Intrinsic Interference between Different Enveloped RNA Viruses

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SUMMARY

Intrinsic interference is not limited to Newcastle disease virus: vesicular stomatitis (VSV) and orthomyxoviruses can be similarly inhibited at the stage of RNA synthesis. Detailed investigations of the interference of fowl plague virus (FPV) by VSV show: (1) adsorption of the interfering virus is not responsible; (2) the synthesis of all FPV virus components is affected, if the interfering virus is present during the initial stages of FPV replication and (3) competition of the interfering RNA polymerase with heterologous templates can be excluded by experiments carried out in vitro.

INTRODUCTION

Several unrelated viruses have been found to induce an inhibition of the multiplication of Newcastle disease virus (NDV) as determined by the haemadsorption-negative plaque test (Marcus & Carver, 1965, 1967; Beard, 1967; Wainwright & Mims, 1967; Seto & Carver, 1969; Marcus & Zuckerbraun, 1970). The NDV-refractory state develops only in those individual cells of a population actually infected by the inducing virus, and was termed intrinsic interference. Intrinsic interference thus differs from mutual exclusion at the adsorption step. It is defined as `a viral genome-induced cellular state of resistance to challenge by high multiplicities of NDV, coexistent with a state of susceptibility to a broad spectrum of other viruses' (Marcus & Carver, 1967). Unlike interferon-mediated interference, intrinsic interference does not depend on new cellular RNA synthesis.

We have found that this kind of interference is not confined to NDV. Semliki Forest virus (SFV) can induce intrinsic interference also of vesicular stomatitis (VSV) and fowl plague viruses (FPV). Furthermore, influenza viruses are inhibited by VSV, SFV and NDV.

METHODS

Virus strains. The various influenza strains used were the same as described recently (Scholtissek & Rott, 1969). Furthermore, the NDV strain ITALIEN, the SFV strain OSTERRIETH, and the VSV, type Indiana, were investigated.

Tissue cultures and virus multiplication. Primary chick fibroblasts at a density of $2 \times 10^7$ cells in Petri dishes of 9 cm in diameter were infected 46 h after seeding. In a few experiments BHK-cells were used. The cells were infected with an input multiplicity of 10 to 50 p.f.u./cell. At the times after infection indicated, virus yields were determined. Intracellular virus was assayed after breaking the cells by 3 cycles of freezing and thawing. Since the titres of the virus activities in the culture medium were as a rule only about 10% or less of that of the cell-associated ones, only the latter have been listed in the Tables and Figs. Thus there was no significant interference with virus release.

Virus infectivity was determined by the plaque technique in primary chick fibroblasts. In order to determine the yield of p.f.u. of either virus after a double infection, one virus was
neutralized by mixing samples of the cell extracts with specific antiserum directed against one of the two viruses used. The extract–antibody mixtures were left at room temperature for 30 min and diluted serially for the plaque test.

*Haemagglutination tests* were performed in the usual manner with chick erythrocytes (for influenza viruses at room temperature, for NDV at 4 °C). Haemagglutinating units (H.A.U.) represent the reciprocal of the haemagglutination titre.

*Neuraminidase activity* was determined as before (Drzeniek, Seto & Rott, 1966) using bovine sialolactose as substrate. The amount of neuraminidase which liberated 1 /μM-sialic acid from the substrate/min at 37 °C was defined as one enzyme unit (EU).

*RNP-antigen* was determined by complement fixation (Schmidt & Lennette, 1965).

*Sera.* The following antisera were used for virus neutralization: FPV and NDV specific antisera were prepared in chicken according to Rott (1965); SFV and VSV specific antisera from rabbits were obtained by Dr M. Mussgay, Tübingen. Volumes of 0.1 ml of the respective antisera neutralized at least 10^8 p.f.u./ml. Monospecific antisera against RNP-antigen was prepared in rabbits. Contaminating antibodies against host material and envelope components were removed with solid immunabsorbents (Becht, 1971).

**Determination of virus RNA.** The RNA polymerase test was performed according to Scholtissek (1969). Cytoplasmic fractions of four pooled cultures were prepared at the times after infection as listed in Tables 3 and 5. The RNA labelled during a 20 min pulse at 36 °C was isolated and dissolved in 2 ml of 5 mM-tris HCl, pH 7.4, containing 1 mM-EDTA. Samples were heated for 5 min at 100 °C and rapidly chilled. To one sample RNase was added immediately, to a second one saturating amounts of non-labelled fowl-plague particle RNA (7 μg/sample) and to a third one an excess of non-labelled fowl-plague complementary RNA was added prior to hybridization. A fourth sample was self-annealed.

