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Quantitative Interaction of *Ricinus communis* Agglutinin and Concanavalin A with Influenza and Vesicular Stomatitis Viruses and Virus-Infected Normal and Polyoma-Transformed Cells†

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ABSTRACT: The interactions of concanavalin A (Con A) and *Ricinus communis* agglutinin (RCA_I) with purified influenza and vesicular stomatitis virus (VSV) and virus-infected cells were examined using ^{125}I-labeled lectin binding and ferritin-lectin labeling. In quantitative binding experiments the number of Con A and RCA_I receptors per influenza virion was greater than for VSV, and there was a close correlation between the number of influenza lectin receptors and viral "spikes." Electron microscopic localization of ferritin–Con A and –RCA_I on purified influenza and VSV virus particles suggested that the viral spikes bind these lectins. Using ferritin–Con A labeling to influenza virus, Klein and Adams (*J. Virol. 10,* 833 (1972)) came to similar conclusions. When BHK cells were infected with influenza or VSV, there was an increase in Con A agglutinability within 30–120 min after infection (as reported by Rott *et al., Z. Naturforsch. B 27,* 227 (1972)), although the number of Con A-binding sites remained constant during the course of infection. RCA_I agglutinability was also enhanced, but the number of RCA_I-binding sites increased approximately sixfold during influenza infection; 75% of this increase was prevented by protein synthesis inhibitors and was probably due to viral component appearance and/or modification of the host cell surface. The remainder of the increase appeared during the early stages of infection, and it was not prevented by protein synthesis inhibitors, suggesting that this increase was due to modification of existing cell membrane components. Polyoma-transformed BHK cells were examined during influenza infection, and although the cells remained highly agglutinable at all times, the increase in RCA_I receptors per cell during infection was much lower than the corresponding increase in BHK cells.

Animal cells that have been transformed to the neoplastic state by a variety of agents usually agglutinate with plant lectins more readily than their normal counterparts (reviews: Lis and Sharon, 1973; Terner and Burger, 1973; Nicolson, 1974a,b). The increased lectin-mediated agglutinability of transformed cells has generally not correlated with increased numbers of lectin-binding sites after transformation (Arndt-Jovin and Berg, 1971; Cline and Livingston, 1971; Ozanne and Sambrook, 1971; Inbar *et al.,* 1971; Nicolson, 1973a; Nicolson and Lacorbiere, 1973), although in one laboratory this finding has been questioned (Noonan and Burger, 1973). It has been proposed on the basis of lectin receptor localization experiments that the increased agglutinability may be due, in part, to the disposition of the lectin-binding sites on the cell surface, an aggregation of lectin receptors being more likely to lead to cell agglutination than a dispersed distribution (Nicolson, 1971; Martinez-Palomo *et al.,* 1972; Nicolson, 1973b; Rosenblith *et al.,* 1973). However, other factors are also important in determining cell agglutinability such as cell zeta potential, cell re-

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ceptor location, cell structures such as microvilli, cell deformability, cytoplasmic interactions with the membrane, etc. (Nicolson, 1974a,b). Infection of animal cells by nononcogenic viruses also results in increased lectin agglutinability (Zarling and Tevethia, 1971; Poste, 1972; Poste and Reeve, 1972; Becht *et al.*, 1972; Tevethia *et al.*, 1972).

The assembly and release of membrane-containing animal viruses requires the formation of cell surface aggregates of viral components to form viral "buds" which eventually pinch off yielding the intact virions (reviews: Choppin *et al.*, 1971, 1972). The released viruses contain host lipids from the cell plasma membrane (Klenk and Choppin, 1969), but the proteins and glycoproteins are entirely virus coded (Holland and Kiehn, 1970). Animal viruses can be agglutinated by lectins such as concanavalin A (Con A),[1] a lectin that binds to oligosaccharides containing α-D-glucose or α-D-mannose residues (Agrawal and Goldstein, 1967). Orthomyxoviruses, paramyxoviruses, arboviruses, rhabdoviruses, and others have been shown to contain receptors for Con A (Oram *et al.*, 1971; Calafat and Hageman, 1972; Klein and Adams, 1972; Okada and Kim, 1972; Becht *et al.*, 1971, 1972). Lectin agglutinability of virus-infected cells increases in certain systems before viral components can be detected at the cell surface (Zarling and Tevethia, 1971; Poste, 1972; Poste and Reeve, 1972; Becht *et al.*, 1972; Tevethia *et al.*, 1972). Reeve *et al.* (1974) recently found that the number of Con A-binding sites does not increase during Newcastle disease virus infection, although Con A agglutinability increases dramatically (Poste and Reeve, 1972). In the present study, we have examined the quantities of lectin-binding sites for Con A and *Ricinus communis* agglutinin (RCA_I) (specific for β-D-galactose and sterically similar residues [Nicolson and Blaustein, 1972; Nicolson *et al.*, 1974]) on purified influenza and vesicular stomatitus viruses (VSV) and on virus-infected normal and polyoma-transformed cells.

