

Cell Fusion by Various Strains of Newcastle Disease Virus and Their Virulence

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Some properties of eight strains of Newcastle disease virus (cell-fusing ability, hemolysin, heat stability of hemagglutinin or of hemolysin) do not correlate with virulence of these strains.

Newcastle disease virus (NDV), like some other paramyxoviruses, induces fusion in a variety of animal cells (2, 3), and this property can be accurately measured (3). In this study, it was correlated with other properties of this virus, such as hemolysin, hemagglutinin, heat stability, and virulence.

The strains used in this study are described in Table 1. All viruses were grown in the allantoic fluid of 10-day-old chick embryos, starting from limiting dilution inocula. One strain (K) was also obtained in the form of infected calf-kidney culture fluid. All strains were centrifuged and purified as follows: (i) $600 \times g$ for 20 min (International);

of hemagglutinin and hemolysin at 56 C, chick embryo infectivity, intracerebral pathogenicity index in 1-day-old chicks, hemolysin, and fusing ability. Hemolysin and fusing ability of LU-106 cells were measured, by the method of Kohn (3), in viral suspensions adjusted to the same hemagglutinating (HA) activity (1,600 HA units per 0.2 ml). Hemolysin was expressed as the percentage of optical density (OD) at 650 nm of a virus-treated red blood cell (RBC) suspension in relation to OD corresponding to total hemolysis of the same amount of RBC. Fusing activity of the viral preparations was expressed as fusion index, i.e., the average number of nuclei per

TABLE 1. *Virus strains and sources*

Virus strain	Designation	Source	Date	Virulence class
B1-Hitchner	B	Brandly	1963	Lento
Komarov	K	Hornstein	1967	Lento
Mass-MK-107 (53)	M	Hanson	1960	Meso
Israel-1968	SP	Kohn	1968	Velo
Iowa 125 (1947)	NI	Hanson	1964	Meso
California (RO) 1944	RO	Hanson	1964	Velo
England-Herts (1933)20	EH	Hanson	1964	Velo
Italy, Milan (1945)41	IM	Hanson	1964	Velo

(ii) $80,000 \times g$ for 60 min (Spinco L, head 30); (iii) resuspension of pellets in a 1/30th volume in phosphate-buffered saline (PBS); (iv) $12,000 \times g$ for 60 min [Spinco L, SW 29.1 rotor, on 65% sucrose in D_2O overlaid with 15% sucrose in buffer composed of 2 ml of 5 M NaCl, 1 ml of 1 M tris(hydroxymethyl)aminomethane, and 1 ml of 0.1 M ethylenediaminetetraacetic acid in 100 ml of water]. The final product collected from the interface of 65/15% sucrose was suspended in PBS and frozen at -60 C .

The following properties of these virus strains were examined: hemagglutinin, plaque formation on chick fibroblast monolayers (1), heat stability

syncytium (or cell) formed in a confluent monolayer of LU-106 or in human amnion FL cells after 3 hr of incubation with 0.2 ml of virus in 60-mm plastic petri dishes. Heat stability of hemagglutinin and hemolysin were measured after incubation of the viral suspensions at 56 C for different periods of time. The time, in minutes, required for 90% inactivation was arbitrarily chosen as the indicator of heat stability. For intracerebral pathogenicity index, 1-day-old white Leghorn chicks were intracerebrally inoculated with 0.05 ml of 8 HA units of each virus. Symptoms and death were registered daily, and the data

were calculated by the procedures of the Poultry Disease Subcommittee (5).

The various properties of these viruses are represented in Fig. 1. There seems to be no correlation between the various properties of these viruses and their virulence or classification. The fusing and hemolytic abilities of several strains of NDV are independent of HA activity. Heat stability of either hemagglutinin or hemolysin is also independent of the other properties of these viruses. Other properties of various strains of

NDV, such as sensitivity to ether, serological differences, and the amount of neuraminidase, do not seem to correlate with virulence (7). The only property of NDV strains which may be indicative of their virulence is plaque size on chick fibroblast monolayers (6; A. Ganoff, *Bacteriol. Proc.*, 1955, p. 74-75).

In our study, plaque size was roughly correlated with virulence. Strains B and Komarov did not produce any plaques.

The acquisition of fusing property or of hemolysin seems to be host-dependent. For example, strains B and RO, strongly hemolytic when grown in chick embryos, were only weakly hemolytic when harvested from the allantoic fluid of duck embryos, but their fusing properties were not changed. The fusion ability of Komarov strain grown in eggs was high as compared to that associated with the same strain cultivated in calf kidney, although the hemolytic activities of these two substrains were similar.

All attempts to separate the fusion factor from the virions by chromatography on a hydroxyapatite column or by density gradient centrifugation (*unpublished data*) were unsuccessful. That the integrity of the virion envelope is essential for its fusing activity and that hemolysin, fusion factor, hemagglutinin, neuraminidase, and adenosine triphosphatase are separate entities were indicated when these properties were selectively destroyed by freezing and thawing, heating, phospholipase A, proteolytic enzymes, and ultrasonic treatment (3, 4).

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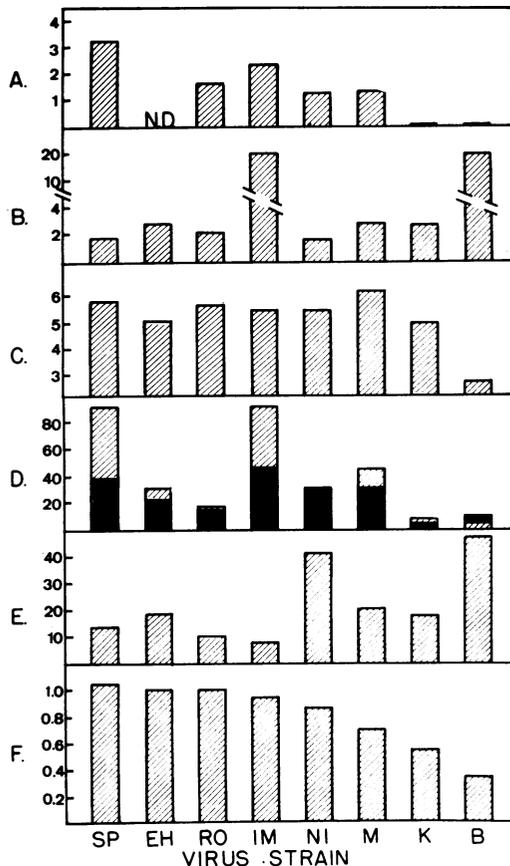


FIG. 1. Comparison of various properties of NDV strains. Strain designation as in Table 1. (A) Plaque size, diameter in millimeters (ND, not done). (B) Fusion index ($\times 4$). (C) Infectivity associated with 1 HA unit of virus in 10-day-old chick embryos. (D) Heat stability expressed as minutes required for 90% inactivation at 56 C; dark area, hemolysin; light area, hemagglutinin. (E) Hemolytic activity in per cent of total hemolysis of a 2% RBC suspension. (F) Intra-cerebral pathogenicity index in 1-day-old chicks.