FPV particle and complementary RNA was determined in the presence of newly synthesized cellular RNA by specific hybridization with an excess of non-labelled virus-specific RNA (Scholtissek & Rott, 1970). For each time point in Table 4 the cells of four culture plates were pooled. To each culture 25 μCi [3H]-uridine was added at the times after the first infection as indicated. The cells were processed 1 h later and the RNA was isolated from the cytoplasmic extract. Samples of the dissolved RNA were treated as described above. The non-labelled particle RNA of FPV used for hybridization contained 0.7 mg/ml virus RNA. The absolute concentration of the complementary RNA is not known. The preparation was titrated before use in order to be sure to work under saturating conditions. In a preliminary experiment it was found that after swelling of the infected cells in a hypotonic buffer and homogenization, about 90% of virus RNA could be isolated from the cytoplasmic extract while only 50% of the radioactive cellular RNA was found in the fraction. In this way the background of RNase-resistant cellular RNA was negligible. NDV–RNA in the presence of heterologous virus RNA was determined by specific hybridization with RNA isolated from NDV particles according to Kingsbury (1966).

**Labelling of the proteins of the infected cells.** Confluent monolayers were inoculated with an input multiplicity of 10 to 50 p.f.u./cell. After an adsorption period of 30 min, the inoculum was removed and replaced by minimal medium (Eagle & Habel, 1956). The medium was removed 6 h after infection and replaced by medium containing radioactive isotopes. These were used at the following concentrations: [14C]-protein hydrolysate, 10 μCi/ml; [3H]-amino acids, 100 μCi/ml. The medium containing the isotopes was left on the cells for 1 h. The monolayer was washed 3 times with cold phosphate buffered saline (PBS), scraped off in 1 ml PBS with a rubber policeman, and stored at −20 °C.

**Polyacrylamide gel electrophoresis.** The cells were disrupted by ultrasonic vibration.
Samples of 100 μl were used for gel electrophoresis. Proteins were dissociated with SDS and mercaptoethanol. They were separated by electrophoresis on 10% acrylamide gel as described (Caliguiri, Klenk & Choppin, 1969). The slicing and processing of gels for the determination of radioactivity by liquid scintillation has been reported previously (Klenk, Caliguiri & Choppin, 1970).

Radioisotopes. Guanosine-5'-triphosphate-8[3H] (9 Ci/mmol) was obtained from Schwarz Bio Research, Orangeburg, N.Y., U.S.A. Uridine-5-[3H] (30 Ci/mmol), protein hydrolysate-[14C] (U), L-leucine-4,5-[3H] (1.0 Ci/mmol), L-valine-2,3-[3H] (1.5 Ci/mmol), and L-tyrosine-3,5-[3H] (1.0 Ci/mmol) were purchased from Radiochemical Centre, Amersham, Buckinghamshire, England.

RESULTS

Multiplication of various RNA-containing viruses in mixedly infected cells

Table 1 shows the results of mutual cross-infections of chick fibroblasts with SFV, VSV, NDV and FPV. It can be seen that SFV interferes with the synthesis of all the other viruses tested. In addition, VSV prevents the multiplication of FPV. VSV does not affect NDV and SFV, NDV does not interfere with VSV, FPV and SFV, neither does FPV with NDV, VSV and SFV. Essentially the same results were obtained, when the primary infected cells were super-infected 2 h later with the exception that in this case preinfection with NDV, too, caused