Materials and Methods

Cell Cultures and Media. BHK 21 (BHK) and polyoma-transformed BHK 21 cells (PyBHK) (from Dr. W. Eckhart, The Salk Institute) were grown in monolayers in 32-oz. prescription bottles in Dulbecco's modified Eagles medium (Gibco #H21) containing 15 mM Hepes buffer with 10% calf serum. MDCK cells (from Dr. J. J. Holland, University of California, San Diego) were grown in the same medium with 10% fetal calf serum. For all studies cells were infected during late-logritic growth before confluency (approximately 5×10^6 cells/32-oz. bottle).

Plant Lectins. Most of the plant agglutinins were purified by affinity chromatography. Con A, obtained as a twice crystallized product (Calbiochem, San Diego), was further purified by absorption onto a 2.5 × 30 cm column of Sephadex G-75. After washing with 0.5 M NaCl–0.05 M sodium phosphate buffer (pH 6.5), the agglutinin was eluted with 0.2 M sucrose in the same buffer (Agrawal and Goldstein, 1967) and extensively dialyzed to remove bound saccharides. *R. communis* agglutinins were purified by the procedures of Nicolson and Blaustein (1972). The dialyzed ammonium sulfate precipitated preparation (0–60% ammonium sulfate fraction) of the *R. communis* agglutinins in 0.2 M NaCl–0.005 M sodium phosphate buffer (pH 7.2) was applied to a 4 × 40 cm column of Bio-Gel A-0.5m agarose (Bio-Rad). After washing the column with buffer, the agglutinins were eluted in a single peak with the same buffer containing 0.2 M D-galactose. RCA_I was separated from RCA_{II} on a 2 × 50 cm column of Sephadex G-100 or Bio-Gel P-100 (Nicolson *et al.*, 1974). Wheat germ agglutinin (WGA) (Burger and Goldberg, 1967) was affinity purified on an ovomucoid-Sepharose column (Burger, 1969, 1970). Crystallized ovomucoid (Sigma Chemical Co.) was coupled to Sepharose by the cyanogen bromide activation method of Cuatrecasas (1970). After extensive washing to remove noncovalently bound protein, the ovomucoid-Sepharose was used to make a 2 × 20 cm affinity column. A crude wheat germ alkaline phosphatase preparation (Worthington) was heat inactivated at 58° for 10 min and then quickly cooled to 5°. After centrifugation to remove precipitated protein, the partially purified WGA preparation was applied to a 2 × 100 cm Sephadex G-75 column and eluted with 1 mM Tris-HCl (pH 7.5). The agglutinating fractions were pooled, concentrated, and applied to the ovomucoid-Sepharose column. After washing with buffer, 0.1 M acetic acid (pH 2) was used to elute the agglutinin, and the eluted fractions were immediately neutralized. *Ulex europaeus* agglutinin was extracted and precipitated in the 0–40% ammonium sulfate fraction according to Matsumoto and Osawa (1969, 1970). The dialyzed, partially purified lectin was used without further purification. *Dolichos biflorus* agglutinin was extracted and affinity purified according to Etzler and Kabat (1970) on polyleucyl A + H hog blood group substance and was provided by Dr. M. E. Etzler (University of California, Davis).

Lectins were radioiodinated in the presence of 0.1 M of the appropriate saccharide inhibitor by the lactoperoxidase method of Arndt-Jovin and Berg (1971) or the iodine monochloride method of McFarlane (1958) and were extensively dialyzed at 4°. No loss in specificity or activity occurred during iodination (Nicolson, 1973a). Some of the ^{125}I-labeled lectins, notably [^{125}I]Con A, were repurified on affinity columns before use.

