Evaluation of Disinfection Techniques for, and Their Effects on, Rectal Thermocouple Catheters

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ABSTRACT

Maher, J. T. (Quartermaster Research and Engineering Command, Natick, Mass.), M. R. Rogers, and D. W. Peterson. Evaluation of disinfection techniques for, and their effects on, rectal thermocouple catheters. Appl. Microbiol. 9:273–278. 1961.—The antibacterial activities of an iodophor (Wescodyne G), a quaternary ammonium compound (Roccal), and an iodine tincture as agents for the cold disinfection of rectal catheters contaminated in vitro were determined. Following thorough cleaning with an alcoholic solution of soft soap, each of the three disinfectants tested showed satisfactory results (100% kill) in 5 min against the enteric test bacteria (Escherichia coli and Salmonella typhosa) as well as a test species of the genus Pseudomonas, among the bacteria most resistant to surface-active agents.

An aqueous solution of Wescodyne G containing 75 ppm available iodine was used both as a wiping solution and for subsequent disinfection of rectal catheters contaminated in vivo. Total bacterial destruction was found to follow a 60-min soak preceded by the wiping procedure.

Rectal catheters subjected to prolonged immersion in each of the test disinfectants were found to be essentially unaffected, retaining their initial calibrations within a permissible tolerance. Neither Roccal nor Wescodyne G solutions were found to measurably attack bare thermocouples. Alcoholic iodine 0.5% did, however, exert a deteriorating effect on bare thermocouples in a short time, as measured by change in resistance characteristics.

The results of this study have led to the recommendation that Wescodyne G containing 75 ppm available iodine be used in standing operating procedures for the initial cleaning and subsequent disinfection of rectal thermocouple catheters.

The rectal thermocouple catheter has proved to be an invaluable laboratory and field instrument in gathering physiological data (Mead and Bommarito, 1948; Davidzick, Harvey, and Goddard, 1953). This deep body temperature-sensing device is now worn by resting or active volunteer military test participants with physiological and psychological acceptability even under extreme temperature conditions (+120 to −65°F).

Although the results of studies on disinfecting rectal thermometers are available (Gershenfeld, Greene, and Witlin, 1951; Sommermeyer and Frohisher, 1953), there is little or no information about disinfecting rectal catheters. Since there is danger of rectal catheters transmitting infectious agents, and considering their unique structure and function, it is advisable to standardize a technique for disinfecting catheters. This study was begun with two objectives, namely:

Phase I. To establish an efficient technique for the disinfection of rectal catheters, and

Phase II. To investigate the effects, if any, of disinfectant action on the components of rectal catheters, specifically in regard to functioning and use-life.

MATERIALS AND METHODS

Phase I. Bacteriological Studies

A) Studies in vitro. 1) Rectal thermocouple catheters. The catheters used in this study (Fig. 1) are temperature measuring devices constructed of modified Dow Corning® 9711 silicone rubber covering a 30 gauge copper-constantan thermocouple. The thermometer is fused with silver solder to a brass tip. The tip is crimped to the end of a 31/2 by 0.16-in. catheter body.

2) Disinfectants tested. (i) Wescodyne G® (Federal stock no. 6840-526-1129) solution containing 75 ppm available iodine (4.7 ml of Wescodyne G to 1 liter distilled water). Wescodyne G® is a brand of iodophor containing as active ingredients: polyethoxy polypropoxy polyethoxy ethanol-iodine complex, nonylphenoxy polyethoxy ethanol-iodine complex, and hydrogen chloride; (ii) alcoholic iodine solution, 0.5% (5 g iodine in sufficient 70% isopropyl alcohol to make the product measure 1 liter); (iii) alcoholic Roccal® solution, 1–1,000 (10 ml of 10% Roccal to 990 ml of

1 The views and conclusions herein contained are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

2 Dow Corning Corporation, Midland, Mich.
3 West Chemical Products, Inc., Long Island City, N. Y.
4 Sterwin Chemicals, Inc., New York 18, N. Y.
70% isopropyl alcohol). Roccal is a brand of alkylidimethylbenzylammonium chloride.

The test concentrations of the commercial disinfectants were those recommended by the respective manufacturers. The efficiency ascribed by Sommermeyer and Froisher (1953) to a 0.5% alcoholic iodine solution in disinfecting rectal thermometers was the basis for its selection. Isopropyl alcohol was used as the carrier for Roccal since tinctures of the quaternary ammonium compounds have been found to be much more effective than aqueous solutions. Aqueous Zephiran, 0.1%, is, in fact, used to isolate Mycobacterium tuberculosis from various body fluids (Patterson, 1956).

