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# Studies on the Cytopathogenicity of Newcastle Disease Virus: Effect of Lectins on Virus Infected Cells

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SUMMARY

The infection of chick embryo (CE) and baby hamster kidney (BHK) cells with Newcastle disease virus (NDV) strains HERTS, WARWICK, TEXAS, FIELD PHEASANT, LAMB ESSEX or BEAUDETTE C increased their susceptibility to agglutination by the lectins, concanavalin A (Con A) and wheat germ agglutinin (WGA). The agglutination reaction with Con A and WGA was inhibited by α-methyl-D-glucopyranoside and *N*-acetyl-glucosamine, respectively. The agglutination of purified virus particles from these strains by the lectins indicated that lectin-receptors on the cell surface were incorporated into the virus envelope.

A correlation was found between the change in cell agglutination behaviour after infection and the virus-induced alterations of the cell coat material. In contrast, CE and BHK cells infected with the avirulent QUEENSLAND or ULSTER strains of NDV were not agglutinated by Con A or WGA and no changes were detected in the cell coat material. The greater susceptibility to agglutination by lectins found for cells infected with the more virulent NDV strains required the synthesis of virus-induced proteins. The relationship between agglutination and changes in the cell coat material is discussed.

## INTRODUCTION

Several lectins have been shown to agglutinate cells transformed by oncogenic viruses but not normal cells (Aub, Sanford & Cote, 1965; Burger & Goldberg, 1967; Inbar & Sachs, 1969; Biddle, Cronin & Sanders, 1970; Sela *et al.* 1970). Normal cells possess similar lectin-receptor sites but are only susceptible to agglutination after treatment with proteolytic enzymes (Burger, 1969, 1971; Inbar & Sachs, 1969; Ozanne & Sambrook, 1971). This difference led Inbar & Sachs (1969) to propose that the lectin-receptor sites on normal cells are present in a 'cryptic' form and are exposed only after transformation or trypsinization. However, recent experiments with radioactively labelled (Cline & Livingston, 1971; Ozanne & Sambrook, 1971; Arndt-Jovin & Berg, 1971) or fluorescein-conjugated lectins (Fox Sheppard & Burger, 1971) have demonstrated that identical amounts of lectin are bound by normal and transformed cells. This suggests that agglutination of cells by lectins requires a change in the topographical distribution of the lectin-binding sites.

Immunofluorescence (Mallucci, 1971) and electron-histochemical studies (Bernhard & Avrameas, 1971; Nicolson & Singer, 1971) of normal and transformed cells have established

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that lectin-receptor sites are situated in the cell coat material. Since lytic infection of normal cells with different strains of Newcastle disease virus (NDV) causes digestion and structural alteration of the cell coat material (Reeve *et al.* 1972) we investigated whether these changes would increase the susceptibility of NDV-infected cells to agglutination by lectins.

The lytic infection of normal chick embryo (CE) or baby hamster kidney (BHK) cells with virulent and mesogenic strains of NDV rendered them susceptible to agglutination by lectins. Virus-induced modifications of the cell coat material and the synthesis of virus-specific proteins were essential to the subsequent change in cell agglutination behaviour.

## METHODS

*Viruses.* The origins and properties of the different NDV strains and the methods of infection of cell cultures have been described (Alexander, Reeve & Allan, 1970; Reeve, Rosenblum & Alexander, 1970; Reeve & Poste, 1971; Reeve *et al.* 1971).

*Cell cultures.* Normal BHK cells were grown in reinforced Eagle's medium (Dulbecco modification) supplemented with 10% foetal calf serum. Primary CE cells were grown in Eagle's Basal Medium supplemented with 10% calf serum. Cultures of normal NIL 2E cells (baby hamster kidney cell line), NIL 2E cells transformed by polyoma virus (NIL-PV) and BHK cells transformed by polyoma virus (BHK-PV) (kindly provided by Dr I. Macpherson) were grown in Eagle's medium (Dulbecco modification) supplemented with 10% foetal calf serum. Unless stated otherwise, cells were grown in 50 mm plastic Petri dishes (NUNC: Denmark) at 37 °C in air + 5% $CO_2$. Cell viabilities were measured as described previously (Poste, 1971).

*Lectins.* Wheat germ agglutinin (WGA) was isolated and purified from wheat germ lipase (Calbiochem, London; Batch no. 101166 and Sigma Chemical Co., London; Batch no. 120C–8030) by the method of Burger & Goldberg (1967). Concanavalin A (Con. A) was obtained as a twice crystallized preparation in saturated NaCl (Miles-Yeda, Israel; Batch no. 47) and used without further purification.

