

# Attempts to Demonstrate Hemagglutination and Hemadsorption by Respiratory Syncytial Virus

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Extensive attempts to demonstrate a hemagglutinin of respiratory syncytial virus by both hemagglutination and hemadsorption tests failed. Hemagglutination tests were performed with red blood cells from 12 different animal species and at various temperatures and pH.

Although respiratory syncytial (RS) virus contains surface projections morphologically similar to the myxoviruses (11, 15), a hemagglutinin (HA) has never been demonstrated. The search for an HA has not been extensive (1-3, 12, 13). In fact, only supernatant fluids of one RS virus-infected cell line were tested with human type O, chicken, and guinea pig red blood cells (RBC), and the effect of temperature and pH were not evaluated. It is known that the hemagglutination of some viruses depends upon specific RBC, pH, and temperature requirements, e.g., arboviruses (4), adenoviruses (7), rabies (8), and rubella viruses (9, 19). It has also been shown that some viruses require serum-free media (8, 9) for HA development in cell culture or media supplemented with serum treated to remove nonspecific inhibitors to hemagglutination (19). In view of the clinical importance of RS virus and the technical advantage of a hemagglutination-inhibition test over complement fixation and neutralization tests, attempts were made to apply to RS various preparative techniques which recently have been successful for HA production of other viruses. In addition, the search for an HA was also determined by applying the hemadsorption technique (18) to RS virus-infected cell cultures.

In these experiments, several methods of HA production were evaluated. Furukawa et al. (6) found that rubella virus HA in infected BHK-21 cell culture could be released from serum inhibition by binding divalent cations with ethylenediaminetetraacetic acid (EDTA). Norrby (14) showed that Tween-80-ether treatment would increase HA activity of measles virus. Stewart et al. (19) prepared rubella virus HA by using kaolin-treated serum to remove nonspecific inhibitors. Halonen et al. (8) prepared rabies virus HA by employing serum-free media to maintain the cell cultures. We routinely treat sera with ether for

removal of nonspecific inhibitors because we find this method superior to kaolin treatment. Suspension cultures were used to increase virus-cell contact, hopefully to achieve high titering virus pools, and to facilitate testing of both concentrated cell packs and supernatant fluids.

Accordingly, four cell lines [i.e., MA-104 (embryonic rhesus monkey kidney), MA-160 (adult human prostate), WI-38, and HEp-2], which represented the most susceptible cell cultures for RS virus growth, were infected with the Bennett strain of RS virus. Since isolation, the Bennett strain has been passaged four times in primary human embryonic kidney cells and nine times in HEp-2 cell cultures. It was decided to concentrate our efforts exclusively on the Bennett strain because (i) by neutralization tests RS virus strains have exhibited very limited antigenic heterogeneity (5) and (ii) the Bennett strain grew to high titers in cell cultures and it was expected that HA production would most likely parallel infectivity. Daily harvests were made of both virus-infected and uninfected control cultures, and, in addition to obtaining culture fluids, 20 times-concentrated cell packs were prepared (16). Timed harvests of fluid and cells and cell-free supernatant fluids were made of RS virus-infected suspension cultures of MA-160 and HEp-2 cells grown in media containing fetal calf serum treated with ether by the method of Hana and Styk (10) to remove nonspecific inhibitors. Samples of some harvests were treated with EDTA or Tween-80-ether. All untreated and treated viral and control antigens were examined for the presence of HA. The untreated viral harvests were tested for RS virus infectivity in MA-104 cells, with some specimens showing as high as  $10^7$  TCID<sub>50</sub> per ml.

Hemagglutination tests were conducted in microtiter equipment at various temperatures (4, 23, and 37 C) and at a pH range of 5.8 to 7.4

by previously described methods (4, 17). The following RBC types were employed: human type O, single donor; rhesus (*M. mulatta*) monkey, five individual donors; African green (*C. aethiops*) monkey, five separate donors; Hartley guinea pig, pool of five animals; NIH albino rabbit, five donors; golden Syrian hamster, pool of five animals; Swiss NIH random bred general purpose strain mouse, pool of 15 animals; Sprague-Dawley albino rats, pool of 3 animals; White Rock cockerel, 10 separate donors; 1-day-old White Leghorn chick, pool of 10 birds; White Embden gander, 10 separate animals; Hampshire sheep, single donor; and Hampshire swine, single donor. The plates were observed at 15-min intervals and read when the cell controls had sedimented into compact buttons, generally after 30 to 90 min, depending upon the temperature. No HA was found.

