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EXPERIMENTAL INVESTIGATION OF SUBSTANCES FOR DISINFECTING OBJECTS INFECTED BY AGENTS CAUSING ANAEROBIC INFECTIONS

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EXPERIMENTAL INVESTIGATION OF SUBSTANCES FOR DISINFECTING OBJECTS INFECTED BY AGENTS CAUSING ANAEROBIC INFECTIONS

by

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EXPERIMENTAL INVESTIGATION OF SUBSTANCES FOR DISINFECTING OBJECTS INFECTED BY AGENTS CAUSING ANAEROBIC INFECTIONS

Phenol is a widely employed disinfectant, used by many bacteriological institutes. The use of a 3 to 5% solution of phenol for disinfecting separators, tanks and containers, bottles for natural plasma, tubing and all manner of gauze bandages, viscose bags and cellophane, as well as for preliminary treatment of utensils, is specified by industrial regulations for obtaining dry diagnostic antigangrene and antibotulin serum. The instructions for preparing and checking purified tetanic anatoxin and sorbed botulin pentaanatoxin (including Type A, B, C, D and E anatoxins) specify the use of a 5% solution of caustic soda for making gloves, aprons, and sleeves germ-free and for swabbing operating rooms after an operation. The use of a 5% solution of chloramine is prescribed in the instructions for working with tuberculosis microbacteria.

Until now, a 5% solution of phenol has been used at the Control Institute of Medical and Biological Preparations in working with pathogenic anaerobic organisms. Data in the literature indicates, however, that this substance exerts its sterilizing action primarily on vegetative forms without killing spores. Thus, Nishida and Imaizumi [1] found that a 5% phenol solution can be used to select the strains of *Cl. histolyticum* and find those which have the most resistant spores; this is important for obtaining toxigenic cultures, since (as the authors point out) there is a direct relationship between the resistance of the spores and the toxigenicity.

The Central Scientific Research Disinfection Institute recommends the use of hydrogen peroxide and soap for the purpose of continuous and final disinfection in the infectious diseases section of a hospital for intestinal infections and running sores, as well as for prophylaxis in connection with suppurative ailments. In the instructions compiled by the workers at the Institute pertaining to the use of hydrogen

peroxide, it is pointed out that 1-3% solutions of hydrogen peroxide have a bactericidal effect, while a 6% concentration has sporicidal properties as well. Solutions with these concentrations are non-toxic to man.

Our preliminary experiments showed that a good growth was observed in the case of washed microbial cells of *Cl. histolyticum* (Strain No. 5), resuspended in a 5% solution of phenol and kept in the latter for 1, 2 and even 3 days, on a thioglycol medium. In connection with these observations, we studied the problem of comparing various disinfectant solutions in terms of their effect on sore-bearing anaerobic microbes. We investigated 5% solutions of phenol, chloramine and NaOH, a 5% solution of phenol and oxalic acid with a 1% solution of hydrochloric acid, and a 6% solution of hydrogen peroxide.

The basic experiments on the effect of disinfectant solutions used *Cl. histolyticum*, since these microorganisms are able to form spores rapidly and in rather large numbers. In smears of cultures of many strains of this type, growth of 20 to 80% of the spores was already visible after 18 to 24 hours.

Twenty-four-hour cultures on a medium consisting of pieces of meat and 0.1% agar were placed in 1 ml. batches in penicillin flasks and suffused from above with disinfectant solutions. After various intervals of time, 0.5 to 0.7 ml. of each batch was poured onto a medium with thioglycolic acid and incubated at 37° for 18 to 20 hours. As the experiments showed, a medium containing thioglycolic acid is more favorable for doing this work. Thus, in a medium containing pieces of meat and 0.1% agar, the microbes treated with disinfectant solutions often failed to grow, while they grew well in a medium containing thioglycolic acid. Since several disinfectant solutions caused turbidity of the thioglycol medium, a condition which made it more difficult to estimate growth in such media, the culture was transferred after 24 hours to a medium containing pieces of meat and 1% agar. The inoculated agar was incubated at 37°. The results of the experiment were evaluated after 24 to 48 hours.

Following treatment of *Cl. histolyticum* with a phenol solution for 24 hours, only one strain (No. 141) out of the 19 investigated lost its capacity for growth; two other strains (No. 17 and No. 144) also failed to grow when reinoculated after being treated for 48 hours. The addition of a 1% solution of hydrochloric acid to the phenol solution increased its disinfectant properties. No growth was observed in 63 cases out of 144 in experiments with 24 and 72 hour cultures of *Cl. histolyticum*. Sixteen out of 19 strains retained their viability after

treatment for 5 hours, while 10 strains survived after being treated for 24 hours. One strain (No. 344) proved to be hardier and grew after a 72-hour treatment with this solution.

