

EFFICIENCY OF PHENYLMERCURIC ACETATE AS A PRESERVATIVE IN ANTIGENS

IDAMAE WILLIAMS, B.S., AND GEORGE PINESS, M.D., LOS ANGELES, CALIF.

SINCE it is necessary to store antigens without loss of initial potency for considerable periods of time, the selection of a preservative is an important problem. This preservative should be bacteriostatic for all contaminants; it should not be irritating or painful when injected intracutaneously; it should not destroy or inhibit antigenicity; it should not be toxic.

The antiseptics generally used by pharmaceutical houses¹ and allergists² who manufacture their own antigens are 0.4 per cent phenol, 0.5 per cent phenol, 0.4 per cent cresol, Chloro-trilinal, or Merthiolate. These agents have been employed in our laboratory, and although they have proved bacteriostatic, they have been found deficient in one or more of the above mentioned requisites.

Phenylmercuric Acetate³ is usually prepared by direct treatment of the benzene nucleus with mercuric acetate. It is a white powder whose mercury content is 59 per cent to 60 per cent. It becomes a clear melt at 150° C. to 155° C., and is soluble in water one to 600 parts. It is inexpensive and readily attainable* in highly purified grades. The compound is stable, although in solution on long standing in the presence of sunlight, reducing agents, alkali, or at a high temperature, a gradual decomposition takes place whereby mercuric and mercurous ions and some free mercury are released. This gradual decomposition does not concern us here as these conditions need never occur in the manufacture and storage of antigens. Studies that have been made of the phenylmercuric ion demonstrate that it more closely resembles the monovalent silver ion than the mercuric ion.³ However, the phenyl mercuric compounds do not react appreciably with proteins as do the silver salts. Therefore, they are not denaturing to protein extracts.

EXPERIMENTAL

It was decided that the standard methods of judging antiseptics, such as the phenol coefficient, were too limited in their scope to evaluate properly the preservative powers of Phenylmercuric Acetate. As Allen and Wright⁴ point out in their article on the determination of the value of antiseptics, it is necessary to test an agent in the environment in which it is going to be used. Thus, it must be tested against the organisms it is to destroy, and the studies in vitro should be correlated with the clinical results.

To determine the effectiveness of Phenylmercuric Acetate against all contaminants encountered in the manufacture and storage of antigens, plain agar plates were exposed in various parts of the laboratories. Also, a collec-

*Edwal Laboratories, Inc., Chicago, Ill. Metalsalts, Inc., Patterson, N. J.; San Francisco, Calif.

tion was made of the organisms that were found in contaminated antigens. These organisms were isolated and pure cultures were obtained. They were *Staphylococcus aureus*, *Staphylococcus albus*, *Escherichia coli*, *Bacillus subtilis*, and an *Aspergillus* mold.

Next, under aseptic conditions 5 groups of 5 tubes were prepared, each containing 10 c.c. of Difco's nutrient broth and the desired concentration of Phenylmercuric acetate. In each group the tubes contained preservatives in the following dilutions:

Tube A contained 1-25,000 Phenylmercuric Acetate

Tube B contained 1-50,000 Phenylmercuric Acetate

Tube C contained 1-75,000 Phenylmercuric Acetate

Tube D contained 0.5 per cent phenol

Tube E contained no preservative

The Phenylmercuric Acetate solution (one gram in 1,000 c.c. distilled water) was added to the broth in proper amounts to make the required dilutions. This addition was made after the broth was autoclaved as it was feared the intense heat might cause some decomposition of the preservative. Since antigens are always sterilized by filtration this would not be an objection to the use of this preservative.

The tubes with no preservative and with phenol served as controls. The phenol was used as control only, and not for the purpose of performing a phenol coefficient.

After the preparation of these groups 0.1 c.c. of a 24-hour nutrient broth culture of *Staph. aureus* was added to each of the tubes in Group I; 0.1 c.c. of a 24-hour culture of *Esch. coli* was added to each of the tubes in Group II; and 0.1 c.c. of a 24-hour culture of *B. subtilis* was added to each of the tubes in Group III. To ascertain the bacteriostatic ability of Phenylmercuric Acetate in the presence of a very heavy growth of spores, Group III was repeated with a 120-hour culture of *B. subtilis*.

These groups were all prepared in duplicate. They were then allowed to stand at room temperature. Transfers (one standard loopful taken after the tube was thoroughly stirred to insure homogeneity) were made from each tube into Difco's semi-solid brain-liver-heart broth at the end of one-half hour, 24 hours, 48 hours, and one week. These subcultures were incubated at 37° C. and checked for growth daily for one week.

Group IV which was to be inoculated with an *Aspergillus* mold was prepared with Sabouraud's media instead of the broth for fear the nutrient broth might not properly support growth. Further, instead of subcultures being made to determine sterility, it was more feasible to check daily the tubes which were kept at room temperature.

