germfree and conventional animals, especially among older animals. All groups of 12-week-old animals had serum complement levels 2 to 3 times those of 1-week-old animals.

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Role of Inhibitors in Hemagglutination Behavior of Mumps Virus.* (25884)

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Mumps virus agglutinates red cells (rbc) of several animal species. However, it has been reported that the rbc of some species (1,2,3,4) are not agglutinable by mumps virus. Special attention has been paid to the inability of some mumps strains to agglutinate human rbc(5,6,7). According to Sohier *et al.* (7), 9 mumps virus strains fell into 2 groups as regards their hemagglutination (HA) behavior. The first group (Enders type), including 3 of the strains, agglutinated equally well fowl and human erythrocytes, the second group of 6 strains (Habel type) agglutinated human rbc only feebly or not at all. Sensitivity of mumps virus to HA inhibitors present in tissues and fluids of chick embryos is known(8,9). The role of these inhibitors in determining ability of some strains of mumps virus to agglutinate human rbc is the object of this study. It will be shown that all the studied virus strains (also Habel types) agglutinated human erythrocytes almost as well as fowl cells after elimination of inhibitors from the virus fluids.

Materials and methods. The following strains of mumps virus were employed in this study; number of passages undergone in this laboratory in the allantoic (all.) or amniotic (amn.) cavities of the embryonate egg are given in parentheses: Enders strain (68-149 all.), Habel strain (1-12 amn., 1-82 all.) obtained from Dr. V. J. Cabasso(6), KS strain (1-8 amn., 1-32 all.) isolated in this laboratory(10), Chopin strain (1-4 amn.) supplied by Dr. R. Sohier(7). For HA tests blood was taken only from fowls whose rbc were found to give uniformly high titers with mumps virus. Human erythrocytes were always taken from the same person of O blood group. Red cells washed 3 times were stored as a 10% suspension and used at a concentration of 0.5% in the assay procedure. Titrations were carried out at room temperature (18-20°C) on plastic plates by the pattern method(11) and read after 2 hours. Amniotic and allantoic fluids containing virus were treated by various procedures to free them of inhibitor activity. Some were dialyzed for 24 hours against phosphate buffered saline (pH 7.4) at 4°C; others were diluted 5-fold and sonicated for 30 minutes at ice temperature (20 KC. MSE Mullard) while some were merely incubated 2 days at 35°C. Enzymatic treatment was effected by incubating at 37°C for 18 hours a mixture of 2 parts of cholera filtrate (Philips-Roxane) and one part of fluid containing virus. In all HA titrations of the virus preparations treated with cholera filtrate, sodium citrate was added to the reagents to give a final concentration of one per cent. To remove inhibitors by differential centrifugation, one ml of virus fluid and 9 ml of buffered saline were combined and centri-

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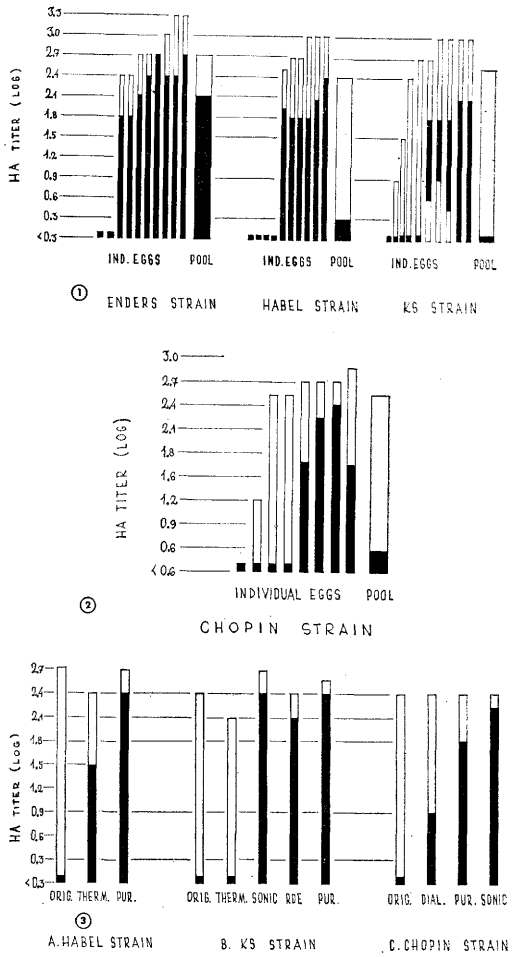

FIG. 1. HA titers with fowl and human red cells of individual and pooled allantoic fluids infected with various mumps strains.

FIG. 2. HA titers with fowl and human red cells of individual and pooled amniotic fluids infected with Chopin strain of mumps virus.

