

The *A. agile* tested was far more tolerant of 2,4-D than the *A. chroococcum* isolates.

It was possible to increase the tolerance of both species of *Azotobacter* to the herbicide by serial subculturing in the presence of the agent.

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## Studies on Disinfection of Clinical Thermometers

### I. Oral Thermometers

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A nation-wide survey of procedures currently employed for disinfecting thermometers in hospitals and public health agencies has revealed the use of a wide variety of substances and methods. For many of these procedures there exists no known experimental basis; their effectiveness is unknown and in some instances seems dubious. In an attempt to evaluate these procedures neither condemnation nor recommendation of any was possible because of lack of exact experimental data. In view of this situation, and because of a wide demand for a method of disinfecting clinical thermometers which could be recommended, on the basis of experimental evidence, as reasonably safe from the standpoint of transmission of communicable diseases, the present study was undertaken. The objectives of the study were (a) to obtain data on which to base evaluations of one or more currently used methods of disinfecting clinical thermometers, and (b) to develop, if necessary, one or more new methods which could be recommended in place of existing procedures.

As a first step, the literature on the subject was care-

fully reviewed. It became evident that, while in-vitro tests of the action of numerous disinfectants against pure cultures of organisms suspended in broth media, saline solutions, or distilled water have been carried out, the action of disinfectants against infectious organisms protected in body secretions or excretions, such as often contaminate clinical thermometers, has been neglected. Cognizance is taken, of course, of the work of Chick and Martin (1908); Klarmann and Wright (1944), Horton and Kitchen (1933), McCulloch and Fuller (1941), and Rahn (1945) on the effect of serum and other organic materials on the disinfectant value of bichloride of mercury, phenol, and related compounds. However, these substances are not widely used in the disinfection of clinical thermometers. Therefore, it was decided to undertake an investigation of the efficacy of various bactericidal agents for disinfecting clinical thermometers artificially contaminated with sputum containing *Mycobacterium tuberculosis* var. *hominis*, *Corynebacterium diphtheriae*, streptococci, and staphylococci. It was felt that these organisms would be sufficiently representative of the pathogenic bacteria common in the mouth. Because of limitations of time, equipment, space, and personnel, no cultural tests were made for *Borrelia*, *Treponema*, *Hemophilus*, *Neisseria*, or *Diplococcus*. No attempt was made in this laboratory to determine the susceptibility of any

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of the viruses, fungi, or rickettsiae to any disinfectants. It is hoped that data on these groups of microorganisms will become available later.

We recognize that artificial contamination of thermometers with sputum, as carried out in these experiments, is not identical with actual contamination of thermometers by patients, but it was felt that the laboratory procedure which was adopted closely simulated the most extreme conditions likely to be encountered in routine practice. It was also thought that this type of laboratory study must precede field testing.

#### MATERIALS

Heart infusion broth (Difco) (HIB) containing 0.1 per cent glucose and approximately 0.5 per cent sterile, defibrinated blood was used to test for the presence of viable staphylococci, streptococci, and diphtheria bacilli on thermometers before and after disinfection.

Slants of Lowenstein-Jensen medium (LJM) (Holm and Lester, 1947) were used to recover *M. tuberculosis* before and after disinfection of contaminated thermometers. Not more than 24 hours before use, 0.1 ml of water containing 1,000 units of penicillin was placed on the surface of each LJM slant. The tubes were stored in the refrigerator until used the next day. This procedure was based on experiments in which penicillin, used in the manner described, was found to be an effective decontaminating agent for culturing *M. tuberculosis* when acid or alkali decontamination could not be used. Other workers (Kirby and Dubos, 1947; Abbott, 1951; Zuckerman and Rantz, 1951; Smith, *et al.*, 1949; Phillips and Hanel, 1950; Whiffen, 1948) have published favorable reports on similar procedures for diagnostic purposes.