<table>
<thead>
<tr>
<th>Viruses assayed</th>
<th>SFV (p.f.u./ml)</th>
<th>VSV (p.f.u./ml)</th>
<th>NDV (p.f.u./ml)</th>
<th>H.A.U.</th>
<th>FPV (p.f.u./ml)</th>
<th>H.A.U.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFV</td>
<td>--</td>
<td>&lt; 10⁶</td>
<td>1.5×10⁶</td>
<td>4</td>
<td>3×10⁶</td>
<td>&lt; 4</td>
</tr>
<tr>
<td></td>
<td>(3.2×10⁹)</td>
<td>(2.5×10⁹)</td>
<td>(246)</td>
<td></td>
<td>(1.2×10⁹)</td>
<td>(1042)</td>
</tr>
<tr>
<td>VSV</td>
<td>3×10⁸</td>
<td>--</td>
<td>2×10⁷</td>
<td>98</td>
<td>3.5×10⁶</td>
<td>&lt; 2</td>
</tr>
<tr>
<td></td>
<td>(5×10⁸)</td>
<td>(2×10⁷)</td>
<td>(224)</td>
<td></td>
<td>(1.3×10⁹)</td>
<td>(1024)</td>
</tr>
<tr>
<td>NDV</td>
<td>1×10⁸</td>
<td>1×10⁷</td>
<td>--</td>
<td>--</td>
<td>9×10⁶</td>
<td>770</td>
</tr>
<tr>
<td></td>
<td>(2×10⁹)</td>
<td>(1.7×10⁷)</td>
<td>--</td>
<td></td>
<td>(4.3×10⁷)</td>
<td>(512)</td>
</tr>
<tr>
<td>FPV</td>
<td>5×10⁷</td>
<td>7.5×10⁷</td>
<td>2.5×10⁷</td>
<td>512</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>(1.5×10⁸)</td>
<td>(1.1×10⁸)</td>
<td>(770)</td>
<td></td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Mixtures were prepared with paired viruses; the cells were infected with this mixture; 8 h after infection yield of the viruses listed in the upper line was determined by plaque assays employing specific antisera to suppress growth of the other viruses in the test. Controls in parentheses: p.f.u. or H.A.U. for the respective virus grown without any second virus.

Table 2. Double infection of FPV and u.v.-inactivated VSV

<table>
<thead>
<tr>
<th>FPV</th>
<th>H.A.U.</th>
<th>p.f.u./ml</th>
<th>VSV (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV</td>
<td>1024</td>
<td>1.5×10⁶</td>
<td>--</td>
</tr>
<tr>
<td>VSV</td>
<td>--</td>
<td>--</td>
<td>1×10⁸</td>
</tr>
<tr>
<td>VSV (u.v.)</td>
<td>--</td>
<td>--</td>
<td>4×10⁸</td>
</tr>
<tr>
<td>FPV + VSV</td>
<td>&lt; 2</td>
<td>3×10⁴</td>
<td>5.5×10⁸</td>
</tr>
<tr>
<td>FPV + VSV (u.v.)</td>
<td>256</td>
<td>5×10⁷</td>
<td>--</td>
</tr>
</tbody>
</table>

U.v.-inactivated VSV and infectious FPV were mixed and applied to chicken fibroblasts; 8 h later the virus yields were determined.
Fig. 1. Formation of p.f.u. in the presence of VSV. VSV was added to FPV infected cells at the time indicated. Infectious FPV was assayed both at the time of addition of VSV (●—●) and 8 h after FPV infection (○—○).

Fig. 2. Formation of neuraminidase, haemagglutinin and RNP-antigen in the presence of VSV. Cultures were mixedly infected as in Fig. 1. Assays and controls were made 8 h after infection. As a control (×) the virus titre without any addition of VSV 8 h after infection of FPV was determined. ○—○, neuraminidase; ●—●, haemagglutinin; △—△, RNP-antigen.

a considerable reduction of infectious FPV (about 2 log units). This delayed effect can be explained by the fact that in comparison to SFV and VSV, NDV grows more slowly. On the other hand, the rapidly multiplying SFV inhibited the growth of the other viruses even when added 2 h after the primary infection. In the following, the inhibition of FPV by VSV was studied in more detail.

**Requirement of active VSV for the inhibition of FPV**

VSV inactivated with u.v. to a survival level of $4 \times 10^4$ p.f.u./ml was compared with active virus ($1 \times 10^8$ p.f.u./ml) in its capacity to prevent FPV multiplication. The results are presented in Table 2. As can be seen, the infectivity of VSV particles is essential to inhibit the growth of FPV. This shows that adsorption of the interfering virus cannot be responsible for the effect.

**Influence of VSV on various stages of FPV multiplication**

In order to obtain some information on the intracellular effect of VSV on FPV replication, VSV was added at different stages of the infectious cycle. The production of RNP-antigen, haemagglutinin, neuraminidase and p.f.u. was examined.

As shown in Figs. 1 and 2, VSV blocked the formation of p.f.u., neuraminidase and haemagglutinin completely only when added simultaneously with FPV. Under these conditions small amounts of RNP-antigen were still detectable. When VSV was added 2 h before FPV infection, RNP-antigen synthesis was completely blocked. The yield of virus material increased as the period between FPV infection and superinfection with the interfering VSV was extended until a minimum inhibitory effect was reached at 4 h. It should be stressed that when VSV was added 1 h after primary infection, all FPV components were detectable although in highly reduced amounts.