The results of these studies are recorded in Table I. Any growth found in a subculture was carefully checked by microscopic and staining techniques to make certain that the turbidity was due only to the organism under study.

Turbidity did not appear in the 24-hour subculture from the test samples containing 1:50,000 Phenylmercuric Acetate and *Staph. aureus* until they had been incubated for 4 days. Thus, 1:50,000 Phenylmercuric Acetate inhibited the growth of this organism but did not kill it. There was no growth in the subculture of the 1:25,000 dilution of Phenylmercuric Acetate.

TABLE I. RESULTS OF STERILITY TESTS

		½ HOUR	24 HOURS	48 HOURS	1 WEEK
<i>E. coli</i>	No preservative	+	+	+	+
	No preservative	+	+	+	+
	1-25,000 P.M.A.	+	-	-	-
	1-25,000 P.M.A.	+	-	-	-
	1-50,000 P.M.A.	+	-	-	-
	1-50,000 P.M.A.	+	-	-	-
	1-75,000 P.M.A.	+	-	-	-
	1-75,000 P.M.A.	+	-	-	-
	0.5% phenol	+	-	-	-
	0.5% phenol	+	-	-	-
<i>Staph. aureus</i>	No preservative	+	+	+	+
	No preservative	+	+	+	+
	1-25,000 P.M.A.	+	-	-	-
	1-25,000 P.M.A.	+	-	-	-
	1-50,000 P.M.A.	+	+	-	-
	1-50,000 P.M.A.	+	-	-	-
	1-75,000 P.M.A.	+	+	-	-
	1-75,000 P.M.A.	+	+	-	-
	0.5% phenol	+	-	-	-
	0.5% phenol	+	-	-	-
<i>B. subtilis</i> (24-hour culture)	No preservative	+	+	+	+
	No preservative	+	+	+	+
	1-25,000 P.M.A.	-	-	-	-
	1-25,000 P.M.A.	-	-	-	-
	1-50,000 P.M.A.	-	-	-	-
	1-50,000 P.M.A.	-	-	-	-
	1-75,000 P.M.A.	-	-	-	-
	1-75,000 P.M.A.	-	-	-	-
	0.5% phenol	-	-	-	-
	0.5% phenol	-	-	-	-
<i>B. subtilis</i> (120-hour culture)	No preservative	+	+	+	+
	No preservative	+	+	+	+
	1-25,000 P.M.A.	+	+	+	+
	1-25,000 P.M.A.	+	+	+	+
	1-50,000 P.M.A.	+	+	+	+
	1-50,000 P.M.A.	+	+	+	+
	1-75,000 P.M.A.	+	+	+	+
	1-75,000 P.M.A.	+	+	+	+
	0.5% phenol	+	+	+	+
	0.5% phenol	+	+	+	+
<i>Aspergillus</i> mold	No preservative	turbid	turbid	mold	mold
	No preservative	turbid	turbid	mold	mold
	1-25,000 P.M.A.	-	-	-	-
	1-25,000 P.M.A.	-	-	-	-
	1-50,000 P.M.A.	-	-	-	-
	1-50,000 P.M.A.	-	-	-	-
	1-75,000 P.M.A.	-	-	-	-
	1-75,000 P.M.A.	-	-	-	-
	0.5% phenol	-	-	-	-
	0.5% phenol	-	-	-	-

+ = growth

- = no growth

P.M.A. = Phenylmercuric acetate

To determine whether Phenylmercuric Acetate was irritating or painful when injected intracutaneously, comparative skin tests were done on a representative number of patients in the clinic. For these tests 1:50,000 and 1:25,000 Phenylmercuric Acetate in buffered saline diluting fluid were used. Buffered saline diluting fluid which contained no preservative served as a control. Note was taken of any local skin irritation. There was none. Further, the patients could not differentiate between the 3 intracutaneous injections when asked which was the most painful. They reported there was little or no pain connected with any of them.

To determine whether Phenylmercuric Acetate destroys or inhibits the antigenicity of the biologicals, again comparative skin tests were performed. It was found that there was no difference in the potency of a freshly prepared buffered saline-glycerine extract and one that had been stored for several years.

That Phenylmercuric Acetate is not toxic is clearly demonstrated by the fact that although Phenylmercuric Acetate has been used in at least 2,000 antigens prepared in our laboratories there never has been a patient who suffered a reaction that was related to the presence of this agent.

SUMMARY

1. A survey was made of the preservatives on the market in view of finding an ideal one for antigenic solutions.

2. Laboratory and clinic studies covering a period of 3 years have proved a 1:25,000 dilution of Phenylmercuric Acetate most satisfactory.

3. Carefully controlled bacteriological and clinical tests were performed, and the results are given.

4. Phenylmercuric Acetate is readily attainable, inexpensive, and possesses all the properties needed to maintain the sterility, potency, and non-toxicity of antigenic solutions.

REFERENCES

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