Penicillin solution was prepared from Penicillin G Potassium buffered with sodium citrate, and contained 10,000 units per ml. Penicillin solutions were kept in the refrigerator and were not used after they were one week old.

All tubes of all media were incubated for 48 hours before use to test for sterility.

In pilot experiments on the various disinfectants and procedures, smooth glass rods, 100 to 110 mm long and 2 to 3 mm in diameter, were used instead of thermometers for ease of handling. In several instances when, after the pilot tests, it appeared that any disinfection procedure might prove to be satisfactory in actual use<sup>4</sup>, oral thermometers without mercury were used to complete the study. The thermometers were included to determine whether the contours and ridges on thermometers would interfere with the cleaning

<sup>4</sup> The statistical basis for decision to include or exclude a given disinfectant was adapted from Wald (1947) for this study by Mr. Myron G. Willis, Communicable Disease Center, Atlanta, Georgia.

and disinfection procedures. The glass rods and thermometers were sterilized with dry heat, or autoclaved with a small amount of moisture, in groups of 10 to 20 in 18 by 150 mm tubes.

Special metal racks were designed and made to transfer 10 thermometers from the disinfectant solutions through two rinses of water or neutralizing solution. These racks had notches on two sides to support the thermometers, open bottoms to prevent transfer of excessive disinfectants, and a ring attached to the center bar to permit transfer of the tray without contaminating the thermometers.

All disinfectant solutions were prepared aseptically immediately before use, using C.P. chemicals unless otherwise indicated, and adding sterile distilled water in the necessary amount for the desired dilutions. All 70 per cent ethyl alcohol solutions were made volumetrically from 95 or 100 per cent alcohol.

Potassium iodide (10 per cent solution) was used at first for removing iodine from glass rods and thermometers when the disinfectant solutions contained iodine. One per cent sodium thiosulfate was substituted later.

Soap solutions were prepared aseptically. The liquid soap used in Procedure 3 was prepared as a 50 per cent aqueous solution from Soap-Liquid-Toilet, Federal Spec. P-S-618a. The tincture of green soap solution used in Procedure 4 was prepared as a 50 per cent aqueous solution from tincture of green soap, U.S.P. The tincture of green soap solution used in Procedure 5 was prepared by adding equal parts of 95 per cent ethyl alcohol to tincture of green soap, U.S.P.

Specimens of fresh sputum (less than 24 hours old) were obtained from patients who had active pulmonary tuberculosis and who were not receiving any chemotherapy. Only those specimens which revealed acid-fast bacilli on direct examination of stained smears were used. Specimens were used individually and not pooled. Each specimen (approximately 10 to 15 ml) was contaminated with 0.1 ml of a 24-hour broth culture of *C. diphtheriae* and was stirred carefully to mix the broth culture with the sputum. Streptococci and staphylococci were invariably present in the sputa. No specimen of sputum was used for more than five tests with each disinfectant. Several different disinfectants were frequently tested simultaneously with one specimen.

#### METHODS

In each of 539 preliminary tests, 12 culture tubes were arranged for each disinfectant as follows:

Tube No.	Purpose	Media
A and B	Sterility tests on sample	HIB 0.1 per cent glucose, with blood used.

Tube No.	Purpose	Media
1	Culture for presence of staphylococci, streptococci, and <i>C. diphtheriae</i> on rod before disinfection.	HIB 0.1 per cent glucose, with blood
2	Culture for presence of <i>M. tuberculosis</i> on rod before disinfection.	Lowenstein's medium with 1,000 U Penicillin
3 and 4	Cultures for presence of test organisms on rods after rinsing with water only.	(Same as 1, 2, respectively)
5 and 6	Cultures for presence of test organisms on rods after disinfection.	(Same as 1, 2, respectively)
7 and 8	Tests on sterility of first rinse water before use.	(Same as 1, 2, respectively)
9 and 10	Tests on sterility of second rinse water before use.	(Same as 1, 2, respectively)

Each test in this series consisted, therefore, of a group of control cultures and test cultures on pairs of glass rods, all of which were contaminated with one specimen of sputum and submitted simultaneously to the same disinfection procedure. In these 539 pilot experiments, each disinfectant was tested as described at least 15 times, using a different specimen of sputum each time.