The replication cycle of VSV was not influenced in mixedly infected cells.
Fig. 3. Polyacrylamide gel electrophoresis of VSV- and FPV-specific proteins in simultaneously infected cells.

Upper panel. Co-electrophoresis of doubly infected cells labelled with a mixture of $[^{14}C]$-amino acids (---) and of VSV-infected cells labelled with a mixture of $[^{3}H]$-amino acids (○---○). The arrows indicate the virus-specific polypeptide peaks in VSV-infected cells. In fractions 10 to 50 the difference between the curve of the doubly infected cells and the VSV-infected cells has been determined. The resulting curve (△—△) has a single peak corresponding to the nucleocapsid protein (NP) of FPV.

Lower panel. Co-electrophoresis of doubly infected cells labelled with a mixture of $[^{14}C]$-amino acids (---) and of FPV-infected cells labelled with a mixture of $[^{3}H]$-amino acids (○---○). The arrows indicate the virus-specific polypeptide peaks in FPV-infected cells. In each case cells were labelled 4 h after infection by pulse with $[^{14}C]$-amino acids (10 µCi/ml) or $[^{3}H]$-amino acids (100 µCi/ml) for 1 h.

Polypeptide pattern of cells doubly infected with FPV and VSV

Fig. 3 shows that cells simultaneously infected with VSV and FPV contained 4 distinct polypeptide peaks. The pattern corresponds well to that of cells infected only with VSV (Wagner, Snyder & Yamazaki, 1970). It reveals the VSV-specific envelope glycoprotein G, the nucleocapsid protein N, the non-structural protein NS, and the carbohydrate-free surface protein S. In addition, a comparison of FPV-specific proteins with the polypeptides of doubly infected cells shows that most FPV-proteins were absent. The internal protein P, the haemagglutinin proteins HA₁ and HA₂, the carbohydrate-free envelope protein M, and the non-structural proteins HA and NS of FPV could not be detected in doubly infected cells. However, the peak with the slowest migration rate in doubly infected cells was located between FPV-protein P and FPV-protein NP. This suggests that this peak in doubly infected cells
Fig. 4. Polyacrylamide gel electrophoresis of virus-specific proteins in cells infected with FPV prior to infection with VSV. Co-electrophoresis of doubly infected cells labelled with a mixture of \[^{14}C\]-amino acids (○—○) and of FPV-infected cells labelled with a mixture of \[^{3}H\]-amino acids. The arrows indicate the virus-specific proteins in FPV-infected cells. The VSV-specific proteins G, N and S co-migrate with FPV proteins NP, HA1, and M, respectively; VSV protein NS corresponds to the peak between FPV proteins HA1 and HA2. FPV-infected cells were labelled 4 h after infection by a pulse with radioactive amino acids for 1 h. Doubly infected cells were inoculated with VSV 2 h after the infection with FPV. The cells were labelled 4 h later by a pulse with radioactive amino acids for 1 h.

contained both the VSV and the FPV protein. This finding was further substantiated when the VSV-polypeptide pattern was subtracted from the pattern of doubly infected cells. The differential pattern clearly showed that doubly infected cells contained in addition to the VSV-proteins some nucleocapsid protein NP as the only FPV-protein.

When the cells were infected with FPV 2 h before the infection with VSV, all structural proteins of FPV as well as of VSV were produced (Fig. 4). This is in accordance with the biological data and shows once more that under these conditions both viruses did not interfere with each other.

**FPV-RNA polymerase and virus RNA synthesis in doubly infected cells**

Table 3 presents data which show that only a trace amount of FPV-RNA polymerase was produced in doubly infected cells. If VSV was added 2 h after the primary infection, the production of FPV-RNA polymerase activity was not significantly inhibited. The activity of FPV-RNA polymerase could be determined in the presence of that of VSV-RNA polymerase by specific hybridization of the radioactive *in vitro* product with an excess of non-labelled FPV particle or complementary RNA, respectively, because self-annealing of the VSV-RNA was relatively low. Most of the *in vitro* synthesized FPV-RNA had a base sequence complementary to particle RNA.