3) Test organisms and media. The test organisms used were Escherichia coli ATCC 26, Salmonella typhosa Hopkins strain, and Pseudomonas aeruginosa ATCC 13,388. Stock cultures of E. coli and S. typhosa were carried on slants of Bacto stock culture agar, whereas P. aeruginosa was maintained on Sabouraud maltose agar (Davis et al., 1959). These organisms are considered among the bacteria most resistant to surface-active agents (Glassman, 1948).

A transfer from the stock culture to 25 ml of BBL7 Fluid Thioglycollate medium, followed by three daily subcultures prepared each organism for use. Fluid Thioglycollate was also used to recover each of the test organisms after disinfection. However, this transplant medium for Roccal-subjected catheters contained 3% Tween 808 and 0.2% lecithin. Eosin methylene blue agar was used as a confirmatory medium for E. coli from tubes of broth showing growth. Broth cultures suspected to be S. typhosa were subcultured on BBL bismuth sulfate agar. The strain of P. aeruginosa used produced the characteristic blue-green pigment in abundance, and this was considered an adequate criterion for identification.

The bacterial pick-up by lubricated catheters was determined by plate counts using Bacto Tryptone glucose extract agar, recommended for the standard plate count according to Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (APHA, 1955).

4) Wiping solution. An alcoholic solution of soft soap containing 75 g soft soap in 1 liter of 70% isopropyl alcohol.


6) Temperature. The tests were performed at a range of 29 to 30 C.

7) Techniques. Since frequent sterilization by steam under pressure may adversely affect the properties of rubber, all catheters used throughout the study were subjected to dry heat at 110 C for 30 min. This process effectively destroyed vegetative contaminants without killing contaminating spores. Somewhat limited data, then, could be obtained about the sporicidal activity of the disinfectants.

A surface film of the sterile, water-soluble lubricant was applied to the catheters to simulate the condition found to exist upon removal of a catheter from the rectum. After lubrication, the catheters were contaminated by immersing in 25 ml of a 22- to 26-hr thioglycolate broth culture of one of the organisms, and then wiped with cotton moistened with the alcoholic solution of soft soap described above. The soap was then removed by rinsing with sterile distilled water, and the catheters transferred to a 600-ml beaker containing enough disinfectant to permit complete im-

\[ \text{FIG. 1. Catheter, thermocouple type} \]

\[ \text{LEAD} \]
\[ \text{BASE} \]
\[ \text{BODY} \]
\[ \text{TIP} \]
\[ \text{CONNECT, CANNON} \]
mersion. After a 5-min immersion in the disinfectant, the catheters were given 2 rinses; first with sterile sodium thiosulfate solution, 1% to inactivate the iodine carried over from the Wescodyne G and alcoholic iodine solutions, then with sterile distilled water. The catheters were then aseptically transferred by sterile forceps to tubes of culture media. The tubes were incubated at 37 C until growth was visible, or for 3 days if there was no apparent growth. Catheters from tubes showing no growth 3 days after disinfection with R occult were checked for bacteriostasis by transferring them to other tubes of broth. Such transfers were not considered necessary after iodine activity, since sodium thiosulfate effectively inactivates the antibacterial action of iodine.

B) Studies in vivo. A lubricated, nonsterile rectal catheter was inserted into each of ten test subjects. The catheters were worn for a 5-hr period, 1 hr of which included treadmill activity at 3 mph to insure maximal rectal contact. Catheters were removed during this 5-hr contact period only for defecation, when necessary, and reinserted immediately thereafter.

Immediately upon removal from the rectum, catheters were wiped thoroughly with cotton moistened with an aqueous solution of Wescodyne G, rather than the alcoholic soap solution used during the in vitro studies. After cleaning and subsequent treatment with aqueous Wescodyne G, the catheters were processed in the same manner as described under A) Studies in vitro.

This procedure was repeated for 6 days; the amount of time the catheters were exposed in the disinfectant was the only variable. Catheters worn on the first, second, third, and fourth days were exposed for 5, 10, 30, and 60 min, respectively. When the efficacy of the 60-min exposure became apparent, the catheters worn on the fifth and sixth days were also subjected to the 60-min exposure to enhance the reliability of the results.

Phase II. Effect of Disinfectants on Rectal Catheters

Three tests were made to determine the possibly harmful effect of test disinfectants on: (i) possible swelling of silicone rubber of the catheter, (ii) the complete or intact catheter, and (iii) the bared silver-soldered thermocouple of the catheter. Methods for each of these are described below.

