On the Efficacy of "Merthiolate" as a Biological Preservative After Ten Years' Use

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Introduction

The ideal antigen or antibody preparations for active or passive immunization of humans would comprise unpreserved native suspensions or solutions of determined potency, and thus the artificial process would closely simulate natural immunization. Since there are so many hazards connected with non-preservation, practically the entire commercial usage of vaccines and antisera has been confined to preparations containing added antiseptics. Such antiseptics must be tolerated constantly by the vaccine or serum for their action in preventing chance contamination. Criteria of their usefulness, therefore, include both the extent of undesirable action on the preserved substance and the extent of desirable action upon the chance organisms which may be encountered. Obviously a combination of characteristics, including in addition to a low degree of toxicity a minimum of injury to antigen or antibody and a maximum bacterial devitalizing action, would indicate the most useful preservative inasmuch as no perfectly selective ideal preservative has been described.

Review of Preliminary Reports

In early observations upon biological properties of "Merthiolate" (Sodium Ethyl Mercuri Thiosalicylate, Lilly), we (1) noted rather strong antiseptic effects on several pathogens and a marked degree of freedom from precipitating action against blood proteins, egg albumin, etc. Upon injection into mice, guinea pigs, rats, rabbits, dogs, and humans, "Merthiolate" was found to be much better tolerated than other similar substances. These properties indicated the use of "Merthiolate" as an antiseptic for human application and as a better preservative for vaccines and sera than the phenoloid compounds, the toxicity of which was reported upon several years ago by Leake and Corbitt (2). It may be mentioned that phenoloid toxicity has been emphasized recently in a critical discussion of preservatives for biological products (3). Also, a moderately rapid disappearance of phenoloid preservative from biological products through combination with rubber stoppers has been shown very recently (4).

In reports of early experiments with "Merthiolate" as a preservative, we (5) have called especial attention to the better keeping qualities of vaccines and antisera when preserved with "Merthiolate" instead of the phenoloid preservatives. Subsequent experimental and routine use of "Merthiolate" in this way has been made by various laboratories.

Our own biological preservative practice comprises mainly the use of "Merthiolate" 1-10,000 concentration in various vaccines and sera and

to a lesser extent the use of "Merthiolate" 1-500,000 concentration in a series of Undenatured Bacterial Antigen preparations.

Our stock preservative solution is 1% "Merthiolate." From this stock the desired quantities are pipetted into the vaccines or sera to be preserved, and in attaining a "Merthiolate" 1-10,000 preservative concentration for example, 1 cubic centimeter of the stock solution suffices for each 100 cubic centimeters of material to be preserved. While any convenient stock preservative solution of "Merthiolate" may be used, even 1-1,000, a 1% stock solution, is sufficiently concentrated to avoid introducing appreciable dilution of antitoxin etc. in the process of adding the preservative.

"Merthiolate" has a rather strong bacterial inhibitory property. Due regard for this should be made in culturing "Merthiolate"-preserved vaccines and sera into the necessary media for sterility examination, and some of the inocula should be near or above the range of one one-hundredth the volume of the medium into which they are planted. If the original preservative concentration, therefore, is 1-10,000, the final concentration of preservative in the sterility-test culture medium of infusion broth will be 1-1,000,000, which will scarcely inhibit the growth of viable organisms, if present, when the sterility-test cultures are incubated the usual seven days. It has been reported (**6**) that in some cases agar may be better than broth for use in sterility proving of vaccines and sera.

It is preferable that rubber stoppers to be used in vaccine and serum vials be free of excess sulphur. This may be made certain by first boiling them in dilute solution of sodium hydroxide and then in several changes of wash water. Excess of sulphur, if left in rubber stoppers, will occasionally cause discoloration of solutions preserved with "Merthiolate" through interaction with the preservative. A recent note on such discoloration has been published by Sickles (**7**).