In each test, six sterile glass rods were slanted in a Petri dish containing a specimen of sputum so that one end of each rod was in contact with the sputum and the other end remained clean. The glass rods were immediately contaminated with the sputum by means of a sterile wooden applicator. An effort was made to deposit a thin film of sputum of uniform thickness over the rods. After the glass rods had been contaminated, they were placed in a sterile, covered, dry, enamel pan using aseptic technic. The pan containing the contaminated glass rods was placed in the incubator for 30 minutes at 37 C to permit some drying. This step was included to simulate many practical situations in which nurses take temperatures and do not clean and/or disinfect thermometers until 20 to 30 minutes later.

After drying at 37 C for 30 minutes, two glass rods of each group of six were removed with sterile forceps to tubes Nos. 1, 2 (contaminated controls). All glass rods which were placed on slants were rolled on the slant to permit contact of all sides of the glass rod with the medium.

Two more of the contaminated rods were placed in a metal rack in an enamel pan. Sterile distilled water, in lieu of disinfectant, was poured gently into the dish beside the rods until the fluid completely immersed them. The time of contact was the same as the time of disinfection in the test run described below.

Two more contaminated rods were handled exactly as above but, instead of water, disinfectant solution

was poured into the dish with the glass rods. A stop watch was started immediately after the glass rods were covered with the water or disinfectant. Three hundred ml of sterile distilled water were then poured into each of four sterile enamel pans; two successive pans for removing, inactivating, or diluting the disinfectant, and two for the parallel water control. As a control on the sterility of the water in the pans 0.5 ml from each pan was pipetted into each of four tubes (Nos. 7 to 10). These never showed growth.

After exactly 10 minutes the racks containing the glass rods were removed from the water and disinfectant solutions, respectively, and quickly rinsed by dipping each into two successive water pans. As quickly as possible, the two water-treated glass rods were then removed from their rack with sterile forceps and placed in tubes Nos. 3 and 4, and the two disinfectant-treated rods were placed in tubes Nos. 5 and 6.

When solutions of iodine were used as disinfectants, the first of the two successive rinse pans contained a 10 per cent aqueous solution of potassium iodide. Rinsing was continued for a sufficient time to remove the iodine before final rinsing. The time necessary to remove all iodine in potassium iodide solution was carefully determined with starch paper before this step was included in the procedure. It varied from 15 minutes for glass rods which were not mechanically cleaned prior to disinfection, to two minutes for those glass rods from which sputum was mechanically removed in some way.

In testing starch paper with solutions of iodine, it was found that an iodine solution of 0.00025 per cent concentration gave a positive test. Weaker solutions gave negative results with the starch paper. Fifty glass rods were rubbed over the surface of the starch paper after being contaminated with sputum and then processed through 0.25 per cent iodine, 10 per cent potassium iodide, and finally water. These 50 glass rods gave no evidence of the presence of iodine when the test with the starch paper was used. Twenty-five thermometers were similarly tested with negative results. However, with certain specimens of sputum, it was evident that the yellow color of iodine was still present after the glass rods and thermometers had been completely processed. When one per cent solution of sodium thiosulfate was substituted for 10 per cent potassium iodide, the rods and thermometers lost the yellow color of the iodine completely, regardless of the specimen of sputum used.

The solutions of iodine in aqueous potassium iodide were apparently removed with two rinses of water. For this reason, neither sodium thiosulfate nor potassium iodide was used in the first rinse pan after disinfection in aqueous iodine solutions. However, in later tests, sodium thiosulfate rinse was introduced.

