# Preliminary Evaluation of an Activated Glutaraldehyde Solution for Cold Sterilization \*

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## Introduction

THE INCREASED INCIDENCE of hospital-acquired infections has initiated renewed interest in their contributory factors. Nahmias<sup>2</sup> has summarized much of this information. The incidence of infection would be decreased if an effective germicide were available to sterilize materials rapidly which are difficult to sterilize by conventional heat or chemical methods. There are many disinfectant and antiseptic compounds available, but these are often corrosive to metal surfaces, react chemically with rubber, plastics, or various cements, are readily inactivated by organic materials, or are slow-acting. Spaulding 5 has recently commented upon the various uses for which the various solutions are best suited.

Snow *et al.*<sup>4</sup> have been concerned with the possibility of infections being transferred by anesthetic equipment used in the operating rooms, and he has reported using ethylene oxide sterilization procedures to disinfect this equipment. This gas compound is a very effective sterilizer but the procedure takes time and special equipment, and in a busy operating-room suite many items of equipment must be cleaned, stored, packed and redistributed after sterilization.

An activated solution of 2 per cent glutaraldehyde (Cidex<sup>®</sup>\*\*) has been found to be extremely effective against various vegetative bacteria (Staphylococcus aureus, Diplococcus pneumoniae, Escherichia coli, Pseudomonas aruginosa, Proteus vulgaris, and in Klebsiella pneumonia) spores (Bacillus globigii, Bacillus subtilis, Clostridium tetani, and Clostridium welchii), several fungi and the tubercle bacillus. The results of a series of in vitro tests on these organisms under AOAC conditions reveal that vegetative forms were killed with less than 5 minutes exposure to this solution and spores were killed with less than 3 hours exposure. The tubercle bacillus was killed within 10 minutes as were poliomyelitis, influenza and mouse hepatitis viruses. Other tests show that the material did not harm cement holding lenses in several instruments, was adsorbed onto rubber surfaces rather than absorbed, and could be almost completely rinsed from these materials with either tap water or saline. In addition, endotracheal tubes and urethral catheters soaked with pure activated glutaraldehyde solution were placed in dogs for 4 to 6 hours and none of the histologic sections taken of these areas showed any evidence of irritation.1

It was obvious from these results that this material has a potential for wide application within a hospital environment to disinfect many pieces of equipment otherwise difficult to effectively treat.

### **Bacteriologic Methods**

All cultures taken in preliminary studies now being reported were made with thioglycollate broth-moistened swabs that were

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**<sup>\*\*</sup>** Cidex<sup>®</sup>, (Activated Dialdehyde), supplied by Arbrook, Somerville, New Jersey.

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TABLE 1. Cidex<sup>®</sup> Study. Results of Cystoscope Cultures

	Solution Used					
- N	Iercuric	Chloride	Cidex ®*			
	Wet	Dry	Wet	Rinsed		
No. cultures taken % negative culture	62 71.0	62 80.6	76 96.1	74 96.1		

\* Arbrook (2% activated glutaraldehyde solution).

then quickly placed into a tube containing 5.0 ml. of the same broth and incubated. Before being used, all culture tubes had been preincubated for 24 hours to exclude contaminating bacterial growth.

Culture tubes were inspected for visible bacterial growth after the initial period of incubation, and when growth was seen the organisms were identified by standard subculturing technics. If no growth were visible after the first 24 hours incubation, the tubes were re-incubated for 24 hours and then checked by subculture to 5 per cent blood agar plates. Control cultures were undertaken in each instance to check for contamination. A sample was considered sterile if there was no bacterial growth after two 24-hour periods of incubation in thioglycollate broth and after subculture to a 5 per cent blood agar plate incubated for 24 hours.

#### **Preliminary Studies**

These studies were made to determine whether or not previous results could be reproduced by testing the effectiveness of this compound on several items needed in a hospital.

In Vitro Laboratory Tests. A preliminary test was carried out to determine the effective sterilization of surgical instruments that were contaminated with whole blood containing a known quantity of viable bacteria. Five ml. of citrated whole blood were contaminated with about 2 billion organisms of either E. coli, Staph. aureus, Pseu-

domonas, or Proteus mirabilis. Heat-sterilized hemostats were then dipped into the contaminated blood and placed within a sterile container and the blood was allowed to dry. Control cultures were then taken from the serrated portions of the hemostats, and in each instance the contaminating bacterial strain was recovered. Initially these contaminated instruments were to be exposed to activated glutaraldehyde solution for periods of 5, 10 and 15 minutes. However the cultures taken from the first set of instruments soaked 5 minutes in Cidex<sup>®</sup> were negative for all test organisms, and no further time studies were conducted. Cultures were taken from the crevices and serrated surfaces on the hemostat. This showed that activated glutaraldehyde solution could effectively and rapidly penetrate dried organic material, was not inactivated by it, and retained germicidal activity.

Sterilization of Cystoscopes. The standard solution used to prepare cystoscopes was a mercuric chloride solution kept in open containers next to the cystoscopic tables. Cystoscopes were removed from the solution by the circulating nurse wearing sterile gloves. A culture was taken while the instrument was either still wet with mercuric chloride solution or after it had been dried with a sterile towel. Each instrument was soaked for a minimum of 20 minutes before it was cultured and used. All instruments had been scrubbed with a detergent solution before being placed in the mercuric chloride solution. Two per cent activated glutaraldehyde solution was then substituted for the mercuric chloride solution, and the instruments were now soaked for only 5 minutes and rinsed with sterile water before use. All instruments were used on patients, and no excessive irritation of the urethra or bladder could be attributed to the use of Cidex<sup>®</sup>.