Virus Growth and Purification. Influenza virus strain NWS used previously (Etchison *et al.*, 1971) was passaged at low multiplicity in MDCK cells before working stocks were grown in 10-day old chicken embryos. The Indiana strain of vesicular stomatitis virus (VSV) was grown in BHK cells, and stocks were prepared by infection of these cells at low multiplicity. Purified influenza virions for quantitative binding studies were grown in 10-day old chicken embryos (Kimber Farms, Fremont, Calif.) for a period of 40 hr. At this time the allantoic fluid was harvested and subjected to centrifugation at 5,000*g* for 10 min. The supernatant was centrifuged in a Beckman-Spinco Type 19 rotor for 3 hr at 19,000 rpm (37,500*g*), and the pellet was gently resuspended in 10 mM Tris-Cl–100 mM NaCl–1 mM EDTA (pH 7.5) (TSE). The resuspended pellet was layered onto a 10–40% Na^+-K^+ tartrate gradient in TSE containing 0.5 mg/ml of bovine serum albumin, and centrifuged at 30,000 rpm in a Beckman-Spinco SW41 rotor for approximately 3 hr. The light-scattering band containing influenza virus was recovered and diluted with the same buffer, and the virus was pelleted by centrifugation at 75,000*g* in the same rotor for 2 hr. The pellet was suspended in a small volume of the tartrate–TSE buffer, applied to a 10–40% sucrose gradient and centrifuged for 75 min at 22,500 rpm in a Beckman-Spinco SW25.1 rotor. Purified influenza virus was collected from the gradient, repelleted as above, and finally suspended in TSE at a concentration of 2–3 mg of protein/ml. VSV was purified in an identical manner except that the starting material consisted of medium from infected BHK cells.

[1] Abbreviations used are: Con A, concanavalin A; RCA_I, *Ricinus communis* agglutinin of mol wt 120,000; VSV, vesicular stomatitis virus; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; RCA_{II}, *Ricinus communis* agglutinin of mol wt 60,000; WGA, wheat germ agglutinin; TSE, 10 mM Tris-Cl–100 mM NaCl–1 mM EDTA (pH 7.5) buffer; TEM, 10 mM Tris-Cl–100 mM NaCl (pH 7.4) buffer.

Binding of ^{125}I-Labeled Lectins to Purified Virions. A saturating amount of ^{125}I-labeled lectin (10 μg of protein in 10 μl) was added to approximately 10 μg of viral protein in phosphate-buffered saline (total volume, 220 μl) and incubated for 15 min at 4°. After the incubation, the mixture was layered onto a 15–45% Na^+·K^+ tartrate–TSE gradient and centrifuged in an SW41 rotor at 30,000 rpm for 90 min; 25-drop fractions were collected from the bottom of the tubes and radioactivity was monitored by counting the fractions in a Packard γ scintillation counter. Controls in identical reaction mixtures contained 0.1 M D-glucose (Con A) or 0.1 M β-lactose (RCA_I). For calculations on the stoichiometry of lectin binding to the purified viruses, we assumed a particle weight of 250 × 10^6 daltons for influenza (Scholtissek *et al.*, 1969) and 630 × 10^6 daltons for VSV (Bishop and Roy, 1972), a molecular weight of 120,000 for RCA_I (Nicolson *et al.*, 1974) and 110,000 for tetrameric Con A (Agrawal and Goldstein, 1967; Kalb and Lustig, 1968).

