

GLYCERITE OF HYDROGEN PEROXIDE

I. COMPARISON OF ITS BACTERIOTOXIC ACTION WITH THAT OF MERCURIAL SOLUTIONS

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A new modification (a glycerite) of an old antiseptic, hydrogen peroxide, was described by Brown, Krabek, and Skiffington (1946) with the results of some preliminary tests of antiseptic action. The glycerite of hydrogen peroxide is derived from a solution of urea peroxide (4 per cent) in substantially anhydrous glycerol with 8-hydroxyquinoline (0.1 per cent) as an added stabilizer. A solution which, for practical purposes, is similar, may be made by dissolving pure, relatively nonaqueous hydrogen peroxide (92 per cent) in glycerol with 8-hydroxyquinoline (oxine) and with or without added urea. It was considered desirable to amplify these preliminary bacteriological findings with an extended comparison with a number of the more common antiseptics readily available commercially, in order to determine how the newer antiseptics, together with control solutions, compared with these well-known solutions. The results of the comparison of the new antiseptic solution with a number of organic mercurial solutions are reported in this paper.

The cup plate technique has been suggested by Ruehle and Brewer (1931) as a method for testing substances intended for continued application to tissue. Although the method is by no means a perfect test for antiseptic action under clinical conditions, it does give some information not so readily obtainable by other means concerning the bacteriotoxic action of a solution when continually applied. Because of errors, resulting from the testing of certain antiseptic solutions while using the FDA technique, it was found necessary to evolve a method utilizing the same principle but using paraffined cylinders instead of the open cup. A more complete description of the method, together with some of its advantages and disadvantages, is being submitted in another paper.

As Nye (1937) and others have pointed out, some antiseptic solutions, as available in commercial strengths, cannot be used undiluted because of either irritation to tissue or other undesirable effects. The peroxide solutions reported here have been used undiluted on extensive lesions, with little or no undesirable reaction. This phase of the work has been reported upon by Brown (1946a) and expressed or implied in other papers on the application of the peroxide-glycerol solutions in various pathological conditions. All solutions were used in the tests undiluted, in order to observe the maximum effect that may be obtained.

The physiological properties of the peroxide-glycerol solutions have been described by Brown (1946b, 1946c). The properties of the mercurial solutions have been summarized by McCulloch (1945).

The solutions tested comprised the following:

Urea peroxide (4 per cent) in glycerol.

Urea peroxide (4 per cent), 8-hydroxyquinoline (0.1 per cent) in glycerol (glycerite of hydrogen peroxide).

Hydrogen peroxide (1.46 per cent) in glycerol (corresponding to a 4 per cent urea peroxide solution).

Hydrogen peroxide (1.46 per cent), 8-hydroxyquinoline (0.1 per cent) in glycerol.

Merbromin (N. F), surgical 2 per cent and solution (aqueous) 2 per cent.

Merseptal, tincture, 1:500, and aqueous, 1:1500.

Mercurochrome, surgical 2 per cent, and solution (aqueous) 2 per cent.

Mercresin, tincture, 1:1,000.

Merthiolate, tincture, 1:1,000, and aqueous, 1:1,000.

Metaphen, tincture, 1:200, and aqueous, 1:500.

The controls consisted of "tincture solvent" (ethyl alcohol 50 per cent; acetone 10 per cent; water 40 per cent), glycerol, glycerol saturated with urea, and 8-hydroxyquinoline (oxine) 0.1 per cent in glycerol. Under the conditions of the test, the control solutions, with the exception of the oxine in glycerol, showed negligible effect, as indicated by a failure to demonstrate a zone. The results with the oxine solution are shown in the tables.

