COMPARATIVE STUDIES ON MERTHIOLATE WITH REFERENCE TO LABORATORY EVALUATION AND HUMAN TISSUE ANTISEPSIS

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The study of materials intended for use as germicides includes a voluminous list of publications and reports dealing with many substances and various methods of technique. It is not the aim in this paper to analyze these reports in detail, or to deal in a comprehensive way with this mass of data. In a brief way, however, attention should be drawn to the main ideas embodied in this literature, the general nature of experimentation, and the conclusions arrived at, followed by the examination of our experimental material.

I. **The Natural Antibacterial Mechanism.** One may safely state that of all antibacterial mechanisms thus far studied, the natural resistance of the body cells is the most effective. Countless times during the life of the individual, invading bacteria and foreign materials are successfully dealt with by the normal or immune antibacterial mechanism of the individual. The success of this mechanism is attested by the survival of the species, and the balance between disease organisms on the one hand and the host on the other. One may well note the change in the notion of infection in the last generation, involving a shift of attention from the original simple study of the virulence of the bacteria to considerations of the various lines of defense presented by the host. In other words, the mere bringing together of virulent bacteria and an apparently susceptible host does not regularly result in infection. The conditions under which so-called carriers develop include a nice balance of offensive and defensive agencies. In all of this natural resistance to infection as just set forth, one may attribute survival of the host to antibacterial reactions, including the normal integrity of epithelial and other barriers, the influence of antibodies on the infectious agent followed by an accelerated phagocytic action and digestion, and the influence of fever temperature on this whole mechanism.

II. **Fortification of Body Immunity.** The practical utilization of germicides has appeared most desirable as a method of fortifying natural resistance. Considerable interest is attached to the possibly successful artificial medication of an infecting bacterium, and this no doubt has been the incentive in the development of many drugs and chemicals to serve as germicides, as has been the case in parallel studies on various specific antisera.

III. **General Evaluation of Germicides.** The main method of examination of substances intended as germicides in the past has been an

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in vitro or test tube test in which certain bacteria are exposed to a solution of chemical for a time, following which cultivation of any remaining viable organisms is attempted. The medium in which this medication has been brought about has usually been a very watery mixture and one cannot surely interpret the results in terms of effective-