*Cell agglutination.* The agglutination of cells by Con A or WGA was assayed by the methods of Burger (1969) and Inbar & Sachs (1969) using plastic agglutination trays with flat-bottomed wells (Linbro; Biocult Laboratories, Scotland). Confluent cultures of CE and BHK cells were infected with NDV in Eagle's medium without serum. At various times after infection the cells were washed twice with calcium-magnesium-free balanced saline (CMF-S), pH 7·2, detached from the Petri dish with 0·02% disodium versenate in CMF-S, washed once and suspended in CMF-S at a concentration of $2 \times 10^6$ cells/ml. For the agglutination assay, 0·5 ml samples of the cell suspension were mixed with 0·5 ml of CMF-S containing either Con A or WGA at the concentrations listed in the text. Agglutination was assessed after incubation for 20 min at room temperature and scored as 0, 1, 2, 3 or 4 for 0, 25, 50, 75% or > 90% of cells agglutinated, respectively. Mock-infected control cell cultures were treated in the same way. The specificity of the agglutination reaction was tested by agglutination inhibition using the specific lectin inhibitors; α-methyl-D-glucopyranoside for Con A (Inbar & Sachs, 1969) and *N*-acetyl-glucosamine for WGA (Burger & Goldberg, 1967).

*Agglutination of virus particles by lectins.* Virus harvested from embryonated eggs was concentrated and purified by density gradient centrifugation (Reeve & Alexander, 1970). A sample of 0·2 ml of the purified virus suspension, containing 4000 to 16000 haemagglutinating units (H.A.U.), was mixed with 0·2 ml of CMF-S containing either Con A (1000 μg/ml) or WGA (500 μg/ml) in the wells of a standard WHO haemagglutination tray. Agglutination

to visible floccules was measured after 30 min incubation at room temperature (Becht, Rott & Klenk, 1972).

*Inactivation of virus.* Non-infective NDV was obtained by u.v. irradiation (Poste & Reeve, 1971).

*Measurement of cell coat thickness.* The term cell coat is applied to the material composed predominantly of acidic glycoproteins which exists external to the outer electron-dense leaflet of the trilaminar plasma membrane structure (for reviews see Martínez-Palomo, 1970; Rambourg, 1971; Parsons & Subjeck, 1972). Cell coat thickness was measured by ellipsometry in a 43603-200E automatic photoelectric ellipsometer (O. C. Rudolph and Sons, Inc., Caldwell, New Jersey, U.S.A.) using the methods described by Poste (1970, 1971). Using this technique, the thickness of the cell coat material is measured at the interface between the outer leaflet of the plasma membrane and the glass substrate to which the cell is attached. The optical basis of ellipsometry and its use in the measurement of cell coat materials have been reviewed by Poste & Moss (1972).

*Reagents.* Cycloheximide, α-methyl-D-glucopyranoside, and *N*-acetyl-glucosamine were obtained from the Sigma Chemical Company, London, and trypsin (twice crystallized) from Koch–Light Laboratories, Colnbrook, Bucks. Anti-NDV serum prepared in chickens was kindly provided by Mr W. H. Allan, Central Veterinary Laboratory, Weybridge, Surrey.

## RESULTS

To test the specificity of the cell agglutination assay used in later experiments, normal BHK and CE cells and transformed BHK-PV and NIL-PV cells were tested for agglutination by Con A (1000 μg/ml) and WGA (250 μg/ml). The effect of treating normal BHK and CE cells with 0·01% trypsin for 5 min at 37 °C on their susceptibility to agglutination by Con A or WGA was also investigated. In agreement with previous reports (Burger, 1969, 1971; Inbar & Sachs, 1969; Sela *et al.* 1970; Ozanne & Sambrook, 1971), Con A and WGA agglutinated the transformed and the trypsinized cells, but did not agglutinate normal cells. The specificity of the observed agglutination reactions was confirmed by the finding that Con A (1000 μg/ml) with 0·1 M-α-methyl-D-glucopyranoside, or WGA (250 μg/ml) with 0·1 M-*N*-acetyl-glucosamine, failed to agglutinate either the transformed or the trypsinized cells.

### *Agglutination of NDV-infected cells by Con A and WGA*

BHK cell cultures were infected with virulent or mesogenic strains of NDV and their susceptibility to agglutination by Con A (1000 μg/ml) or WGA (250 μg/ml) measured at intervals after infection. Measurements of cell coat thickness were made in duplicate infected and uninfected control cell cultures.

