STUDIES WITH THE ELECTRON MICROSCOPE ON THE INTERACTION OF RED CELLS AND INFLUENZA VIRUS

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The influenza virus, red cell agglutination phenomenon, first observed by Hirst (1941) and McCelland and Hare (1941), has proved to be a reaction of considerable importance. In addition to its practical value this phenomenon offers an opportunity to study a virus and cell interaction of general theoretical interest. It is generally conceded that adsorption of virus on the susceptible cell surface constitutes the first step in the process of infection by a virus. The adsorption of influenza virus on red cells has been amply demonstrated by indirect methods and strengthens the analogy between the virus-red-cell reaction and the initial phase of virus infection (Hirst, 1942; Francis and Salk, 1942). It is the purpose of this paper to present direct evidence of the interaction between virus and red cell.

In work with multicomponent biological materials the electron microscope technique presents considerable difficulties. For example, in the demonstration of an antigen-antibody reaction, for which relatively high concentrations of materials may be needed, the problem of clearing the specimen background arises in obtaining electron micrographs of satisfactory quality. With such a system it is necessary that care be exercised in the washing process for clearing the supporting film, in order that the desired features of the preparation may not be lost.

Even more difficult to study with the electron microscope is the interaction between proteins and cells. Here osmotic equilibrium must be maintained, since the removal of salt may result in cell destruction. This problem is found with systems involving human red cells, as hemolysis is initiated easily and the subsequent liberation of inner structural material alters the experimental conditions. In such cases it is easier to work with cell ghosts, provided that they still retain the desired interacting properties. In general, with a knowledge of the difficulties involved and a suitable handling of the system, satisfactory electron micrographs can be obtained at various stages of virus and red cell interaction.

MATERIALS

Virus samples were obtained from chick allantoic fluid and purified by the use of the Sharples centrifuge. Both active influenza virus type B and formalin-inactivated A and B mixtures were employed in the experiments.[2]

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[2] I am indebted for virus samples to Dr. B. Hampil of the Sharpe and Dohme Company, Inc.

Human red cell ghosts were prepared by the following procedure: (1) Washed cells were hemolyzed in distilled water for 30 minutes and sedimented by low-speed centrifuging. (2) The sedimented cells were washed twice in saline. (3) The final sediment was stored at 5 C in saline.