The following bacterial species are reported upon in this paper: *Staphylococcus aureus* (FDA209); *Staphylococcus albus*, coagulase-positive; *Staphylococcus pharyngis*; *Streptococcus liquefaciens*, alpha hemolytic; a diphtheroid; *Bacillus subtilis*; *Escherichia coli*, var. *communior*; *Proteus mirabilis*; *Pseudomonas aeruginosa*, and *Aerobacter cloacae*. The organisms were, with the exception of *Staphylococcus aureus* (FDA209) which came from the Food and Drug Administration, strains freshly isolated from lesions of clinical interest. Other strains of organisms and of streptococci and staphylococci, in particular, were examined. Only one of each is included here, since the others gave essentially the same results, although the clinical strains of *Staphylococcus aureus* tended to be slightly more resistant to some of the mercurial solutions. It is intended to report, at a later time, a statistical examination of the results obtained with a number of strains of different organisms as affected by different antiseptics. All organisms demonstrated the characteristic biological reactions as given by Bergey *et al.* (1939). The *Bacillus subtilis* strain used is apparently not a very resistant strain, since solutions which show little or no action against other bacteria show a definite zone with this strain.

A cylinder agar plate method was used, since its prototype, the agar cup plate, has been suggested as a method for testing substances used for prolonged clinical application. Harris and Prout (1940) considered that this type of method gave a somewhat better correlation between *in vitro* and *in vivo* applications than some other methods, although there is some disagreement on this score. The method was essentially as follows:

Difco glucose agar, with or without 10 per cent added horse serum, was pre-inoculated with 0.2 ml of an undiluted 22- to 26-hour glucose broth culture of the

organism, for each 25 ml of melted agar. The inoculated agar was then dispensed in 25-ml amounts into sterile 100-mm petri dishes with unglazed clay covers. After hardening of the agar, slightly warmed "penicylinders" of 8-mm outside diameter, the rims of which had been paraffined, were placed on the surface of the agar. The solution to be tested was then pipetted in 0.2-ml amounts into the cylinders with a sterile pipette. The plates were immediately placed in the incubator at 37 C and incubated for 18 hours. At the end of this time, the plates were removed and subcultures of approximately 3 mm in cross section were taken at intervals of 1 to 3 mm radially and placed in modified Brewer's thioglycolate medium (Baltimore Biological Laboratories, list 135). The subculture tubes were then incubated at 37 C until growth occurred, or for at least 7 days. Before the subcultures were discarded, those tubes showing no growth were reinoculated with a small inoculum (50 organisms or fewer) of the same strain tested and incubated for 3 days, or less if growth occurred sooner. This technique determined that the test solution was not carried over into the subculture tubes in amounts that were inhibitive to growth of normal bacteria.

After subculturing, the distance of the outer edge of the clear zone (if any) from the outer edge of the cylinder was measured to the nearest 0.5 mm and recorded for each test. At the same time, the distance from the edge of the cylinder of the inner and outer edge of the indentation left by the removal of materials for subculturing was measured and recorded. Because of space limitations, the tables show only the average distal measurements of the subculture area closest to the cup that showed growth in the subculture tube. This obviously favors the antiseptic. At least three, and in some cases more, replicate tests were made with each antiseptic solution.

The values in the tables have been rounded off to the nearest whole number, since this is sufficient for comparative purposes. Table 1 lists the zones obtained with gram-positive organisms, and table 2 those obtained with gram-negative organisms. The first column (A) indicates the apparent visible zone, and the second column (B) the zone as determined by subcultures for plates without serum. The corresponding values for plates containing 10 per cent horse serum are given in columns (C) and (D). In addition to the visible changes as shown by the zones, there are a number of miscellaneous observations which may be of interest in relation to the possible modes of action of the antiseptics on the organisms tested. These are given briefly, in order that the record may be complete.

Opaque zones within the zones of apparent inhibition were noted in the case of the following: surgical merbromin with *S. pharyngis* and *E. coli* in serum plates; aqueous merbromin with staphylococci in both serum and plain plates; surgical mercurochrome with some organisms, particularly the staphylococci. Control plates without organisms show no opaque zones, either with or without serum. The opacity may be due to acid formation by the organism with concomitant precipitation of constituents of the solution.

