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A Comparison of the Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. I. Merthiolate.

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Lambert[1, 2] bathed fragments of human tissues in a dilute broth culture of *Staphylococcus aureus* for a few minutes and then in dilutions of the germicides for one hour. The tissue fragments were then washed in physiological salt solution, embedded in plasma and examined for growth of bacteria and tissue after several days of incubation. The chemicals included alcohol, iodine, argyrol, mercuric chloride, potassium mercuric iodide, potassium cyanide, hypochlorites, phenol, tri-cresol and hydrogen peroxide. He found that human connective tissue cells and histiocytes, were, in general, more easily killed than *Staphylococcus aureus*. Of the compounds tested iodine approached most closely the theoretically perfect disinfectant, killing bacteria in strengths that did not seriously injure human tissue cells.

* C represents a complete, P a preliminary manuscript.

1 Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

2 Lambert, R. A., *J. Am. Med. Assn.*, 1916, **67**, 1300.

In a later communication Lambert and Meyer[3] modified the above procedure. Rabbit spleen was used instead of human tissue. Also, the tissue fragments were bathed in a culture of *Staphylococcus aureus* for one minute followed by 20-minute exposures to the dilutions of the germicides, before being embedded in plasma. The chemicals tested included alcohol, iodine, mercuric chloride, mercurochrome, acriflavine, protargol, albargin, gentian violet, neosalvarsan and hexylresorcinol. Iodine was again found to approach nearer the theoretically perfect disinfectant. It is possible that in the favorable cultures the cells may have come from the center of the explant where they were partially protected. In another series the chemicals were added directly to the culture medium. Lambert and Meyer arrived at the same conclusions as in the first series.

German[4] bathed fragments of chick tissues in broth cultures of *Streptococcus hemolyticus, Staphylococcus aureus* and *Bacillus coli,* after which they were transferred to the antiseptic solutions. After periods of one and 5 minutes they were planted on agar plates. Fragments of chick tissues were also bathed in various dilutions of the chemicals for one and 5 minutes, after which they were washed in Locke's solution and embedded in plasma. He believed that the efficiency of an antiseptic to be inversely proportional to its harmful action on tissues and directly proportional to its bacteriostatic effect

Buchsbaum and Bloom[5] prepared chick tissue cultures in which the various dilutions of the antiseptics were embedded in chick plasma. The test organism, *Staphylococcus aureus* was added to the overlying embryonic fluid. The cultures were observed for bacterial and tissue growth after 24 and 48 hours' incubation. They tested phenol, iodine, mercurochrome, metaphen and merthiolate. They stated that an antiseptic killing the bacteria at concentrations that would not harm the cells would have an index of 1.0 or greater (greatest dilution that killed the organisms divided by the greatest concentration in which cells show approximately normal growth). Merthiolate was given an index of 0.9, the highest and phenol 0.2, the lowest.

Our work was devoted entirely to a study of phenol and merthiolate. The test organisms were *E. typhi* and *Staphylococcus aureus.* The organisms were at no time in contact with the tissues. Phenol

[3] Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

[4] German, W. J., *Arch. Surg.*, 1929, **18**, 1920.

[5] Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

coefficients for merthiolate were first determined, using the method of Reddish (as recommended by the A. P. H. A.).

The *E. typhi* phenol coefficient was found to be 50 and that for *Staph. aureus* 70. Identical results have been reported by Eli Lilly and Company, manufacturers of merthiolate.

Chick hearts obtained from 9-day-old embryos furnished the tissue. The tissue fragments were embedded in guinea pig plasma diluted with 3 parts of Tyrode solution and one part of embryonic fluid. After coagulation of the plasma the flasks were washed with Tyrode solution to remove the uncoagulated constituents of the plasma.

Dilutions of phenol and merthiolate were made in chick embryonic fluid and then added to the flasks. All fluids were accurately measured so that the final concentrations of the chemicals could be determined. Cultures were examined after 48 hours of incubation.

The results are given in Table I.

TABLE I.

Germicide	Highest Dilution Showing no Tissue Growth=A	Highest Dilution Showing no Growth of *E. typhi*=B	Highest Dilution Showing no Growth of *S. aureus*=B	Toxicity Index=A/B
Phenol	1-840	1-80		10.5
"	1-840		1-70	12.0
Merthiolate	1-176,400	1-4,000		44.1
"	1-176,400		1-5,000	35.3

Theoretically the smaller the toxicity index, the more efficient the germicide. In our tissue culture experiments phenol showed a smaller toxicity index than merthiolate.

The above results are just the reverse of those reported by Buchsbaum and Bloom. It is believed that the introduction of the organisms in the tissue cultures with the dilutions of the germicides by the above workers was responsible for the differences in results. Since the chemical was embedded in the plasma and the organisms were suspended in the overlying embryonic fluid it is very questionable if a uniform mixture of germicide and organisms was obtained. Also chick rather than human plasma was used. Chick plasma may exert a bactericidal action on the organisms which may be wholly lacking, or almost so, in human plasma.