

## SEPARATION OF HEMAGGLUTININ FROM SONICALLY VIBRATED NEWCASTLE DISEASE VIRUS (NDV)

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Several animal viruses have recently been fractionated into infectious and non-infectious components by chromatography on DEAE-cellulose<sup>1-4</sup>). This study of the chromatographic behavior of sonically vibrated NDV on DEAE-cellulose columns has been undertaken to examine the separation of hemagglutinin from infectious virus particles.

The experiment was set up as follows: Concentrated suspensions of virus in phosphate buffer saline at pH 7.2 were prepared through many steps. The virus concentrate was vibrated in a cup of Kubota's KMS-100 sonic vibration (frequency, 10 kilocycles per second; total power, 100 watts) for 1 hr. The cup was chilled with flowing ice-cold water throughout the vibration. Titrations of hemagglutinin (HA) were performed with chicken erythrocytes according to the pattern method of Salk<sup>5</sup>). Infectivity titers for eggs were expressed in terms of EID<sub>50</sub> per ml according to the method of Reed and Muench<sup>6</sup>).

Typical findings observed with Miyadera strain are shown in Table 1.

TABLE 1. Effect of Sonic Vibration on HA and Infectivity of NDV

Sonic vibration (hr.)	HA/ml	LogEID <sub>50</sub> /ml	EID <sub>50</sub> /HA ratio
0	20496	10.3	10 <sup>6.0</sup>
1	40992	7.4	10 <sup>2.3</sup>

Following sonic vibration for 1 hr., HA titer of NDV suspension was raised up to twice that of the starting material, while the infectivity titer was dropped to one thousandth.

Then, 2.0 ml of the sonically vibrated NDV suspension, previously dialyzed against 0.05 M NaH<sub>2</sub>PO<sub>4</sub> solution at pH 6.0, was run onto columns (1 × 15 cm) packed with DEAE-cellulose (Brown Co., U.S.A.), and eluted with the same

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solution containing increasing concentrations of NaCl and finally with distilled water. The final NaCl concentration was 0.8 M. Aliquots (5.0 ml) of eluate were collected and assayed for HA and infectivity.

Results of a typical experiment are shown in Fig. 1. Two fractions of

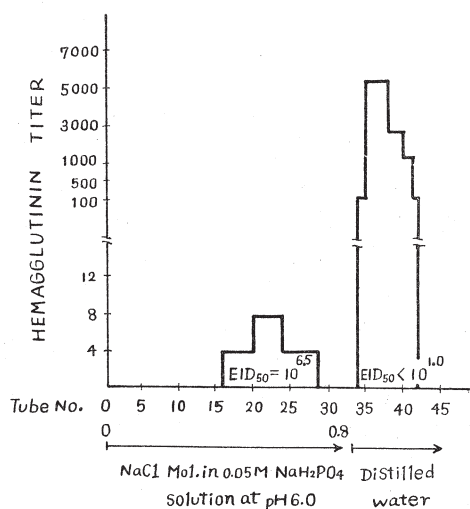


FIG. 1. Separation of hemagglutinin and infectious particles from 2.0 ml sonically vibrated NDV suspension by chromatography on DEAE. Hemagglutinin titer reciprocal of dilution giving "one-plus" agglutination. Elution with distilled water started tube 33.

hemagglutinating activity were observed. One fraction contained  $10^{6.5}$  EID<sub>50</sub> per ml and had a EID<sub>50</sub>/HA ratio  $10^{5.7}$  (infectious virus), and the other fraction contained  $<10^{1.0}$  EID<sub>50</sub> per ml, reaching maximum titer 5120 HA per ml (hemagglutinin).

These results showed the disruption of NDV by sonic vibration for 1 hr.

Separation of hemagglutinin from sonically vibrated NDV conveniently achieved on DEAE-cellulose columns, might be useful in the manufacture of NDV vaccine, and for physical, and immunological studies on this material.

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