FIG. 3 (A, B and C). Effect of various treatments of virus preparations on their capacity to agglutinate human red cells. ORIG. = original virus preparation; THERM. = incubated for 2 days at +35°C *in vitro*; DIAL. = dialysed overnight against buffered saline; PUR. = partially purified by ultracentrifugation; SONIC = treated in ultrasonic disintegrator; RDE = treated with cholera filtrate. Details in text.

fuged (Spinco model L) for 60 minutes at 26, 530-56, 550 x G. The supernatant was discarded and the sediment suspended in one ml of buffered saline.

Results. In our first passages of the Habel strain, it behaved as described by Cabasso and Cox(6), *i.e.*, it did not agglutinate human rbc at any temperature. During the first amniotic passages, however, some virus fluids had a slight ability to agglutinate human erythrocytes. When the strain was passed allantoically, virus fluids giving high HA titers with human rbc began to appear rather frequently. The same was found with the KS strain. Conversely, with the Enders strain it was seen that some low titered virus preparations lacked ability to agglutinate human rbc. These observations suggested that this ability was influenced by some extrinsic factors. Further information was obtained by determining HA titers in individual infected eggs. A typical experiment of this type is given in Fig. 1. About 10^4 infective doses of Enders, Habel and KS strains were each inoculated into 10 eggs allantoically. After 5 days' incubation at +35°C the allantoic fluids were collected separately and the HA titer of each one was determined with fowl and human rbc. Also equal amounts of the allantoic fluids from individual eggs were pooled and HA titers determined. The figure shows that with the Enders strain the pool titers corresponded quite well with those of individual eggs. The Habel strain when pooled lost the ability to agglutinate human rbc almost entirely, while the titer with fowl cells remained high. With the KS strain, the reciprocal ratio of fowl titer and human titer in individual eggs varied greatly. Some infected allantoic fluids agglutinated human cells rather well, others only patchily (a phenomenon often seen in HA titrations with human erythrocytes), while others not at all, in spite of their high titers with fowl cells. The pool gave a high titer with fowl cells but no agglutination at all with human cells.

In the following experiments different methods were used to remove the activity of HA inhibitors which might be present in the virus preparations and then HA titers obtained with human and fowl cells were compared. These experiments included the Chopin strain which has been reported to agglutinate very weakly human rbc(7). In our laboratory pooled amniotic fluids infected with this strain gave the same results but some individual amniotic fluids agglutinated human erythrocytes to high titer (Fig. 2).

In Fig. 3 (A, B, and C) are shown HA titers obtained with fowl and human cells using preparations of mumps virus treated in various ways. All virus fluids included in this experiment originally failed to agglutinate human cells. However titers with these cells were not far below those with fowl cells once the virus fluids were treated with ultrasonic waves (B, C) or cholera filtrate (B) or partially purified by ultracentrifugation (A, B, C). Slight ability to agglutinate human rbc was revealed in the Chopin strain by dialysis against buffered saline (C). Thermal treatment (2 days at +35°C *in vitro*) of the Habel strain (A) but not of the KS strain (B) also brought out some human cell agglutination.

Discussion. According to Buzzel and Hanig(9) the HA behavior of mumps virus is affected by its heavy contamination with inhibitors, which by giving the virus a strong negative charge hamper attachment to the red cell. On the other hand, it is known that the inhibitor in normal allantoic fluid affects to greater degree the attachment of mumps virus to human than fowl rbc(12,5). From this, one could draw the conclusion that the ratio between fowl titer (F) and human titer (H) of a given mumps virus preparation is directly proportional to inhibitor contamination. Our results are consistent with this conclusion. The methods which raised the F/H ratio of the virus preparations destroyed or removed inhibitors(9,13). Inversely, the pooling of individual egg fluids tended to depress the F/H ratio by incorporating the inhibitors from low titer and negative eggs, which are typically present in mumps infected embryonated eggs(10). Variations of F/H ratio in individual eggs may be explained as also being caused by inhibitors, bearing in mind the above-mentioned variation in production of hemagglutinins by individual eggs and the differences in amount of inhibitors in individual allantoic fluids(8,14).

Obviously contamination of mumps virus by inhibitors is not an irreversible process but a balanced condition where the essential factors are virus, inhibitor, and rbc(14,15). When this system of 3 factors prevails variations in HA results are to be expected depending on quality and concentrations of these factors.

The results obtained disagree with the supposition that certain mumps virus strains are characterized by certain F/H ratios. In fact, F/H ratio could vary in individual eggs infected simultaneously and also in different pooled virus fluids. However, the Habel and Chopin strains generally gave higher F/H values than the Enders strain. The inhibitor sensitivity of different strains and their ability to destroy them thus still demand further elucidation.

Summary and conclusion. 1) Low ability of some strains of mumps virus to agglutinate human rbc was studied. Some individual-infected amniotic and allantoic fluids and pooled virus preparations agglutinated human erythrocytes nearly as well as fowl erythrocytes. When virus fluids, failing to agglutinate human cells, were treated by various procedures capable of removing or destroying inhibitors, they became positive. 2) Failure to agglutinate human erythrocytes is not a stable intrinsic characteristic of certain strains of mumps virus but is due to inhibitors present in virus preparations.

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