Increased absorption of pigment at the edge of the zone of inhibition was shown by *Aerobacter cloacae* with surgical merbromin and by *P. aeruginosa* with

aqueous merbromin, tincture of mercresin, surgical mercurochrome, tincture of merseptal, and aqueous merbromin. Increased colony size distal to the area of inhibition was shown by *P. aeruginosa* with aqueous merbromin and tincture of metaphen, and by *Proteus mirabilis* with tincture of Merthiolate. Raised wet growth (mucoid) appeared in the case of *Aerobacter cloacae* with surgical mer-

TABLE 1

Comparison of action of glycerite of hydrogen peroxide and mercurial antiseptic solutions on gram-positive organisms

| GLYCEROL SOLUTIONS | | S. AUREUS | | | | S. ALBUS | | | | S. PHARYNGIS | | | | S. LIQUEFACIENS | | | | DIPHTHEROID | | | | B. SUBTILIS | | | |
|---------------------------|----|-----------|----|----|----|----------|----|----|----|--------------|----|---|----|-----------------|----|---|----|-------------|----|----|----|-------------|----|----|----|
| | | A* | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Urea peroxide + oxine | G† | 13 | 13 | 13 | 11 | 19 | 20 | 14 | 13 | 13 | 10 | 9 | 12 | 11 | 15 | 8 | 12 | 16 | 12 | 14 | 11 | 13 | 13 | 11 | 11 |
| Urea peroxide | G | 14 | 15 | 13 | 10 | 18 | 18 | 11 | 12 | 12 | 12 | 9 | 9 | 10 | 8 | 8 | 8 | 15 | 13 | 17 | 5 | 13 | 13 | 7 | 7 |
| Hydrogen peroxide + oxine | G | 13 | 15 | 17 | 9 | 22 | 23 | 20 | 17 | 12 | 15 | 7 | 12 | 11 | 14 | 9 | 10 | 9 | 12 | 5 | 5 | 13 | 13 | 11 | 14 |
| Hydrogen peroxide | G | 13 | 10 | 6 | 8 | 18 | 18 | 11 | 12 | 10 | 4 | 7 | 7 | 12 | 13 | 6 | 5 | 12 | 13 | 9 | 6 | 14 | 13 | 9 | 4 |
| Oxine alone | G | 10 | 6 | 7 | 7 | 15 | 14 | 10 | 11 | 5 | 4 | 2 | 3 | 6 | 3 | 5 | 2 | 16 | 8 | 6 | 5 | 15 | 16 | 13 | 10 |
| MERCURIAL SOLUTIONS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Merbromin | T | 12 | 16 | 10 | 12 | 15 | 15 | 10 | 13 | 6 | 5 | 6 | 6 | 8 | 5 | 6 | 5 | 13 | 13 | 8 | 13 | 15 | 17 | 11 | 13 |
| | A | 6 | 11 | 5 | 4 | 7 | 5 | 3 | 5 | 4 | 4 | 4 | 4 | 6 | 5 | 2 | 2 | 8 | 8 | 6 | 5 | 9 | 12 | 5 | 9 |
| Mercresin | T | 11 | 10 | 7 | 8 | 14 | 13 | 7 | 10 | 9 | 5 | 6 | 4 | 7 | 6 | 5 | 5 | 16 | 15 | 10 | 13 | 15 | 15 | 12 | 12 |
| Mercurochrome | T | 10 | 9 | 8 | 11 | 14 | 13 | 7 | 8 | 8 | 5 | 5 | 4 | 6 | 3 | 5 | 5 | 11 | 13 | 6 | 8 | 10 | 13 | 7 | 10 |
| | A | 6 | 5 | 9 | 7 | 8 | 6 | 6 | 8 | 8 | 4 | 3 | 4 | 6 | 4 | 3 | 2 | 8 | 9 | 5 | 8 | 8 | 11 | 5 | 8 |
| Merseptal | T | 12 | 8 | 7 | 4 | 16 | 15 | 9 | 7 | 10 | 5 | 5 | 4 | 9 | 6 | 6 | 4 | 20 | 14 | 9 | 11 | 15 | 16 | 11 | 10 |
| | A | 2 | 4 | 8 | 4 | 17 | 16 | 9 | 10 | 3 | 5 | 3 | 3 | 9 | 5 | 5 | 3 | 13 | 14 | 7 | 11 | 15 | 15 | 9 | 8 |
| Methiolate | T | 16 | 11 | 10 | 6 | 21 | 15 | 11 | 8 | 9 | 7 | 6 | 5 | 8 | 5 | 5 | 5 | 25 | 13 | 7 | 8 | 23 | 17 | 18 | 15 |
| | A | 16 | 4 | 10 | 2 | 21 | 9 | 12 | 3 | 9 | 3 | 6 | 3 | 9 | 4 | 7 | 3 | 19 | 5 | 11 | 15 | 17 | 15 | 11 | 11 |
| | G | 15 | 5 | 8 | 3 | 17 | 7 | 10 | 3 | 7 | 3 | 3 | 3 | 8 | 3 | 1 | 3 | 15 | 4 | 9 | 3 | 13 | 7 | 7 | 6 |
| Metaphen | T | 10 | 14 | 9 | 6 | 12 | 11 | 8 | 8 | 6 | 8 | 6 | 5 | 8 | 10 | 7 | 7 | 11 | 12 | 6 | 11 | 14 | 15 | 11 | 12 |
| | A | 10 | 5 | 5 | 5 | 13 | 12 | 7 | 7 | 9 | 3 | 4 | 3 | 8 | 6 | 1 | 3 | 11 | 13 | 7 | 9 | 11 | 13 | 6 | 6 |