Hemadsorption tests were performed as initially described by Shelokov et al. (18) by using RS virus-infected MA-104 cell cultures when cytopathic effect involved 25 to 50% of the cell sheet. RBC were obtained from more than one animal of each species and tested individually to counteract any variation in susceptibility which might have existed between animals. The following RBC and number of each species were employed: rhesus, 9; African green monkeys, 7; chimpanzee (*Pan satyrus*), 4; gelada, 2; guinea pig, 4; rat, 5; hamster, 5; Swiss NIH inbred strain mouse, 3; Swiss NIH random bred general purpose strain mouse, 3; NIH CFW white strain mouse, 3; Pekin duck, 2; goose, 5; White King pigeon, 2; cockerel, 10; and a single pool of 1-day-old chick. Unless otherwise stated, the species of animals were as listed above. Hemadsorption could not be demonstrated in any of the preparations.

Although not successful, we feel the results of our extensive experiments may benefit other workers engaged in similar attempts.

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#### LITERATURE CITED

1. Beem, M., F. H. Wright, D. Hamre, R. Egerer, and M. Oehme. 1960. Association of the chimpanzee coryza agent with acute respiratory disease in children. *N. Engl. J. Med.* 263:523-530.
2. Bennett, C. R., Jr., and D. Hamre. 1962. Growth and serological characteristics of respiratory syncytial virus. *J. Infect. Dis.* 110:8-16.
3. Chanock, R., B. Roizman, and R. Myers. 1957. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation properties, and characterization. *Amer. J. Hyg.* 66:281-290.
4. Clarke, D., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Amer. J. Trop. Med. Hyg.* 7:561-573.
5. Coates, H. V., D. W. Alling, and R. M. Chanock. 1966. An antigenic analysis of respiratory syncytial isolates by a plaque reduction neutralization test. *Amer. J. Epidemiol.* 83:299-313.
6. Furukawa, T., S. Plotkin, W. D. Sedwick, and M. L. Profeta. 1967. Studies of hemagglutination of rubella virus. *Proc. Soc. Exp. Biol. Med.* 126:745-750.
7. Ginsberg, H. S. 1962. Identification and classification of adenoviruses. *Virology* 18:312-319.
8. Halonen, R. E., F. A. Murphy, B. Fields, and D. Rose. 1968. Haemagglutinin of rabies and some other bullet-shaped viruses. *Proc. Soc. Exp. Biol. Med.* 127:1037-1042.
9. Halonen, P. E., J. Stewart, and A. D. Hall. 1967. Rubella hemagglutinin prepared in serum-free suspension culture of BHK 21 cells. *Ann. Med. Exp. Fenn.* 45:182-185.
10. Hana, L., and B. Styk. 1960. Influence of delipidization on the hemagglutination inhibition activity of rat serum against influenza type-C viruses. *Acta. Virol.* 4:342-343.
11. Joncas, J., L. Berthiaume, and V. Pavilanis. 1969. The structure of the respiratory syncytial virus. *Virology* 38:493-496.
12. Kisch, A. L., K. M. Johnson, and R. Chanock. 1962. Immunofluorescence with respiratory syncytial virus. *Virology* 16:177-189.
13. Morris, J. A., R. E. Blount, Jr., and R. E. Savage. 1956. Recovery of a cytopathogenic agent from chimpanzees with coryza. *Proc. Soc. Exp. Biol. Med.* 92:544-549.
14. Norrby, E. 1962. Hemagglutination by measles virus. 4. A simple procedure for production of high potency antigens for hemagglutination-inhibition (HI) tests. *Proc. Soc. Exp. Biol. Med.* 111:814-818.
15. Norrby, E., Marusyk, and C. Örvell. 1970. Morphogenesis of respiratory syncytial virus in a green monkey kidney cell line (Vero). *J. Virol.* 6:237-242.
16. Schell, K., R. J. Huebner, and H. C. Turner. 1966. Concentration of complement fixing viral antigens. *Proc. Soc. Exp. Biol. Med.* 121:41-46.
17. Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. *J. Immunol.* 88:320-329.
18. Shelokov, A., J. E. Vogel, and L. Chi. 1958. Hemadsorption (adsorption-hemagglutination) test for viral agents in tissue culture with special reference to influenza. *Proc. Soc. Exp. Biol. Med.* 97:802-809.
19. Stewart, G. L., P. D. Parkman, H. H. Hopps, R. D. Douglas, J. P. Hamilton, and H. M. Meyer, Jr. 1967. Rubella virus hemagglutination-inhibition test. *N. Engl. J. Med.* 276:554-557.