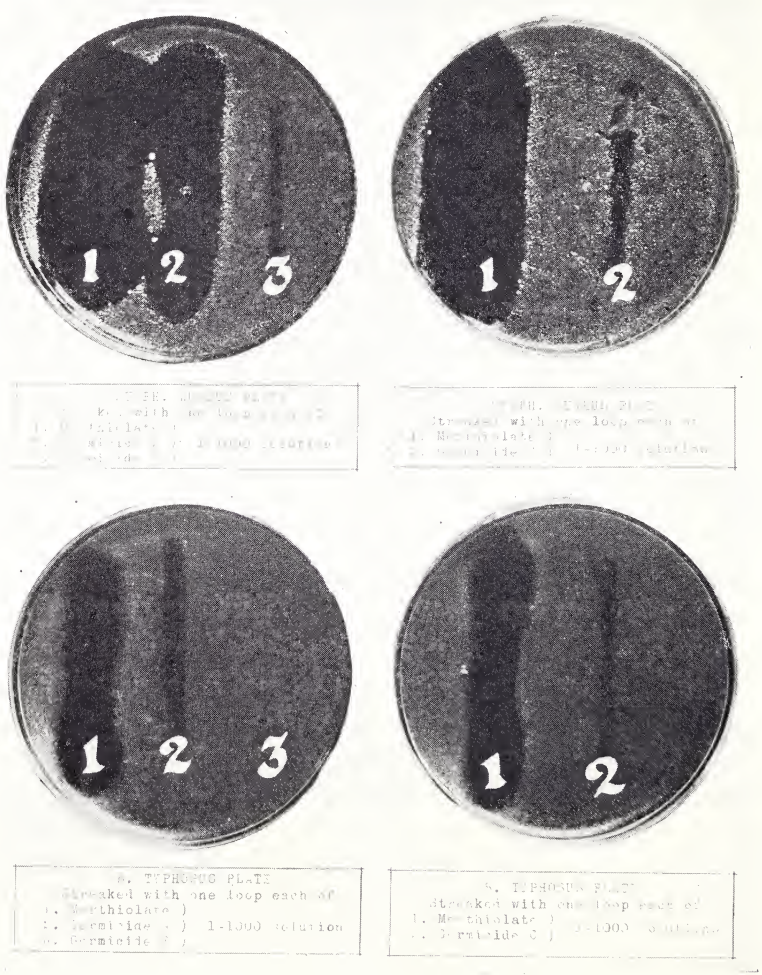

Fig. 1

ness in tissue antisepsis. The actual manner of killing of exposed bacteria may be coagulation of the bacterial substance, rupture of vital parts, or poisoning of respiratory or reproductive mechanisms. There has been little information as to the exact time of death of medicated bacteria, and negative results in cultivation attempts mean only that such bacteria will no longer proliferate in the usual way. Whether all

of the vital functions of such treated bacteria, in addition to reproduction, have ceased at the end of the medication period is not, of course, determined by the usual culture experiments.

IV. **Modifications of Germicide Evaluation.** In general one finds that too few species of bacteria have been examined in germicide

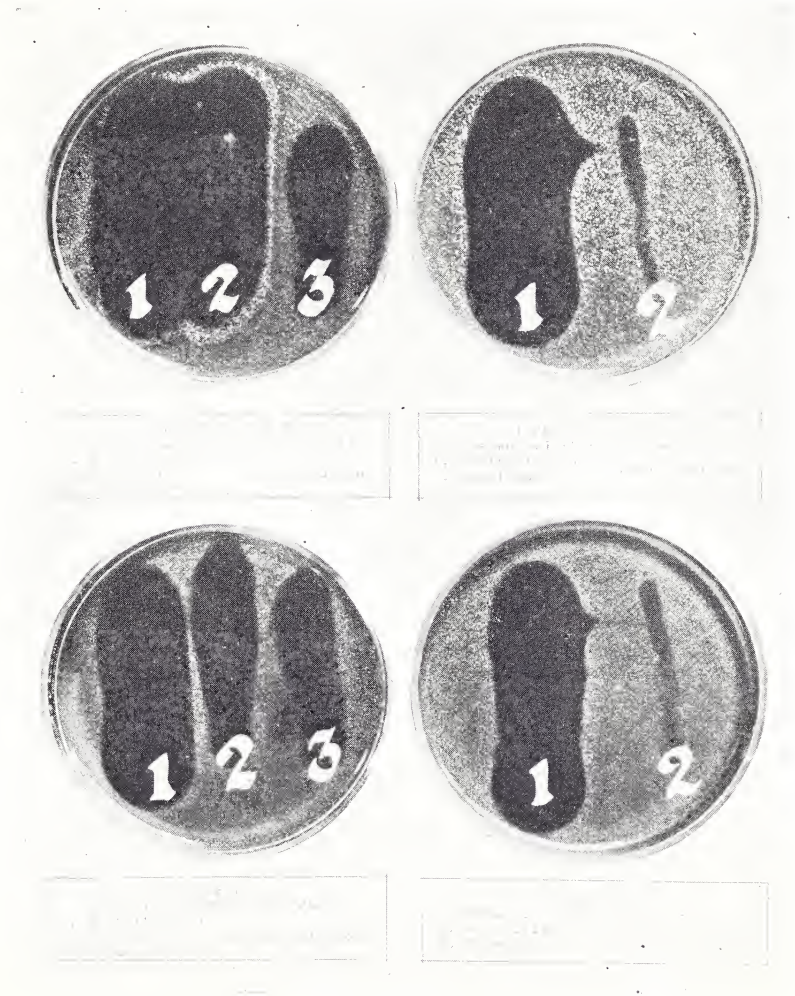

Fig. 2

studies. It would be much safer to base conclusions on wider studies including many different bacteria. In many studies, even though many bacteria have been used, there is an almost total disregard of the question as to whether the mechanism of bacterial killing brought about in a very watery medium in a test tube would be at all applicable to actual tissue antisepsis in which the medium of medication is a semi-

solid of high protein content. A third important criticism of a large amount of previous work is that due attention has not been paid to the compatibility of germicide and body tissues and whether the use of such germicide may not outrage already insulted and inflamed tissues. Certainly one cardinal property of a germicide should be its freedom