The experiments with NaOH showed that of the 19 strains of *Cl. histolyticum* investigated, 18 survived a 5-hour exposure and 16 survived a 24-hour exposure. Growth was observed in 75 out of 112 experiments. A total of 114 experiments were performed with a chloramine solution. Growth was observed in 15 of them. Six strains withstood treatment with chloramine for 5 hours, while 2 strains survived a 24-hour treatment. There were 114 tests conducted with oxalic acid and 1% hydrochloric acid.

A sterilizing effect was observed in 98% of the experiments. A total of 114 experiments was performed with hydrogen peroxide. In all of the experiments, even a 5-hour exposure led to the death of all of the microbes.

Hence, the most powerful of the chemical substances tested was found to be hydrogen peroxide and oxalic acid with a 1% solution of HCl.

Additional experiments conducted with the No. 5 and No. 334 strains of *Cl. histolyticum* showed that even a 30-minute treatment with a 6% solution of hydrogen peroxide was sufficient to render all of these cultures completely harmless.

In addition to the tests on rendering *Cl. histolyticum* harmless, other tests were devoted to determining the resistance of other forms of anaerobes to these chemical substances. It was found that the agents causing gas gangrene *(Cl. perfringens, oedematiens, septicum)*, tetanus and botulism, as well as spore-bearing anaerobes, were resistant to the action of phenol and alkali for 48 hours. Strain No. 79 of *Cl. oedematiens* was found to be particularly resistant to the action of phenol solutions containing 1% HCl; it withstood the action of this disinfectant solution for 3 days.

We also studied the effect of a 6% solution of hydrogen peroxide and a 5% solution of oxalic acid with 1% HCl, on cultures of 7 anaerobes of various types *(Cl. tetanus, perfringens, oedematiens, botulinum, histolyticum, septicum, sordellii)*, kept in sealed test tubes in liquid media for periods of 1 to 2 years. Observations showed that this treatment led to the death of all cultures in the spore stage.

In our further experiments, we attempted to determine how long the disinfectant properties of a 6% solution of hydrogen peroxide are retained. For this purpose, the above solution was poured into a deep container covered with black paper and then placed in a box. After 2 to 4 weeks, this solution was used to treat 7 strains of *Cl. histolyticum* for a period of 24 hours. No growth of the cultures was observed after such a treatment.

We then attempted to determine the manner in which the disinfectant properties of hydrogen peroxide are retained after its exposure to light. The hydrogen peroxide was placed in a 100-ml. cylinder and set on a window sill. A pipet containing 0 5 ml of a culture of Strain No. 334 of *Cl. histolyticum* was inserted into the cylinder daily for two weeks; this was the strain which had proven to be most resistant to other disinfectant solutions. Reinoculation took place after 5 and 24 hours. The peroxide retained its disinfectant properties during this period of time.

Titration of the peroxide according to the method recommended by the Ninth Pharmacopaea (1961) showed that the decomposition rate of H_2O_2 depends on the amount of dirty glassware submerged in it; when the solution was overloaded with dirty pipets, the titre of the peroxide decreased approximately by a factor of 2 during an interval of 2 weeks.

Experiments in determining the minimum concentrations of H_2O_2 which shows a sterilizing effect on anaerobes indicated that daily contact with a 1% solution of hydrogen peroxide also ensured the death of all strains of *Cl. histolyticum*.

Hence, the experiments which we conducted indicated that the practice of using phenol or chloramine as disinfectants is not justified. Phenol has less disinfectant power than hydrogen peroxide or oxalic acid. In addition, phenol is not entirely harmless to the human organism; by saturating the air of boxes and other containers or areas treated with it, it may have a harmful effect on those working in such locations.

From the economic standpoint, the use of a solution of medical perhydrol is completely justified, since its cost is negligibly greater than that of phenol.

RESULTS

1. Hydrogen peroxide in a 6% solution is much superior to 5% solutions of phenol, alkali, and chloramine as far as sterilizing properties are concerned.

2. It was found that a 6% solution of hydrogen peroxide, when allowed to stand in an open vessel in a box, retained its disinfectant properties and could be used for a period of 10 to 14 days.

3. It would be advantageous to replace the phenol solution in current use by a solution of hydrogen peroxide; this change should be made in all Interrepublic Technical Specifications for making bacterial preparations.

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Comparison studies were made of the bactericidal properties of common disinfectants. A 6% solution of hydrogen peroxide was found to be superior to similar solutions of phenol, chloramine and alkali, both in terms of arresting bacterial growth and in terms of non-toxicity for mar

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