After double-infection, FPV-RNA synthesis *in vivo* was also almost completely inhibited, while superinfection 2 h after FPV-infection had rather little effect on FPV-RNA production (Table 4). VSV-RNA synthesis interfered in this kind of experiment more severely with the
Intrinsic interference in RNA viruses

Table 3. Activity of FPV- and VSV-RNA polymerase in doubly infected cells
d.p.m. in RNA

<table>
<thead>
<tr>
<th>Harvest after first infection</th>
<th>FPV</th>
<th>FPV</th>
<th>VSV</th>
<th>VSV mixed</th>
<th>FPV/VSV 2 h later</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV/VSV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total RNA</td>
<td>2 h</td>
<td>4 h</td>
<td>4 h</td>
<td>4 h</td>
<td>78 000</td>
</tr>
<tr>
<td>Immediately RNase</td>
<td>16800</td>
<td>60000</td>
<td>80000</td>
<td>52 000</td>
<td></td>
</tr>
<tr>
<td>Self-annealed</td>
<td>400</td>
<td>3600</td>
<td>1200</td>
<td>1 560</td>
<td></td>
</tr>
<tr>
<td>Particle RNA added</td>
<td>5400</td>
<td>36000</td>
<td>6100</td>
<td>2 400</td>
<td></td>
</tr>
<tr>
<td>Complementary RNA added</td>
<td>13000</td>
<td>45800</td>
<td>5 700</td>
<td>29 800</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2900</td>
<td>11 700</td>
<td>6050</td>
<td>8 800</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Synthesis of fowl-plague RNA in doubly infected cells
d.p.m. in RNA

<table>
<thead>
<tr>
<th>Start of the pulse</th>
<th>FPV</th>
<th>VSV</th>
<th>VSV mixed</th>
<th>FPV/VSV 2 h later</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPV/VSV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total RNA</td>
<td>3 h</td>
<td>1.5 h</td>
<td>3.5 h</td>
<td>150 000</td>
</tr>
<tr>
<td>Immediately RNase</td>
<td>305 000</td>
<td>146000</td>
<td>120 000</td>
<td>210 000</td>
</tr>
<tr>
<td>Self-annealed</td>
<td>9 500</td>
<td>3 000</td>
<td>3 700</td>
<td>4 400</td>
</tr>
<tr>
<td>Particle RNA</td>
<td>78 000</td>
<td>6 9000</td>
<td>29 000</td>
<td>76 000</td>
</tr>
<tr>
<td>10 #1 particle RNA</td>
<td>34 000</td>
<td>6 2000</td>
<td>30 000</td>
<td>71 000</td>
</tr>
<tr>
<td>25 #1 particle RNA</td>
<td>32 000</td>
<td>6 0000</td>
<td>31 000</td>
<td>70 000</td>
</tr>
<tr>
<td>50 #1 complementary RNA</td>
<td>106 000</td>
<td>6 1000</td>
<td>29 400</td>
<td>73 000</td>
</tr>
<tr>
<td>100 #1 complementary RNA</td>
<td>103 000</td>
<td>6 0000</td>
<td>29 000</td>
<td>76 000</td>
</tr>
</tbody>
</table>

Table 5. Interaction of VSV- and FPV-RNA polymerase in vitro
d.p.m. in RNA

<table>
<thead>
<tr>
<th>FPV</th>
<th>VSV</th>
<th>FPV/VSV mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA</td>
<td>80 000</td>
<td>40 000</td>
</tr>
<tr>
<td>Immediately RNase</td>
<td>2 000</td>
<td>200</td>
</tr>
<tr>
<td>Self-annealed</td>
<td>44 000</td>
<td>4 000</td>
</tr>
<tr>
<td>Particle RNA added</td>
<td>63 000</td>
<td>4 400</td>
</tr>
<tr>
<td>Complementary RNA added</td>
<td>8 700</td>
<td>3 900</td>
</tr>
</tbody>
</table>

For each sample five cultures were pooled and cytoplasmic fractions were prepared 4 h after infection. In the mixed incubation an equal volume of each cytoplasmic fraction was used, while in the single incubation the cytoplasmic fraction was mixed with an equal volume of minimal buffer (Schottissee, 1969) before incubation.

detection of FPV-RNA, since the self-annealing of VSV-RNA 4 h after infection was quite high. Only the failure to influence this self-annealing by mixing with an excess of non-labelled FPV particle or complementary RNA gave an indication that in mixedly infected cells no FPV-RNA was being synthesized. When VSV was added 1.5 h before the [³H]-uridine pulse was started there was very little VSV-RNA detectable in any case. Thus, the RNA as determined after superinfection with VSV 2 h after the primary infection by hybridization with non-labelled FPV-specific RNA must be exclusively FPV-RNA.