Subsequent Comparative Laboratory Experiments

The earlier reports on better keeping qualities of "Merthiolate" as against phenoloid preserved antigens and antibodies have been verified and amplified by several experimenters. This is true of particularly careful studies of Rosenstein and her associates (**6**). These authors have dealt with the problem of rendering the vaccine or serum self-sterilizing against varied amounts of artificially introduced contaminations. For this combined use they preferred "Merthiolate" 1-10,000 for antigen preservation, and, rather than use "Merthiolate" 1-5,000, which alone was necessary for sterilizing and preserving their antisera, they preferred a mixture of "Merthiolate" 1-20,000 plus 0.25% phenol. Still weaker mixtures were later experimented with, and the mixed preservatives appeared to fortify each other. Rosenstein and Levin believed "Merthiolate" 1-5,000 was contraindicated in antisera since these are frequently administered in large doses. In view of the fact that human adults repeatedly tolerate over 250 milligrams of "Merthiolate" intravenously and that one would scarcely attain a toxic dose of "Merthiolate" 1-5,000 in antiserum until theoretically after more than 1,000 cc. of such serum had been used, we first utilized this concentration as wider margin

of preservative safety in the commercial preparation of antisera. A routine use for two years of "Merthiolate" 1-5,000 in this way elicited no reports of undesirable preservative reactions in humans. Later, however, we have used "Merthiolate" 1-10,000 since experience has indicated little need and some contraindication of making these antisera so strongly self-sterilizing. It appears that large numbers of contaminants or their products should be prevented from appearing in antiserum previous to the addition of the preservative. If this is not done, initially contaminated sera, even if subsequently rendered sterile through the action of the preservative, on injection often produce added chills and fever.

Wadsworth and his associates (8) in a report on purification of diphtheria toxoid have dealt in part with "Merthiolate" 1-10,000 and phenol 0.4% as preservatives. Data on comparative preservative effects on stability over a long period of time or age are lacking. These authors indicate, however, that, when frozen at –10° C. for twenty hours, the flocculating value of phenol-preserved toxoid was almost completely destroyed, while that of "Merthiolate"-preserved toxoid was unaffected. Watson and Langstaff (9) previously observed this phenomenon with phenol-preserved toxoid, and related phenomena have been observed in toxin-antitoxin mixtures.

Scherp and Rake (10) in a report on concentration and standardization of antimeningococcus serum have noted better keeping qualities of serum preserved with "Merthiolate" 1-10,000 as compared to serum preserved with 0.3% tricresol. For example, the former serum showed no change in antibody content after storage at 0°–4° C. for six months as compared to losses of 13% to 19% appearing in the tricresol-preserved serum under the same conditions.

Douglas and Hartley (11) have shown in comparative experiments that "Merthiolate" 1-5,000 devitalizes tubercle emulsions containing 1 mg. moist tubercle bacilli per cc. in less than one day and that "Merthiolate" 1-10,000 is effective in this way in four days. This is to be compared with phenol 0.5%, which is not effective up to fourteen days. As proof of devitalization, these authors injected fifty-nine guinea pigs with various doses of the medicated emulsions and observed these for a year. At this time the test animals reacted negatively to OT and subsequently when autopsied showed no tubercular lesions.

Eldering and Kendrick (12) have reported upon the preparation of Phase I pertussis vaccine and have dealt with its keeping qualities and the effects of the preservatives in the course of time in bringing about depression in immunizing action. These authors have noted that "Merthiolate"-preserved vaccine did not show as marked changes in agglutinin production after storage as did phenol preserve vaccine. At this time no information is available regarding the comparative human protection conferred by the "Merthiolate"- versus phenol-preserved vaccines made by Eldering and Kendrick. Previous to knowledge concerning phase relationship of *H. pertussis*, we had observed that stronger agglutinins were produced by "Merthiolate"-devitalized than by heat-devitalized pertussis vaccine.

Krueger and Nichols (13) in experimenting with undenatured bacterial antigens prepared from staphylococci and preserved with phenol,

tricresol, and "Merthiolate," have found "Merthiolate" caused the least denaturation. Tricresol 0.3% and phenol 0.5% produced 50% and 48% denaturation, respectively, as compared to "Merthiolate" in concentrations of 1-10,000, 1-20,000, and 1-50,000, which produces in these three concentrations 37%, 27%, and 24% denaturation, respectively. These native antigens are labile to the extent that thermal denaturation begins to be significant at 40° C. and hence responds very rapidly to denaturing influences.

Use of "Merthiolate" in Special Antigens, Sera, and Solutions

During the last few years many experimenters have used "Merthiolate" in preserving vaccines and antisera in small amounts for laboratory purposes. Although little comparative data are available in most of these brief references, little or no denaturation has been reported in any instance. However, several reports including quantitative data may be referred to as follows.

