A Human Placental Fluid Inhibitor to Hemagglutination by H-1 and HB Viruses: II. Electron Microscope Studies.* (30545)

SHUICHI KARASAKI, HELENE W. TOOLAN AND MAGDALENA USATEGUI-GOMEZ
Putnam Memorial Hospital Institute for Medical Research, Bennington, Vermont

The preceding paper(1) reported on the purification of a substance in human placental fluids that inhibits hemagglutination of 2 of the H-viruses: namely H-1 and HB. The inhibitor, which was described as a glycoprotein, does not affect hemagglutination of H-3 or RV viruses, 2 other agents of the H group. The present paper describes an electron microscope study of the effect of the placental inhibitor on all 4 of these agents, and compares these findings with those seen when neutralizing antibody is added to its specific virus.

Materials and methods. Purified placental inhibitor. The effect of whole placental fluid or any of its purified fractions on the H-viruses, as observed by electron microscopy, was similar. The observations described herein concern virus treated with only one of these substances, *i.e.*, the centrifugation pellet of procedure B, described in detail elsewhere (1). This material was employed since it had had most of the protein removed; electron microscopy thus was not impaired by extraneous protein-caused artifacts. It also had a high hemagglutination inhibition (HA-I) titer, and was available in greater quantity than the final eluate.

Antibody. The anti-H-virus globulins were prepared from sera of young hamsters injected subcutaneously 3 times at weekly intervals with one of the viruses. The animals were bled from the heart one week after the last injection and the sera obtained were combined with equal amounts of cold saturated ammonium sulfate. The resulting precipitate was dissolved in distilled water, reprecipitated with saturated ammonium sulfate and dialyzed overnight against saline. The H-virus globulins thus prepared had titers of 1:1280 or more, both as HA-I and as neutralizing antibodies. Their capacity as neutralizing antibodies was determined by protection tests in newborn hamsters.

Viruses. All the viruses employed were prepared, as previously described(2), as distilled water filtrates of liver homogenates from baby hamsters infected with the 4th to 8th passage of virus. Several strains of each virus were used; all had hemagglutination titers with guinea pig cells of 1:1280 or more (expressed as the highest dilution that completely agglutinated all the red cells). Several tests were done with H-1 virus grown in tissue cultures of Salk monkey heart cells. The results were the same as those obtained for the hamster liver filtrates.

Electron microscope preparations. The virus suspensions, placental fraction, and antibodies were each kept in a disposable 1 ml syringe fitted with a 26G needle. A drop of the virus suspension was placed on a plastic spot plate and a drop of the placental fraction or the antibody was subsequently added. The latter solutions were prepared by diluting the stock material 1:50 or 1:100 with distilled water. On occasion, higher (1:200) or lower (1:2) dilutions were used. After standing for 1 hour at 23°C, a drop of the mixture was put on a carbon-Formvar coated grid and one drop of 2% phosphotungstic acid solution, pH 7.0, added. After one minute, excess fluid was gently withdrawn with wet filter paper. The grid was then placed in the JEM 7 electron microscope. Micrographs were taken at an initial magnification of 50,000 with double condenser illumination.

Observations. Control virus suspensions. The control preparation of each virus suspension which did not contain either the placental inhibitor or the homologous antibody, showed a sparse distribution of virus particles. In most cases, there were less than 30 per 1 μ^2 area. They were single or in small groups of 2 to 4 particles. No large clumps were observed.

* Aided by Grant E-343 from Am. Cancer Soc., and Grant CA-07826-01 from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