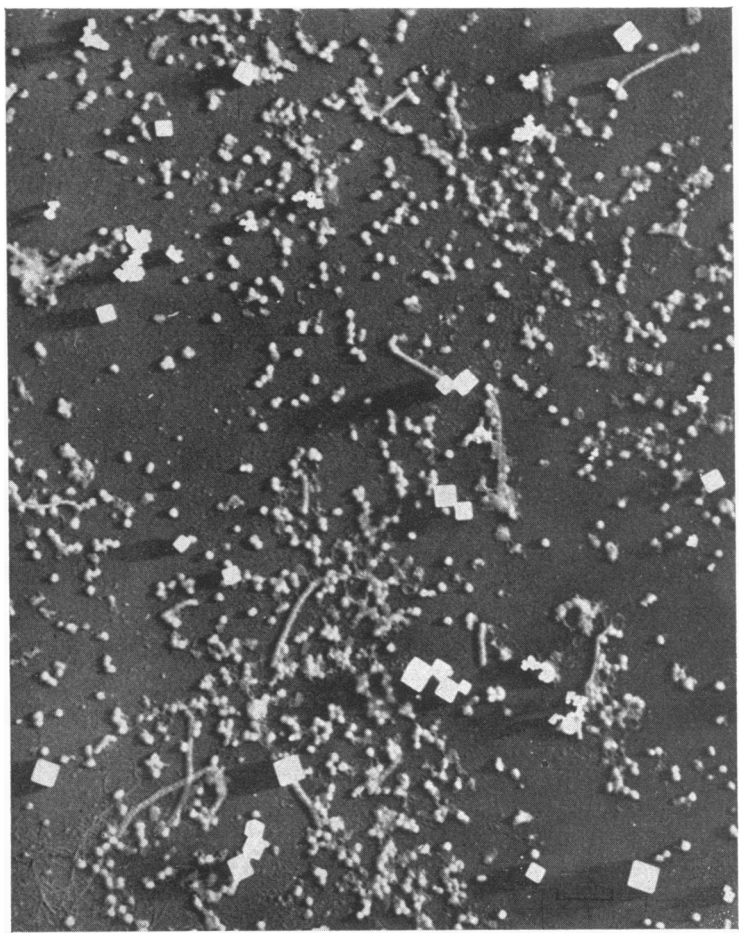

FIG. 1. INFLUENZA VIRUS A AND B VACCINE MIXTURE, DEMONSTRATING VARIOUS STAGES OF AGGREGATION AND DISINTEGRATION OF PARTICLES.

The length of the scale is 1 micron.

Washed chicken red cells can be used directly, since they are less easily hemolyzed during the washing of the specimen. The R.C.A. type B electron microscope, without objective aperture, was used.

METHODS

Electron microscope specimens were prepared in the following manner: (1) Virus solution was diluted in distilled water and a drop of the suspension was placed on the specimen screen. After a few minutes the excess fluid was re-

moved and the specimen screen dried. The screen was then dipped several times in distilled water and, after again drying, gold shadow casting was performed (Williams and Wyckoff, 1945*a*). The same technique was also used for human red cell ghosts. Intact human and chick red cells were suspended in saline and handled in the usual manner. The human red cells were very sensitive to hemolysis, and the single dipping of the specimen into water for the removal

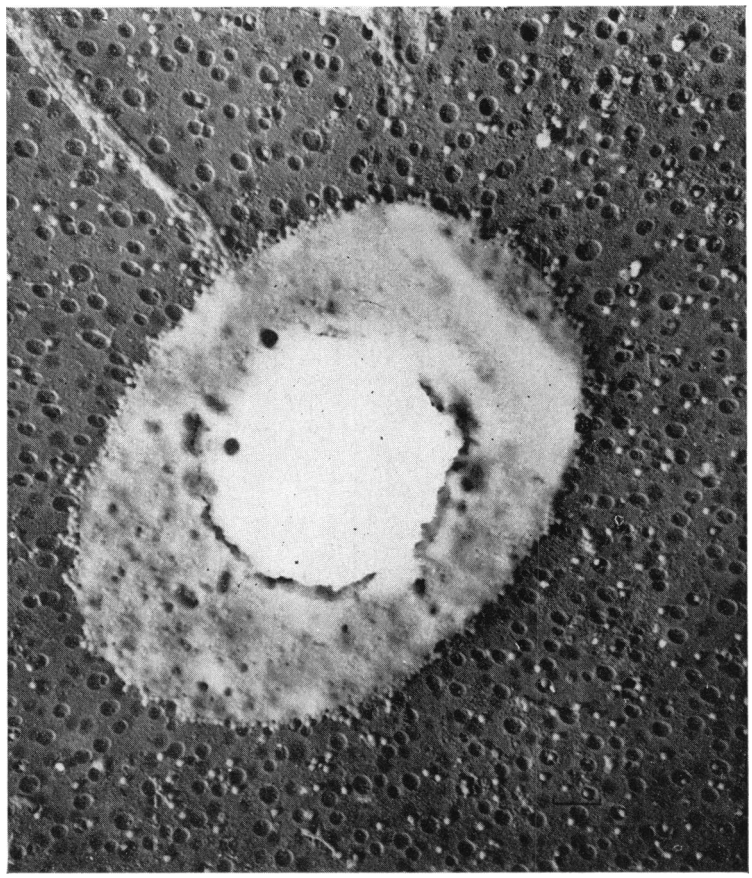

FIG. 2. INFLUENZA B VIRUS ADSORBED ON CHICK RED CELL AFTER 12 MINUTES IN CONTACT IN SALINE SOLUTION (TEMP. 6 C)

The craters on the film are due to defects in the supporting film. The length of the scale is 1 micron.

of salt caused partial hemolysis. (2) Virus and cells (or red cell ghosts) were mixed and kept in contact at a constant temperature for the required time. The suspension was then diluted in saline, or in distilled water, and one drop was placed immediately on the specimen screen and handled in the previously described manner. The washing has to be done carefully in this case; otherwise the cells are easily washed off the screen.