Table 1 compares results obtained when these solutions were used for disinfecting

Equipment Cultured	Results Previous Study		Results Cidex <sup>®</sup> * Study				
	% Positive		No.	% Positive		No. Used on	
	Cultured	Bacteria	Pathogens	Cultured	Bacteria	Pathogens	Patients
Anesthesia masks Corrugated tubing—	106	83	20	52	11.5	5.8	20
Old New	97	68	60	8 58	50 3.4	37.5 0.0	4 11
Suction catheters Endotracheal tubes	45 46	45 40	5 12	53 11	5.6 0.0	3.7 0.0	13 10

TABLE 2. Cidex<sup>®</sup> Study. Results of Anesthesia Equipment Cultures

\* Arbrook (2% activated glutaraldehyde solution).

cystoscopes. With mercuric chloride solution only 71 per cent of instruments cultured while wet and 81 per cent of those cultured after drying were negative for bacterial growth.

Of cultures taken from 150 cystoscopes sterilized in glutaraldehyde 96.1 per cent were negative for bacterial growth. This is significantly different from results obtained using the mercuric chloride solution (p << .001). There were no instances of irritation to either nursing personnel, physicians carrying out cystoscopic procedures, or patients.

Food Container Study. A pathogen-free environment has been constructed<sup>3</sup> and patients kept within this area must be fed a presterilized diet. The methods used to sterilize the outside of commercial food containers were not completely satisfactory due to the time needed, the possibility of the foods being contaminated by residual germicides when the container was opened, and, in some instances, incomplete destruction of bacteria present on the container. Therefore, 120 cans of different types of fruit juice were placed in groups of six into a container containing the 2 per cent activated glutaraldehyde solution. After being left in the container for 5 minutes the cans were removed using sterile gloves, the outside was rinsed with a small amount of sterile water, and the can was opened with a sterile can opener. The contents were then cultured, and in no instance was bacteria found in these cultures. Patients reported no change in odor or flavor of the juice.

Anesthesia Equipment Study. Previous bacterial cultures have been taken of the various items of equipment used by staff anesthesiologists. The results of these cultures are shown in part in Table 2.

The equipment studied was formerly cleaned and washed with a detergent solution at various intervals according to the desire of the anesthesiologist. In the present study all these items were soaked in 2 per cent activated glutaraldehyde solution for 5 minutes, removed with sterile gloves and rinsed under tap water, and cultures were then taken from the surfaces.

Eighty-three per cent of masks, 68 per cent of corrugated tubing, 45 per cent of suction catheters and 40 per cent of endotracheal tubes had been contaminated in the earlier study. More significant is the percentage of pathogenic organisms recovered, which varied from 5 per cent for suction catheters to 60 per cent for pieces of old corrugated tubing still used. The effect of the glutaraldehyde solution is shown on the right side of Table 2. None of the endotracheal tubes and only 5.6 per cent of suction catheters, 3.4 per cent of new corrugated tubing and 11.5 per cent of anesthesia masks yielded bacteria. Old corrugated tubing contained many cracks and crevices which influenced the action of glutaraldehyde solution-four of eight tubes so cultured showed bacterial growth and three of these contained pathogenic bacteria (*Pseudomonas*); the percentage of pathogenic bacteria recovered was even less. No patients showed evidences of irritation following the use of any of the items. No staphylococci were recovered in any of the cultures.

It is probable that more attention to the initial cleaning of these items would result in an even lower number of positive cultures from their surfaces. The use of Cidex<sup>®</sup> obviates the need for extra personnel to clean, package, sterilize and redistribute these items; for a prolonged sterilizing procedure either with other germicidal solutions or with ethylene oxide; and for hospital space wherein the sterilizing unit could be kept. The only equipment used to sterilize items that were cultured was a long plastic column in which corrugated tubing was suspended and a large stainlesssteel container in which masks, catheters and endotracheal tubes were placed.

#### Summary

A preliminary study has been made on the effectiveness of a new germicidal solu-

tion in rapidly sterilizing various items of equipment routinely used in a hospital.

Results show that this solution (Cidex<sup>®</sup>) is very effective, does not require prolonged exposure to effect sterilization and is practically not irritating or toxic to either personnel or patients. One case of dermatitis occurred in a practical nurse using this solution, but repeated skin tests with the material did not show that activated glutaraldehyde solution was the allergen.

There has been no damage to any equipment exposed to this agent.

## Bibliography

- 1. Ethicon, Inc. Cidex<sup>®</sup> Activated Dialydehyde Solution. Research Reports, 1962.
- Nahmias, A. and T. Eickhoff: Staphylococcal Infections in Hospitals. New Engl. J. Med., 265:74, 120, 177, 1961.
- Rittenbury, M. S., D. M. Hume and M. E. Hench: "Pathogen-Free" Patient-Care Area. Antimicrob. Agents Chemother., May 1962. pp. 51-65.
- Snow, J., A. Mangiaracine and M. Anderson: Sterilization of Anesthetic Equipment with Ethylene Oxide. New Engl. J. Med., 266:433, 1962.
- Spaulding, E.: Recommendation for Chemical Disinfection of Medical and Surgical Materials. Nat. Cong. of Operating Room Nurses, Washington, D. C., 1963.