CONGLUTINATING COMPLEMENT ADSORPTION TEST AS APPLIED TO FOOT AND MOUTH DISEASE

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The phenomenon of conglutination was first reported by Ehrlich and Sachs (1902), and subsequently received attention from Bordet and Gay (1906) and Bordet and Streng (1909). However, a systematic study of the mechanisms involved did not appear until Coombs and associates, in a commendable series of papers, reviewed the earlier work (Hole and Coombs, 1947a), applied the test to certain virus systems (Stoker, Coombs, and Bedson, 1950), and studied the components and their interactions (Coombs, Blomfield, and Roberts, 1950; Coombs and Coombs, 1953; Coombs, 1954). These studies have demonstrated that each serum acts differently to various species of complement and the level of antibody measured may be influenced by the amount of complement adsorbed. Certain antigen-antibody complexes adsorb one complement to a greater degree than another, and if that complement is conglutinating rather than hemolytic the conglutinating complement adsorption test is more sensitive.

Solovieff (1943) and Bier, Furtado, and Cisalpino (1957) investigated the application of the conglutination test to foot and mouth disease systems. Solovieff reported satisfactory results for diagnosing foot and mouth disease, giving fewer doubtful reactions than the hemolytic complement fixation test. Only one dilution of serum was used and a description of the antigen was not available. Bier et al. developed a micro plate technique which was satisfactory for virus typing. Neither method was utilized to assay antibody content of immune serum.

This paper describes the applicability of the conglutinating complement adsorption test to various foot and mouth disease antigen-antibody systems and reports the relative value of the conglutinating complement adsorption and hemolytic complement fixation tests as methods to assay the antibody content of rabbit, guinea pig, bovine, and swine antisera. The hemolytic complement fixation test has been found unsatisfactory for titration of swine sera for foot and mouth disease antibodies, and the search for a satisfactory assay method stimulated much of this work.

MATERIALS AND METHODS

Antigens. Infected bovine tongue epithelium and guinea pig metatarsal pad epithelium, homogenized and suspended in tryptose broth (Difco), at pH 7.3, were used as tissue antigens. These preparations were found to be anticomplementary for horse serum; however, this activity was successfully eliminated by combining 1 ml of horse complement with 9 ml of antigen. The mixture was held at 4 C for 2 hr and then the excess complement was destroyed by heating at 56 C for 30 min. Infective guinea pig and bovine vesicular fluids and fluids containing virus grown in cultured bovine kidney cells were also used as antigens with excellent results. These latter preparations did not need preliminary treatment to remove anticomplementary activity. The optimal dose of antigen was determined by cross block titration of varying antigen and serum dilutions.

Antibody. Cattle, swine, guinea pig, and rabbit antisera were titrated for antibody against foot and mouth disease virus. Antisera diluted 1:10 in veronal buffered saline (Oslor, Strauss, and Mayer, 1952) were inactivated by heating at 56 C for 30 min.

Complement. Fresh horse serum appears to be the most sensitive conglutinating complement (Coombs and Hole, 1948, and Blomfield, Coombs, and Hole, 1949, 1950) and was used throughout this study. Since horse complement is easily destroyed at warm temperatures, it was not allowed to clot fully before the serum was removed and stored at -28 C. It was found that horse complement used in the conglutinating comple-

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ment adsorption test has to be accurately titrated prior to each antibody assay.

**Indicator system.** The conglutinating indicator system consisted of sheep erythrocytes, conglutinin, and anti-sheep cell antibody. Sheep erythrocytes were washed, suspended in buffered saline, and standardized according to the method of Savan (1959). Normal bovine serum, inactivated at 56°C for 30 min, contains conglutinin and natural anti-sheep cell antibody. In the test, equal parts of the optimal dilution of inactivated bovine serum and a 1:5,000 dilution of commercially obtained hemolysin (Difco) were mixed and added to a volume of red blood cell suspension equal to this mixture. The addition of 1:5,000 hemolysin has been recommended by several workers (Rice and Avery, 1950) to supplement the natural antibody in the event it is of low titer in the bovine serum.