"Tween 80" (polyoxyethylene sorbitan monooleate)

was added to solutions of iodine in aqueous potassium iodide in an attempt to prepare an aqueous iodine solution having low surface tension. The "Tween 80" apparently produced a colloidal suspension of the iodine, because an opaque, dark-brown mixture resulted. This mixture did not seem to have any advantages as a disinfectant over aqueous or alcoholic iodine solutions. It stained containers badly, and is not recommended for use in disinfecting thermometers. However, Terry and Shelanski (1951) have shown that, for many routine purposes of disinfection, iodine in combination with a nonionic, surface-active carrier (ethylene oxide condensate of a  $C_9$  alkyl phenol) similar in some respects to "Tween 80", is very effective and has lower volatility and irritative effect than iodine in tincture.

All LJM slants were held in a nearly horizontal position for 48 hours in the incubator so that penicillin could have contact with all the organisms on all parts of the slant. The tubes were then kept upright for 6 weeks. Tubes were examined at the end of the second week and every week thereafter for 6 weeks. Smears stained with Ziehl-Neelsen stain were made from all tubes as soon as growth was observed. Similar stained smears were also made from all apparently negative tubes at the end of 6 weeks. When stained smears from these negative tubes showed acid-fast organisms, the smears were examined carefully for numbers of organisms present, and the culture tubes were re-examined for possible missed colonies. No confusion between original inoculum and incipient growth arose.

At the end of 2 weeks, all glass rods were removed from the LJM tubes and were stained with Ziehl-Neelsen stain by a procedure similar to that used in preparation of slides for recognizing serpentine cords, as described in a report on slide-culture technic (Berry and Lowry, 1949, 1950). The rods were examined under  $100\times$  magnification for serpentine cords.

All HIB tubes (tests and controls) were examined for growth every 24 hours for one week. Gram-stained smears, blood-agar plates and cystine-tellurite plates were made from all HIB tubes showing growth. Blood agar slants were also made so that the growth could be rechecked if necessary. Colonies were picked for pure-culture identification of organisms that had escaped disinfection.

After the first 256 tests, in addition to testing the action of disinfectants on glass rods and thermometers contaminated with sputum but not wiped before disinfection, the value of four different preliminary wiping procedures was studied. The five procedures, with and without wiping were as follows:

Procedure 1.—Not wiped before disinfection.

Procedure 2.—Wiped with dry, sterile, cotton ball (approximately 2 inches in diameter) before disinfection.

Procedure 3.—Wiped with a cotton ball moistened with 50 per cent aqueous solution of liquid soap.

Procedure 4.—Wiped with a cotton ball moistened with 50 per cent aqueous solution of tincture of green soap.

Procedure 5.—Wiped with a cotton ball moistened with solution consisting of equal parts of tincture of green soap and 95 per cent ethyl alcohol.

Each glass rod and thermometer which was wiped with any soap solution was carefully rinsed by placing it in approximately 50 ml of sterile distilled water after wiping and before other disinfection.

Beginning with test No. 540, the extensive controls on the sterility of rinse water and glass rods were discontinued because these controls had never yielded any growth. The controls using water in lieu of disinfectant were discontinued also because there were only small discrepancies between them and the contaminated controls, except after wiping with soap. This seemed unimportant because the final results of the cleaning and disinfection procedures used on contaminated thermometers are the essential data. In order to control contaminants on LJM medium, both actidione and penicillin were used in the tests beginning with No. 540. Actidione (Upjohn Company, Kalamazoo, Mich.) solution was prepared so that 0.1 ml contained 1 mg actidione. One mg of actidione was placed on the surface of each LJM slant (Phillips and Hanel, 1950; Whiffen, 1948).

