# TECHNICAL PAPERS

## Effect of Swine Influenza Virus on the Viscosity of the Egg-white Inhibitor of Hemagglutination[1]

Edward A. Eckert, Frank Lanni, Dorothy Beard, and J. W. Beard

*Department of Surgery,*
*Duke University School of Medicine,*
*Durham, North Carolina*

Egg-white (EW) contains a component which is capable of combining with influenza virus and inhibiting the agglutinative reaction of virus with *RBC* (*3, 5–6*). Furthermore, untreated purified swine influenza virus is capable of destroying the EW inhibitor, but suitably heated virus is essentially devoid of such destructive capacity (*7*). Thus far, the destruction of inhibitor by virus, which has aspects of an enzymatic action, has been inferred only from indirect experiments based on the hemagglutination phenomenon. Recently, it has been observed, as described briefly in the present report, that virus exerts a profound effect on the viscosity of solutions of semipurified EW inhibitor.

Semipurified inhibitor preparations were obtained from EW by repeated precipitation at pH 5.7 and extraction of the precipitate with 0.06 M phosphate buffer at pH 7.2 (*8*). Typical extracts, which were 40 to 60 times as active as EW on a nitrogen basis, contained 25 to 150 γ N and 50 to 125 γ carbohydrate[2] per ml; one preparation, A178 PIII EI, which was 41 times as active as EW, contained 150 γ N per ml and 12.5% N on the basis of dry weight. The inhibitor was nondialyizable and gave qualitative tests for protein. The swine influenza virus was a freshly purified preparation, obtained as previously described (*10*), containing 400 γ N per ml and characterized by a 50% endpoint infectious unit of $10^{-14.4}$ g N. The viscosity experiments were carried out in capillary viscometers (*9*) at 29.78° C.

The relative viscosity of preparation A178 PIII EI was found to be linear with respect to concentration over the range from 0 to 150 γ N per ml and had the value 1.255 at 100 γ N per ml. Assuming a density of 1.33 for the viscous component of this preparation, one may calculate a minimal viscosity increment (*4*) of approximately 400, a value considerably greater than that which is obtained with ordinary proteins (*4*). From this result one may infer the presence of a highly asymmetric component.

[1] This work was aided by a grant to Duke University from Lederle Laboratories Inc., Pearl River, N. Y., and by the Dorothy Beard Research Fund.

[2] Determined with the orcinol reagent and calculated as glucose.

The effect of untreated, purified swine influenza virus on the viscosity of inhibitor solutions is shown in Fig. 1. The rate of decrease in viscosity is initially rapid, falling to a low value within a few minutes. The terminal viscosity (after 24 hr) of virus inhibitor mixtures was approximately 1.01, corresponding to a reduction of 80 to 90% in the specific viscosity (relative viscosity −1). This terminal value was essentially independent of the initial virus concentration.

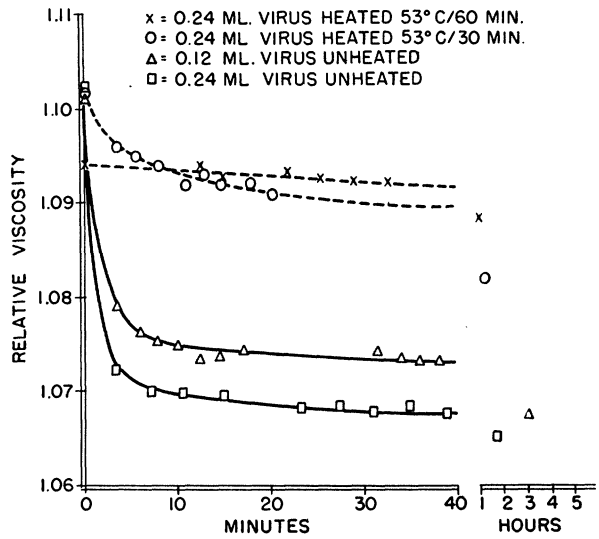


FIG. 1. Effect of heated and unheated purified swine influenza virus on the viscosity of solutions of purified EW inhibitor. The virus preparation contained 200 γ N per ml (2.2 mg virus per ml). The volume of virus preparation indicated in the chart was added to 10 ml of the inhibitor preparation A180 PEI containing 30 γ N per ml.

In an experiment of a different sort, 0.24 ml virus at a concentration of 200 γ N, or 2,200 γ virus per ml, was mixed with 10 ml inhibitor solution containing 30 γ N per ml with a relative viscosity of 1.0815, and the reaction was followed in the viscometer. After 25 min, when the relative viscosity was 1.0685 and was changing slowly, 5 ml reaction mixture was mixed with 5 ml fresh inhibitor solution. This process was repeated three more times. With each addition of fresh inhibitor there occurred a burst of activity as manifested in a rapid initial fall in viscosity. While it is difficult to interpret the results precisely at the present time, the experiment gave no evidence of saturation of the viscosity reducing capacity of the virus preparation. The activity of the fresh inhibitor solution was such that a quantity of 10 ml would have been capable of inhibiting the hemagglutinating activity of approximately 30 mg of virus heated 30 min at 53° C in concentration of 1 γ N per ml.

Fig. 1 shows also that heating the purified virus preparation for different periods at 53° C progressively reduces the capacity of the virus to cause a decrease in viscosity. These results with heated virus have a correlate in the reduction of the inhibitor destroying capacity of virus consequent to heating (*7*).