EXPERIMENTAL RESULTS

Virus. The influenza virus used for interaction experiments appeared in the electron microscope to be in the same particle size range as estimated from electron microscopic data by Taylor *et al.* (1943), Williams and Wyckoff (1945*b*), and others, the particles being round and of diameters ranging from 75 to 130 mμ. It was observed that during storage in 0.9 per cent saline at 5 C inactivated virus started to change its configuration and a variety of shapes appeared, sug-

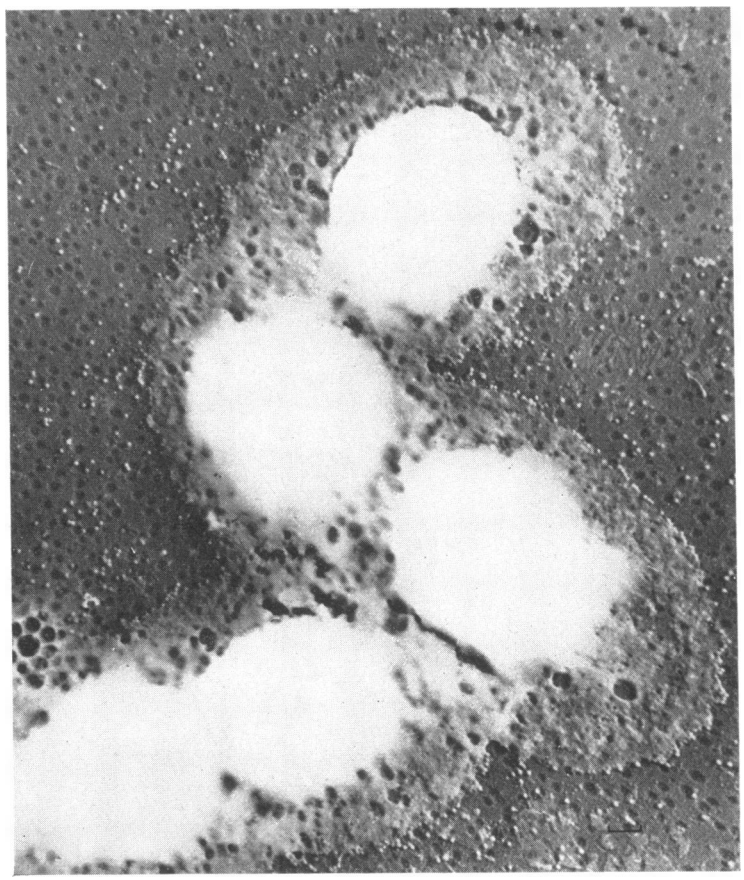

FIG. 3. CHICK RED CELL AGGLUTINATION IN THE PRESENCE OF INFLUENZA B VIRUS; SAME SAMPLE AS FIGURE 2 WITH 45-MINUTE CONTACT

The length of the scale is 1 micron.

gestive of products of disintegration and aggregation. Examination of the same virus sample during a 4-month period, at weekly intervals, revealed that definite changes in the external geometry were taking place. Figure 1 shows a micrograph of a sample taken in the early phase of storage. Here the virus is seen in the form of single particles, filaments, and various aggregates. Most of the single particles are well defined, but some flat particles, with increased surface

area, are also present. It has been observed from many other similar micrographs that disintegration of particles into smaller fragments takes place when the surface area has increased too extensively. Generally there are many modes of disintegration and aggregation. White rectangular particles on the micrographs are magnesium oxide crystals, introduced on the specimen in the form