After the completion of the first 539 tests, the data available were reviewed by the Statistical Section of the Communicable Disease Center. In view of these results, a binomial sequential sampling plan developed by Wald (1947) was adopted and the following probability values were selected:

$P_1 = 0.01$  (maximum allowable failure for disinfectant to be worthy of further tests)

$P_2 = 0.05$  (maximum allowable failure, above which disinfectant is unworthy of further tests)

$\alpha = 0.10$  (risk of rejecting a satisfactory disinfectant; that is one which fails 0.01 of the time or less)

$\beta = 0.01$  (risk of accepting an unsatisfactory disinfectant; that is one which fails 0.05 of the time or more)

This sequential plan was followed for the remainder of the tests in this study as a guide to rejecting a procedure or continuing to test it further.

In all, 9,202 tests were done in this manner, that is, contamination, wiping (or not wiping); disinfection; rinsing twice; culturing one rod or thermometer of each pair in HIB for staphylococci, streptococci, and *C. diphtheriae*; and the other on a slant of LJM with penicillin and actidione for tubercle bacilli. The data obtained in these experiments are shown in table 1.

Since the different wiping procedures were not always tested simultaneously, or with the same specimens of sputum, the results of the tests of the various procedures, while listed in table 1 opposite the appropriate disinfectants, are totalled in separate columns for the wiping procedures. Thus, all tests done with a given disinfectant are strictly comparable as to technic but not necessarily as to time of test, specimen of sputum, or wiping procedure.

*Disinfectants tested.* In the course of this work the following substances were tested for suitability as disinfectants for oral thermometers:

1 per cent, 0.5 per cent, 0.25 per cent, and 0.05 per cent.

Solutions of iodine 0.25 per cent and 0.05 per cent in 1 per cent KI; same with "Tween 80".<sup>5</sup>

Formalin 10 per cent and 4 per cent.

Quaternary A (cetyl-pyridinium chloride) 1:1000 aqueous and 1:1000 tincture (50 per cent alcohol).

Quaternary B (a mixture of alkyl-dimethyl-benzyl ammonium chlorides) 1:1000 aqueous and 1:1000 tincture (50 per cent alcohol).

50 per cent aqueous solution of tincture of green soap.

TABLE 1. Results of treatment of glass rods and thermometers with various disinfectants, without wiping, and after various wiping procedures

DISINFECTANTS	SURVIVAL OF TEST ORGANISMS*								
	No. of Tests	No wiping		Tests	Dry Wiping		Tests	Soap wiping (combined data)	
		Tbc†	Others‡		Tbc	Others		Tbc	Others
<i>Quaternaries (combined data A and B)</i>									
1:1000 aqueous.....	50	100	—	50	100	—	149	53.7	—
1:1000 tincture.....	50	0	—	50	0	—	150	0	—
<i>Ethyl alcohol (70 per cent)</i>									
Alcohol.....	30	6.7	0	225	0.4 (3.6)	1.8	424	0.7	4.0
Alcohol + 0.05 per cent iodine.....	140	5.7	2.9	270	0 (0.4)	2.2	489	0.6	1.0
Alcohol + 0.25 per cent iodine.....	265	7.2 (0.4)§	4.9	275	0	0.7	494	0.4	0.8
Alcohol + 1.0 per cent iodine.....	—	—	—	20	0	0	—	—	—
<i>Isopropyl alcohol (70 per cent)</i>									
Alcohol.....	60	10.0 (1.7)	10.0	15	66.7 (6.7)	60.0	224	0 (0.9)	1.3
Alcohol + 0.05 per cent iodine.....	155	10.3 (1.9)	7.7	15	20.0 (6.7)	13.3	424	0.4 (0.4)	1.9
Alcohol + 0.25 per cent iodine.....	265	1.9	3.5	255	0 (0.4)	1.6	424	0.2	0.2
Alcohol + 0.5 per cent iodine.....	45	0	0	50	0	0	100	0	1.0
Alcohol + 1.0 per cent iodine.....	45	0	2.2	50	0	0	100	0	0
<i>Formalin</i>									
4 per cent.....	25	88.0	88.0	100	89.0	61.0	200	52.0 (2.0)	21.0
10 per cent.....	25	44.0 (8.0)	88.0	100	18.0	9.0	200	5.0 (2.5)	3.5
<i>Water controls**</i> .....	190	86.3 (5.3)	99.5	25	84.0 (12.0)	100	74	67.6 (10.8)	50.0

\* Percentage of tests positive.