Kuhns (14) has used "Merthiolate" 1-10,000 to devitalize meningococcus cultures and to preserve various culture antigens used for diagnostic and immunizing purposes in several hundred human subjects. As shown in a comparative way with suitable controls, this preservative caused no "non-specific" or undesirable reactions in any of the subjects. Comparative information on the effect of the preservative on the various antigens was not shown; however, the degree of stability of the antigens appeared to indicate their clinical usefulness.

Hecht, Rappaport, and Briggs (15) have experimented with "Merthiolate" as a sterilizing agent for protein solutions prepared for allergic skin tests and treatments. They have used "Merthiolate" 1-3,000 successfully for this purpose, and after exposure to this concentration of antiseptic for twenty-four hours a 50% dilution of the preparation is accomplished by the usual addition of glycerin, giving a stock solution containing "Merthiolate" 1-6,000. These authors have also found in controlled precipitin tests with rabbit antisera that concentrations of "Merthiolate" even up to 1-1,000 caused no denaturation or alteration of 1% egg albumin solution during three months time.

Davis (16), in investigating the preparation of sterile solution, has given some attention to the action of "Merthiolate" as a preservative. He states that the alkalinity of this antiseptic makes it unadapted to the preservation of hypodermic drug solutions but that it lends itself to use as a biological preservative. His tests indicate that "Merthiolate" 1-100,000 would be a more potent germicide than 0.5% phenol or 0.3% tricresol.

Laidlaw, Smith, Andrews, and Dunkin (17), in preparing horse antiserum against human influenza virus, have used "Merthiolate" 1-20,000 as a preservative. This has at least a twenty-fold margin of safety in noninterference in serum-virus neutralization tests. In other words, the preservative does not in itself inactivate the virus in any serum-virus mixtures prepared in laboratory tests.

In this connection it may be mentioned that "Merthiolate" also does not markedly inactivate vaccine virus or poliomyelitis virus, while rabic virus and phage appear to be only moderately inactivated, and in turn

horse encephalomyelitis virus appears to be readily inactivated by "Merthiolate" treatment. Apparently the antiviral action differs from one virus to another just as with other antiseptics. For example, phenol 0.5% has been used for many years as a preservative of commercial vaccine virus, which in the form of a "live" virus is scarcely affected in potency by the phenol. Similar application of phenol preservative to phage results in critical loss in titer.

In experiments bearing partly on intravenous antisepsis and somewhat on the use of "Merthiolate" as a preservative, Smith, Czarnetzky, and Mudd **(18)** have indicated a large amount of coupling and inactivation of mercurials, including "Merthiolate," with serum. Their main interest appeared to be on the use of conventional antiseptics intravenously, and their results contraindicated such use. However, we believe such use has not been proved in direct experiments in infections, and hence "Merthiolate" has not been recommended in this way. It might be stated that, in light of the rather feeble "test tube" potency of sulfanilamide and the phenomenal "in vivo" curative action of this drug, intravenous excellence could hardly be proved or disproved by the technique used by Smith and her associates.

In experiments bearing directly on the comparative lasting qualities of "Merthiolate" as a preservative, we have shown in a separate report **(19)** that "Merthiolate" lasts in this way fully three years and that diminished activity is demonstrable after seven years. This is to be contrasted to the rapid drop in titer of phenoloid preservatives.

Summary

About ten years ago we started to experiment in a comparative way with the use of "Merthiolate" as a preservative agent for vaccines and sera. The results were sufficiently good to lead us to introduce routine biological preservation with "Merthiolate" two years later. Eight years have now elapsed since "Merthiolate" was first used commercially in this way. The degree of its usefulness in this field is attested by the experiences of other workers as well as ourselves. References to these have been made in the preceding paragraphs, and most of these reports are quantitative and comparative. It is believed that the papers which have been cited verify conclusively the usefulness of "Merthiolate" as a biological preservative first introduced ten years ago.

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The following, included in the titles of papers in the foregoing references, is a trademark which identifies a product of Eli Lilly and Company:
"Merthiolate" (Sodium Ethyl Mercuri Thiosalicylate, Lilly).