TECHNICAL PAPERS

Antiseptic Action of Glycerite of Hydrogen Peroxide on *Mycobacterium tuberculosis* (var. *hominis*)

ETHAN ALLAN BROWN
Department of Medicine, Tufts Medical School

L. W. SLANETZ
Department of Biology, University of New Hampshire

In a previous communication, the bactericidal effects of glycerite of hydrogen peroxide on gram-positive and gram-negative bacteria, as measured by the cup-plate technique, were described (*1*). A second report, concerned with oral organisms, demonstrated the solution as extremely bactericidal by both *in vitro* and *in vivo* studies (*4*). The present paper describes the effects of the solution on *Myco. tuberculosis* (var. *hominis*) as measured by laboratory methods, and clinically on patients with tuberculous lesions of the body surface.

The solution consists of hydrogen peroxide (1.5 per cent) as derived from urea peroxide (4 per cent) dissolved in anhydrous glycerol. Although such solutions are relatively stable (*1*), a secondary stabilizing agent, 8-hydroxyquinoline, is present in concentrations of 0.1 per cent.

To obtain a uniform suspension of cells, the organism is grown in Long's broth medium in 22-ml. tubes for 5 days at 37° C. The cultures are placed on a Kahn shaker for 30 minutes and the clumps permitted to settle. The upper portion of the liquid, containing the suspended bacteria, is pipetted into sterile tubes. The organisms from several tubes (usually two or three) are pooled. Of this suspension, 0.5 ml. is inoculated into tubes containing 20 ml. of liquefied, cooled, Long's agar medium. After thorough mixing, the agar is poured into a Petri dish and permitted to solidify. The bacterial content of each plate averages 9,000,000 organisms as determined by plating 1:10,000 and 1:100,000 dilutions of the bacterial suspension in Long's agar.

Penicylinders (porcelain) are placed centrally on the agar and into each is pipetted 0.1 ml. of the solutions to be tested. These consist of glycerite of hydrogen peroxide, in serial dilutions of 0.1, 0.5, 1, 2, and 4 per cent (given as total urea peroxide); phenol, 2, 3, 4, and 5 per cent; penicillin, 10, 50, 100, and 1,000 units; sulfaguanidine, 1 per cent; sulfadiazine, 5 per cent; and sulfamethazine, 10 per cent.

The plates are incubated at 37° C. for 3 days. The zones of inhibition are measured in millimeters from the edge of the cup to the edge of the clear zone. Evidence of either bacteriostasis or bactericidal potency is determined by transfer of pea-sized portions taken from the center to the periphery of the clear zone to slants of Long's agar and to tubes of Long's broth. These are incubated for 14 days at 37° C.

As was expected, penicillin exhibited no measurable effects. Partial inhibition for the entire plate, with apparent clear zones, resulted from exposure to phenol. There was partial inhibition, with no clear zones, when the sulfonamides were used. For all of these solutions, however, the action was one of bacteriostasis, since all samples of transferred agar, by subculture technique demonstrated viable organisms.

For the glycerite of hydrogen peroxide in the 0.5, 1, 2, and 4 per cent concentrations there were clear zones containing no viable organisms; that is, there was bactericidal action. For the 0.1 per cent solution there was a clear zone with bacteriostasis. The average radial measurements were, successively, 5, 13, 17, 20, and 24 mm., repetition demonstrating statistically acceptable equivalent results.

In a separate set of experiments (*3*), a 17-day-old culture of *Myco. tuberculosis*, as grown on Petragnani's medium, was used. Ten-mm. cubes of this medium are cut and immersed in glycerite of 8 per cent hydrogen peroxide (total urea peroxide) for periods of 1, 2, and 24 hours. The cubes containing the bacteria are removed from the antiseptic solution at the end of the specified time, and, in order to counteract the residual peroxide solution, are placed in Brewer's thioglycolate medium for 1 hour. The cubes are then mixed with sterile saline and streaked on Petragnani's medium. A growth-control cube is placed into the thioglycolate medium and the saline and streaked on the same medium.

Following immersion for 1 hour, the cubes demonstrated slight growth visible by the 9th day of subculture. Following immersion for 2 or 24 hours, there was no growth for periods up to 21 days. The growth control showed a moderate growth by the 9th day.

From this it may tentatively be concluded that the solution is probably bactericidal for *Myco. tuberculosis* in periods of about 2 hours and with certainty in periods of more than 1 but less than 24 hours.

Since other substances will act *in vitro* on *Myco. tuberculosis* but are ineffective *in vivo*, clinical experiments were performed with four patients studied by Dr. Joseph Goldberg, of the Essex County Tuberculosis Sanatorium, Danvers, Massachusetts. All demonstrated "cold abscesses," which were positive by smear and culture for *Myco. tuberculosis*. In one patient the sites were multiple, involving the chest, thigh, buttock, and leg. In the remaining three, the chest wall alone was affected. None had responded to previous treatment, and, as is generally known, this condition does not heal spontaneously, and no local measures have so far been effective.

