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PRESERVATION OF *M. GALLISEPTICUM* ANTIGEN +*

by

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During the last ten years in which *Mycoplasma gallisepticum* (MG) has been used as an antigen for agglutination and hemagglutination tests, it has been noted that variations in sensitivity occur upon storage. This was attributed arbitrarily to physical and chemical factors. In studies of the osmotic sensitivity of mycoplasma, Leach (4) found that the most sensitive mycoplasma to osmotic shock was *M. gallisepticum*. Adler (1) determined that MG antigens were heat labile antigens when used for the agglutination and complement fixation tests. Morowitz *et al.* (3) showed that the major components of MG were protein. There was little polysaccharide and lipid, heat stable antigens. It is known that formalin treatment would protect protein against moderate heat treatment. The purpose of the present paper is to determine the effect of preservations on the stability of MG. Greatest emphasis will be given to the use of formalin for this purpose.

EXPERIMENTAL

Antigens. The *M. gallisepticum* antigens used in the following experiments were prepared according to the method of Adler *et al.* (2). In all instances the cultures were incubated 96 hours before harvest and then standardized to a density of 2X the opacity of the No. 10 McFarland density standard. Aliquots of antigen were distributed in 50 ml. quantities, and the various concentrations of preservatives added. The final concentrations of formalin were 1, 4, 5, 10 and 20% respectively. Two levels of phenol were used, 0.5 and 1.0%. Since in previous studies, 0.01% merthiolate was satisfactory, it was used at this concentration as a standard.

Formalin treatment. Various quantitives of undiluted formalin were placed in a collodion bag and then lowered into the stirred antigen. The formalin was allowed

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to diffusion for 24 hours at 4°C. Treated antigens were centrifuged and the sedimented organisms resuspended in phosphate buffered saline with 0.01% merthiolate as a preservative. These antigens were used for the hemagglutination (HA) and agglutination tests. In Table 1 may be seen the results of the agglutination and HA with these antigens. It is evident that 4 to 20% formalin destroyed the HA ability of the MG antigens. The two concentrations of phenol (0.5 and 1.0%) markedly reduced HA of the antigen. Merthiolate did not affect HA beyond 1 doubling dilution as compared to an untreated antigen. A remarkable finding was that the 20% formalin treatment did not alter the sensibility of the antigen for saline agglutination. Phenol and merthiolate were satisfactory preservatives for a saline antigen.

Heat treatment. To test the effect of formalin on the preservation of the MG antigen, various heat treatments were applied. 3 ml. of antigen were pipetted into 13 x 100 mm tubes; rubber stoppered and placed in an enclosed water bath. Temperature ranging from 50 to 85°C for 30 minutes were used and the antigens tested for saline and HA reactions. In Table 2 may be seen the results of the heat treatment on subsequent HA and saline agglutination. Formalin fixation protected the antigen against the adverse effects of heat. This protection was limited to the saline agglutination and not the HA procedure. It was unexpected that 0.5% phenol provided an environment comparable to the formalin treatment.

Sonic disruption. To invesigate the effect of physical disruption on MG antigens 2 batches of antigen were sonicated for 7 minutes with a Branson sonifier Model 575 (Stanford, Connecticut), at peak frequency. With the first batch of antigen the HA titer was 1:160 before and after sonication. The saline agglutination reactions also remained unchanged following sonication of the antigen.

There was a question as to whether the HA was associated with mycoplasma wall substances. Sonicated organisms were centrifuged at a high speed, and the supernatant and sediment tested for HA activity. Only 1 doubling dilution loss of HA occured using the sediment; however, no HA was observed with the supernatant. Using the second batch of antigen, sonication did not adversely influence HA, or saline agglutinins and the reciprocals were 1280 in both samples. After 10 days of storage at 4°C, the sonicated antigen lost almost all its HA activity, but the saline agglutination titer was the same as it was initially.

DISCUSSION

Evidently the major antigenic component of MG is a heat labile protein. Heating MG destroyed all antigenicity for complement fixation and saline agglutina-

tion procedures. Formalin treatment through gradual diffusion of the fixative, protected the antigen until 70°C was attained. The procedure of adding formalin to the antigen may be important. In previous studies the direct addition of formalin at concentrations above 1% resulted in granular selfaggregating product. Formalin levels of 4% and higher destroyed the HA activity of the MG antigen. Low heat treatment also had an adverse effect on the HA antigen. All of the latter results suggest that the HA antigen is much more vulnerable to physical and chemical factors. The antigenic components of MG seem to reside in the membrane as indicated by the sonication experiment. Loss of HA in the sonicated preparation following 10 days storage, was unexpected since in previous studies antigens preserved with 0.01% merthiolate had HA activity for at least 6 months. Possibly the sonic disruption caused some molecular rearrangement of the protein of the antigen resulting in adverse effects upon storage.

SUMMARY

Mycoplasma gallisepticum (MG) antigens were treated with concentrations of formalin ranging from 1 to 20% using a slow diffusion system. High concentrations of formalin did not affect the saline agglutination antigens; however, 4% formalin destroyed hemagglutination (HA) activity. The formalin fixed antigens had greater heat stability for saline agglutination than merthiolate preserved MG. The HA of all antigens was lost at 50°C. Sonic disruption of MG did not alter either HA or saline agglutination reactivity of the antigens, and the HA activity seemed to be associated with the membranes of the organisms. Storage of the sonicated MG resulted in lose of HA activity.

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TABLE 1. Results of agglutination and hemagglutination tests using M. gallisepticum antigens treated with different concentrations of formalin or phenol.

Antigens	Reciprocal slide agglutination titers	Reciprocal H.A. titers
1% Formalin	320	160
4% Formalin	640	10
5% Formalin	640	10
10% Formalin	640	0
15% Formalin	640	0
20% Formalin	320	0
0.5% Phenol	320	40
1% Phenol	320	40
0.01% Merthiolate	320	160
Frozen	ND+	320

ND = Not done

TABLE 2. Results of agglutination and hemagglutination test with M. gallisepticum antigens following various heat treatments.

Antigens	Slide Agglutination								Hemagglutination				
	Heat treatment in degrees Centigrade												
	4	50	55	60	65	70	75	80	4	50	55	60	65
1% Formalin	640+	640	320	160	160	80	40	—	160++	20	—	—	—
4% Formalin	320	320	320	320	320	160	80	—	10	—	—	—	—
5% Formalin	320	320	320	320	320	160	80	—	10	—	—	—	—
10% Formalin	320	320	320	320	320	160	80	—	—	—	—	—	—
15% Formalin	320	320	320	320	320	160	80	—	—	—	—	—	—
20% Formalin	320	320	320	320	320	40	20	—	—	—	—	—	—
0.5% Phenol	640	640	320	320	320	160	40	—	40	10	—	—	—
1% Phenol	640	320	80	40	10	—	—	—	40	10	—	—	—
0.01% Merthiolate	640	640	160	160	80	20	10	—	160	20	—	—	—

+ = 2+

++ = Reciprocal of the Hemagglutination titer.