Studies of the effect of convalescent antiswine influenza swine serum showed that a quantity of serum capable of neutralizing completely the hemagglutinative activity of the virus prevented completely the typical effect of virus on inhibitor viscosity. The same amount of normal swine serum was somewhat less effective. The dependence of the rate of viscosity reduction on virus concentration and on the state of the virus, i.e., whether the virus was heated or not heated, as well as the absence of viscosity changes in the presence of immune serum, suggests that the reduction in viscosity is related to an interaction of the virus particles themselves with the viscous component of inhibitor solutions.

There are several reasons for supposing that the relationship of the EW inhibitor of hemagglutination to the viscous component of solutions of purified inhibitor is one of identity: (a) both the inhibitor and the viscous component appear capable of interacting with virus; (b) both suffer a change when treated with unheated virus, and both are less susceptible to action by heated virus; (c) the viscous component has probably a high molecular weight, and it is likely that any material of high molecular weight which is capable of combining with virus would be capable also of inhibiting virus hemagglutination; and (d) the specific viscosity of the two purified inhibitor preparations, A178 PIII EI and A180 PEI, which have been studied in detail, is proportional to the inhibitory activity. Calculated on a nitrogen basis, the activities of these preparations were 41 and 60 times, respectively, that of EW, and the specific viscosities at 30 $\gamma$ N per ml were 0.075 and 0.105. This proportionality becomes a valid test when it is considered that the viscosity of purified inhibitor solutions is contributed chiefly by a component (or components) which is susceptible to virus action, as inferred from the low terminal viscosities of virus inhibitor mixtures.

The reduction in viscosity induced by virus may be interpreted as a reduction in the asymmetry of the molecules (or particles) susceptible to virus action. There are several obvious and quite different ways by which such a change in shape could be achieved: (a) the molecules could be fragmented across the long axis; (b) they could be made to fold or coil without change in size; (c) they could be made to condense with one another with simultaneous increase in size and decrease in asymmetry; and (d) they could form suitable stable complexes with virus particles. Of these ways, the first three could be thought to depend on an enzymatic action of the virus, while the fourth could be regarded as a process of stable aggregation of inhibitor molecules and virus particles. Support for the latter explanation is provided by the observation (*6*) that a precipitate forms at interfaces between EW and purified virus preparations. No definite evidence of precipitation has been obtained, however, in the present experiments with dilute solutions of purified inhibitor; and since inhibitor virus mixtures which have been incubated for long periods possess considerable hemagglutinative activity, comparable to that of uninhibited virus, it is likely that the virus separates from the viscous component after interaction. Accordingly, an enzymatic hypothesis of virus function offers the most reasonable explanation at the present time of the virus induced reduction in viscosity of solutions of purified inhibitor. This hypothesis is compatible also with the relative independence of the terminal viscosity on initial virus concentration and with the failure of repeated additions of fresh inhibitor solution to affect appreciably the viscosity reducing capacity of virus.

If the above interpretation is correct, the present observations provide the most direct demonstration, so far as we are aware, of an enzymatic action of influenza virus on a relatively pure and simple substrate. It should be mentioned that several previous attempts (*1, 2*) at such a direct demonstration have failed.[3]

### References


1. Briody, B. A., and Hanig, M. *Proc. Soc. exp. Biol. Med.*, 1948, **67**, 485.
2. Burnet, F. M. *Lancet*, 1948, **254**, 7.
3. ———. *Austr. J. exp. Biol. Med. Sci.*, 1948, **26**, 371.
4. Cohn, E. J., and Edsall, J. T. *Proteins, amino acids and peptides as ions and dipolar ions.* New York: Reinhold, 1943.
5. Hardy, P. H., Jr., and Horsfall, F. L., Jr. *J. exp. Med.*, 1948, **88**, 463.
6. Lanni, F., and Beard, J. W. *Proc. Soc. exp. Biol. Med.*, 1948, **68**, 312, 442.
7. Lanni, F., and Beard, J. W. *J. Immunol.*, in press.
8. Lanni, F., *et al. J. biol. Chem.*, in press.
9. Neurath, H., Cooper, G. R., and Erickson, J. O. *J. biol. Chem.*, 1941, **138**, 411.
10. Taylor, A. R., Sharp, D. G., McLean, I. W., Jr., Beard, D., and Beard, J. W. *J. Immunol.*, 1945, **50**, 291.


## A New Human Hereditary Blood Property (Cellano) Present in 99.8% of all Bloods


**Philip Levine, May Backer, Milton Wigod, and Ruth Ponder**

*Blood Testing Laboratory, Ortho Research Foundation, Raritan, New Jersey,* and *the Nassau Hospital Laboratory, Mineola, New York*


A new agglutinogen of human blood was recently observed with the aid of an immune agglutinin produced by a mother of an infant with a mild form of hemolytic disease.[1] This antibody, which behaves like a "warm" agglutinin (*4*), is remarkable because of the unusually

[1] The diagnosis was made by Dr. Eric Ponder on the basis of clinical and hematologic studies.

[3] While this paper was in press, D. W. Wooley (*J. exp. Med.*, 1949, **89**, 11) reported changes in the viscosity of erythrocyte extracts on treatment with influenza virus A (PR8 strain).