† *Mycobacterium tuberculosis*.

‡ Streptococci, Staphylococci, *C. diphtheriae*, etc.

§ Percentages in parentheses refer to the additional rods which were positive for serpentine cords when tubes of Lowenstein-Jensen medium were negative.

\*\* Water used in lieu of disinfectant.

Ethyl alcohol 100 per cent<sup>5</sup>, and 70 per cent.

Ethyl alcohol 70 per cent with iodine 1 per cent, 0.25 per cent and 0.05 per cent.

Ethyl alcohol 70 per cent with 0.5 per cent and 0.1 per cent NaOH<sup>5</sup>.

Isopropyl alcohol C.P. (99 per cent)<sup>5</sup>.

Isopropyl alcohol 70 per cent (prepared from C.P. and also used, as purchased, as commercial "rubbing alcohol").

Isopropyl rubbing alcohol 70 per cent with iodine

<sup>5</sup> Rejected after preliminary tests based on the binomial sequential sampling plan described in the text.

Tincture of green soap plus equal volume of 95 per cent ethyl alcohol.

Phenol, cresols, hypochlorite solutions and bichloride of mercury were not included because of their physical and chemical properties make their use for clinical thermometers undesirable. On the basis of the 539 preliminary screening tests referred to above, several of the disinfectants were eliminated from further testing, since cultures from too large a percentage of the rods or thermometers subjected to them contained growth of the test organisms. The cultural data concerning most of those eliminated were not included in table 1, as they would have made the table too large.

Data on a few of them (quaternaries A and B and formalin) were included, however, as they seemed to be of special interest.

Data on those substances which were more extensively tested and which might appear to be suitable for use in disinfecting oral thermometers with proper wiping procedure are shown in table 1.

### RESULTS

The results of all final tests, with exceptions as noted above, have been included in table 1. The results obtained with the two quaternaries were virtually identical. Therefore, the data obtained with the two quaternaries were combined for presentation in table 1. Because there were no significant differences between the results obtained with glass rods and those yielded by thermometers, the data for these have been tabulated together. There were no significant differences between the results of disinfection following the three soap-wiping procedures. These data have, therefore, also been tabulated together.

While every effort was made to reduce to a minimum the error due to contaminants, it is possible that some few of the HIB cultures positive for staphylococci were due to contamination. This could account for the tests positive for staphylococci but negative for tubercle bacilli. In any event, the small error involved favors none of the disinfectants. In addition, while disinfectants were evaluated only on the basis of staphylococci, streptococci, *C. diphtheriae*, and *M. tuberculosis*, and while only these were recorded in the table, a few additional broth test cultures occasionally contained sporeforming bacilli. Higher fungi were sometimes found in additional LJM cultures. Some of the broth cultures also occasionally yielded stains of *Alcaligenes*, *Lactobacillus*, and unidentified diphtheroids when the test organisms were not present. The numbers were small and introduced no real error into the results.

#### *Wiping Procedures*

It is of especial importance to note the improved results obtained with nearly all of the disinfectants when disinfection was preceded by wiping. It has long been known that organic material interferes with the action of disinfectants, especially coagulative disinfectants such as formalin, phenol, and bichloride of mercury. The results presented here demonstrate the good effect of mechanically removing coagulable material (sputum) from thermometers (and glass rods) prior to disinfection. It appears that formalin very quickly precipitates a protective coating of coagulum which it does not penetrate<sup>6</sup>. Therefore, it becomes ineffective in the situations described in this paper. The effectiveness of

liquid soap or green soap in the data under "Soap Wiping" in table 1 is particularly striking, except in the case of formalin. In view of these data, it may be said that wiping is an essential part of the disinfection of clinical thermometers. That it is not in itself sufficient is shown by the fact that, even after careful wiping, without further disinfection the test organisms were not infrequently demonstrable. (See "Water Controls" under "Soap Wiping" in table 1.)

