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Production of a Less Sensitive Rapid Antigen for Diagnosis of *Brucella* Infections.

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(Introduced by Herman C. Lichstein.)

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The production of oversensitized antigens for use in diagnosis of *Brucella* infections has created a problem in the interpretation of agglutination tests. According to Huddleson,[1] the presence of dissolved agar in dilutions as high as 1-50,000 in the antigen can cause nonspecific agglutination of the organisms. Tallman[2] employed cellophane sheets over Bordet-Gengou agar for the cultivation of *Hemophilus pertussis* with considerable success in ridding suspensions of blood cells and possibly of dissolved agar. Later, Moore and Mitchell[3] suggested the use of a dialysing membrane layered over broth absorbed in cotton. Growth was obtained in this manner and used in the production of a less sensitive antigen. Harmsen and Kolff[4] also employed cellophane as a dialysing membrane for growth of bacteria nourished by fluid on the opposite side. They pointed out that the membranes were impermeable to bacteria and viruses, but allowed passage of water and solutes, except those of very large molecular weight.

The present paper is concerned with a cultural method designed to eliminate oversensitivity of rapid antigen by using a collodion dialysing membrane in conjunction with agar plates as described.

Method and Equipment. A BAI strain of *Brucella abortus* exhibiting normal agglutinability was employed throughout. The organisms were grown on a sterile collodion membrane* superimposed on the surface of an agar plate. The collodion sheets were cut so as to give a circular sheet of approximately 130 cm in diameter to fit over a large Petri dish. Each sheet was layered between a piece of flat wrapping paper backed by cardboard in order to maintain the flatness of the membrane and sterilized by autoclaving at 121° C, for 30 minutes.

The medium used was Bacto-tryptose agar. To obtain larger areas of growth, the medium was poured into sterile 150 cm Petri dishes and allowed to solidify. The collodion membrane was then placed on the surface of the agar by using a pair of sterile forceps, flaming after each plating. A tendency for the membrane to curl was noted. This objectionable feature was rectified by allowing plates to stand inverted in the refrigerator for several hours after which time a smooth surface was obtained. Extreme caution was taken at the time of placing the membrane on the agar surfaces as possibilities for contamination during this procedure were very great.

A 24 hour culture of *Brucella abortus* was used. To this culture was added 5 ml of tryptose broth and the growth removed from

1 Huddleson, I. Forrest, *Brucellosis in Man and Animal*, 1939, 192.

2 Tallman, A. W., *Personal Communication*, 1942.

3 Moore, T., and Mitchell, C. H., *J. Am. Vet. M. A.*, 1945, **107**, 226.

4 Harmsen, G. W., and Kolff, W. J., *Science*, 1947, **105**, 582.

* There are two types of collodion membrane that might be used. The first is covered with a fine coating of plastic film. This coating prevents the free passage of nutritional fluids through the membrane and thus must be removed before use. This can be done readily by boiling for several minutes in a 5% solution of bicarbonate. Commercial cellophane is permeated with oil, which renders it unsatisfactory. The other type of cellophane is that which is free from either a coating film or oil and readily permits free passage of nutrients. This type was used throughout the work.

the slant with the aid of a sterile inoculating needle. A sterile cotton swab dipped into the culture was used for the inoculation of each plate by more of less "painting" the surface of the membrane. The pressure exerted on the swab was also useful in removing any air bubbles that might have been entrapped under the membrane. Again extreme care was exercised to prevent possible contamination.