Electron Microscopy. Lectins were conjugated to ferritin by the techniques of Nicolson and Singer (1971, 1974) using the following final concentrations: ferritin, 4–5%; lectins, 1.5–2%; glutaraldehyde, 0.02–0.03%; saccharide inhibitors, 0.1 M. Ferritin–conjugates were affinity purified before use (Nicolson and Singer, 1974). Purified influenza virions were labeled with the ferritin–conjugates after mounting on thin films as follows: 1 drop of the virus suspension (~0.5 mg/ml of protein) was placed on a carbon-strengthened collodion-coated electron microscopy grid, and the grid was quickly floated face down on a large drop of TEM to remove unattached viruses. The grid was conditioned with a solution of 5% bovine serum albumin in TEM for 1–2 min, touched to a drop of TEM, and then a large drop of ferritin–Con A or ferritin–RCA_I was applied. After 1–2-min incubation at 22°, the grid was rinsed by floating face down on several drops of TEM buffer. The washing steps were complete in approximately 2–3 min. Excess buffer was removed and the grid was quickly rinsed once in distilled water and air dried. Some of the specimens were briefly fixed in 0.5% glutaraldehyde and negatively stained with 1% uranyl acetate (pH 4.8) just prior to drying. Other purified virus samples were labeled with ferritin–Con A or ferritin–RCA_I in solution, washed through a 15–45% Na^+·K^+ tartrate gradient as above, and fixed in 1% glutaraldehyde. The labeled viruses were pelleted, post-fixed in osmium tetroxide and dehydrated, and finally embedded in Epon 812. Thin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Specimens were examined at 75 kV in a Hitachi Model HU-12 transmission electron microscope.

Agglutination and Cell Binding of ^{125}I-Labeled Plant Lectins. Cell agglutination assays were performed in plastic trays with 16-mm wells (Linbro FB-54). Cells (2 × 10^6 per ml) and serial dilutions of the agglutinins were added to each well (total volume, 0.4 ml), and the trays were placed on a rotary table (Henkart and Humphreys, 1971) at 1–2 Hz for 20 min at room temperature. Agglutination was scored by light microscopy (Nicolson, 1973a). Control experiments contained 0.1 M saccharide inhibitors (methyl α-D-mannoside for Con A, β-lactose for RCA, and *N*-acetyl-D-glucosamine for WGA). During these procedures the cells remained >90% viable, as judged by Trypan Blue dye exclusion. Virus agglutination was performed in 20-μl capillaries by the ring test method. Positive agglutination was recorded when massive flocculation was visible under a dissecting microscope. Controls containing inhibitory saccharides were all negative.

For quantitative labeling, 2 × 10^6 cells per ml were incubated for 10 min at 4° with saturating concentrations (usually 100 μg/ml) of [^{125}I]Con A, -WGA, or -RCA_I (total volume, 0.3 ml). Occasionally, 2 mM (final) sodium azide was included in the incubation medium, but the results were generally the same. After the incubation, the cells were washed twice by centrifugation at 4°, and the final pellet was counted in a Packard γ scintillation counter. Controls were treated in the same manner except that the incubation and washing solutions contained 0.1 M of the appropriate saccharide inhibitor. Specific binding was calculated as the average CPM of [^{125}I]lectin bound to quadruplicate or triplicate cell samples minus the average CPM of [^{125}]lectin bound to triplicate controls with 0.1 M saccharide inhibitor present (Nicolson, 1973a; Nicolson and Lacorbiere, 1973).

TABLE I: Number of Con A and RCA_I Receptors on Influenza and Vesicular Stomatitis Viruses.

Virus	Lectin	μg of Lectin Bound per μg of Virus	Molecules Lectin Bound per Virion[a]
Influenza	Con A	0.32	730
Influenza	RCA_I	0.40	830
VSV	Con A	0.04	130
VSV	RCA_I	0.06	320

[a] The following specific activities were used for calculations: [^{125}I]Con A, 5800 cpm/μg; [^{125}I]RCA_I, 87,000 cpm/μg.

Results

Quantitative and Ultrastructural Analysis of Lectin–Virus Interaction. Purified influenza and VSV were examined for lectin binding by agglutination in a ring test assay. Influenza virus agglutinated strongly with Con A, RCA_I, and wheat germ agglutinins as shown by massive flocculation at lectin concentrations less than 100 μg/ml. Influenza virus agglutinated weakly with *Dolichos biflorus* and *Ulex europaeus* agglutinins at lectin concentrations of 500–1000 μg/ml. VSV agglutinated strongly with RCA_I and wheat germ agglutinin at lectin concentrations of 100 μg/ml, weakly with Con A at 500 μg/ml, and showed no reactivity with *D. biflorus* or *U. europaeus* agglutinins. To determine the number of lectin-binding sites on influenza and VSV, preparations of purified virions were incubated with [^{125}I]RCA_I or [^{125}I]Con A as described in Methods and then repurified by equilibrium centrifugation in Na^+·K^+ tartrate gradients. The results of the gradient analyses for influenza virus show that a significant amount of radioactivity from a solution containing excess [^{125}I]lectin was bound to the purified virus band and that the binding was specific as indicated by the ability of saccharide inhibitors to essentially abolish lectin binding (Figure 1).[2] Similar results were obtained for VSV (not shown). The average number of lectin molecules bound per influenza or VSV particle was calculated (see Materials and Methods), and the results of these calculations are presented in Table I. It is interesting to note that the average number of Con A or RCA_I receptors per influenza virion is very close to the number of "spike" glycoproteins in the virus