The results indicated that BHK cells infected with NDV strain HERTS, TEXAS, WARWICK, FIELD PHEASANT, LAMB ESSEX or BEAUDETTE C became susceptible to agglutination by either lectins at different times after infection (Fig. 1). Measurements of the thickness of the cell coat in cells infected with these strains showed a close temporal relationship between the observed changes in cell agglutination behaviour and the reduction in cell coat thickness (Fig. 1). Similar results were obtained with CE cells infected with these strains of NDV.

Measurements of cell agglutination in the later stages of infection with these NDV strains were frustrated by the formation of small polykaryocytes by virus-induced cell fusion. The possibility that the observed agglutination was due to polykaryocyte formation rather than to specific agglutination was discounted by the finding that the agglutination of NDV-infected cells by Con A or WGA was inhibited by 0·1 M-α-methyl-D-glucopyranoside or

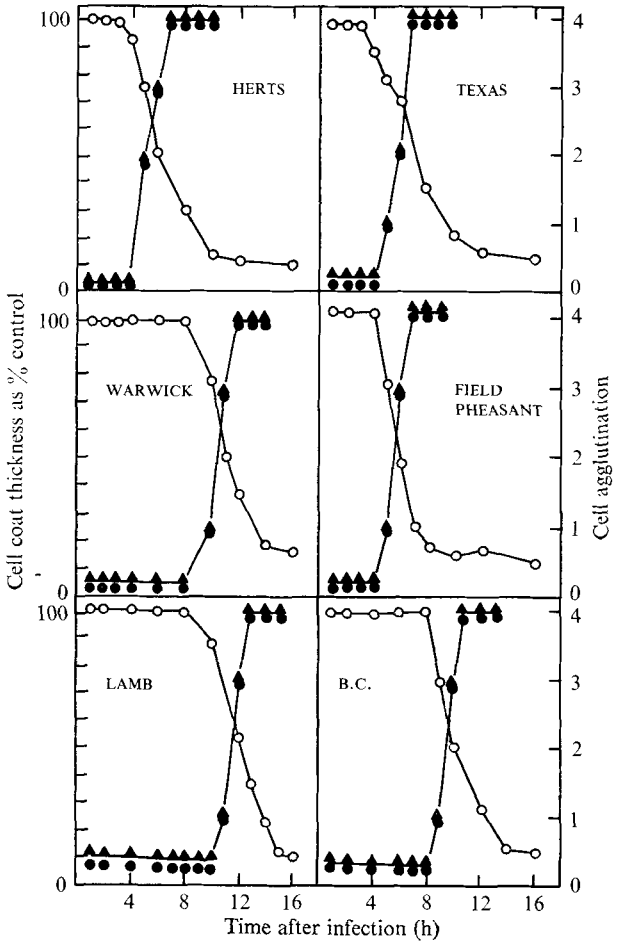


Fig. 1. Cell coat thickness (○——○) and agglutination of baby hamster kidney cells infected with different strains of Newcastle disease virus. Agglutination by 1000 μg/ml concanavalin A (●——●) or 250 μg/ml wheat germ agglutinin (▲——▲). The extent of cell agglutination by lectins is scored from 0 to 4 according to the proportion of cells agglutinated. Cell coat thickness in virus-infected cells is expressed as a % of the value in uninfected control cells.

0·1 M-*N*-acetyl-glucosamine, respectively. No agglutination was observed in mock-infected cell cultures tested over the same time period.

BHK cells infected with NDV strain HERTS or TEXAS inactivated by u.v. irradiation were not susceptible to agglutination by either Con A or WGA. Similarly, BHK- cells treated with NDV strain HERTS plus anti-NDV serum were not agglutinated.

The relationship between the development of susceptibility to agglutination and the time of reduction in coat thickness found in cells infected with these NDV strains suggested that changes in the cell coat might be responsible for the altered cell agglutination. To test this possibility, BHK cells were infected with the avirulent NDV strain QUEENSLAND or ULSTER which do not produce changes in the cell coat material (Reeve *et al.* 1972) but replicate as well as the more virulent strains used above (Reeve & Waterson, 1970).