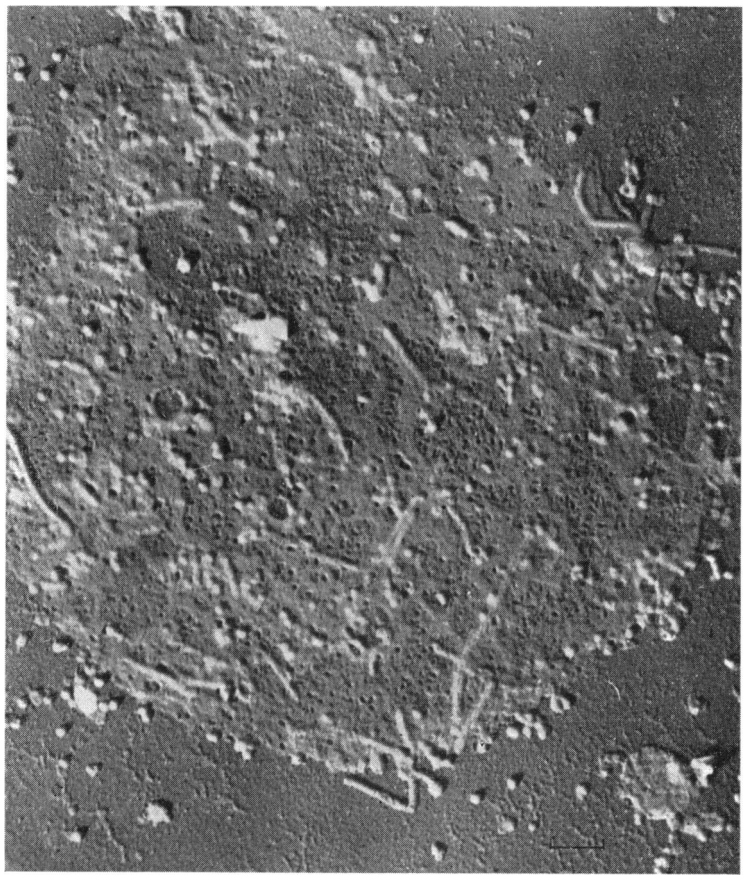

FIG. 4. INFLUENZA VACCINE A AND B MIXTURE, ADSORBED ON HUMAN RED CELL GHOST AFTER 20 HOURS' CONTACT AT 4 C

The length of the scale is 1 micron.

of smoke. They were used for determining the height of the particles. This subject will be discussed elsewhere.

In the most orderly instances of aggregation particles seem to form filaments of various lengths. The examination of samples stored for longer periods of time showed further increase of filamentous particle forms and general loss of definition of individual particles.

Virus and chick red cells. Figure 2 shows a chick cell with a dense nuclear area and a relatively less dense peripheral region. This outer region is sufficiently

transparent for virus interaction studies. Virus is seen to be attached to the border area of the cell and to the surface of certain areas of the less dense cell region. Other areas of the cell surface are too dense for the resolution of individual virus particles.

The fibrous material seen on one side of the cell also has some virus particles attached. The background craters are defects of the supporting film. Figure 3

FIG. 5. AGGLUTINATION OF HUMAN RED CELL GHOSTS BY INFLUENZA A AND B VACCINE

The length of the scale is 1 micron.

is a micrograph of chicken red cells agglutinated by influenza virus, again demonstrating the surface adsorption of the virus particles.

Virus and human red cells. The adsorption of inactivated virus on the ghost surface is shown in figure 4, where virus particles and filaments of various lengths cover both sides of the ghost. Particles on the upper surface of the cell are more clearly defined and are not covered by the cell wall. Figure 5 demonstrates the agglutination of ghosts by virus.

Surface saturation of a ghost cell by active virus is shown in figure 6, this screen having been prepared by adding relatively few ghosts to a concentrated

virus solution. As can be seen, the average concentration of virus on the ghost surface is much larger than that on the background film.