[2] Occasionally in the [^{125}I]Con A labeled virus preparations a small radioactive peak appeared behind the main virus peak that was not abolished by saccharide inhibitors (Figure 1B). This may indicate that a minor contaminant nonspecifically binds [^{125}I]Con A or the Con A still has some reversible affinity to the virus particles in inhibitor solutions and some of it is displaced from the top of the gradient.

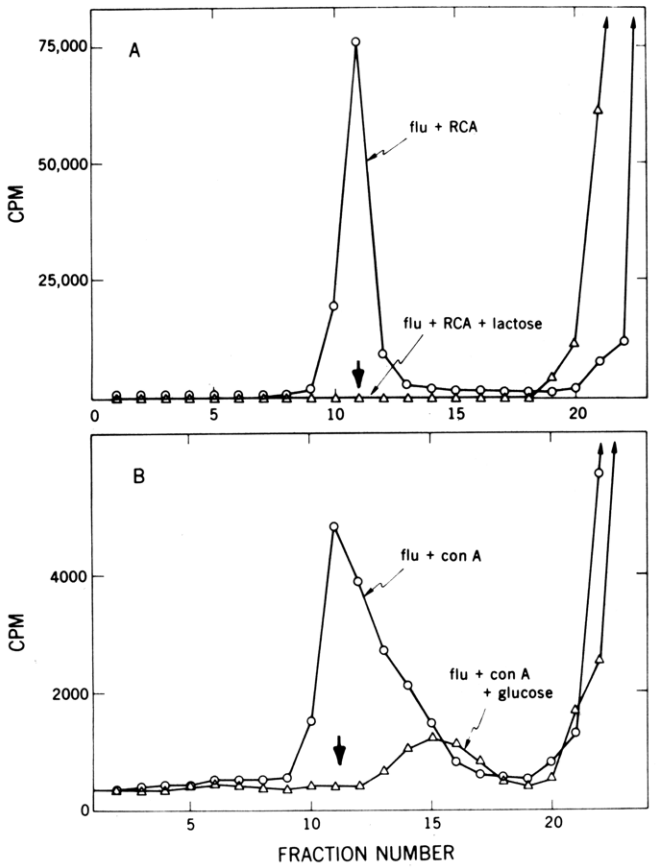

FIGURE 1: (A) Influenza virions were reacted with excess ^{125}I-labeled RCA_I in the presence (Δ) or absence (O) of β-lactose as indicated in Materials and Methods. The reaction mixture was layered onto a linear 15–45% Na^+·K^+ tartrate gradient and centrifuged in a Beckman-Spinco SW41 rotor at 30,000 rpm for 30 min; 0.5-ml fractions were collected from the bottom of the tube, represented at the left of the figure, and counted in a γ radiation counter. (B) Influenza virions were reacted with [^{125}I]concanavalin A in the presence (Δ) or absence (O) of α-methyl-D-glucose or D-glucose. The arrows indicate the positions of maximum pfu in duplicate samples run under identical conditions.