Zones were measured radially, in mm, from the edge of cylinder to the edge of clear zone.

* Column headings: A = apparent visible zone of inhibition—no serum; B = zone as determined by subculture—no serum; C = apparent visible zone of inhibition—10% serum; D = zone as determined by subculture—10% serum.

† Abbreviations: T = tincture; A = aqueous; G = glycerol.

bromin and glycerite of merthiolate, and with *B. subtilis* with surgical mercurochrome.

Changes in pigmentation were observed with *P. aeruginosa*, normally a blue-green culture, with the following: surgical merbromin showed an increased production of pigment; aqueous merthiolate produced a 2-mm zone of decreased pigmentation; glycerite of merthiolate caused a 6-mm pink zone; tincture of metaphen showed a 3-mm yellowish zone followed by a 4-mm pink zone distal to the yellow zone.

The glycerol solution of the urea peroxide and oxine resulted in a zone of raised wet mucoid growth in the case of *Aerobacter cloacae*. The organisms within the zone were coccoid in nature instead of the normal, small, rodlike shape. In one series of subcultures they maintained this characteristic for several transfers. With the same solution *P. aeruginosa* showed a reaction to the antiseptic by

TABLE 2

Comparison of action of glycerite of hydrogen peroxide and mercurial antiseptic solutions on gram-negative organisms