A) Resistance of silicone rubber to test disinfectants.

The phenomenon of rubber products swelling in liquids is of practical importance not only because of dimensional changes, but also because of the effect on physical properties. The swelling of a rubber in a liquid is taken as an indication of its resistance, or lack of resistance to a fluid (Wilson, Griffis, and Montermoso, 1958).

The method used in determining the percentage of swelling of silicone rubber specimens exposed to each of the test disinfectants conforms to that described in Federal Test Method Standard No. 601 (U. S. Government Printing Office, 1955).

B) Effects of exposure of intact rectal catheters to test disinfectants. The first step in evaluating these effects consisted of totally immersing six precalibrated rectal catheters in each of the test disinfectants. After exposure times of 5, 10, 30, 60 min, 24 and 48 hr, catheters were removed and rinsed with tap water. The catheter tips were then fastened with a rubber band to the bulb of a National Bureau of Standards certified mercury thermometer, enclosed in a water-tight polyethylene bag, and immersed in a well stirred water bath. After a more than adequate temperature equilibration period at both 95 and 98 F, the emf of each catheter thermocouple was measured in millivolts with a model 8662 Potentiometer and converted to degrees Fahrenheit. Confirmation of measurements was made with a type K-3 Universal Potentiometer. A series of five readings was made on each catheter at a specific temperature, and a mean reading was recorded.

In all tests, a nonexposed, standardized reference thermocouple was used as a control.

C) Effects of exposure of bared, silver-soldered thermocouples to test disinfectants. Since it is conceivable, although unlikely, that a disinfectant could penetrate the catheter core (thermocouple) without measurably altering the true emf, it was considered desirable to investigate this possibility. A rectal catheter of proper thermoelectric characteristics was cut apart and sections of copper and constantan wire cut from it were made into thermocouples. The junctions were silver-soldered, and care was taken to remove all trace of flux. The free ends of a thermocouple so made were attached to the binding posts of a Wheatstone bridge and the silver-soldered junction immersed in a beaker containing a test disinfectant in such a way that 2 in. of both copper and constantan above the junction were exposed to the disinfectant. An initial resistance measurement was made, and subsequent readings were recorded at various intervals throughout a 24-hr exposure.

Erratic readings were obtained when catheters were directly immersed in the water bath. Enclosure in polyethylene effectively eliminated this, presumably by insulating the catheters from the battery action of the water bath.

Results

Phase I. Bacteriological Studies

A) Studies in vitro. Upon incubation of the catheter-containing tubes of broth, growth of a gram-positive, aerobic, spore-bearing organism frequently appeared. It has been shown in this laboratory that each of the test bacteria can survive and multiply without apparent antagonistic action by this spore-bearer under the incubation conditions of the test. Recovery, then, did not pose a problem. It was noted, however, that none of the disinfectants tested could destroy the contaminating spore-bearer during the 5-min exposure.

Total destruction of each of the test organisms (10⁴ or more per catheter) resulted from a 5-min soak in any of the three disinfectants whether or not wiping preceded the immersion. However, data showed that wiping per se is not invariably adequate for complete bacterial destruction. P. aeruginosa was found to survive wiping alone in each of the three replicates. E. coli was once found to survive, whereas S. typhosa was twice recovered.

B) Studies in vivo. Whether or not defecation interrupted the period of rectal contact, most catheters were found to have heavy deposits of fecal matter. It has been demonstrated that minute amounts of fecal matter may be lodged in the fissure formed at the junction of the brass tip and catheter body. As was expected, the wiping procedure did not completely remove this organic and inorganic material. Although the amount of extraneous matter remaining on the catheter after wiping was not enough to significantly diminish the antimicrobial capacity of the disinfectant, it was apparently enough to give protection to surviving organisms during short-term exposure. As a result, it was found necessary to lengthen the exposure time until the free iodine ultimately permeated the debris and reached the cells. As can be seen (Table 1), this necessary exposure time was invariably 1 hr.

Smears were made from all tubes showing growth and treated with a Gram stain. Microscopic examination revealed the most frequently present survivor to be an enterococcus bearing a morphological resemblance to Streptococcus faecalis.

