Adsorption Studies With Herpes Simplex Immune Sera as Evidence for Specificity¹

FRANCES G. FELTON AND L. VERNON SCOTT Department of Microbiology, University of Oklahoma School of Medicine and Veterans Administration Hospital,

Oklahoma City

It has been shown by Scott *et al.* (1957) in a previous paper that tannic acid treated sheep erythrocytes sensitized with herpes simplex virus will agglutinate when exposed to specific immune serum. The technics used were modified from the methods of Boyden (1950) and Stavitsky (1954).

Whether this hemagglutination is a true antigen-antibody reaction and if so, whether the antigen is identical with the virus-neutralizing antibody is the subject of investigation in this paper.

Materials used in the procedure were prepared as previously described (Scott et al., 1957). Tannic acid-treated and virus-sensitized sheep ery-throcytes were mixed in equal amounts with herpes immune serum and allowed to incubate overnight at 25° C. The serum was removed from the cells and used in the experiments as tannic acid virus cell-adsorbed serum (TVCS). Another aliquot of the immune serum was mixed and incubated with tannic acid-treated sheep erythrocytes, which were not sensitized with the virus. This serum was used in the experiments as tannic-acid cell-adsorbed serum (TCS).

The herpes simplex virus hemagglutination test was performed using the two sera, TVCS and TCS, in two-fold dilutions. Typical negative reactions were obtained with TVCS, demonstrating that the antibodies which react in the hemagglutination reaction had been removed. Positive hemagglutination reactions were obtained with TCS, demonstrating that the specific antibodies had not been removed.

Dilutions of herpes simplex virus were made and mixed in equal volume with each of the undiluted sera, TVCS and TCS. These mixtures were inoculated onto the chorio-allantoic membranes of developing chick embryos. They were incubated 72 hours, harvested, and the infectivity of the membrane was determined. The presence of herpetic lesions on the membrane was recorded as positive and the absence of lesions as negative. The virus adsorbed serum (TVCS) did not neutralize the virus, demonstrating that no demonstrable neutralizing antibodies were present. As a com-

¹ This investigation was supported in part by a research grant B-964C-2) from the National Institutes of Neurological Diseases and Blindness, U.S. Public Health Service.

parison, the immune serum adsorbed with tannic acid treated-cells with virus (TCS) neutralized the virus.

The results of these adsorption studies on the hemagglutination reaction and virus infectivity add evidence to support the fact that a specific antigen-antibody reaction is involved in the hemagglutination test with herpes simplex virus. Since the antibodies which react in the hemagglutination and neutralization reactions are both removed by the same adsorption procedure, it appears that the neutralizing and hemagglutinating antibodies in the immune serum may be identical.

SUMMARY

The antibody in herpes simplex immune serum can be removed by adsorption with tannic acid treated and virus sensitized sheep erythrocytes.

The removal of this antibody as demonstrated by both hemagglutination and infectivity technics indicates that the herpes simplex neutralizing and hemagglutinating antibodies are probably identical and that a specific antigen-antibody reaction is involved in the hemagglutination test with herpes simplex virus.

LITERATURE CITED

- Boyden, S. V. 1950. The Adsorption of Proteins on Erythrocytes Treated with Tannic Acid and Subsequent Hemagglutination by Antiprotein Sera. Proc. Soc. Exper. Biol. and Med. 73: 289-295.
- Scott, L. V., F. G. Felton and J. A. Barney. 1957. Hemagglutination with Herpes Simplex Virus. J. Immunol. 78: 211-213.
- Stavitsky, A. B. 1954. Micromethods for the Study of Proteins and Antibodies. J. Immunol. 72: 360-368.

38