| GLYCEROL SOLUTIONS | | E. COLI | | | | P. MIRABILIS | | | | P. AERUGINOSA | | | | AEROBACTER CLOACAE | | | |
|---------------------------|----|---------|----|---|----|--------------|----|---|----|---------------|----|---|----|--------------------|----|----|----|
| | | A* | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Urea peroxide + oxine | G* | 7 | 6 | 6 | 7 | 10 | 9 | 7 | 7 | 5 | 3 | 4 | 4 | 8 | 10 | 8 | 8 |
| Urea peroxide | G | 5 | 5 | 7 | 5 | 7 | 6 | 7 | 6 | 4 | 3 | 4 | 5 | 6 | 6 | 6 | 9 |
| Hydrogen peroxide + oxine | G | 4 | 5 | 3 | 3 | 7 | 6 | 6 | 3 | 5 | 3 | 4 | 3 | 6 | 6 | 7 | 7 |
| Hydrogen peroxide | G | 3 | 3 | 7 | 6 | 5 | 3 | 5 | 6 | 1 | 2 | 1 | 3 | 6 | 6 | 6 | 6 |
| Oxine | G | 4 | 3 | 0 | 0 | 7 | 3 | 2 | 3 | 0 | 3 | 0 | 3 | 6 | 5 | 0 | 3 |
| MERCURIAL SOLUTIONS | | | | | | | | | | | | | | | | | |
| Merbromin | T | 6 | 18 | 5 | 13 | 8 | 18 | 7 | 16 | 10 | 14 | 7 | 13 | 15 | 15 | 14 | 14 |
| | A | 4 | 7 | 3 | 4 | 6 | 6 | 3 | 7 | 5 | 7 | 4 | 9 | 3 | 7 | 4 | 6 |
| Mercresin | T | 4 | 10 | 1 | 7 | 8 | 9 | 5 | 6 | 5 | 9 | 5 | 8 | 7 | 14 | 8 | 8 |
| | A | 3 | 5 | 2 | 3 | 6 | 8 | 4 | 8 | 5 | 7 | 5 | 6 | 3 | 7 | 4 | 6 |
| Mercurochrome | T | 6 | 14 | 3 | 11 | 7 | 7 | 6 | 8 | 9 | 10 | 6 | 9 | 6 | 12 | 8 | 9 |
| | A | 3 | 5 | 2 | 3 | 6 | 8 | 4 | 8 | 5 | 7 | 5 | 6 | 3 | 7 | 4 | 6 |
| Merseptal | T | 6 | 8 | 3 | 8 | 9 | 6 | 6 | 7 | 7 | 11 | 6 | 7 | 7 | 7 | 6 | 5 |
| | A | 6 | 10 | 2 | 3 | 5 | 5 | 4 | 4 | 5 | 6 | 3 | 5 | 6 | 5 | 4 | 3 |
| Merthiolate | T | 7 | 13 | 5 | 11 | 11 | 12 | 7 | 6 | 9 | 13 | 9 | 9 | 13 | 13 | 14 | 15 |
| | A | 6 | 6 | 3 | 5 | 6 | 7 | 6 | 3 | 10 | 8 | 7 | 10 | 11 | 11 | 8 | 10 |
| | G | 5 | 4 | 4 | 3 | 5 | 5 | 4 | 3 | 9 | 5 | 7 | 6 | 6 | 6 | 6 | 7 |
| Metaphen | T | 7 | 13 | 5 | 11 | 9 | 9 | 8 | 17 | 10 | 14 | 7 | 10 | 7 | 14 | 11 | 15 |
| | A | 2 | 3 | 1 | 3 | 4 | 3 | 4 | 3 | 7 | 4 | 5 | 5 | 3 | 2 | 2 | 3 |

Zones were measured radially, in mm, from the edge of cylinder to edge of clear zone.

* See footnotes to table 1.

turning from a normal bluish-green culture to an emerald-green in plates without serum. In plates with serum, there was a yellow zone, followed by an emerald zone extending peripherally from the cup.

Urea peroxide without oxine caused a deeper pigmentation in *S. aureus* cultures, with an undercutting of the clear zone. In *Proteus mirabilis* cultures, there was a raised moist growth immediately distal to the clear zone. Hydrogen

peroxide with oxine, in glycerol, caused a pink zone immediately distal to the clear zone with *P. aeruginosa*, the remainder of the plate remaining an emerald green.

The peroxide solutions evidently showed greater activity than did the mercurial solutions against gram-positive organisms. The addition of oxine results in somewhat greater activity of the solutions in the presence of serum, but not much difference in the absence of serum. The values resulting from oxine alone are normally overshadowed by the effects of the hydrogen peroxide. In addition, slightly more bacteriostatic action, as indicated by decreasing density of the colonies, is shown by solutions containing oxine as compared with the solutions not containing the stabilizing agent. The solutions of hydrogen peroxide in glycerol, without urea, demonstrate somewhat less activity than did the solutions with urea. It will be noted that there is apparently more effect on the bacterial metabolism, as shown by variations in the type of growth, by the solutions containing oxine and urea than with hydrogen peroxide alone in glycerol.