The plates were then inverted and incubated at 37° C for 4-5 days. The cells were harvested by scraping the growth with a sterile spatula and were deposited into a previously weighed flask. Ordinarily about 15% of the plates were contaminated and therefore discarded. Others showed slight contamination under the collodion membrane or had only one or two contaminating colonies on the surface of the membrane. When plates of this nature were used, care was taken in removing the growth so as not to pick up contaminating colonies. After the growth was removed, the flask was reweighed to ascertain the amount of bacterial mass present. To the flask was added enough suspending solution (12% NaCl-20% glycerine and 0.5% phenol) to make a suspension of cells that was between 18 to 20% by net weight. To this suspension was added crystal violet, and brilliant green in a dilution of 1:25,000, to inhibit growth of possible contaminants. The cells were killed by heating in a water bath at 60° C for one to 2 hours. The antigen was then filtered through a sterile cotton filter and dispensed into clean dropping bottles. Sensitivity of the antigen was titered according to the method of Huddleson.[5]

Results. Three types of antigen, namely rapid antigen and test tube antigen as described by Huddleson[6] and the collodion membrane rapid antigen described in this paper were used in each test. All agglutination tests were performed in accordance with the standard procedure recommended by Huddleson.[7]

TABLE I.

A Selective Number of Samples Showing the Type of Agglutination with Each Antigen.

Sample	1:25			1:50			1:100			1:200		
	Regular rapid	Collodion rapid	Test tube	Regular rapid	Collodion rapid	Test tube	Regular rapid	Collodion rapid	Test tube	Regular rapid	Collodion rapid	Test tube
1	+	+	±	±	±	+						
2	+	+	+	±	±	+						
3	+	+	+									
4												
5	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+			
7	+	+	+	+	+	+	±	±	±			
8	+	+	+	+	+	+	±	±	±			
9	+	+	+	+	+	+	±	±	±			
10	+	+	+	+	+	+						

\+ Positive.
— Negative.
± Incomplete positive.

[5] Huddleson, I. Forrest, *Op. Cit.*

[6] *Ibid.*

[7] *Ibid.*

TABLE II.
Number of Tubes Showing Complete Agglutination with Each Antigen.

Antigen employed	Dilution			
	1:25	1:50	1:100	1:200
Regular rapid antigen	19	19	11	30
Test tube antigen	16	11	8	29
Collodion rapid antigen	16	9	3	30

Of the 301 blood samples tested, using the three sets of antigens, 38% of the specimens gave evidence of agglutination. This agglutination ranged from very slight in the 1-25 dilution of serum to complete agglutination in dilutions to 1-200. Actually only 15% were reported as "positive." Table I lists a selective number of samples and the types of reaction given with each antigen. Table II shows the number of reactions called "positives" in each dilution. The collodion membrane rapid antigen proved to be less sensitive in 65 of the samples when compared with regular rapid antigen, and was less sensitive than the test tube antigen in 19 samples. In 3 samples collodion membrane rapid antigen was more sensitive than regular rapid antigen, and in 13 samples more sensitive than test tube antigen. All 3 antigens were in agreement in 32 of the samples. In 3 samples only collodion membrane rapid antigen and the regular rapid agreed, whereas in 37 samples only collodion membrane rapid antigen and test tube antigen were in agreement. In 54 samples regular rapid antigen showed more sensitivity than did test tube antigen.

Discussion. Rapid antigen obtained from growth on collodion membranes, appears to be superior to regular rapid antigen as indicated by closer agreement with test tube antigen reactions, particularly in dilutions of serum below 1:200. References to the sensitivity of rapid antigen imply that the presence of dissolved agar may be partially responsible for oversensitivity. We made no test for permeability of the collodion membrane to dissolved agar, but the fact that collodion membrane rapid antigen gave more reliable results than regular antigen indicates that the factor responsible for oversensitivity was not present in sufficient amount to be significant.

Therefore, the use of a collodion membrane as a mechanism for eliminating factors responsible for oversensitization of antigens appears worthy of application to the production of *Brucella* and other rapid antigens showing a tendency to oversensitivity.

Summary. The preparation of a rapid antigen for use in the agglutination reaction for the diagnosis of *Brucella* infection is described. The essential difference between this antigen and former preparations is the growth of the organism on collodion membranes layered over nutrient agar.

When compared with regular rapid antigen, the collodion membrane rapid antigen showed markedly less sensitivity in serum dilutions of 1:100 or less. The collodion membrane rapid antigen and test tube antigen were more frequently in agreement than test tube antigen and regular rapid antigen.