THE ANTIVIRAL ACTIVITY OF NEWCASTLE DISEASE IMMUNE SERA

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The diagnostic value of the hemagglutination inhibition test for Newcastle disease has been established for some years (Brandly et al., 1946). The question of the relationship of the hemagglutination inhibition activity (HI) of immune sera to virus-neutralizing activity (SN) or to the refractivity of the host to challenge infection is still one of both fundamental and practical importance. Data have been gathered in this laboratory that make it difficult to ascribe the various expressions of antiviral activity to a single antibody.

EXPERIMENTAL METHODS AND RESULTS

One strain of Newcastle disease virus (NDV), a chicken-embryo-adapted culture of low virulence for chickens, designated KD-NJ-1945, was used in both the living and killed state, in all the original experiments. The living inocula consisted of infected allantoamnionic fluids having an HI titer of 1:1,280 and an embryo infectivity titer of $20 \times 10^{-9}$ per ml. Killed virus preparations were obtained by treating infected allantoamnionic fluids with 0.4 per cent formalin and subsequently holding them at 4 C for 7 days.

White Leghorn hens and mature Holstein cows were used as sources of sera. The route of administration of virus was intravenous in all cases.

The HI titer and the SN titer were determined according to the procedure outlined previously (Brandly et al., 1947). The HI test for NDV, aside from a few minor modifications in the size of the tube and the concentration of red blood cells, was essentially the same as that of Salk (1944) for influenza virus. The SN test was also similar to the one used for influenza virus and described by Burnet (1943). The 50 per cent end point, however, was always estimated on the basis of death of the embryo and not on the presence or absence of hemagglutinin.

A dilute volume modification of the low-temperature alcohol precipitation method was used to prepare the serum fractions for study. This phase of the work was done in the Department of Physical Chemistry, University of Wisconsin. The methods and the electrophoretic studies have been reported by Hess and Deutsch (1949).

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Evaluation of chicken sera. Four days after 6 hens had received 5 ml of formalin-activated NDV intravenously, HI titers of 1:20 and SN titers of 10⁴ were demonstrable. Peak titers of 1:80 and 10⁵ for HI and SN, respectively, were obtained at the eighth day. The HI titers decreased after that time, and within 6 weeks all HI activity had disappeared without, however, there having occurred much loss in the SN titer, which was still 10⁴. In 3 of 4 remaining birds SN titers of 10⁴ or greater could be demonstrated as long as 6 months after injection.

Another group of 6 hens received 1 ml each of live virus. The resultant titers persisted longer and differences among individuals were more apparent, yet the same general trend developed. Six months after injection all birds had high SN titers but only one retained a HI titer. In one instance a peak SN titer (10⁶) was attained after the HI activity could no longer be demonstrated.

In field trials of 3 commercial live virus vaccines about 5 per cent of 51 sera tested were negative to the HI test 6 months after vaccination, and 20 per cent had very low titers.

Fractionation of immune bovine sera. Two cows that had received repeated injections of both formalin-killed and living NDV were bled and the sera pooled. The serum was separated initially into supernatant and crude globulin portions. From the latter were prepared gamma- and beta-globulins. The gamma-globulin was subsequently fractionated into gamma-1 and gamma-2 components. The final fractionation of gamma-1 globulin yielded gamma-1a and gamma-1b. All the fractions could be differentiated serologically and electrophoretically, as pictured in figure 1.

Figure 1. The activity of various fractions of bovine serum against Newcastle disease virus. Prepared from hyperimmune sera by precipitation with alcohol in the cold.
For the study of serological properties, the lyophilized fractions were reconstituted in a pH 7.2 phosphate buffer to a concentration comparable to that in which the fraction existed in whole serum. The antibody activity appeared to be essentially unaltered by the purification process since the crude globulin and gamma-globulin retained the full activity of the original serum. The subsequent fractionation procedure resulted in a separation of the HI and SN expressions of NDV antibody activity. The SN titer was demonstrated in both gamma-1 and gamma-2, but HI function was evident only in gamma-1. Of the subfractions of gamma-1, gamma-1b exhibited SN activity but lacked HI activity, and gamma-1a lacked both activities.

**DISCUSSION**

Since individual response to various antigenic stimuli is not uniform, the level of antibody and the degree of immunity will vary from host to host. It therefore may be presumed that a certain activity, e.g., HI, may disappear from the serum of an individual before another activity, e.g., SN, and that both functions may subside simultaneously in another animal. The rather rapid decrease in HI antibodies has been noted by others in the face of the general assumption that lack of HI indicated inexperience with NDV. This may not be the case, nor does it appear that refractivity to infection will persist for the life of the hen following natural infection. Borstein et al. (1949) found that a year after vaccination with live virus most hens had a low or negative HI titer and were susceptible to reinfection.

Whether the HI activity is the result of an antibody or function distinct from the one that results in the neutralization of the virus in vitro (SN) cannot be determined from the evidence obtained by serum fractionation. It would nevertheless appear possible that the HI activity or manifestation requires both SN antibody or capacity and some unknown factor. However, other laboratories had reported the appearance of HI activity before SN (Osteen and Anderson, 1948; Fabricant, 1949), and we have obtained two such sera among hundreds examined. A chicken receiving strain CG179 live virus intravenously yielded sera having an HI titer of 1:320, yet lacked neutralizing capacity. Similarly, another chicken receiving strain 11914 live virus intravenously produced 1:160 HI titer at 7 days and lacked neutralizing activity. The problem is complicated by certain dilution and time factors of antibody-virus interaction that have not been standardized.

The HI activity may be the result of an antibody distinct from the one producing neutralization of the virus. Whether either HI or SN function is comparable to neutralization in vivo or a disease-resistant state of the bird is not known.

**SUMMARY**

Hemagglutination inhibition and serum-neutralizing activity against Newcastle disease virus generally appeared simultaneously following exposure of chickens to living or formalin-killed Newcastle disease virus.

In general, the peak titers of hemagglutination inhibition and serum neutralization were not reached simultaneously or maintained equally.
The serum-neutralizing titers in chickens studied persisted much longer than the hemagglutination inhibition titers.

Gamma-globulin fractions of immune bovine serum were obtained that contained high levels of serum-neutralizing activity and that lacked hemagglutination inhibition activity.

It appears possible that hemagglutination inhibition and serum-neutralizing activities may be the function of separate antibodies.

REFERENCES


