Studies on the Cytopathic Effects of Newcastle Disease Virus:
RNA Synthesis in Infected Cells

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Many viruses, in addition to the induction of c.p.e., produce profound biochemical alterations in the cell, particularly by the inhibition of cellular macromolecular synthesis (for review see Roizman & Spear, 1969). Reeve et al. (1971) have shown that the ability of different Newcastle disease virus (NDV) strains to inhibit cellular protein synthesis is related directly to their virulence for cells in vitro and for eggs and chickens in vivo.

Certain NDV strains can also inhibit cellular RNA synthesis, but the relationship of this property to the virulence of the infecting strain is not clear (Wheelock & Tamm, 1961; Scholtissek & Rott, 1965; Wilson, 1968). Moore, Lomniczi & Burke (1972) examined 13 strains of NDV but were unable to establish a relationship between virulence and the inhibition of host cell RNA synthesis.

The cell culture techniques and most of the virus strains have been described (Alexander, Reeve & Allan, 1970; Reeve & Poste, 1971). Virus strains were obtained from W. H. Allan, Central Veterinary Laboratory, Weybridge, Surrey, except strain ULSTER which was obtained from J. B. McFerran, Ministry of Agriculture, Stormont, Belfast, N. Ireland. The LAMB-ESSEX 70 (LAMB) and BUXTED-ESSEX 70 (BUXTED) strains are virulent strains which were isolated from two disease outbreaks in the 1970 NDV epizootic in Great Britain. Strain AG68 is a virulent strain from the Middle East producing a similar clinical disease to LAMB and BUXTED (W. H. Allan, personal communication). Coverslip monolayers of chick embryo (CE) or baby hamster kidney – 21 clone 13 (BHK) cells were infected at a multiplicity of 20 to 50 EID₅₀/cell for 30 min at 37 °C. Incorporation of [³H]-uridine into a trichloracetic acid (TCA)-insoluble fraction was measured as described (Reeve et al. 1971, 1972). TCA-soluble incorporation was estimated by washing the cell cultures three times with ice-cold phosphate-buffered saline (PBS), pH 7.2, and adding 1 ml of ice cold 5 % (w/v) TCA which was removed after 10 min. Thereafter, 0.1 ml samples of the TCA were placed in oxitol scintillation fluid and radioactivity estimated (Reeve, Rosenblum & Alexander, 1970).

The virulent strains all induced a significant inhibition of incorporation of uridine into the TCA-insoluble fraction of CE cells, while avirulent strains and strains of low virulence produced little, if any, effect (Fig. 1). There was slight increase in incorporation into the TCA-insoluble fraction 12 to 14 h after infection with the virulent strains, but this apparent recovery was only temporary (Fig. 1). Cells infected with virulent strains also showed a reduction in TCA-soluble incorporation which was most marked after 12 h (Fig. 1), suggesting that the incorporation of [³H]-uridine into both fractions may be inhibited after 12 h because of cell damage and death. Cell cultures infected with QUEENSLAND and B₁ showed some inhibition of uridine incorporation but to a lesser degree and much later after infection than cells infected with virulent strains.

The accumulative incorporation of [³H]-uridine into the TCA-insoluble fraction of NDV-infected cells in the presence of actinomycin D can be regarded as incorporation into virus-specified RNA (Bratt, 1969). The rate of incorporation of [³H]-uridine in the presence of
actinomycin D (5 μg/ml) over the first 12 h after infection was found to be related to the virulence of the infecting strain both in CE and BHK cells (Figs. 2, 3). The reduction in the rate of incorporation into cells infected with virulent strains is probably due to the progression of c.p.e. and cell death.

