# 17101 P. Interaction of Swine Influenza Virus and Egg-White Inhibitor of Virus Hemagglutination.*

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The interaction of swine influenza virus and egg-white (EW) inhibitor of virus hemagglutination resembles an enzymatic reaction with the virus acting as enzyme and the inhibitor as substrate.[1] Attempts to follow the kinetics of inhibitor inactivation by virus have revealed that the inhibitor present during the intermediate period of the reaction differs in behavior from untreated inhibitor. This observation and its possible significance are the subjects of the present report.

*Materials and Methods.* The swine influenza virus was the purified preparation SF, previously described,[1] which contained about 7,200 hemagglutinating doses (HD) and 0.234 mg N per ml. The preparation of semipurified inhibitor, A200 PII EI, was obtained by precipitating EW with 7 volumes 0.1 M $KH_2PO_4$ and extracting the precipitate, after washing, with 0.06 M phosphate buffer at pH 7.2.[2] The extract contained 120 $\gamma$ N per ml and was about 30 times as active as EW on a N basis. The residual inhibitory activity of virus-inhibitor mixtures was titrated against 4 HD heated swine virus (53°C, 30 minutes) by the constant virus-varying inhibitor method.[2] Preliminary to titration, the virus-inhibitor mixtures were immersed in boiling water for 2 minutes to destroy the hemagglutinating activity of the virus; the activity of the inhibitor was not significantly affected by this treatment. Buffered saline, consisting of 0.81% NaCl and 0.005 M phosphate at pH 7.3, was the diluent throughout.

*Experimental.* In a typical experiment, mixtures of equal volumes of 1:5 inhibitor and 1:200 SF were incubated for varying periods at room temperature (27°C), immersed in boiling water, cooled, and titrated for residual inhibitory activity with virus heated at 53°C; inhibitor controls devoid of virus were included. The complete titration data are shown in Fig. 1, in which both the conventional designations, O to ++++, denoting minimal to maximal hemagglutination, and the corresponding chicken red blood cell concentrations in the reference red-cell suspensions are used as ordinates. In the present experiment, the designation +++± corresponds to zero inhibition. From the results it is evident that virus-treated inhibitor cannot properly be described as diluted untreated inhibitor, since the inhibition curves cannot be superimposed by translation along the axis of inhibitor dilution; therefore, no simple kinetic analysis is possible. Hirst[3] has re-

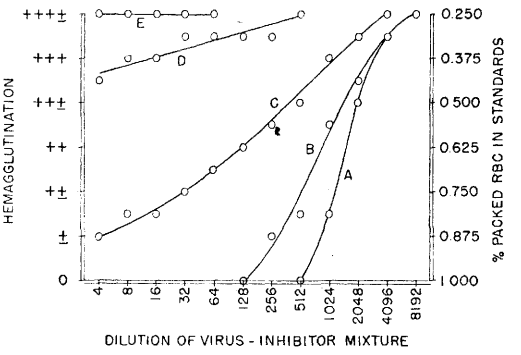


FIG. 1.

Inhibitory activity of purified EW inhibitor (A200 PII EI) after incubation with purified swine influenza virus (SF) for varying periods at 27°C, as tested with heated (53°C, 30 minutes) virus. Period of incubation: curve A, 0 minutes; B, 3 minutes; C, 10 minutes; D, 30 minutes; E, 100 minutes. Curve A applies also to untreated inhibitor control and to inhibitor control heated in boiling water for 2 minutes.

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[1] Lanni, F., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 442.

[2] Lanni, F., Sharp, D. G., Eckert, E. A., Dillon, E. S., Beard, D., and Beard, J. W., *J. Biol. Chem.*, in press.

[3] Hirst, G. K., *J. Exp. Med.*, 1948, **87**, 315.

ported a similar result with normal serum inhibitor.

Experiments to clarify the origin of the progressive decrease in slope induced by virus (Fig. 1) have shown that (a) the slope effect is not attributable to the concentration of impurities (cf. 2) or to the accumulation of non-inhibitory reaction products; and (b) the inhibitor characterized by shallow slope does not sediment appreciably in centrifugal fields sufficient for sedimenting virus.

*Discussion.* The slope of the inhibition curves (Fig. 1) in the region of transition from zero to maximal agglutination may be tentatively interpreted in terms of the firmness of attachment of inhibitor to the particles of the *titrating* virus. Accordingly, virus-treated inhibitor may be described as a "weak" inhibitor in comparison with untreated inhibitor.

Available evidence suggests that the weak inhibitor is a free inhibitor, unattached to virus. Such an inhibitor could pre-exist in a heterogeneous inhibitor population and could be made manifest through a preferential action of virus against strong inhibitor. An attractive alternative is that the weak inhibitor is the product of a progressive, rather than all-or-none, action of virus. If the latter explanation should be correct, the demonstration of a free, weak inhibitor, intermediate between fully active and inactive inhibitor, would provide strong support for the enzymatic hypothesis of inhibitor inactivation by virus. The possibility that the action of virus is not all-or-none has been previously suggested by Burnet and collaborators[4,5] from related, but somewhat more complicated, experiments.

*Summary.* During the interaction of swine influenza virus and egg-white inhibitor of hemagglutination, there appears a weak inhibitor, which has titrating properties different from those of untreated inhibitor. This result renders impossible a simple kinetic treatment of the data. The significance of the weak inhibitor for the mechanism of virus-inhibitor interaction is discussed.

[4] Burnet, F. M., *Austr. J. Exp. Biol. and Med. Sci.*, 1948, **26**, 389.

[5] Anderson, S. G., Burnet, F. M., Fazekas de St. Groth, S., McCrea, J. F., and Stone, J. D., *Austr. J. Exp. Biol. and Med. Sci.*, 1948, **26**, 403.

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## 17102. Role of Adrenal in Uptake of $I^{131}$ by the Thyroid Following Parenteral Administration of Epinephrine.

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In a previous paper[1] we reported that the parenteral injection of epinephrine into both the intact and the totally thyroidectomized dog resulted in an increase in secretion of thyrotropin from the adenohypophysis. In the intact dog this was evidenced by the development of hyperplastic changes in the thyroid following the daily injection of adrenalin-in-oil. Serum obtained from similarly treated totally thyroidectomized animals, when injected subcutaneously into young guinea pigs not exceeding 200 g in weight, resulted in hyperplastic changes in the thyroids of the treated guinea pigs.

It was further reported that the increase in circulating thyrotropic factor resulting from the injection of epinephrine in totally thyroidectomized dogs reached its peak approximately 4 to 6 days following the beginning of treatment, and thereafter began to diminish despite the continued injection of epinephrine.

The present report is concerned with a study of the role of the adrenals in the above described phenomena. In place of the biological assay method previously employed for the determination of circulating thyrotropin, we used the percentage uptake of parentally administered $I^{131}$ as an index of thyroid activity. This technique has the relative ad-

[1] Soffer, L. J., Volterra, M., Gabrilove, J. L., Pollack, A., and Jacobs, M., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 446.