Ferritin-tagged Antibody Cross-reactions among Rinderpest, 
Canine Distemper, and Measles Viruses

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SUMMARY

Rinderpest, canine distemper, and measles viruses were all grown in Vero cell tissue cultures. Each virus was treated with ferritin-tagged antibodies to each of the viruses. The cross-reactions and electron micrographs showed that they all are antigenically and morphologically similar.

The examination of the development and morphogenesis of the viruses of rinderpest, canine distemper and measles with the electron microscope has been reported in several publications (Matsumoto, 1966; Nakai & Imagawa, 1969; Nakai, Shand & Howatson, 1969; Lawn, 1970; Tajima et al. 1971; Tajima & Ushijima, 1971; Cornwell et al. 1971). In these, the appearance and developmental stages of the three viruses have been described either singly or in pairs, pointing out the very definite similarities in their morphology and the uniqueness of their mature structure. At maturity, the particles found in the intercellular spaces are very pleomorphic, very large (as much as 500 µm across) and completely lacking any core structure. The final particles apparently consist of nucleoprotein strands surrounded by a membrane containing virus-specific antigens. Since all these viruses are antigenically related, it was proposed that the three of them be grown in a single tissue-culture system and that the developing and mature particles be treated with ferritin-tagged antibodies to each of the three. This report describes the results of these experiments.

The Vero line of African green monkey kidney cells was received from Dr A. J. Kniazeff, Naval Biological Laboratory, Oakland, California, in 1967 and had undergone 220 passages since that time. These cells were passed weekly and seeded into disposable plastic flasks (Falcon Plastics, Oxnard, California, no. 3012) of 25 cm² area. The cells were grown in Eagle’s balanced salt solution with 0.5% lactalbumin hydrolysate, 0.005% yeast extract, and 10% foetal calf serum which had been inactivated at 56 °C for 30 min. One hundred units of penicillin and 100 µg streptomycin per ml of medium were used to suppress contamination. After infection the maintenance medium used was the same as that described above but with only 2% foetal calf serum.

The Kabete O strain of virulent rinderpest virus was received from the East African Veterinary Research Organization, Maguga, Kabete, Kenya, passed 3 times in primary bovine kidney cells and then passed 3 times in Vero cells. The final infectious fluid was stored at −40 °C for the stock virus and had a titre in Vero cell cultures of 10⁴ TCID₅₀/0.5 ml.

Canine distemper virus was supplied by Dr J. A. Gourlay, National Animal Disease Laboratory (NADL), Ames, Iowa, and passaged directly into Vero cells. After three passages, infectious fluid was clarified by centrifuging and frozen at −40 °C. The titre was 10⁴ TCID₅₀/0.5 ml.

Measles virus, Enders strain, was supplied by Drs J. C. Wagner and E. B. Seligmann, Division of Biologics Standards, National Institutes of Health (DBS, NIH), Bethesda, Maryland, as freeze-dried lot 2 of April 1964. It was resuspended and passed directly into Vero cells. Infectious fluid from the 2nd passage was clarified by centrifuging and frozen at −40 °C. It had a titre of 10⁴ TCID₅₀/0.5 ml.

Reference antiserum to measles lot 1 and reference immune globulin (human) lot 175 was
Table 1. Cross-reactions of rinderpest, canine distemper and measles viruses with ferritin-tagged antibody

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Rinderpest</th>
<th>Canine Distemper</th>
<th>Measles</th>
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<tbody>
<tr>
<td>Rinderpest</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Canine distemper</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Measles</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

supplied by Drs Wagner and Seligmann, DBS, NIH, Bethesda, Maryland. Antiserum to canine distemper from dogs, lot 102, was supplied by Dr Gourlay, NADL, Ames, Iowa. The rinderpest antiserum was prepared at Plum Island by inoculation of colostrum-deprived calves with Kabete O rinderpest virus passed in primary bovine embryo kidney cells at the East African Veterinary Research Organization. Serum obtained from these animals 28 days after an inoculation with virulent virus, 23 days after the initial inoculation with attenuated virus, neutralized 100 TCID₅₀/ml of rinderpest virus at a dilution of 1:1600.

Infected tissue culture flasks were examined for c.p.e. after inoculation of the cell layers with 1 ml of virus suspension followed by a 60 min absorption period at 37 °C and the addition of 5 ml of maintenance medium and incubation at 37 °C. Cells were ready for electron microscopic examination 7 to 12 days after infection depending on the virus.