Our results show that the ability of different NDV strains to inhibit cellular RNA synthesis is related to their virulence. The inhibition of cellular RNA synthesis reported here by the virulent strains HERTS, TEXAS, FIELD PHEASANT and LAMB is similar to that described in previous studies with these strains (Wilson, 1968; Moore et al. 1972). We have been unable to confirm, however, the inhibition of host cell RNA synthesis by strains of low virulence reported by Moore et al. (1972). In a study of 13 NDV strains, Moore and his colleagues found that strain B1 was the most efficient in inhibiting cellular RNA synthesis, whereas in our study the inhibitory effect of this strain was slight. Similarly, strain ULSTER, which in our study did not affect cellular RNA synthesis, was found by Moore et al. (1972) to induce almost complete inhibition by 17 h after infection. It should be noted that Moore et al. (1972) reported that their ULSTER strain was able to occasionally plaque and cause c.p.e. In this respect it is pertinent to note that in other studies where the in vitro cytopathogenicity of NDV strains and their virulence were investigated the ability to plaque and cause c.p.e. were reliable indicators of virulence in other host-systems while the failure to produce these cellular changes was found only with natural strains of low virulence or attenuated strains (Granoff, 1964; Daniel & Hanson, 1968a, b; Schloer & Hanson, 1968a, b; Estupian & Hanson, 1969; Kohn & Fuchs, 1969; Beard, Spalatin & Hanson, 1970; Reeve & Alexander, 1970; Singh, Saad & El Zein, 1970; Reeve & Poste, 1971; Schloer & Hanson, 1971; Poste et al. 1972a, b).

The strain ULSTER used in the present study did not form plaques, did not fuse cells and did not consistently kill eggs.

The present results have also shown that the rate of synthesis and intracellular accumula-

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**Fig. 1.** Incorporation of [³H]-uridine into TCA-insoluble (solid line) and TCA-soluble (broken line) fractions of chick embryo cells infected with different strains of NDV. Cell cultures were treated with 1 ml [³H]-uridine (1 μCi/ml) 1 h prior to the times specified. The results are expressed as % incorporation into similar fractions of uninfected cells. The highly virulent strains (closed symbols) and the strains of low virulence (open symbols) are placed in order of virulence from the left. Absolute incorporation: 100 % TCA-insoluble was approx. 18000 ct/min, 100 % TCA-soluble was approx. 54000 ct/min/ml.
Fig. 2. The accumulative incorporation of $[^{3}H]$-uridine into an acid-insoluble fraction of chick embryo cells in the presence of actinomycin D. Actinomycin D (5 $\mu$g/ml) and $[^{3}H]$-uridine (1 $\mu$Ci/ml) were added 30 min after infection. The strains used were, in order of decreasing virulence, HERTS (○○○), TEXAS (■■■), BUXTED (▲▲▲), BEAUDETTE C (▼▼▼), ULSTER, (□□□) and QUEENSLAND (○○○). Control cells (broken line) were uninfected. At 12 h the incorporation into uninfected, untreated cells was approx. 36000 ct/min.

Fig. 3. The accumulative incorporation of $[^{3}H]$-uridine into an acid-insoluble fraction of baby hamster kidney cells in the presence of actinomycin D. Actinomycin D (5 $\mu$g/ml) and $[^{3}H]$-uridine (1 $\mu$Ci/ml) were added 30 min after infection. The strains used were, in order of decreasing virulence, HERTS (○○○), TEXAS (■■■), BUXTED (▲▲▲), AG68 (▲▲▲), B6 (○○○), and F (□□□). Control cells (broken line) were uninfected. At 12 h the incorporation into uninfected, untreated cells was approx. 33000 ct/min.

tion of virus-specified RNA is related to the virulence of the infecting strain. Virulence is a measure of the speed to kill or cause c.p.e and would necessarily relate to the rate of production of virus components if all the strains were cytopathic. However, some strains of NDV never cause death or c.p.e although eventually reaching extracellular titres of virus as high as virulent strains when grown in eggs or cells (Liu & Bang, 1953; Drain, 1969). These results, together with the present findings and those of Reeve et al. (1970), suggest it is not necessarily a faster synthesis of virus products that causes c.p.e and cell death, but rather it is the imbalance between the production of virus components and their release from the cell surface. Thus, if the rate of intracellular production of virus components exceeds the rate of virus release then accumulation takes place and cytopathogenicity is the likely outcome. Conversely, if the potential rate of virus release exceeds or is equal to the rate at which virus products are produced then cytopathogenicity would be absent.
Short communications

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