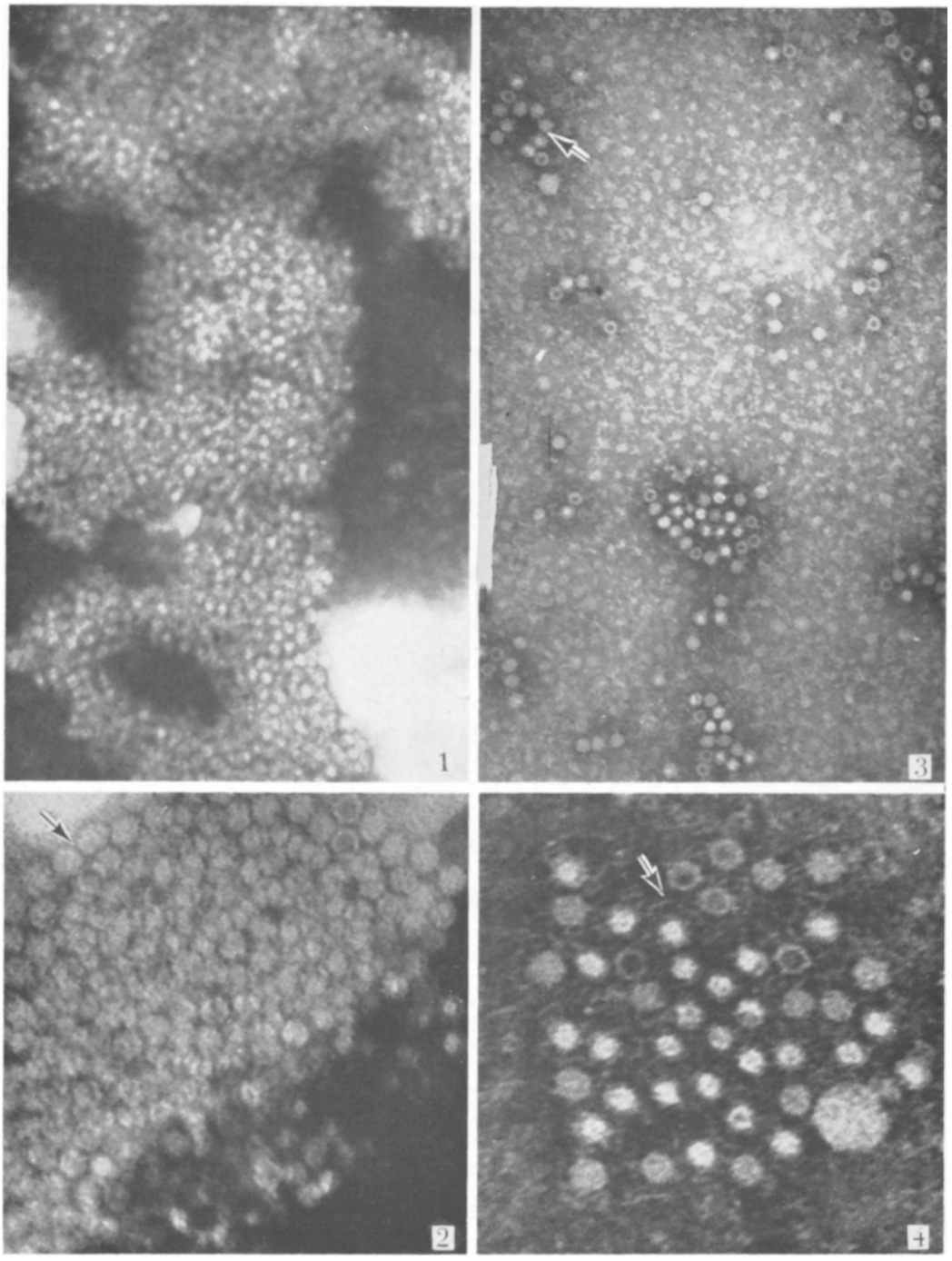

FIG. 1. Large aggregate of H-1 virus particles after treatment with a human placental fluid fraction. × 90,000.

FIG. 2. H-1 virus plus placental fluid inhibitor. Note closely packed particles at a periphery of aggregate. Distances between particles are 15Å (arrow). × 210,000.

FIG. 3. Clusters of H-1 virus particles after treatment with anti-H-1 virus globulin. Note chains (arrow). × 90,000.

FIG. 4. H-1 virus plus anti-H-1 virus globulin. Antibody molecules (arrow) associated with virus particles. × 210,000.

Virus mixed with the placental fraction. As Fig. 1 and 2 show, when the H-1 virus suspension was mixed with the placental fraction, large aggregates of particles were formed. Some of the clumps measured as much as 1.5 μ. Each aggregate consisted of angular particles about 230Å in size with the characteristic morphology of the H-1 virus as previously described(2,3,4). Both complete and "empty" forms were present. The proportion of "empty" particles was about 5% of the total, approximately the same as in the control preparations. In most of the aggregates induced by the placental fluid fraction, numerous virus particles were closely packed and stacked in a 3-dimensional pattern. The distance between each virus, as far as could be determined by measurements taken at the edge of a large clump (Fig. 2, arrow) was 15 to 25Å. Particles were not arranged in a crystalline pattern, although hexagonal arrangement of particles was observed in limited areas (Fig. 2). Under high magnification, it could be seen that virus particles were attached by finely textured fibrils with a diameter of 10Å or less. These fibrils were the only visible components of the placental inhibitor itself.

The placental fraction was also added to 3 other H-viruses. When mixed with HB virus, it produced the aggregation pattern described for H-1. However, when added to either H-3 or RV viruses, no aggregation of any type occurred although various concentrations from 1:2 to 1:200 were applied.

Virus mixed with antibody. The anti-H-1 globulin incubated with the H-1 virus suspension also produced an aggregation of H-1 virus. As shown in Fig. 3 and 4, the pattern was very different from that induced by the placental fraction. The relatively small aggregates consisted of particles loosely arranged in a 2-dimensional manner as seen in Fig. 3. Numbers of the particles in each aggregate varied from two to hundreds. Small groups of virus particles were linked by fibrillar material in linear chains. Large aggregates appeared to be composed of a convoluted chain of particles which were connected and often cross-linked by fibrous material (Fig. 4). The distances between virus particles in the chains were 20 to 250Å and, most frequently, less than 50Å. The fibrous material appeared to be the anti-H-1 virus globulin molecule. In some instances, individual antibody molecules, possibly at their full length, measured 250 to 290Å in length and 35 to 40Å in diameter (arrow in Fig. 4). These dimensions agree with those described by Almeida *et al*(5) for the antibody molecule of polyoma virus. It is interesting that the virus-antibody complex did not show any 3-dimensional arrangement even in an aggregate consisting of hundreds of particles. The proportion of the "empty" forms in these complexes was 15 to 30% of the total virus. This proportion was significantly higher than that observed in the control preparation or in the virus suspension treated with the placental fluid fraction although the conditions for negative staining were constant.

