17149. Electrical Precipitation of Egg-White Inhibitor of Influenza Virus Hemagglutination.*

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During electrophoresis of solutions of crude egg-white or semi-purified egg-white inhibitor[1] of virus hemagglutination, there frequently develops a voluminous, gelatinous precipitate which contains considerable inhibitory activity. This phenomenon and its possible significance are discussed in the present report.

Materials and methods. Egg-white (EW) was collected from 1-day-old hen's eggs. The thick white was obtained apart from outer and inner thin,[2] diluted 4-fold, and homogenized *lightly* in a Waring blendor. Semi-purified inhibitor was prepared from whole or thick EW by precipitation with 7 volumes of 0.1 M KH_2PO_4 and extraction of the precipitate, after washing, with 0.06 M phosphate buffer of pH 7.2[1]. Electrophoresis was carried out in the Tiselius cell with dialyzed solutions in veronal buffer at pH 8.6 or phos-

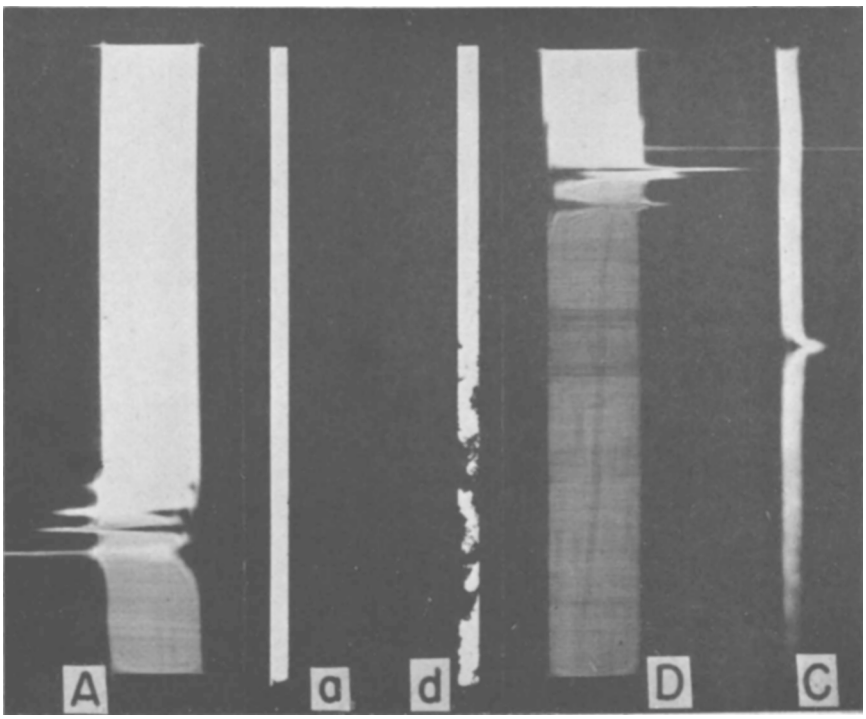

FIG. 1.
Photograph of ascending (a) and descending (d) limbs of Tiselius cell after 3 hr showing precipitate in (d). Diagrams of boundaries and the opacity due to precipitate in ascending (A) and descending (D) limbs after 22 min. The diagram of the slowest component after 215 min. is shown in C.

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[1] Lanni, F., Sharp, D. G., Eckert, E. A., Dillon, E. S., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1949, in press.

[2] Hughes, J. S., and Scott, H. M., *J. Poultry Sci.*, 1936, **15**, 349.

TABLE I.
Properties of Fractions Obtained by Electrophoresis of EW Inhibitor Preparation A200 PII EII in Phosphate Buffer of 0.1 Ionic Strength and pH 7.2.

Fraction	Vol. recovered (ml)	N (γ per ml)	Inhibition titer	Partition of total recovered, N (%)	Partition of total recovered, activity (%)	Purification factor*
Starting material		95.5	12,800			40
Descending limb	6.1	25.8	1,500	19	6.8	17
Ascending "	6.4	17.2	2,700	13	13	47
Bottom cap						
(a) supernatant	2.2	85.6	12,100	22	20	42
(b) precipitate†	1.3	298	62,000	46	60	62
(c) total‡	3.5	165	31,000	68	80	56
Crude EW		16,700	55,500			1.00

* Calculated from the titer/N ratio with reference to the titer/N ratio of crude EW.
† An almost transparent gel. Deducting the nitrogen and inhibitory activity of an equal volume of supernatant, one obtains the value 71 for the purification factor of the solid phase.
‡ Calculated by combining the results for supernatant and precipitate.

phate buffer at pH 7.2, each at 0.1 ionic strength.† Inhibitory activity was titrated against 4 hemagglutinating doses of heated (53°C, 30 minutes) purified swine influenza virus.[1] Nitrogen was determined by direct nesslerization.