The results (Fig. 2) indicated that cells infected with strain QUEENSLAND or ULSTER did not become susceptible to agglutination by either Con A or WGA and showed no reduction

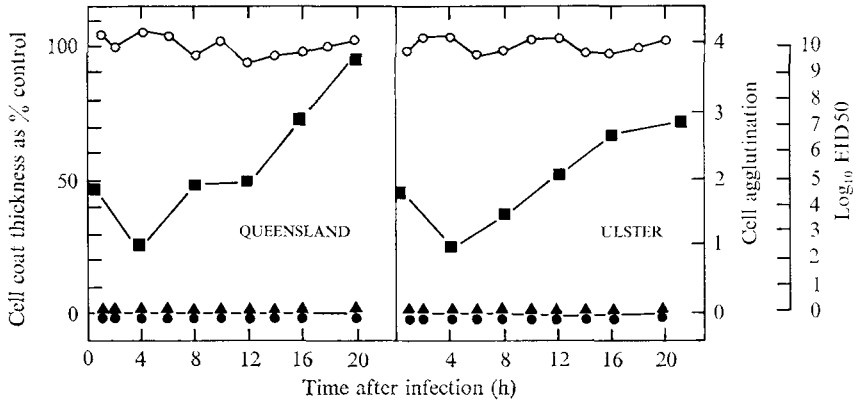


Fig. 2. Cell coat thickness (○——○) and agglutination of baby hamster kidney cells infected with the QUEENSLAND or ULSTER strain of Newcastle disease virus. Agglutination by 1000 μg/ml concanavalin A (●——●) or 250 μg/ml wheat germ agglutinin (▲——▲). Cell coat thickness and the extent of cell agglutination were measured as for Fig. 1. Growth of virus in the cells is shown as released infectivity/ml (■——■).

in coat thickness. These results suggest that the development of susceptibility to agglutination by lectins is related to changes occurring in the coat material.

### *Effect of metabolic inhibitors on agglutination of NDV-infected cells*

Since the cell coat changes found in cells infected with virulent or mesogenic NDV strains require virus-induced protein synthesis (Reeve *et al.* 1971, 1972) it was of interest to test whether similar virus-specific protein synthesis was necessary for agglutination of cells by Con A or WGA.

Treatment of BHK cells with cycloheximide (10 μg/ml), an inhibitor of protein synthesis, at the time of infection with NDV strain HERTS or TEXAS inhibited both virus replication and cell agglutination. Control cells treated with strains HERTS or TEXAS in the absence of cycloheximide became agglutinable by Con A or WGA at 5 h and 7 h after infection, respectively. Agglutination by lectins was inhibited when cycloheximide (10 μg/ml) was added to cells within 3 h of infection by strain HERTS or TEXAS (Fig. 3). When added after 3 h, cycloheximide did not prevent the agglutination of infected cells by WGA or Con A (Fig. 3). A similar inhibition of agglutination by lectins was found in cells infected with strain HERTS or TEXAS in the presence of *p*-fluoro-phenylalanine (500 μg/ml) added at the time of infection or up to 3 h after infection. In view of the evident relationship between NDV-induced changes in cell agglutination and changes in the cell coat, it is noteworthy that the latter also required the synthesis of new proteins within 3 h of infection (Reeve *et al.* 1972).

### *Agglutination of virus particles by Con A or WGA*

The envelope of NDV, and of other paramyxoviruses, contains material derived from the plasma membranes of the host cell at the time of virus release from the cell (Rott *et al.* 1966; Becht *et al.* 1972). Any lectin-receptor sites situated on the cell surface may thus also be present in virus particles released from the cell.

To test whether NDV particles contained such lectin receptor sites, Con A or WGA were added separately to suspensions of purified virus. When Con A (1000 μg/ml) or WGA (500 μg/ml) were added to suspensions of NDV strains HERTS, TEXAS, WARWICK, LAMB ESSEX

