

Inhibition by Egg-White of Hemagglutination by Swine Influenza Virus.*

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Shortly after the discovery of influenza virus hemagglutination^{1,2} it was reported³ that normal serum possessed the capacity to inhibit this phenomenon in low dilution. Subsequent investigation showed that a variety of other materials possessed a similar capacity.^{4,5}

Recently, Francis⁶ demonstrated that the inhibition by normal serum was enhanced if heated virus preparations were used in the titration of inhibitor. The present report is concerned with the preliminary description of an analogous, but much more striking, inhibition phenomenon with egg-white.

Experimental. Preliminary experiments have been carried out with a purified preparation of egg-adapted swine influenza virus⁷ and a derivative obtained by heating a buffered-saline suspension of the virus for 30 minutes at 53°C, at a concentration of 1.0 γ N per ml. Fresh egg-white (EW) was strained through gauze and diluted serially with buffered saline for the tests. Typical inhibition titers, determined by the standard hemagglutination method of this laboratory⁷ and expressed as the reciprocal of the final dilution of EW required to neutralize two to three hemagglutinating units of virus, averaged 170,000 with heated virus; and, with one egg-white which was investigated in detail, the enhancement over the inhibition titer for

unheated virus was 750- to 1,000-fold. With the same virus preparations, a pool of normal rabbit serum gave an inhibition titer of 760 with heated virus and 120 with unheated virus, the Francis enhancement thus being only 6-fold.

A striking susceptibility to inhibition by EW was shown also by an aged formalized swine influenza vaccine,⁷ with which titers of about 54,000 were obtained, but not by a freshly prepared vaccine, which exhibited a behavior identical, in this respect, with that of unheated virus. The ratio of about 1 to 3 for the inhibition of aged vaccine and heated virus was found with each of 13 individual whites, and we may accordingly suppose that these 2 virus preparations presented the same differences to each of the egg-whites tested and that the inhibitory property is ascribable to a characteristic ingredient of EW.

The EW inhibitor is almost completely destroyed by treatment with dilute periodate (Table I), which has been shown to destroy the red cell receptor^{5,8,9} and the normal serum inhibitor.^{4,5,9} In view of the current belief^{4,5} that both the red cell receptor and the normal serum inhibitor are mucoproteins, it is interesting to note that when EW, either undiluted or diluted 5-fold with buffered saline, is treated with an equal volume of a saturated aqueous solution of potassium periodate, there is formed a considerable mucin-like precipitate, consisting possibly of ovomucin.^{10,11}

We have further observed that when undiluted or 20% EW is overlaid with a con-

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¹ Hirst, G. K., *Science*, 1941, **94**, 22.

² McClelland, L., and Hare, R., *Canad. Pub. Health J.*, 1941, **32**, 530.

³ Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

⁴ Burnet, F. M., *Lancet*, 1948, **254**, 7.

⁵ Hirst, G. K., *J. Exp. Med.*, 1948, **87**, 301.

⁶ Francis, T., Jr., *J. Exp. Med.*, 1947, **85**, 1.

⁷ McLean, I. W., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., *J. Immunol.*, 1945, **51**, 65.

[†] Strain 15, originally obtained from Dr. R. E. Shope.

⁸ The Rockefeller Foundation, International Health Division, Annual Report, New York, 1945, 50.

⁹ Burnet, F. M., McCrear, J. F., and Anderson, S. G., *Nature*, 1947, **160**, 404.

¹⁰ Hektoen, L., and Cole, A. G., *J. Infect. Dis.*, 1928, **42**, 1.

¹¹ Young, E. G., *J. Biol. Chem.*, 1937, **120**, 1.

TABLE I.
Effect of Dilute Potassium Periodate on the Capacity of Egg-white (EW) to Inhibit Hemagglutination by Heated and Unheated Swine Influenza Virus.

EW sample	Treatment*	Inhibition titer†	
		Unheated virus	Virus heated 30 min. at 53°C
1	1.0 ml 20% EW (in buffered saline, pH 7.4) + 2.0 ml buffered saline	60	100,000
2‡	1.0 ml 20% EW + 1.0 ml sat. KIO ₄ (<0.05 M); after 2 hrs, add 1.0 ml 10% glucose§	<60	60
3	1.0 ml sat. KIO ₄ + 1.0 ml 10% glucose; after 2 hrs, mix with 1.0 ml 20% EW	60	33,000
4‡	1.0 ml 20% EW + 1.0 ml sat. KIO ₄ ; mix and add 1.0 ml 10% glucose immediately	<60	450
5	1.0 ml 20% EW + 1.0 ml H ₂ O; mix and add 1.0 ml 10% glucose immediately	60	100,000
6‡	1.0 ml 20% EW + 1.0 ml 10% glucose; mix and add 1.0 ml sat. KIO ₄ immediately	<60	3,200

* Carried out at room temperature (29°C).

† Determined with 4 two-plus units of virus. Titers expressed as the dilution of whole egg-white.

‡ Considerable mucin-like precipitate formed immediately on the addition of periodate and was removed by centrifuging at the end of the two-hour reaction period. Judged visually, the greatest precipitate formed in sample 2, next in sample 4, and least in sample 6. The results suggest that part of the decrease in inhibition titer may be attributable to the formation of this precipitate.

§ Glucose was used to inactivate the residual periodate.

centrated suspension (about 10 γ N per ml) of unheated virus or aged vaccine, reaction with virus is directly manifested by the formation of a precipitate at the interface. Furthermore, this property of EW, like its inhibitory property, is destroyed by treatment with periodate. In addition, we have found that the similar capacity of normal rabbit serum to precipitate with concentrated virus preparations^{12,13} is likewise destroyed by treatment of the serum with periodate.

Discussion. The present total of evidence, in particular, the direct observation of reaction between EW and virus, the parallelism in the effect of periodate on the precipitation and inhibition reactions, and the dependence of

inhibitor activity on the state of the virus, suggests that the capacity of EW to inhibit virus hemagglutination is attributable to a component which combines with the virus.

Summary. Egg-white (EW) has been found approximately 200 times as effective as normal rabbit serum in inhibiting hemagglutination by heated swine influenza virus, giving titers of about 170,000. An aged formolized vaccine was also highly susceptible to inhibition by EW, but unheated virus and a fresh vaccine required about 750 to 1,000 times as much EW for similar inhibition. In addition, EW gave an interfacial precipitation reaction with concentrated virus suspensions, and both the precipitating and inhibiting capacities were destroyed by treatment with dilute periodate.

¹² Knight, C. A., *J. Exp. Med.*, 1944, **80**, 83.

¹³ Knight, C. A., *J. Exp. Med.*, 1946, **83**, 281.