<table>
<thead>
<tr>
<th>Exposure time (min)</th>
<th>No. of positive cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9/10</td>
</tr>
<tr>
<td>10</td>
<td>10/10</td>
</tr>
<tr>
<td>30</td>
<td>3/10</td>
</tr>
<tr>
<td>60</td>
<td>0/10</td>
</tr>
<tr>
<td>60</td>
<td>0/10</td>
</tr>
<tr>
<td>60</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Catheters exposed to Wesco G for 5, 10, and 30 min invariably showed growth of a gram-positive, aerobic, spore-bearing organism resembling that described in the studies in vitro. However, of the 30 catheters exposed to Wesco G for 60 min, recovery was made in only seven tubes. This sporicidal activity, although greatly to be desired was, nevertheless, unexpected since chemical disinfectants are not expected to destroy spores in normal use concentration (Frobisher and Sommerrmeyer, 1956).

Phase II. Effects of Disinfectants on Rectal Catheter Components

A) Resistance of silicone rubber to test disinfectants. It is generally agreed that a volume swell of less than 10%, taking into consideration the intended use, is of little significance. The data presented (Table 2) show that the silicone rubber used on rectal catheters described herein possesses a satisfactory resistance to each of the test disinfectants.

B) Effects of exposure of intact rectal catheters to test disinfectants. The thermal emf characteristics of rectal catheters exposed for 48 hr to each of the three disinfectants remained essentially unaltered. Although a +0.1 F error at 95 F and 98 F was observed with each of the 48-hr exposed catheters, as well as with the control catheter, this error is within the permissible tolerance for thermocouple material, and may be

<table>
<thead>
<tr>
<th>Medium</th>
<th>Swell %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic iodine, 0.5%</td>
<td>8.0</td>
</tr>
<tr>
<td>Aqueous Wesco G, 75 ppm available iodine</td>
<td>1.5</td>
</tr>
<tr>
<td>Alcoholic Roccal, 1:1,000</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 2. Resistance of silicone rubber to test disinfectants after 24-hr exposure at room temperature

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Increase in resistance from initial (ohm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0.001</td>
</tr>
<tr>
<td>20</td>
<td>0.002</td>
</tr>
<tr>
<td>30</td>
<td>0.004</td>
</tr>
<tr>
<td>60</td>
<td>0.008</td>
</tr>
<tr>
<td>120</td>
<td>0.014</td>
</tr>
<tr>
<td>180</td>
<td>0.021</td>
</tr>
<tr>
<td>240</td>
<td>0.027</td>
</tr>
<tr>
<td>300</td>
<td>0.034</td>
</tr>
<tr>
<td>360</td>
<td>0.041</td>
</tr>
<tr>
<td>420</td>
<td>0.046</td>
</tr>
<tr>
<td>1,440</td>
<td>—*</td>
</tr>
</tbody>
</table>

Table 3. Effect of alcoholic iodine 0.5% on resistance of silver-soldered copper-constantan thermocouple at room temperature

* A resistance measurement could not be made because corrosion of the constantan caused breakage.
attributed to the sources of error inherent in electrical circuits.

The brass tips of catheters exposed to both Wes-
codyne G and alcoholic iodine solutions became lightly
covered with a white coating, probably cuprous iodide.
In no case did the coating decrease the sensitivity of
heat transfer between the thermocouple and its en-
vironment. Moreover, the coating was easily removed
with crocus cloth or other such abrasive material.

One might expect, upon penetration of a disinfectant
into the catheter core (thermocouple), a partial short
circuit with a resulting reduction of the emf. Also, an
electrolytic emf could be set up between the wires.
Neither phenomenon occurred with intact catheters.

It therefore appears likely, although not conclusive,
that the molded rubber effectively excludes the pen-
etration of test disinfectants into the catheter core.

C) Effects of exposure of bared, silver-soldered thermo-
couples to test disinfectants. The data (Table 3) show
that a short exposure to the alcoholic iodine solution
results in a measurable increase in resistance of the
thermocouple circuit. The resistance is seen to increase
with continued exposure until at 24 hr the thermo-
couple circuit is completely destroyed. No measurable
resistance change was observed during a 24-hr con-
tinuous exposure to either Wescodyne G or Rocal.
However, Wescodyne G caused a mild oxidation of the
silver with the formation of yellow silver iodide coating
the solder. This oxidation was not sufficient to alter the
homogeneity of the circuit so as to produce a meas-
urable amount of resistance.

Since it has been demonstrated that tincture of
iodine, 0.5% visibly and measurably attacks bare
copper-constantan thermocouples in a short time, and
since this action was not seen to occur with intact rectal
catheters following prolonged exposure, it is likely that
properly constructed rectal catheters not only act as
efficient water-tight protection tubes, but also effec-
tively retard the entrance of surface active agents.