#### *Disinfectants*

The summarized results obtained with the several disinfectants, with and without wiping, are compared in table 1.

*Quaternary ammonium compounds.* In considering methods of testing quaternary ammonium compounds the question of an inhibitor arose immediately. A review of several studies in this field (Quisno, Foter and Rubenkoenig, 1947; Armbruster and Ridenour, 1947; Weber and Black, 1948; Idem, 1948a; Idem 1948b; Armbruster and Ridenour, 1949; Stuart, Bogusky and Friedl, 1950; Klarmann and Wright, 1948; Lawrence, 1948) revealed considerable confusion as to choice of inhibitor, concentration, type of subculture medium, effectiveness in tests with various organisms, and other factors. Although "Asolectin" (lecithin in "Tween-80") has rather general support, we were unable to arrive at a suitable concentration in the types of subculture media we were to use; nor did we have any information as to the value of the various organic inhibitors in relation to our test organisms, especially in the concentration of 1:1000 of quaternary, and on the surface of thermometers in contact with solid media. We, therefore, did not include tests with inhibitors.

In view of the work by Smith, *et al.*, (1950) it was anticipated that in aqueous solution these compounds would fail to kill tubercle bacilli and that the inhibitor question would not arise. The anticipation was correct, since the aqueous solutions appeared to be worthless. The contrast between the aqueous solutions and the tinctures of the two quaternaries tested is remarkable.

The tinctures appeared to be highly effective. Since no inactivator was used, it is possible that their effectiveness could be more apparent than real. Further studies, not at present feasible in these laboratories, should be made to establish this point with certainty. However, it seems likely that the results obtained with the tinctures represent true bactericidal action rather than an inhibition which could be released by an inactivator. It should be pointed out in this connection that the solvent, 50 per cent ethyl alcohol, has no significant bactericidal effect *per se* on any of the test organisms. This was repeatedly demonstrated experimentally during this study. If the results obtained with the tinctures were not due to some deceptive bacterio-

<sup>6</sup> Further evidence in support of this is being published.

static action, the tinctures of these quaternaries would certainly seem to be among the best of the agents tested in this study for disinfection of clinical thermometers and similar objects.

*Ethyl alcohol.* Ethyl alcohol, 95 per cent and 100 per cent, were found to be of little value as disinfectants. Under the experimental conditions described, ethyl alcohol 70 per cent (volumetric) was fairly effective. Of 679 rods and thermometers disinfected and cultured, with and without preliminary wiping, only 21 (3.1 per cent) yielded staphylococci, streptococci, or diphtheria bacilli. Of an equal number similarly treated and tested for surviving tubercle bacilli, only 14 (2.1 per cent) yielded these organisms. Considering only those results obtained when a wiping procedure was used, staphylococci, streptococci, and diphtheria bacilli were cultured from 21 of the 649 rods and thermometers (3.2 per cent); tubercle bacilli from 12 (1.85 per cent).

*Ethyl alcohol 70 per cent with iodine.* Addition of iodine to 70 per cent ethyl alcohol increased the disinfectant action. It is seen that, when a preliminary wiping procedure was used, test cultures yielded staphylococci, streptococci, or diphtheria bacilli from only 17 of 1,548 thermometers and rods tested (1.1 per cent); tubercle bacilli from 6 (0.4 per cent).

While it might be supposed that 1.0 per cent iodine would be more effective than 0.25 per cent iodine, and either of them more effective than 0.05 per cent, insufficient comparative tests were made to establish this conclusively. Gershenfeld, *et al.* (1951) have found iodine effective in disinfecting thermometers contaminated, not with sputum, but with broth cultures.