Anti-globulins for HB, H-3 or RV viruses did not affect the H-1 virus, nor did H-1 antibody react with other than H-1 (Table I). As expected, both H-3 and RV antibody reacted with either H-3 or RV. This cross reaction has been previously noted(6,7).

Discussion. The interaction of H-1 and HB viruses with either the placental fraction or with homologous antibody is characterized by the formation of aggregates of the virus particles that have a strong tendency to stick on the surface of the supporting film. The pattern of aggregation parallels the pattern of hemagglutination inhibition. The present observations have demonstrated that 2 types of aggregate are formed. The placental fraction induced large aggregates of virus particles closely packed in a 3-dimensional manner, while antibody produced relatively small linear chains of virus antibody complex convoluted in a 2-dimensional pattern. In either case it would appear that the aggregations leave few free sites on the virus particles. They would thus not be able to react adequately enough with red cells for a hemagglutination to occur; in this manner, both the placental fraction and neutralizing antibody could produce a hemagglutination inhibition of these 2 viruses.

The close packing of viral particles in the aggregates may be due to the cross-linking

TABLE I. Comparison of Interaction of a Human Placental Fraction and Specific Antibody Against the H-Viruses.

Viruses	Human placental fraction	Antibody (hamster)			
		Anti-H-1	Anti-HB	Anti-H3	Anti-RV
H-1	++	+	—	—	—
HB	++	—	+	—	—
H3	—	—	—	+	+
RV	—	—	—	+	+

++ 3-dimensional aggregation of virus particles. + 2-dimensional aggregation of virus particles. — No aggregation.

of virus particles at several sites. It can be assumed that the surface properties of a virus particle are changed after it has been affected by the placental fraction. The result may be a "stickiness" which produces aggregation of particles. Recently Bayer(8) has noted that a glycoprotein from human urine which inhibits the hemagglutination of influenza virus, consists of 3 groups of elementary fibrils 40Å, 20Å and 10Å or less in diameter, and it is his opinion that all these components interact with the virus by close attachment to its surface. In the glycoprotein inhibitor from human placental fluid, only one fibrillar component was seen. This was 10Å or less in diameter and appeared to interact with both H-1 and HB viruses. It is possible that this component itself may account for the "stickiness" of the virus surface. However, it is noteworthy that while the virus observed in control preparations was rather widely dispersed, and even the virus treated with antibody had only moderately large groups of particles, the virus treated with placental inhibitor was clumped together in large 3-dimensional masses. These huge clumps of virus almost suggest that in some manner the placental fraction induced a strong intermolecular attraction between the virus particles.

The morphology of the interaction between the H-viruses and their homologous antibodies is quite similar to that described by Almeida *et al.* Antibody is known to be bivalent in its relation to antigen and the combining sites of antibody in the present system appear to be at the two opposite ends of the elongated fibril-like molecules. Although antigen is believed to be multivalent in reaction to antibody, the absence of 3-dimensional aggregates and the tendency of linear arrangement in the virus-antibody complex suggest that the sites of the virus surface interacting with specific antibody are not as numerous as those interacting with the placental inhibitor. It is also interesting that the number of "empty" virus particles which were penetrated by phosphotungstic acid increased after antibody treatment. This suggests that some conditions of the capsomere architecture were changed by the attachment of the antibody molecule. However, after 48 hours treatment with specific antibody, no decomposition of virus or capsomeres could be observed.

Summary. Treatment of H-1 or HB viruses with a purified hemagglutination inhibitor derived from human placental fluids induces the formation of large three-dimensional aggregates of virus. Fibrils, 10Å or less in diameter, appear to connect the virus though they may not be the cause of the virus clumping. The placental inhibitor does not affect H-3 or RV viruses. When antibody is added to its homologous virus, aggregates are also formed. These are 2-dimensional and much smaller than those formed by the placental fluid fraction. They are characterized by the presence of fibrils, believed to be antibody, which join the individual virus particles.

1. Usategui-Gomez, M., Proc. Soc. Exp. Biol. and Med., 1965, v120, 385.

2. Greene, E. L., Karasaki, S., ibid., 1965, v119, 918.

3. Chandra, S., Toolan, H. W., J. Nat. Canc. Inst., 1961, v27, 1405.

4. Toolan, H. W., Saunders, E. L., Greene, E. L., Fabrizio, D. P., Virol., 1964, v22, 286.

5. Almeida, J., Cinader, B., Howatson, A., J. Exp. Med., 1963, v118, 327.

6. Moore, A. E., Virol., 1962, v18, 182.

7. Toolan, H. W., Proc. Am. Assn. Canc. Res., 1964, v5, 64.

8. Bayer, M. E., J. Cell. Biol., 1964, v21, 265.

Received June 18, 1965. P.S.E.B.M., 1965, v120.