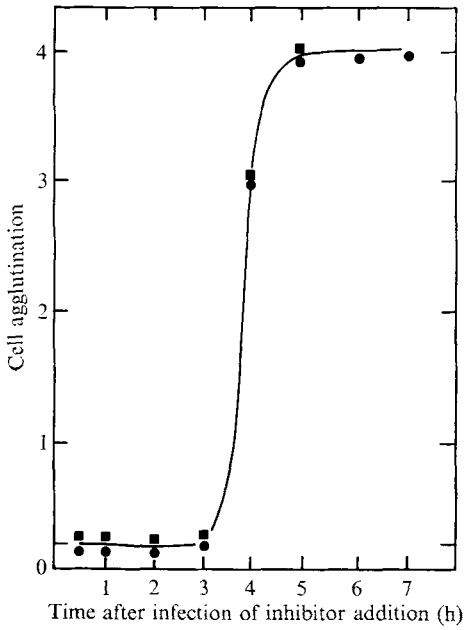


Fig. 3. Effect of adding cycloheximide (10 $\mu$g/ml) at different times after infection of baby hamster kidney cells with the HERTS (■——■) or TEXAS (●——●) strain of Newcastle disease virus on agglutination of infected cells by 1000 $\mu$g/ml concanavalin A or 250 $\mu$g/ml wheat germ agglutinin. Similar results were obtained with both lectins. Agglutination was measured 6 or 8 h after infection with strain HERTS or TEXAS, respectively. Cycloheximide was not added later than 5 or 7 h after infection with strain HERTS or TEXAS, respectively, since cells were then susceptible to agglutination.

or BEAUDETTE C, large floccules and aggregates formed in 5 to 10 min. This is in agreement with the observation by Becht *et al.* (1972) of macroscopic aggregation of fowl plague or $SV_5$ viruses in the presence of Con A. Flocculation of the different NDV strains was not found in preparations treated with Con A + 0·1 M-α-methyl-D-glucopyranoside or WGA + 0·1 M-*N*-acetyl-glucosamine.

In contrast, macroscopic flocculation was not detected in purified suspensions of strain QUEENSLAND or ULSTER treated with similar doses of Con A or WGA.

## DISCUSSION

Our experiments have demonstrated that the agglutination of cells by lectins is not a specific property of transformed cells: lytic infection with certain strains of NDV rendered normal CE and BHK cells susceptible to agglutination by Con A or WGA. These results are similar to those of Zarling & Tevethia (1971) who found that rabbit kidney cells infected with vaccinia virus became susceptible to agglutination by Con A. Becht *et al.* (1972) also demonstrated that the infection of normal CE and BHK cells with a range of RNA-containing enveloped viruses, including the ITALIEN and BEAUDETTE strains of NDV, increased their susceptibility to agglutination by Con A.

Our results have shown that these virus-induced alterations in cell agglutination behaviour are related to changes occurring in the cell coat material. The possibility must also be considered that glycosylated virus-specific proteins present on the surface of infected cells could

act as lectin receptors (Oram *et al.* 1971). However, this is unlikely to be the only mechanism involved in the increased agglutination response of NDV-infected cells. That changes in the cell coat are also involved is suggested by the recent finding that similar alterations in the coat thickness of BHK cells induced by drug and enzyme treatments rendered the cells susceptible to agglutination by Con A or WGA (Poste, 1972).

The specific agglutination of particles of NDV by Con A or WGA suggests that lectin-receptor sites on the cell surface are incorporated into the virus envelope. The failure of virus particles from the avirulent QUEENSLAND or ULSTER strains to agglutinate under similar conditions may be interpreted in two ways. The first is that lectin-receptor sites are not present on the virus. This is considered unlikely since these virus strains are released from the cell surface by budding as are the more virulent strains which contain lectin receptors. The second explanation is that agglutination takes place because the lectin-receptors on the virus are in a specific configuration or distribution. This proposal is prompted by the observation that equal amounts of lectin are bound by normal cells which do not agglutinate and by transformed or trypsinized cells which do agglutinate. To explain these differences in cell agglutination by lectins it has been proposed (Arndt-Jovin & Berg, 1971; Nicolson, 1971) that in cells which are susceptible to agglutination the distribution of the lectin sites is such that lectin molecules bound to the cell surface retain available groups to react with receptors on another cell. In this way, lectins can act as 'bridges' to promote cell agglutination. For virus particles it is suggested by analogy that the lectin sites on particles which do not agglutinate are in a different pattern from those on particles that are agglutinated readily.

There is now considerable evidence that changes in the interaction of lectins with the cell surface are related to the control of cell division and growth regulation (Pollack & Burger, 1969; Benjamin & Burger, 1970; Dulbecco, 1971; Eckhart, Dulbecco & Burger, 1971; Sheppard, 1971). It is therefore of interest to determine whether the alterations in cell agglutination induced by nononcogenic viruses may be used to study the role of the cell surface in growth regulation. Clearly, infection of cells with virulent strains of virus of the type used here would be unsuitable since the cells do not survive. However, the study of virus-cell systems in which the cell coat is modified to ensure cell viability (Poste, 1971; Rubin, 1971) may enlarge our understanding of growth regulation in normal cells. At present this is based largely on information obtained from abnormal, transformed cells.

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