DISCUSSION

Upon completion of the studies in vitro, an investiga-
tion was conducted into the effects of disinfectants
on the components of rectal catheters (phase II). This
work showed no appreciable departures from the
initial calibrations of intact rectal catheters following
prolonged exposure to each of the three disinfectants.
However, bared thermocouples were found to be
particularly vulnerable to attack by alcoholic iodine
solution. Such an attack could occur during disinfection
of an improperly constructed or otherwise defective
catheter which would permit entrance of the iodine
tincture. Therefore, it was decided to eliminate the
tincture as the disinfectant to be used in standing
operating procedures.

Under the conditions of the bacteriological studies
in vitro, both Rocal and Wescodyne G were judged
efficient germicides. Solutions of these surfactant
germinicides have been shown to neither affect thermo-
electric uniformity nor in any other significant manner
contaminate or exert a deteriorating effect upon rectal
catheter components.

On the basis of these findings, it was considered
advisable to choose between Wescodyne G and Rocal
by evaluating other desirable properties, and to subject
catheters contaminated under actual use conditions to
the disinfectant of choice.

Solutions of both Wescodyne G and Rocal are,
from a practical aspect, stable, nontoxic, readily
miscible with water, and relatively inexpensive. There
are, however, certain advantages associated only with
Wescodyne G, including indication of potency by color,
and a broad antimicrobial spectrum (Gershensonfeld,
1955; Bartlett and Schmidt, 1957; Lawrence, Car-
penter, and Naylor-Foote, 1957).

The pronounced advantages ascribed to Wescodyne
by these investigators were considered adequate justi-
fication for choosing this disinfectant for further
evaluation by studies in vivo.

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We are indebted to the enlisted men who served as
volunteer test participants.

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Comparative Effects of β-Propiolactone on Mice, Mouse-derived Cell Cultures, and Venezuelan Equine Encephalomyelitis Virus

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Abstract

Hearn, Henry J., Jr. (U. S. Army Chemical Corps, Frederick, Md.), and Frederick W. Dawson. Comparative effects of β-propiolactone on mice, mouse-derived cell cultures, and Venezuelan equine encephalomyelitis virus. Appl. Microbiol. 9:278–282. 1961.—Studies were made comparing the toxicity of β-propiolactone (BPL) for mammalian (mouse) cells in vitro and for mice and for Venezuelan equine encephalomyelitis (VEE) virus which is highly cytotoxic and pathogenic for each. The mammalian cells grown in tissue culture were found to be adversely affected by BPL in concentrations ranging from 0.001 to 0.1 mg/ml of supernatant fluid. The difference in response was influenced by the menstruum in which the BPL was suspended and the difference in cell types tested. Tenfold less BPL appeared to be required to destroy the cells when it was suspended in a balanced salt solution than when it was suspended in protein-containing solutions such as beef heart infusion broth or medium 199 plus 20% horse serum. Secondary embryonic mouse lung cells seemed slightly more adversely affected by BPL than the established embryonic lung or L cells. BPL given to mice by intranasal instillation and by intracerebral injection was lethal to half of the animals within 2 days at doses of 0.31 and 0.39 mg, respectively. Higher concentrations of BPL were required to rapidly inactivate the virus in vitro than were required to kill mice or to cause a toxic effect on cells in culture. It required 10 mg/ml of BPL to completely inactivate a high-titered VEE virus preparation in 5 min and 1 mg/ml to inactivate most, but not all, of the virus in 15 min. A concentration of 0.1 mg/ml of BPL had only a slight effect on the virus after a period as long as 60 min. Evidence is presented indicating that simultaneous inactivation of all of the properties of the VEE virus particles by BPL aerosols did not occur at the same time but that, after treatment, the virus possessed a limited ability to immunize mice despite a loss in infectivity.

Numerous studies of the effects of liquid preparations of β-propiolactone (BPL) on viruses in tissues and biological fluids have appeared in the literature. These studies and others related to killed vaccines prepared with BPL recently were reviewed by LoGrippo (1960). The number of viruses and rickettsiae reported to be inactivated by BPL vapor was extended by Dawson, Janssen, and Hoffman (1959, 1960) to include Venezuelan equine encephalomyelitis virus and the causative agents of smallpox, yellow fever, psittacosis, and Q fever. No quantitative data seem to be available, however, comparing the virucidal levels of BPL with its toxicity levels for tissues or animals. In the present report, the toxicity levels of BPL for mice and for mouse-derived cell cultures are compared to the concentration of BPL necessary to inactivate a viral agent of known virulence for the same host.

Materials and Methods

Preparation of cell monolayers. The preparation of monolayers of embryonic mouse lung cells was carried