Washed cell suspensions from infected cells were treated with one of the following: (1) 0.2 ml of canine distemper antiserum conjugated to ferritin; (2) 0.2 ml of human gamma globulin against measles conjugated to ferritin; and (3) 0.2 ml of bovine antiserum to rinderpest followed by washing and 0.2 ml of rabbit anti-bovine serum conjugated to ferritin. The treated cells were then washed 3 times with Sorenson’s buffer pH 7.2 and fixed with 2 % glutaraldehyde in Sorenson’s buffer. Further fixation with osmium was followed by dehydration in graded alcohols and embedment in Epon.

In order to observe the effects of changes in the concentrations of reactants, infected cells were treated with one or more dilutions of ferritin conjugate. The thin sections were examined in the electron microscope and random micrographs made of areas showing developing or mature virus particles. These were then examined to determine whether or not the ferritin conjugate had tagged the virus surface and, if so, how intensely; Table 1 shows these results. All three viruses reacted to some degree with each of the antisera. The intensity of reaction is indicated in Table 1 by one or more + signs. The rinderpest antiserum combined with the indirect ferritin conjugate resulted in the heaviest tagging. By comparison, an in vitro neutralization test with equivolume mixtures of 100 TCID₅₀ of rinderpest virus and serial two-fold dilutions of antiserum on primary calf kidney tissue cultures was used. The dilution of serum at which c.p.e. were seen (+) and not seen (−) after appropriate incubation at 37 °C for a period of 2 to 3 weeks is shown in the following table:

<table>
<thead>
<tr>
<th>Virus (100 TCID₅₀)</th>
<th>Rinderpest antiserum dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinderpest</td>
<td>1/1600+, 1/3200−</td>
</tr>
<tr>
<td>Canine distemper</td>
<td>1/10+, 1/40−</td>
</tr>
<tr>
<td>Measles</td>
<td>1/10+, 1/20−</td>
</tr>
</tbody>
</table>

In the electron micrographs (Fig. 1) the budding stages of rinderpest virus are shown along with a portion of the nucleoprotein strands (NP) found in the development of these
Fig. 1. Early stages of developing rinderpest virus specimen treated with canine distemper antiserum ferritin.

Fig. 2. Nucleoprotein tubules of rinderpest infected Vero cell tagged with rinderpest bovine antiserum and rabbit anti-bovine serum conjugated to ferritin.

Fig. 3. Canine distemper virus treated with rinderpest bovine antiserum and rabbit anti-bovine serum conjugated to ferritin.

Fig. 4. Rinderpest virus treated with homologous antiserum and ferritin conjugate as in Fig. 3.

Fig. 5. Variety of antigenic sites shown with canine distemper virus and the rinderpest antibody-ferritin system.

Fig. 6. Human measles virus in Vero cells treated with ferritin conjugated to human gamma globulin against measles.
viruses. The arrows point to antigenic regions which in this example have been tagged with antiserum to canine distemper virus conjugated to ferritin.

Fig. 2 shows a very clear region of nucleoprotein tubules from a disrupted Vero cell infected with rinderpest virus and treated with rinderpest antiserum and a rabbit anti-bovine serum ferritin. The ends of the tubules and those regions around the periphery which have been penetrated by the antiserum show positive tagging. The mature virus particles shown in succeeding micrographs (Figs. 3 to 6) show only fragmented tubule structures. As the viruses mature the tubules become less evident even though all fixation and embedment was the same for all samples.

The tagging of both canine distemper virus (Fig. 3) and rinderpest virus (Fig. 4) with the rinderpest antiserum and ferritin conjugate are of about equal intensity. The degree varies somewhat depending on the concentration of reagents and the amount of washing of the pellets, but the rinderpest antiserum seems almost as effective in both instances.

The many bizarre shapes and sizes of the antigenic structures in these viruses are shown in Fig. 5. In this instance, canine distemper virus is again tagged with the rinderpest antibody-ferritin system. None of the three viruses have any central structure such as a nucleoid and are thereby different in morphology from most other virus groups.

The many variations in morphology may also be illustrated in Fig. 6 where measles virus infected cells were treated with human measles globulin conjugated to ferritin. The antigenic sites (arrows) show a very delicate attachment of ferritin indicating a very low affinity for the human antibody and the virus grown in a non-human cell line. Both spots and short line segments of antigen occur on the developing structures (arrows).

Rinderpest, canine distemper, and measles viruses are morphologically almost indistinguishable. Their structure is very similar to that of mumps virus (Duc-Nguyen & Rosenblum, 1967). When grown in Vero monkey kidney cells thy all have antigenic cross-reactions with antiserum against each of them prepared in the appropriate species. Similar results were shown using fluorescent antibody reactions by Yamanouchi et al. (1970).

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REFERENCES


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