Corresponding results as shown in Table 4 were obtained, when SFV was used for mixed infection or when cells were preinfected with NDV. In this case also no FPV-specific RNA was detectable. In cells mixedly infected with SFV and NDV, SFV-RNA was being synthesized normally, while no NDV-RNA could be detected by specific hybridization with an excess of non-labelled particle NDV-RNA.
Interaction of VSV- and FPV-RNA polymerase in vitro

It has been suggested that intrinsic interference might occur because the polymerase of one virus competes with the polymerase of the second virus for the RNA template of the latter (Marcus & Zuckerbraun, 1970). Therefore an in vitro experiment was performed in which the RNA polymerase preparations of VSV and FPV were mixed before incubation with the radioactively labelled nucleoside triphosphate precursor. As shown in Table 5, the VSV-RNA polymerase did not influence the synthesis of FPV complementary RNA in vitro.

Other influenza viruses and other cells

It was found that inhibition by VSV was not restricted to FPV, but also held equally well for other influenza A virus strains like Asian A2 (SINGAPORE), A1-FMI, A0-PR8, A SWINE and A EQU 2.

If BHK-cells were used instead of chick fibroblasts, VSV also inhibited multiplication of FPV. Thus this effect was not restricted to chick cells.

DISCUSSION

In this paper it is shown that in cells doubly infected with a variety of enveloped RNA viruses some are excluded from multiplication. Thus, SFV interferes with the formation of infectious NDV, VSV and FPV, while its own synthesis is not inhibited. On the other hand, the production of FPV can be prevented by all the viruses tested. It is a very early step which does not function in the replication cycle of those viruses which are inhibited. The virus-specific RNA polymerases are not formed and virus RNA is not produced in measurable amounts. This is not inconsistent with the small amount of FPV-RNP antigen found in doubly infected cells, since even in the presence of actinomycin D the inhibition of the production of RNP-antigen was somewhat leaky (Rott & Scholtissek, 1964).

In the VSV-FPV system, which was studied in more detail, the adsorption of FPV was not influenced by VSV, since u.v.-inactivated VSV had no inhibitory effect. Furthermore, VSV influenced influenza multiplication, even when added 1 h after the primary infection.

Induction of interferon by VSV and its action on influenza production cannot be responsible for this effect for the following reasons: (1) interferon production is a rather slow process; (2) SFV, which is very sensitive to interferon, multiplies normally under analogous conditions; and (3) u.v.-inactivated VSV inhibits interferon synthesis as efficiently as infectious VSV (Wagner & Huang, 1966).

Because of these considerations, the phenomenon described above is in agreement with the intrinsic interference as defined by Marcus & Carver (1967) for NDV.

The mechanism of intrinsic interference is not clear. It has been suggested that the RNA polymerase of the interfering virus might compete for the RNA template of NDV (Marcus & Zuckerbraun, 1970). In vitro experiments with the RNA polymerases of VSV and FPV have shown that such a competition does not occur in this system (Table 5). An alternative explanation would be that interference occurs at the level of translation of the virus mRNA. Evidence for this has been given by Hattman & Hofschneider (1967, 1968) and Hattman (1970). They showed that the exclusion of the multiplication of M12 phage by the T4 phage in Escherichia coli is due to inhibition of translation of M12 RNA on polysomes. Similarly, Saxton & Stevens (1972) found that poliovirus infection prevents the translation of herpes simplex virus specific RNA. Thus, the phenomenon of intrinsic interference seems to be a...
more general phenomenon, although exceptions have been described (e.g. for the paramyxoviruses SV5, Choppin & Holmes, 1967).

An additional explanation has to be considered for intrinsic interference with orthomyxovirus replication. A cellular function seems to be required early after infection, since it is known that the multiplication of orthomyxoviruses can be abolished by interference with cellular DNA-dependent RNA synthesis (Barry, Ives & Cruickshank, 1962; Rott, Saber & Scholtissek, 1965; Scholtissek, Becht & MacPherson, 1970; Rott & Scholtissek, 1970). It is also known that all the interfering viruses block cellular protein synthesis (Wagner & Huang, 1966; Wilson, 1968; Wagner et al. 1970; Kang & Prevec, 1971; C. Scholtissek, unpublished results).

Therefore the inhibition of the multiplication of orthomyxoviruses could be explained by the assumption that the induction of a certain cellular protein, necessary for their multiplication, is prevented. Experiments to clarify this alternative explanation are in progress.

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REFERENCES


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