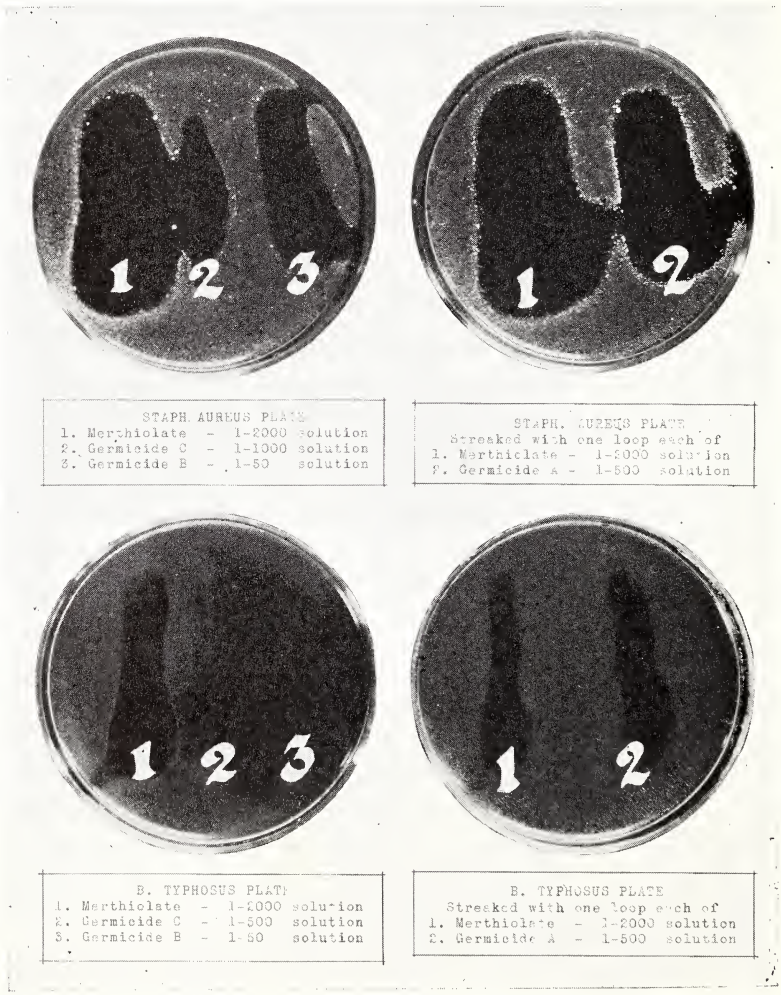

Fig. 3

from tissue-injuring properties. The germicide of choice should at least do no harm. One may now go farther and inquire whether the germicide in question positively has a beneficial effect upon the tissues. This is departing at some length from the older ideas that germicides are proved or disproved in test tube experiments including phenol co-

efficient determinations. The germicide of choice may now be expected to contain components which promote tissue repair and healing.

V. **Properties of the Ideal Germicide.** The ideal germicide should have ample germicidal value as proven in media most nearly like the tissues. In tissue antisepsis this property may be expected to reduce