Experimental. The following experiment is illustrative. Semi-purified inhibitor, dialyzed against phospate buffer, was submitted to electrophoresis at 5 volts per cm. Within 3 minutes, the whole solution, initially almost clear, acquired a ground-glass appearance. Floccules formed gradually and settled toward the bottom cap. Precipitate remaining in the descending limb after 3 hours is shown in Fig. 1d; settling was often complete in the ascending limb (Fig. 1a). The isolated contents of the 2 limbs and bottom cap, the latter after centrifugation for 10 minutes at 870 g, were analyzed for nitrogen and inhibitory activity. The results (Table I) show (a) concentration of inhibitory activity in the bottom cap; (b) association of the activity with the precipitate; and (c) purification of the inhibitor consequent to electrical precipitation.

Precipitation occurred frequently during electrophoresis of solutions of whole or thick EW, with results analogous to those of Table I. The precipitation seemed more pronounced in veronal than in phosphate buffer and could be prevented in the former by vigorous blending without significant decrease in inhibitory activity. With semi-purified inhibitor, vigorous blending delayed the onset of precipitation, making it possible to record boundaries, as shown in Fig. 1 (A and D). In the absence of precipitation, neither inhibitory activity nor nitrogen was concentrated in the bottom cap. In contrast with precipitates obtained chemically,[1] these precipitates dissolved readily in dilute phosphate buffer. Indeed, resolution occurred in the cell if the current was discontinued shortly after the onset of precipitation.

During the initial period of precipitation in thick EW, the precipitate moved in the cell like a protein component too dilute to register as a distinct peak of refractive index gradient but visible because of its light-scattering capacity. The diffuse edges of this material were visible, one in each limb, moving a little slower than the ovalbumin peaks. The peaks located in the region of precipitation were initially obscured, but reappeared as the precipitate settled, and the final diagram did not differ significantly from diagrams of materials in which no precipitation occurred. With purified inhibitor, the precipitate moved well ahead of the slow component.[1]

Discussion. While the mechanism of precipitation during electrophoresis is not yet clear, the electric field, pH, and inhibitor concentration may be recognized as contributing factors. Moreover, the blending experiments indicate that the physical properties of the

† Precipitate containing inhibitory activity frequently developed during dialysis of 25% whole or thick EW and was removed by centrifugation prior to electrophoresis.

inhibitor in solution are important for the phenomenon and that it is possible to modify some of these properties without appreciably affecting biological activity. Solutions of purified inhibitor have a high viscosity, suggesting the presence of a highly asymmetric component.[1,3] Although it has not been possible to observe birefringence during electrophoresis of these solutions, it is possible that predisposition to aggregation results from orientation of asymmetric particles in the electric field, as in the Kerr effect.[4] The application of the electrical precipitation phenomenon to the final purification of the EW inhibitor is being explored.

Summary. Precipitation occurs in solutions of egg-white or purified egg-white inhibitor of virus hemagglutination submitted to an electric field. The precipitate includes a great part of the inhibitor of the preparations in a state substantially purer than that of the starting material. This result suggests application of the phenomenon to the final purification of the inhibitor.

[3] Eckert, E. A., Lanni, F., Beard, D., and Beard, J. W., *Science*, 1949, **109**, 463.

[4] Kerr, J., *Phil. Mag. and J. Sci.*, 4th Ser., 1875, **56**, 446.

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17150. Propagation of Canine Distemper Virus on the Chorio-Allantoic Membrane of Embryonated Hen Eggs.

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Several attempts have been made by various investigators to cultivate canine distemper virus in the tissues of developing chick embryos. Most of these attempts were not too successful. Thus, Mitscherlich[1] found the virus to survive for 6 days after inoculation onto the chorio-allantoic membrane, but not to be active after subinoculation to other eggs. Plummer[2] in two attempts maintained the virus for 5 and 6 passages respectively on the chorio-allantois but failed to carry it further. Beveridge and Burnet[3] obtained no evidence that canine distemper virus multiplied in chick embryo tissues although various routes of inoculation were tried.

However, Haig[4] reported the successful cultivation of Green's distemperoid virus through 30 serial passages on the chorio-allantoic membrane of chick embryos, and later reported[5] that the virus had been maintained on the chorio-allantoic membrane for more than 90 serial passages over a period of 15 months. During this time the egg-adapted virus was found to have become partially attenuated for ferrets and to have lost some of its highly contagious characteristics for this species. A temperature of 37°C seemed to be the most suitable for incubation of the inoculated eggs since both higher virus titers and more pronounced macroscopic lesions on the chorio-allantoic membranes were attained. Attempts to maintain the virus by serial passage by the yolk-sac, allantoic cavity or amniotic cavity routes of inoculation ended in failure.

For some time attempts have been made in this laboratory to adapt canine distemper virus to some host other than dogs or ferrets. In

[1] Mitscherlich, E., *Dtsch. tierarztl. Wschr.*, 1938, **46**, 497.

[2] Plummer, P. J. G., *Canad. J. Comp. Med.*, 1939, **3**, 96.

[3] Beveridge, W. I. B., and Burnet, F. M., Med. Res. Council, Sp. Report Series, No. 256, 1946, p. 76.

[4] Haig, D. A., *Onderstepoort J. Vet. Sci. and Animal Industry*, 1948, **23**, 149.

[5] Haig, D. A., *J. South Afr. Vet. Med. Assn.*, 1948, **19**, 73.