Table 1. ACTIVITY OF NEURAMINIDASE IN TEST PREPARATIONS

| Preparation | μg of N-acetyl neuraminic acid liberated from bovine glycoproteins by 0·2 ml. of preparation in 2 h at 37° C | |
|---|---|---|
| | *p*H 5·8 | *p*H 7·4 |
| Bovine strain PI 3 virus | 1·16 | 0·52 |
| Control tissue culture fluid | 0·07 | 0·02 |
| Neuraminidase | 24·5 | 17·3 |

suspension, and a commercially available preparation of neuraminidase (extract of *Vibrio cholerae*).

Serum from calves deprived of colostrum (globulin-free) and normal adult bovine serum were used as the source of glycoprotein substrate. The activity of neuraminidase was assayed by the incubation at 37° C for 2 h of 0·2 ml. of the test preparation, 0·2 ml. bovine serum (containing 180 μg of total sialic acid[6,7]) and 0·1 ml. of a 0·2 molar phosphate buffer (final *p*H 5·8 or 7·4). After subjection to 100° C for 1 min to inactivate the enzyme, free sialic acid was estimated in the reaction mixture by a method[8] which was specific for N-acetylneuraminic acid. The results obtained with adult bovine serum as the substrate are shown in Table 1. Similar results were obtained when colostrum-deprived calf serum was used.

The suspension of PI 3 virus showed significant enzyme activity at *p*H 5·8 and 7·4. At *p*H 5·8, the optimal hydrogen ion concentration for viral neuraminidases[9], the enzyme activity of the virus suspension was more than twice that observed at *p*H 7·4. The control preparation of neuraminidase, which has an optimal activity at *p*H 5·6 (ref. 10), also showed somewhat greater activity at *p*H 5·8 than at *p*H 7·4. The control, concentrated fluid collected from tissue culture, showed only very slight neuraminidase activity at either *p*H (Table 1).

These results show that *T*1 bovine strain of PI 3 virus possesses neuraminidase activity. If the criteria proposed[1] are accepted, then at least this bovine strain of virus should correctly be considered as a myxovirus.

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## Failure of Function of the "Early Protein" induced by an Influenza Virus in Cells infected by Newcastle Disease Virus

AFTER infection of chick embryo cells with fowl plague virus (FPV), an influenza *A* virus, or with Newcastle disease virus (NDV), a para-influenza virus, "early proteins" are synthesized before viral RNA-synthesis starts[1–4]. These "early proteins" might function as an RNA-dependent RNA polymerase as has been demonstrated for picornaviruses[5]. In contrast to the picornaviruses, the "early proteins" of the myxoviruses are stable[1–4]. The purpose of this investigation was to determine whether or not the "early protein" induced by FPV is able to function for the multiplication of NDV.

The cell system and virus strains used and the procedure of infection have been published previously[1]. *p*-Fluorophenylalanine (FPA) has been used as an inhibitor for virus multiplication and the demonstration of the "early protein"[1,2]. If FPA is added to the tissue cultures immediately after infection, no viral RNA is synthesized. The normal complement of viral RNA is produced, however, if the inhibitor is added 2 h post infection to FPV infected cells or 3 h post infection to NDV infected cells, respectively[1,2,4].

In the present experiments chick embryo cells were infected with FPV and superinfected with NDV 2 h later. After the adsorption of NDV, 300 μg/ml. FPA and 5 μg/ml. actinomycin were added. Actinomycin prevents the synthesis of cellular and FPV–RNA, but not the synthesis of NDV–RNA[6,7]. Six hours after addition of the inhibitors 0·5 μc. $^{14}$C-uridine per culture were added and the radioactivity in the RNA was determined 2 h thereafter as described before[8]. The following controls were used: (1) FPA was omitted; (2) FPA was added 4 h after superinfection with NDV; (3) NDV and FPA were omitted; (4) tissue cultures alone were incubated with the isotope.

Table 1. SYNTHESIS OF NDV-RNA IN CELLS PRETREATED WITH FPV

| FPV | NDV | Actinomycin | Addition of FPA, time after superinfection | C.p.m. in RNA |
|---|---|---|---|---|
| – | – | – | – | 17,000 |
| + | – | + | – | 199 |
| + | + | + | 0 h | 110 |
| + | + | + | 4 h | 1,470 |
| + | + | + | – | 1,041 |

Essentially the same results were obtained in four independent experiments. The results of one representative experiment are given in Table 1. It can be seen that the "early protein" of FPV is not able to stimulate the synthesis of RNA after superinfection with NDV if FPA and actinomycin are added immediately after superinfection. As already mentioned, actinomycin prevents the synthesis of cellular and FPV–RNA and, under the conditions used (addition of FPA immediately after superinfection), FPA interferes with the production of the "early protein" of NDV. Thus the "early protein" of FPV synthesized before FPA is added is not able to act as a substitute for the "early protein" of NDV. The control experiments show that the superinfected cells are able to synthesize NDV–RNA in the presence of actinomycin if FPA is either omitted or added after the "early protein" of NDV is already synthesized.

Two different possibilities might be considered to explain the fact that the "early protein" induced by FPV does not function in the NDV-system. It is known that the RNA of FPV is synthesized within the cell nucleus[9], while NDV–RNA very probably replicates in the cytoplasm[10]. Thus the "early protein" of FPV might not be able to leave the nucleus. As shown with two different RNA-containing phages the RNA-dependent RNA polymerase (RNA replicase) is very specific in the sense that it uses only that RNA as template which has induced its synthesis[11]. A similar situation might apply to different myxoviruses.

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