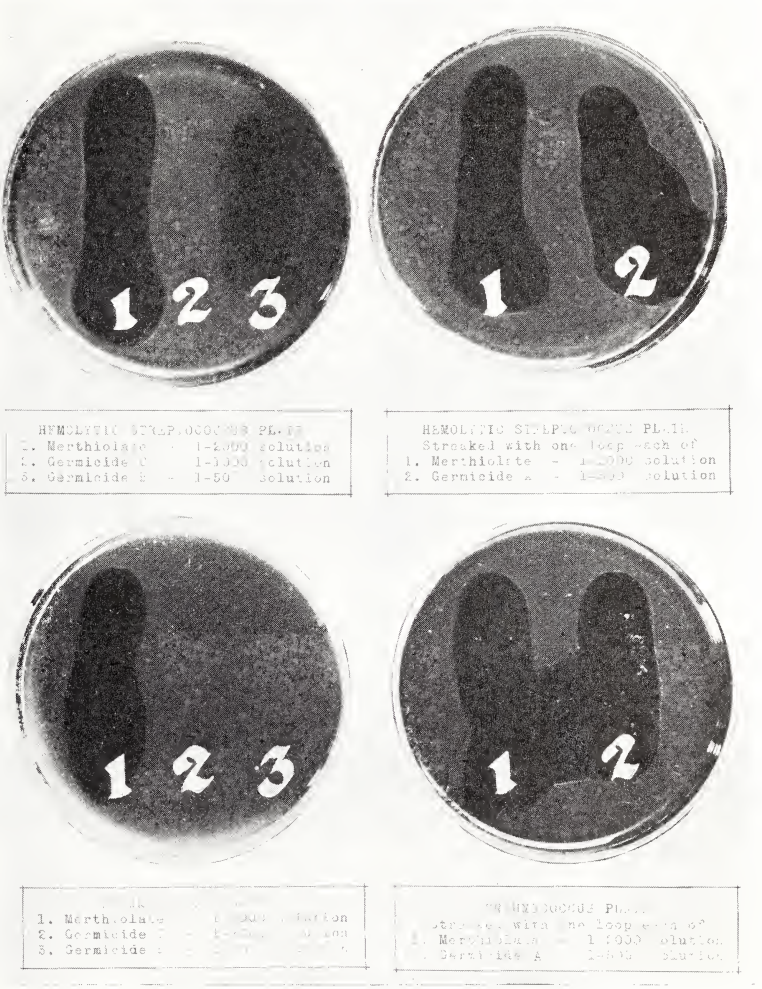

Fig. 4

the number of viable infecting organisms. The ideal germicide should have bacterial inhibitory properties in high dilution, so that the diminished infectious process shall be held in check for several hours instead of reappearing as formidably as ever in a short time after treatment. The ideal germicide should not cause precipitation of proteins, and should dialyze freely and thus penetrate sufficiently easily, as proven

in the laboratory, to indicate usefulness in actual tissue antisepsis. Along with these properties, and that of low toxicity, should be coupled the property of promoting healing.

The considerations just set forth have been recorded in some detail since it appears that tissue antisepsis is a much more complex subject than has usually been appreciated. The subject is an involved one in which ordinary phenol coefficients mean very little, and the ease of accomplishing tissue sterility has been greatly overestimated. Proper importance may now be placed upon the nature of the medium in which medication is attempted, and the optimum relations between the germicide and the tissues.

In the light of this broader conception of the nature of tissue antisepsis and the natural difficulties involved it is proposed to examine the new chemical, *Merthiolate,* as a germicidal agent of a new type.

EXPERIMENTAL

I. **General Properties of Merthiolate.** Merthiolate may be chemically identified as the sodium salt of ethyl mercuri thiosalicylic acid. Evidence has previously been presented (1) showing that Merthiolate has many desirable properties indicating its probable usefulness as a germicide. Chief among these may be mentioned its great solubility in body fluids as well as water, its very low toxicity in large doses for experimental animals and man, and its uniform germicidal effectiveness and bacteriostatic action against many human pathogens. These studies covered a wide range of tests, and placed greatest emphasis on experiments designed to approximate as nearly as possible the physical and chemical conditions obtaining in the tissues. Tissue antisepsis was not confounded with killing bacteria suspended in water. Precautions were used in all tests to evaluate properties possible of utilization and realization in tissue antisepsis. In these studies it was found that the action of some chemicals which are effective germicides in water is almost entirely blocked in serum and fibrin media most closely resembling the tissues. Unwarranted assumptions, either stated or assumed, as to effectiveness in tissue antisepsis should not be made on the basis of test tube results alone.

