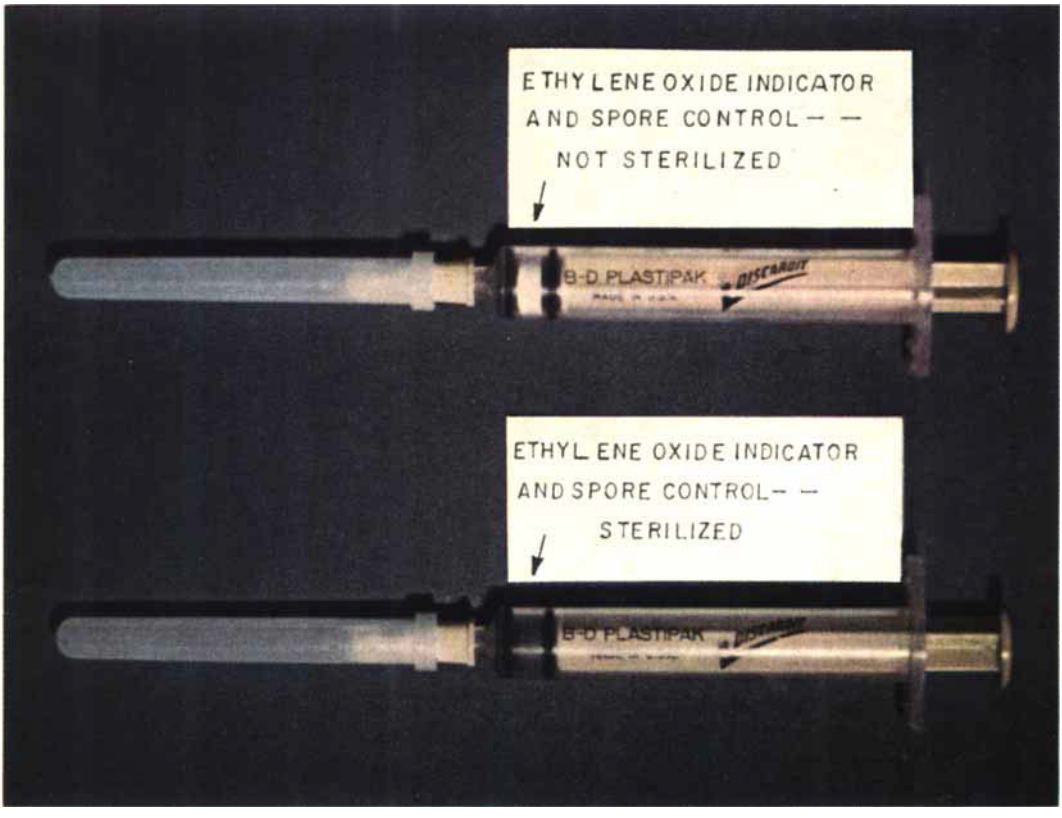


Fig. 1.—Sterilized and unsterilized ethylene oxide indicators and controls.

purposes we have found the thermophilic organism much more satisfactory. One is not likely to encounter this type of organism as a laboratory contaminant in the sterility test, thereby causing a false positive reaction. This would necessitate the retesting of the load and delay production.

A very important consideration in the selection of the chemical indicator was to make sure that it did not inhibit the growth of the control organisms. Bacteriostatic-type tests were made using diluted spore suspensions with the paper strips, both before and after exposure to the sterilizing gas. No inhibition was noted and controls from small inocula grew quite luxuriantly in the test media.

The spores used to inoculate the indicator strips were prepared by suspending washed spores of *B. stearothermophilus* in distilled water containing 1% sorbitol. The suspension was standardized to approximately 5 million spores per ml. Spore counts were determined by appropriate dilution of the suspension and plating on trypticase soy agar. Plates were incubated at 60° for 24 hr. Paper strips were inoculated with 0.02 ml. of the suspension or approximately 100,000 spores. After sterilization, strips were transferred to fluid thioglycollate medium and incubated at 60° for 7 days to determine survival of spores. Control tubes containing indicator strips, both before and after exposure to ethylene oxide and inoculated with diluted spore suspension, showed no inhibition of the control organism.

APPLICATION

In commercial lots, dye-spore strips with 100,000 spores are prepared in a size to fit the item being sterilized. They have been prepared in sizes similar to the commercially available spore strips[2] for use in the index finger and in the cuff of rubber gloves, since it is felt that these are the most difficult places for the gas to reach in this item. These controls in the index finger change color, and the organisms are killed even if a knot is tied in the wrist part of the glove, indicating adequate penetration. In the sterilization of commercial lots of gloves,[3] the use of these indicators throughout the sterilizer load give assurance that the entire lot has been sterilized.

REFERENCES

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(3) Sawicki, E., *et al.*, *ibid.*, **35**, 1479(1963).
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[2] Kilit spore strips, Baltimore Biological Laboratory, Baltimore, Md.
[3] Wilson Rubber Co., Canton, Ohio.

Pharmacokinetic Model for Nalidixic Acid in Man I

Kinetic Pathways for Hydroxynalidixic Acid

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A model which includes the parameters of absorption, metabolism, and excretion of hydroxynalidixic acid (HNA) is presented. Individual rate constants for the absorption and disappearance of HNA in 5 subjects were determined. Theoretical plasma-level curves based upon these constants were calculated, and good agreement with the experimental data was obtained. Rate constants for glucuronide formation, oxidation, and excretion of HNA were calculated.

HYDROXYNALIDIXIC acid (1-ethyl-1, 4-dihydro-7-hydroxymethyl-4-oxo-1, 8-naphthyridine-3-carboxylic acid) is 1 of 4 metabolites of nalidixic acid[1] formed in man. It has been isolated and shown to have an *in vitro* antibacterial spectrum similar to that of nalidixic acid (1). The other 3 metabolites, all of which have not shown biological activity, are nalidixic acid glucuronide, hydroxynalidixic acid glucuronide, and the 3,7-dicarboxylic acid.

A previous article by the authors (2) described a simplified working model which permitted calculation of the apparent kinetics of the biologically active and inactive forms of nalidixic acid as separate groups.

Because nalidixic acid has been found to be clinically effective in the treatment of Gram-negative infections (3, 4), more definitive studies of its pharmacokinetics have been continued. These experiments would also illustrate the mechanisms which enabled the simplified model to describe absorption and elimination parameters.

In order to describe the complete pharmacokinetic profile of nalidixic acid, it is necessary to quantitate all the rate processes, including those occurring after its oxidation to the 7-hydroxy derivative. This article deals with a study of the absorption, metabolism, and excretion of hy-

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[1] Marketed as NegGram by Winthrop Laboratories, New York, N. Y.