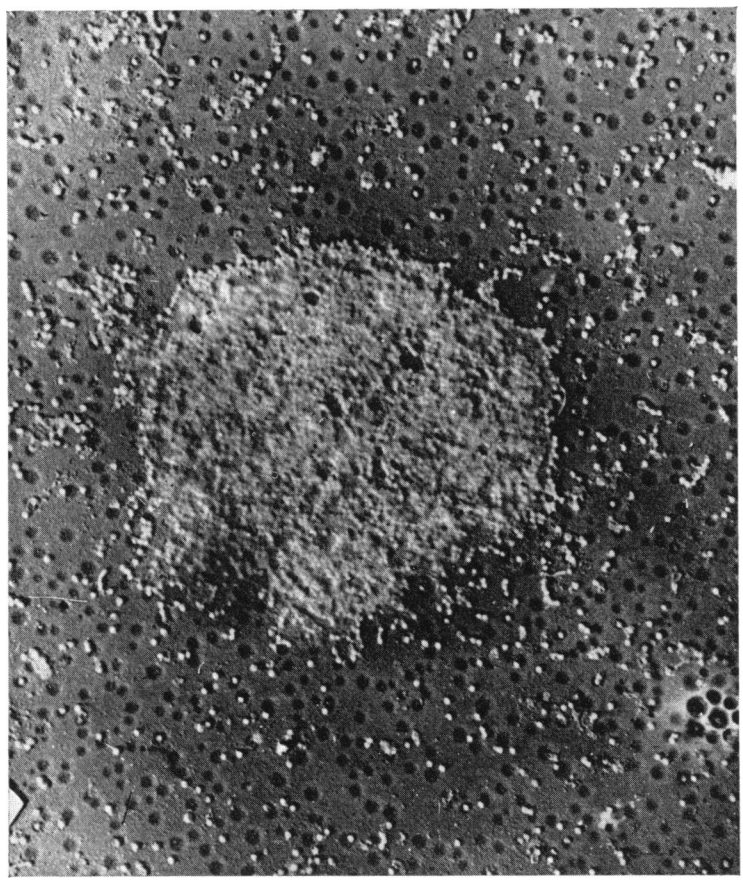

FIG. 6. INFLUENZA B VIRUS ADSORBED ON HUMAN RED CELL GHOST; CONTACT 30 MINUTES AT 4 C; EXCESS VIRUS

The length of the scale is 1 micron.

DISCUSSION

It is of interest to know how the immunochemical properties of a virus particle are affected when a change takes place in its external geometry. It is expected that some information concerning structure can be gained by studying thoroughly the modes of deformation. At the present time, the experimental data are insufficient for such an analysis, but it can be stated that in some cases of aggregation the interacting properties of this virus are not destroyed. As the evidence presented shows, filament type virus particles can still adsorb on the red cell surface. It is uncertain at what stage of disintegration the virus particle fractions become incapable of interacting with cell surfaces. Fractionation

studies, accompanied by chemical experiments, might offer an opportunity to locate the biologically active groups of virus particles.

It is uncertain whether agglutination of red cells is caused by the virus particles directly, forming a linkage between agglutinated cells, or whether the cell surfaces are sensitized by adsorbed virus and subsequently agglutinated. Repeated observations with the aid of the electron microscope indicate that fresh, unstored red cells, when agglutinated by virus, always show its presence on the surface and especially on the contact area of the cells. As revealed by figure 6, a human red cell ghost is also capable of adsorbing virus over practically all of its surface. The observations further indicate that certain red cells that are not agglutinated may still have virus adsorbed on their surfaces. This is usually the case at relatively low virus concentrations. The observation suggests that virus adsorption does not sensitize the cell surface for agglutination, but rather that the most probable explanation, based on a large number of observations, is that the agglutinating action of influenza virus on the red cells is a direct one. The phenomenon appears to take place, however, only when there is a sufficient number of virus particles present to form linkages between the cells. Furthermore, any visible precipitation of red cells indicates rather an extreme case of interaction, since combination between virus and cells can take place without producing any visible agglutination by a standard test-tube technique.

Electron microscopic studies have so far not revealed any visible changes on the red cell ghost surface after the virus elution, but further studies should be done on the subject. The difficulties are considerable because the large number of holes in the cell wall and the extensive disorganization of the surface, even before virus adsorption, make changes difficult to detect. On the other hand, the normal red cell, with its intact cell wall, which would be more suitable in that respect, is too dense for study with the electron microscope. Replica studies might be helpful.

ACKNOWLEDGMENTS

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SUMMARY

Electron microscopic studies suggest that inactive influenza virus undergoes deformation into various forms during prolonged storage. Some aggregation forms, at least, appear to retain the property of interaction with red cells.

Electron micrographs are presented showing the physical relationships between influenza virus and agglutinated red cells or red cell ghosts.

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