*Isopropyl alcohol.* In general, the results of tests with isopropyl alcohol, including "rubbing alcohol," were comparable with those obtained with ethyl alcohol. Differences observed are of doubtful significance. The 99 per cent "C.P." isopropyl alcohol was relatively ineffective.

The unusual results obtained with isopropyl rubbing alcohol 70 per cent and 70 per cent plus 0.05 per cent iodine following dry wiping might have been due to an error in the percentage of isopropyl rubbing alcohol. An error was found later in which a supply of isopropyl rubbing alcohol from the same distributor, actually 99 per cent isopropyl alcohol, was found to be labeled 70 per cent. Except for this group of results obtained with glass rods dry wiped, the 70 per cent isopropyl ("rubbing") alcohol yielded only three cultures of staphylococci, streptococci, or diphtheria bacilli from 224 rods and thermometers tested following wiping procedures (1.3 per cent); in only two cultures were tubercle bacilli isolated from the same rods (0.9 per cent). In table 1 the aberrant results are included in the calculations in order not to give any advantage to any disinfectant.

*Isopropyl alcohol (70 per cent) with iodine.* As in the

tests with ethyl alcohol, addition of iodine to rubbing alcohol increased its value. In 1,918 tests made with iodine, with and without wiping procedures, only 38 rods or thermometers (2.0 per cent) yielded staphylococci, streptococci, or diphtheria bacilli; only 34 (1.8 per cent) tubercle bacilli. The advantages of wiping over not wiping are apparent in the percentages given in table 1. Even though limited numbers of tests were done with 0.5 per cent and 1.0 per cent iodine in 70 per cent isopropyl alcohol, it seems that the higher concentrations of iodine which were tested were more effective than the weaker solutions.

*Soap.* Tincture of green soap mixed with equal parts of 95 per cent ethyl alcohol, when used as a disinfectant solution for 10 minutes, even without previous wiping, yielded a relatively small number of positive culture tubes (9 positive HIB and 3 positive LJM in 200 tests). This solution, used as a disinfectant, was more effective than the 50 per cent aqueous solution of tincture of green soap used as a disinfectant. However, these soap solutions, when used in wiping procedures prior to applying other disinfectants, gave comparable results. Neither would be satisfactory as a disinfectant or if used as a wiping procedure without subsequent disinfection.

*Formalin.* The results obtained with the two solutions of formalin (4 per cent and 10 per cent) indicate that these are unsatisfactory under the conditions described. Even when the contaminated glass rods were first wiped with alcoholic solutions of tincture of green soap, these formalin solutions failed more frequently than any of the other disinfectants tested, possibly excepting the aqueous solutions of the quaternaries.

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### SUMMARY

In this study an evaluation was made of the effectiveness of several common and readily available disinfectants, with and without four different preliminary wiping procedures, in disinfecting clinical thermometers. The thermometers (and simulant glass rods) were contaminated with fresh sputum from patients with open cases of tuberculosis and containing large numbers of tubercle bacilli. Staphylococci and streptococci which were present in the sputa, and diphtheria bacilli which were added to the sputa, were used as additional indicators of the activities of disinfectants.

The data indicate that some sort of wiping procedure, properly used, is essential to effective disinfection of oral thermometers. The following procedure is proposed:

1. Thoroughly wipe the thermometers with a pledget of cotton wet with a mixture consisting of equal parts of tincture of green soap and 95 per cent ethyl alcohol.

2. Immerse the thermometers in 1.0 per cent solution of iodine in 70 per cent (volumetric) isopropyl alcohol ("rubbing alcohol") or 70 per cent (volumetric) ethyl alcohol for 10 minutes.

Almost equally satisfactory results may be obtained by using the 70 per cent ethyl or isopropyl ("rubbing") alcohol without iodine. It seems probable that even better results could be obtained with tinctures of the quaternary compounds tested in this study. A final statement on this point awaits further experiments, using inactivators. These experiments are not at present feasible in this laboratory. No implications are made relative to viruses, rickettsiae, or higher fungi.

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