II. **Comparative Action of Merthiolate in Semisolid High Protein Media.** Certain modified experiments on comparative germicidal action may be described which serve to give a better estimation of germicidal excellence than the usual test tube experiments. Different dilutions of Merthiolate and other germicides have been applied as streaks across poured agar plates of various pathogens. Veal infusion agar has been used alone or with various contents of serum, or fresh plasma plus calcium chloride to cause clotting as the agar solidifies. In this way one has a medium of agar, serum agar, or fibrin agar, including the desired bacteria throughout the mass. Routinely 15 cc. of medium were mixed with 0.1 cc. of a twenty-four hour culture in each plate. Plates prepared in this way were streaked with a three millimeter loop of dilutions of Merthiolate and other well known commercial germicides, after which they were incubated for twenty-four hours at 37° C. At

this time clear zones about the streaks indicated the degree of penetration and antibacterial action. In order to judge whether the substance in question had killed or only inhibited the organisms included in the cleared agar area for a short time, small blocks of the latter were regularly removed and cultured to tubes of fresh broth. In these tests, utilizing a 20 per cent serum agar medium, as shown by plates I to IV,[1] Merthiolate and Germicide A alone were regularly germicidal. It may be mentioned that Germicide A is ten times as toxic as Merthiolate. Germicides B and C were not effective in these tests although they bring about germicidal effects in water. Preliminary watery dilution test tube tests of Merthiolate had shown that this chemical has forty to fifty times the germicidal action of phenol. These results were confirmed by Marshall (2).

III. **Comparative Action of Merthiolate on Living Tissues in Vitro.** It is not assumed that the serum agar plate tests just described are the best that can be designed for obtaining accurate information on the properties of a germicide indicating excellence in tissue antisepsis. Recently Buchsbaum and Bloom (3) have carried out experiments with several well known germicides in which both bacteria and living tissues growing *in vitro* were utilized. The tissues are propagated by well known methods devised by Carrel and others. In this study Buchsbaum and Bloom prepared tissue cultures together with bacteria and various concentrations of germicides, and attempted to find the limits in regard to tissue injury and bacterial killing. Using the score of 1 for an ideal antiseptic, i.e., one which at the weakest concentration would kill the bacteria and yet in this concentration not damage the tissues in the slightest, it was found that Merthiolate would receive a score of 0.9, which was the highest in the list and the closest approach to the ideal.

IV. **Action of Merthiolate on Labile Antigens and Antibodies.** It has been shown (4) that Merthiolate is practically non-injurious to various labile antigens and antibodies. Hemotoxins as well as the more stable toxins are little affected by Merthiolate, and presumably, due to the low ionization rate of this chemical and resulting lack of injury to serum globulins, antibodies are very little if any affected by Merthiolate. For these reasons this chemical is being used quite widely as a preservative for bacterial vaccines and antisera for human use. It may be supposed that Merthiolate would have a less severe action on immunity processes than other germicides having greater tendencies toward destruction of the agents of such processes, namely, antigens and antibodies. Lack of interference with the normal defensive action of the body would constitute a very worth while property of a germicide.

V. **Direct Action of Merthiolate on the Tissues.** Reiman (5) and others have shown the importance of sulphydryl in the multiplication of cells in repair and healing. So pronounced is this effect of sulphydryl in

[1] Fairly clear photographs were obtained for all plates except one, namely the B typhosus plate in the lower left corner for Figure 3. Here germicide B is obscured by a shadow, while only a little of the germicide C streak is seen.

stimulating tissue growth that it amounts to practically a general law. Since Merthiolate contains a substituted sulphydryl group it became of interest to examine the healing properties of this germicide. Results of this study have been completed (6). This work may be summarized as follows:

In tests in which the indirect effect of germicide on tissues was ruled out (i.e., antibacterial action may be confused with tissue stimulating properties) it was found that Merthiolate promoted rapid production of new epithelium and granulation tissue in the same way that other thio compounds acted. Denuded areas were covered with epithelium which rapidly closed in as a ring like the diaphragm on a camera. A non-sulphydryl containing mercurial germicide used as a control did not specifically stimulate healing, while thiocresol used as a second control stimulated rapid healing. It appears that no studies of this type have been reported in connection with any other germicides in common use. Development of germicides with healing properties, as well as antibacterial properties, represents a new and important development in antisepsis.

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