

THE INFLUENCE OF ANTISEPTICS ON PHAGOCYTOSIS

H. M. POWELL and W. A. JAMIESON
(Lilly Research Laboratories, Indianapolis, U. S. A.)

INTRODUCTION

Inasmuch as phagocytosis of invading bacteria and foreign substances is a natural immunity mechanism of the first order, and closely similar to such vital physiological processes as digestion and assimilation, it becomes of interest to study any proposed *artificial* antibacterial methods, such as chemical antiseptics, in relation to possible injurious effects upon this *natural* mechanism. Certain desirable characteristics of the most promising antiseptic have heretofore been pointed out (1, 2, 3, 4, 5). These include quite naturally low tissue toxicity and adequate antibacterial qualities in media of the general nature of tissues. Coupled with these qualities may be added a most favorable bacteria: tissue index according to the refined methods set forth by Buchsbaum and Bloom (6), together with the property of promoting healing and repair (7).

Most of these properties have been qualitatively and quantitatively measured for Merthiolate. The study reported here was made to obtain additional information concerning the comparative effects of this chemical upon the process of phagocytosis.

EXPERIMENTAL

The materials used in these tests included, (1) staphylococcus cultures, (2) antistaphylococcus horse serum, (3) guinea pig leucocytes, (4) a series of dilutions of Merthiolate and other commercial antiseptics prepared with physiological saline and the latter alone as a control, and (5) sufficient capillary pipettes, slides, et cetera, for conducting the test.

The staphylococcus culture always consisted of a beef extract broth culture incubated twenty-four hours, after which it was diluted and shaken up with nine parts of saline to produce a homogeneous suspension of desired turbidity.

The antistaphylococcus horse serum was taken from a lot prepared by administering a series of intravenous and subcutaneous doses of staphylococci and their broth culture products to horses over a period of several months. The serum so obtained was concentrated and purified by well known salting-out methods and the pseudoglobulin fraction was utilized.

Guinea pig leucocytes were obtained just previous to each test by washing out with citrated saline the peritoneal exudate resulting in guinea pigs treated twenty-four hours previously with a 5 cc. intra-peritoneal dose of 2 per cent aleuronat suspension. Following the emulsification of this exudate, largely made up of polymorphonuclear cells, in warm citrated saline, centrifugation was done and the cells resuspended in warm saline and placed in the water-bath until used in the tests.

Three antiseptics listed as follows were used in these tests: "A," Merthiolate Solution No. 45, 1:1000, and saline dilutions of this giving 1:5000 and 1:10,000 concentrations; "B," a widely used mercurial antiseptic 1:500 commercial solution and saline dilutions of this giving 1:5000 and 1:10,000 concentrations; and "C," a widely used non-mercurial anti-

TABLE 1. Influence of Antiseptics on Staphylococcal Phagocytosis

Antiseptic Dilution	Leucocyte Suspension	Staphylococcus Culture 1:10	Staphylococcus Serum 1:10	Phagocytosis Observed
0	1 unit	1 unit	1 unit	Complete; all leucocytes engorged.
Saline 1 unit	1 unit	1 unit	0	Very slight; nearly all cocci free.
Saline 1 unit	1 unit	1 unit	1 unit	Complete; all leucocytes engorged.
Merthiolate Sol. "A" 1:1000; 1 unit	1 unit	1 unit	1 unit	Moderate phagocytosis; many free cocci, however.
Merthiolate Sol. "A" 1:5000; 1 unit	1 unit	1 unit	1 unit	As above
Merthiolate Sol. "A" 1:10,000; 1 unit	1 unit	1 unit	1 unit	Complete; all leucocytes engorged.
Antiseptic "B" 1:500; 1 unit	1 unit	1 unit	1 unit	Slight phagocytosis; all leucocytes shrunk; many free cocci.
Antiseptic "B" 1:5000; 1 unit	1 unit	1 unit	1 unit	As above
Antiseptic "B" 1:10,000; 1 unit	1 unit	1 unit	1 unit	Good phagocytosis but not complete; few free cocci.
Antiseptic "C" 1:1000; 1 unit	1 unit	1 unit	1 unit	No phagocytosis; leucocytes clumped together and disintegrating.
Antiseptic "C" 1:5000; 1 unit	1 unit	1 unit	1 unit	Slight phagocytosis; leucocytes clumped and ruptured; many free cocci.
Antiseptic "C" 1:10,000; 1 unit	1 unit	1 unit	1 unit	Moderate phagocytosis; many free cocci, however.

septic 1:1000 commercial solution and saline dilutions of this giving 1:5000 and 1:10,000 concentrations; also saline for controls.

The technique for conducting the tests was that commonly used in opsonic examinations. Using suitably prepared Wright capillary pipettes, one drop each of leucocyte suspension, staphylococcus culture, staphylococcus serum, and antiseptic dilutions were thoroughly mixed, then drawn up into the pipette again and the tip of the latter sealed and placed at 37° C. for fifteen minutes. A saline control was used for the serum and for the antiseptic dilutions. After incubation, the contents of each capillary pipette was smeared and stained as in blood work.

In these tests it is to be noted that we attempt to evaluate the total coincident effect of the antiseptic upon the three factors in phagocytosis; namely, leucocytes, bacteria, and immune serum, and this would appear to be the condition obtained in chemical tissue antiseptics. Heretofore certain phagocytosis-antiseptic tests have been conducted subjecting at first only the bacteria and serum to the action of the antiseptic, following which the leucocytes were added.

Table I shows the results of these antiseptic-phagocytosis tests. A dilution of immune serum of 1:10 without added complement was sufficient to cause complete phagocytosis of the test dose of culture by the dose of leucocytes used, and practically all leucocytes were engorged with cocci, which were too crowded to count. Substitution of saline for serum resulted in only slight phagocytosis in which a leucocyte here and there was found to contain a pair or a few cocci. Addition of saline to a leucocyte-culture-serum mixture resulted as in the first instance in complete phagocytosis. The additional tests with the series of antiseptic dilutions have been recorded in the table as indicated. In the use of each antiseptic it was aimed to record the pronounced differences in phagocytosis in comparison with the normal control.

In these tests Merthiolate shows less interference with phagocytosis and less injury to the leucocytes than either antiseptic "B" or "C." Antiseptic "B" allowed greater phagocytic activity with less leucocyte injury than Antiseptic "C." Possibly the low surface tension value of Antiseptic "C" (32.1) was partly responsible for its particularly destructive effects. The leucocytes in the stronger Antiseptic "C" solutions were clumped together and quite uniformly ruptured and disintegrated. Naturally under such conditions but slight phagocytosis occurred.

SUMMARY

In the tests described in this paper it is shown that all three antiseptics tested retard phagocytosis in varying degrees. Of the three, Merthiolate interfered least with phagocytosis and causes the least amount of damage to the cells.

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