NOTES

Rubella Virus Hemagglutination with a Wide Variety of Erythrocyte Species

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In HEPES diluents, rubella antigens agglutinated a wide range of species of erythrocytes, and the hemagglutination reaction showed little or no dependence on low temperatures.

The first reports on hemagglutination (HA) by rubella virus indicated that agglutination did not occur with adult chicken, human group O, rhesus monkey, and guinea pig erythrocytes in the dextrose-gelatin-Veronal (DGV) diluent employed by Stewart et al. (6), and adult chicken erythrocytes did not show HA in the bovine albumin-borate saline-phosphate-buffered saline (BABS-PBS) diluent used by Halonen et al. (1). Goose and sheep erythrocytes were agglutinated to a low titer (6), but pigeon (2) or 1-day-old chick (1, 6) erythrocytes were the most sensitive to rubella HA.

Hemadsorption by rubella-infected cell cultures was demonstrated in this laboratory (5) by using pigeon, 1-day-old chick, sheep, goose, bovine, and adult chicken erythrocytes but not with human O, rhesus monkey, guinea pig, horse, goat, or swine erythrocytes. On the other hand, Perlino and Isacson (4) reported that guinea pig,human O, and green monkey erythrocytes were adsorbed onto rubella-infected BHK-21 cells, although they were not hemagglutinated by the virus in vitro.

In the HEPES-saline-albumin-gelatin (HSAG) diluent developed by Liebhaber (3) for rubella hemagglutination-inhibition (HI) tests, or minor modifications of the diluent, rubella antigens have been shown to agglutinate a much wider variety of erythrocyte species than originally reported.

The microtiter system was used for all HA and HI tests. Each species of erythrocyte was used at the lowest concentration at which unagglutinated cells settled into compact buttons after 90 min of incubation at 4° C. Some of the species of erythrocytes agglutinated spontaneously in HSAG, and, for tests with these, the diluent was modified slightly by changing the protein composition or the pH (Table 1). Unless otherwise indicated, tests were incubated at 4° C for 90 min. Sera used in HI tests were treated with kaolin at pH 9.0 to remove rubella HA inhibitors (1) and absorbed with the species of erythrocyte used in the test to remove natural agglutinins.

Table 1 shows the HA titers of a rubella antigen (Tween 80 and ether-treated) against 20 different species of erythrocytes. Only horse, goat, bovine, guinea pig, and fish erythrocytes were not agglutinated by the antigen. Most of the other 15 species of erythrocytes gave antigen titers of 1:128 to 1:512. The specificity of the HA reactions as being due to rubella virus was confirmed by the fact that (i) control antigen, prepared from uninfected BHK-21 culture material in the same manner as the rubella antigen, failed to agglutinate the erythrocytes, and (ii) HA was inhibited by rubella immune rabbit serum but not by preimmunization serum from the same rabbit (Table 1). Human sera containing antibodies to rubella virus also inhibited HA with each species of erythrocyte, but antibody-negative human sera did not.

The HSAG diluent also enhanced rubella HA to the extent that the reaction was not temperature-dependent as it is in DGV (6) and BABS-PBS (1) diluents (Table 2). Most of the antigens showed identical HA titers at 4° C and higher temperatures. Even when a higher titer was obtained at 4° C, appreciable HA occurred at room temperature or 37° C.

These studies show that a wide variety of erythrocyte species possess receptors for rubella hemagglutinins. They also suggest the possibility of developing HI tests for assay of rubella antibodies with erythrocytes more readily available than 1-day-old chick cells; preliminary investigations have suggested that human group O erythrocytes might be suitable for this purpose.

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### Table 1. Species of erythrocytes agglutinable by rubella virus in HEPES buffer: specific inhibition of hemagglutination (HA) by rubella immune serum

| Species of erythrocyte | Conc of erythrocytes (%) | Diluent | HA titer of rubella antigen | Inhibition of HA by Preimmune rabbit serum | Rubella immune rabbit serum |
|------------------------|--------------------------|---------|-----------------------------|------------------------------------------|------------------------------|
| Pigeon                 | 0.25                     | HSAGa   | 1,024b                      | <8                                       | 128                          |
| Chicken, 1-day-old    | 0.25                     | HSAG    | 512                         | <8                                       | 64                           |
| Chicken, adult         | 0.25                     | HSAG    | 256                         | <8                                       | 64                           |
| Goose                  | 0.08                     | HSAG    | 128                         | <8                                       | 32                           |
| Turkey                 | 0.28                     | HSAG    | 128                         | <8                                       | 32                           |
| Human group O          | 0.30                     | HSAG    | 256                         | <8                                       | 64                           |
| Monkey, rhesus         | 0.28                     | HSAG    | 64                          | <8                                       | 128                          |
| Sheep                  | 0.28                     | HSAG    | 128                         | <8                                       | 128                          |
| Swine                  | 0.28                     | Modified HEPESc | 64                      | <8                                       | 32                           |
| Horse                  | 0.28                     | HSAG    | <4                          |                                          |                              |
| Goat                   | 0.28                     | HSAG    | <4                          |                                          |                              |
| Bovine                 | 0.28                     | HSAG    | <4                          |                                          |                              |
| Dog                    | 0.28                     | HSAG    | 32                          | <16                                      | 256                          |
| Cat                    | 0.28                     | HSAG    | 512                         | <8                                       | 128                          |
| Rabbit                 | 0.36                     | HSAG, pH 7.6 | 128                     | <8                                       | 512                          |
| Rat                    | 0.36                     | HSAG    | 256                         | <8                                       | 32                           |
| Hamster                | 0.36                     | HSAG    | 512                         | <8                                       | 32                           |
| Mouse                  | 0.28                     | Modified HEPESd | 128                   | <8                                       | 64                           |
| Guinea pig             | 0.30                     | HSAG    | <2                          |                                          |                              |
| Fish (carp)            | 0.28                     | HSAG    | <4                          |                                          |                              |

a HEPES-saline-albumin-gelatin diluent described by Liebhaber (3): 0.025 M HEPES, 0.14 M NaCl, 10^-3 M CaCl_2, 1% bovine serum albumin, and 0.00025% gelatin, pH 6.2.
b Reciprocal of titer.
c HEPES (0.025 M), 0.14 M NaCl, 10^-3 M CaCl_2, 0.03% bovine serum albumin, and 0.03% gelatin, pH 6.2.
d HSAG without bovine albumin and gelatin, but with 0.5% heparin-MnCl_2-treated fetal bovine serum.

### Table 2. Hemagglutination of rubella virus at various temperatures in HSAG diluent

| Rubella antigen lot | Indicator erythrocytes | HA titer of antigen in tests incubated at | 4 °C | Room temp | 37 °C |
|--------------------|------------------------|-------------------------------------------|------|-----------|-------|
| 545                | Human “O”              |                                           | 256  | 128       | 64    |
| 572-3              | 1-Day-old chick        |                                           | 128  | 128       | NDb   |
| 572-3              | Human “O”              |                                           | 64   | 64        | ND    |
| 589-90             | 1-Day-old chick        |                                           | 512  | ND        | 512   |
| 589-90             | Human “O”              |                                           | 256  | ND        | 128   |

a All antigens treated with Tween 80 and ether.
b Not done.

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