The glycerite of hydrogen peroxide (urea peroxide 4 per cent) was applied as a wet dressing 2–6 times daily. No other treatment was used. All previous treatment is noted as having been without effect. In three patients, the abscesses healed completely; and in the last, who presented multiple lesions and who is still under treatment, the fourth lesion, although greatly diminished in size and obviously healing, shows occasional slight serous discharge. In no patient was there

evidence of irritation or allergic reaction for a treatment period which varied from 4 to 11 months. In order to make certain that these were not temporary remissions, one year was permitted to elapse before the clinical report was made, the first patient having healed by February 1945 and the last by November 1945.

It is hoped that this preliminary communication will stimulate further exploration of the treatment of tuberculous lesions with glycerol peroxide solutions.

References

1. Brown, E. A., Abramson, H. A., Gorin, M., Kauffmann, H. O., and Shanley, E. C. *J. Amer. pharm. Ass.*, 1946, **35**, 304.
2. Brown, E. A., Krabek, W., and Skiffington, Rita. *New Engl. med. J.*, 1946, **234**, 468.
3. Brown, E. A., Krabek, W., and Skiffington, Rita. Unpublished data.
4. Slanetz, L. W., and Brown, E. A. *J. dent. Res.*, 1946, **25**, 223.

Depletion of Vitamin A Reserves in the Livers of Cattle

Paul R. Frey and Rue Jensen

Departments of Chemistry and *Veterinary Pathology and Bacteriology, Colorado Agricultural Experiment Station, Fort Collins*

In a recent publication (*2*) the writers reported on the depletion of vitamin A reserves in the livers of steers while in the feed lot. One-hundred twenty Hereford steers, about 18 months of age, were taken from native grass pasture and placed on a fattening ration consistent with good feeding practice. Table 1 gives the slaughtering data and the average vitamin A content of the livers. Vitamin A was determined by the method of Davies (*1*).

TABLE 1

Days in the feed lot	No. of animals slaughtered	Vitamin A reserves (μg. vitamin A/gram liver)
0	22	51.4
41	19	23.7
76	20	11.9
119	19	5.3
166	40	1.9

A relationship in the above data has recently been pointed out by Norris Embree, of Distillation Products, Inc. It was mentioned (*2*) that the curve obtained by plotting the data in Table 1 shows a decreasing rate of loss of vitamin A reserves throughout the experimental period. Dr. Embree cited an article by K. C. D. Hickman (*3*), also of Distillation Products, Inc., which appeared while the above publication was in press and in which Dr. Hickman proposed the hypothesis that the rate of depletion of the body reserves of a vitamin are directly proportional to the total reserves of that particular vitamin in the body. Further, when an animal is subjected to vitamin therapy, it should be possible to calculate a period of half adjustment.

To determine the validity of Dr. Hickman's hypothesis, the data in Table 1 were subjected to analysis. Vitamin A reserves were plotted against days in the feed lot and vitamin A values determined at intervals of 40 days. Table 2 gives the values obtained.

TABLE 2

Days in the feed lot	Vitamin A reserves (μg. vitamin A/gram liver)	Drop in 40 days (%)
0	51.4	—
40	24.2	47
80	11.1	46
120	5.2	47
160	2.2	42

It will be observed that the percentage drop in vitamin A reserve in the liver at the end of each 40-day period is practically constant. This supports Dr. Hickman's hypothesis as regards the rate of depletion of body reserves of a vitamin.

References

1. Davies, A. W. *Biochem. J.*, 1933, **27**, 1770.
2. Frey, P. R., and Jensen, R. *J. Nutrition*, 1946, **32**, 133.
3. Hickman, K. C. D. *Interne*, April 1946, p. 278.

Thromboplastic Properties of Penicillin and Streptomycin

David I. Macht[1]

Department of Pharmacology, Laboratories of the Sinai Hospital, Baltimore, Maryland

Moldavsky, Hasselbrook, and Cateno (*6*) have described some interesting findings with the blood of patients receiving parenteral injections of penicillin. In a series of human subjects studies on the clotting of blood before and after administration of penicillin revealed that the clotting time was much shorter after injections of the antibiotic than the normal clotting time was before injection. In connection with a study of blood in hemophilia the present writer and Dr. Marcus Ostro repeated the above observations on normal clinical subjects and confirmed the findings made by Moldavsky and his associates, but, curiously enough, when injections of penicillin were given to two patients suffering from hemophilia, no shortening of coagulation time was produced by the drug (*5*).

Inasmuch as it has been discovered experimentally on animals and confirmed by clinical observations that drugs belonging to the digitaloid group show a definite tendency to promote coagulation of blood (*2*, *3*), and inasmuch as more recently certain widely employed mercurial diuretics have also been found to produce such a thromboplastic effect leading occasionally to fatal thrombotic accidents (*4*), an extensive investigation was undertaken to ascertain the frequency and degree of the thromboplastic effects of penicillin on the blood of higher animals. For this purpose not only the ordinary

[1] Grateful acknowledgment is made to the Commercial Solvents Corporation and its medical director, Dr. Lawrence Smith, for valuable aid in carrying out this research.