**Test method.** The conglutinating complement adsorption test was performed according to the method of Hole and Coombs (1947b) with modifications. Briefly, the protocol was:

1. Doubling dilutions (1:10 to 1:160) of antisera were made and 0.1 ml of this was deposited in a row of tubes.
2. Two units of complement contained in 0.1 ml were added to each tube.
3. The optimal dilution of antigen (0.1 ml) was also added to each tube. The racks were shaken vigorously and incubated at 25°C for 1 hr.
4. Two-tenths ml of the indicator system was added with shaking and the racks were again incubated at 37°C for 15 min.

The tubes were then centrifuged at 800 rpm for 4 min and the degree of conglutination was estimated using the following symbols: 0 = no conglutination; 1 = very slight clumping of cells; 2 = approximately one half the cells conglutinated; 3 = nearly complete conglutination; and 4 = complete conglutination.

When the hemolytic test was used, 100% hemolysis was considered the end point. Basically, the complement fixation tests were performed according to the method described by Savan (1959).

**RESULTS**

Table 1 contains the complement fixation and conglutinating complement adsorption titers of antisera of rabbits, cattle, and guinea pigs infected with foot and mouth disease virus, type O.

**TABLE 1**

<table>
<thead>
<tr>
<th>Source of Antibody</th>
<th>CF</th>
<th>CCA</th>
<th>CF</th>
<th>CCA</th>
<th>CF</th>
<th>CCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Guine pig</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bovine</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

* DPI = days postinoculation.

**TABLE 2**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Titer at Postinoculation Day</th>
</tr>
</thead>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
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<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

* The swine were inoculated with strain FMDV-O-M11.

It was not possible to compare the conglutinating complement adsorption and complement fixation tests when swine antisera was the source of antibody. Swine serum is rich in certain components of complement that enhance the lytic effect of the guinea pig complement and cause...
lysis in dilutions where fixation has occurred. Evidence of this procomplementary activity was not seen in the conglutinating complement adsorption test. Table 2 contains the conglutinating complement adsorption titers of sera from several swine bled at 0 to 21 days postinoculation with strain FMDV-O-M11.

**DISCUSSION**

Guinea pig, rabbit, cattle, and swine antisera were examined for foot and mouth disease virus antibodies by the conglutination complement adsorption test and the hemolytic complement fixation test, and the results of the two methods were compared. Each test was based on the 100% end point, i.e., complete hemolysis or complete conglutination. Maximal fixation in the complement fixation test required overnight incubation at 4°C, whereas the adsorption of horse complement was not enhanced by more than 1 hr fixation.

In the titration of rabbit and guinea pig antiserum, both tests appeared to be of equal sensitivity. When bovine antisera were used, the conglutinating complement adsorption test showed slightly higher titers and positive reactions appeared earlier.

The hemolytic complement fixation test is not applicable for assay of swine antiserum for precise antibody studies. This is especially true of freshly prepared antiserum. The conglutinating complement adsorption test, using horse complement, appears not to be affected by the procomplementary activity of swine serum. The procomplementary activity of swine serum may be attributed to its richness in the third component of complement (C'3). It has been generally postulated that in the immune hemolysis reaction, C'1 is first bound to the sensitized red blood cells, followed by C'2 and C'4, and hemolysis occurs when C'3 is adsorbed. A serum with excessive heat-stable C'3 might cause a high degree of hemolysis when combined with a complement rich in C'1, C'2, and C'4. The mechanism of the conglutinating reaction is similar to the hemolytic system in that C'1, followed by C'2 and C'4, is adsorbed to the sensitized cell. However, conglutinin takes the place of C'3 in the final stage to bring about agglutination of the red blood cells (Coombs et al., 1950; Blomfield, 1952).

**SUMMARY**

The conglutinating complement adsorption technique was adapted to the study of foot and mouth disease systems. The antigens investigated were prepared from infected guinea pig metatarsal epithelium, bovine tongue epithelium, and virus grown in tissue culture.

The hemolytic complement fixation and conglutinating tests both gave results of equal sensitivity when antibodies in rabbit or guinea pig sera were titrated. The conglutinating complement adsorption test was slightly more sensitive with bovine antiserum, and much more useful for detecting antibody in swine serum by avoiding the procomplementary activity which renders the hemolytic test unreliable.

**REFERENCES**


Coombs, A. M., and R. R. A. Coombs 1953 The conglutination phenomenon. IX. The produc-