The alcoholic mercurial solutions showed better activity than did the aqueous ones. Some of the tinctures showed larger zones against the gram-negative organisms tested than do the peroxides. The latter, however, showed as good or better results when compared to the aqueous solutions of the mercurials. Thus, since the alcoholic solutions cannot be used on large wound areas because of the irritation, the glycerite of hydrogen peroxide would seem more applicable in such cases. Another disadvantage suffered by the mercurial solutions is the undesirability of the risk of absorption of mercury compounds. Such is not the case with the peroxide solutions of constituents which are nontoxic and nonallergenic. Tissue tolerance studies have shown them to be nonirritating on both normal and infected skin and mucous membranes (Brown, 1946a).

The solutions of merbromin, in general, give consistently larger zones than do mercurochrome solutions. The cause of this is not immediately apparent, since the two are supposedly of the same concentration and of essentially the same composition. The answer may lie, however, in the pH of the two solutions, since the apparent pH of the surgical mercurochrome was 9.6, whereas that of the corresponding merbromin solution was 8.1. The fact that both solutions show as good results as they do on the plates may be due to the long-continued action, since McCulloch's (1945) review indicates that, as ordinarily applied clinically, the solutions leave much to be desired.

SUMMARY

When tested by a modified cylinder plate method, peroxide-glycerol solutions, made from either urea peroxide or hydrogen peroxide, showed a bacteriotoxic action on both gram-positive and gram-negative organisms.

A greater bactericidal effect was noted with gram-positive than with gram-negative bacteria.

The presence of 8-hydroxyquinoline in the peroxide solution did not appear to improve significantly the bacteriotoxic action of the solution in the absence of

serum, but did appear to enhance the activity in the presence of 10 per cent horse serum.

In comparison with 12 mercurial solutions, the glycerol-peroxide solutions showed, in general, greater bacteriotoxic action on gram-positive organisms than did the mercurial solutions. The latter were, in general, the more effective on gram-negative bacteria. In specific cases, however, the peroxide-glycerol solutions were more efficacious than some of the mercurial solutions, particularly when water was the principal solvent for the mercurial compound.

REFERENCES

- BERGEY, D. H., BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1939 Bergey's Manual of determinative bacteriology. 5th ed. Williams and Wilkins, Baltimore.
- BROWN, E. A. 1946a Studies in tissue tolerance to a new glycerol-peroxide antiseptic solution. *Ann. Allergy*, **4**, 33-39.
- BROWN, E. A. 1946b Glycerite of hydrogen peroxide: a correlative review of laboratory and clinical data. *Ohio State Med. J.*, **42**, 600-603.
- BROWN, E. A. 1946c Glycerite of hydrogen peroxide: an evaluation of its clinical effect in mixed infections of the skin and mucous membranes. *J. Maine Med. Assoc.*, **37**, 181-184.
- BROWN, E. A., KRABEK, W. B., AND SKIFFINGTON, RITA E. 1946 A new antiseptic solution for topical application, *New Engl. J. Med.*, **234**, 468-472.
- HARRIS, R. G., AND PROUT, W. A. 1940 Correlation of the evaluation of disinfectants by the agar cup plate method and clinical experience. *J. Am. Pharm. Assoc.*, **29**, 413.
- MCCULLOCH, E. C. 1945 Disinfection and sterilization. 2d ed. Lea and Febiger, Philadelphia.
- NYE, R. N. 1937 The relative *in vitro* activity of certain antiseptics in aqueous solutions. *J. Am. Med. Assoc.*, **103**, 280-287.
- RUEHLE, G. L. A., AND BREWER, C. M. 1931 United States Food and Drug Administration methods of testing antiseptics and disinfectants. U.S. Dept. Agr., Circ. 198.