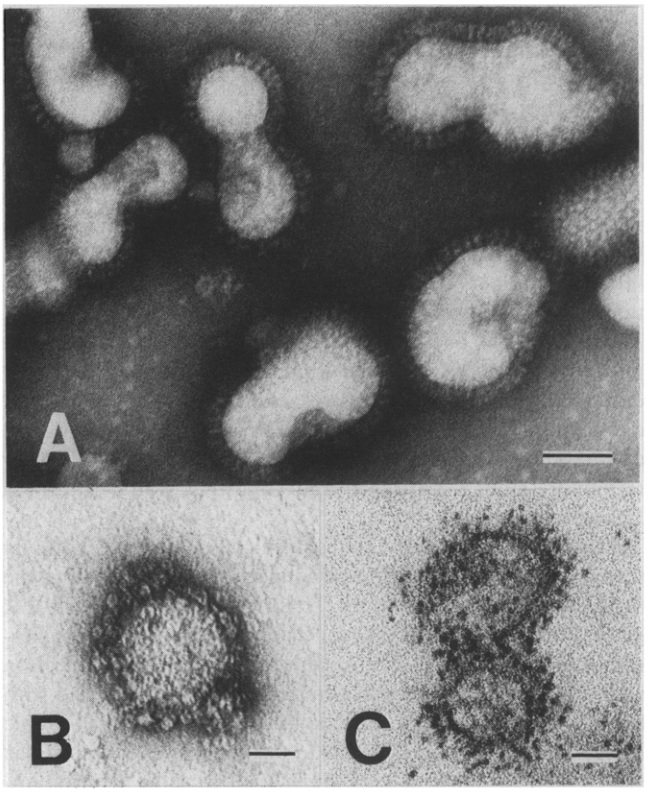

FIGURE 2: (A) Purified influenza preparation negatively stained with uranyl acetate. Bar equals 0.5 μm. (B) Binding of ferritin–RCA_I to purified influenza virions. Viruses were mounted on collodion–carbon films and stained with ferritin–RCA_I. After washing the labeled viruses were fixed in 0.5% glutaraldehyde for 10 min at room temperature and negatively stained with a 1% uranyl acetate solution. Bar equals 0.05 μm. (C) A purified influenza virus preparation was labeled with ferritin–RCA_I and washed through a 15–45% Na^+·K^+ tartrate gradient. The labeled viruses were fixed in glutaraldehyde and post-fixed in osmium tetroxide and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate. Bar equals 0.05 μm.

envelope (Compans *et al.*, 1970).

To confirm that the lectins bind directly to the virus particles and not to possible contaminants in the virus preparation, and to examine the sites of lectin binding in relation to identifiable viral structures, ferritin–Con A and ferritin–RCA_I were reacted with the purified virions in solution or after attachment to thin films. Virus preparations purified as described in Materials and Methods were judged to be highly pure; greater than 90% of the particles were readily identifiable as virus particles in negatively stained preparations (Figure 2A). Virus particles mounted on colloidion–carbon films can be identified by their characteristic size and electron density even without negative staining (McLean and Singer, 1971). When influenza viruses are labeled with ferritin–Con A or ferritin–RCA_I, the ferritin molecules are packed around each virus particle. The labeling was specific, as inclusion of inhibitory saccharides abolished ferritin–lectin binding. In order to visualize the virus spikes, negative staining was used after labeling. Although the ferritin conjugates almost entirely masked the viral spikes (Figure 2B), their distance from the virus capsid surface indicates that they are probably bound to the spikes similar to the finding of Klein and Adams (1972). This was more clearly demonstrated in thin sections of purified virus labeled with ferritin–RCA_I (Figure 2C).

Agglutination and Quantitative Binding of Con A and RCA_I to BHK Cells during Infection with Influenza or VSV. BHK cells grown in monolayer were infected with either influenza virus (NWS strain) or VSV (Indiana strain) at a multiplicity of 10. Various times after infection, cells were removed from the surface of the bottles, and their agglutinability was determined with RCA_I or Con A as described in Materials and Methods. As previously shown by others (Becht *et al.*, 1972; Rott *et al.*, 1972), infection of cells with influenza virus resulted in increased Con A agglutinability within 30–60 min after infection. Similar results were obtained using VSV (Table II). When RCA_I agglutinability was assayed during infection, there was a sixfold increase in agglutination within 30 min post-infection (Table II). It should be noted that the increase in lectin agglutinability of influenza-infected cells was greater than the corresponding increase with VSV and occurred much sooner after infection. To determine whether the increased lectin agglutinability was accompanied by a concurrent increase in the number of available lectin receptors at the cell surface, the number of lectin-binding sites on these cells was monitored before and during infection with [^{125}I]lectins. Our experiments revealed a progressive increase in the number of RCA_I-binding sites on the surface of influenza infected cells as the infection proceeded, while there was only a slight increase in the number of RCA_I-binding sites on VSV-infected cells in comparable time periods (Figure 3). In parallel studies BHK cultures infected with influenza virus were treated with cycloheximide 30 min following infection to inhibit viral pro-

TABLE II: Agglutination of BHK Cells with RCA_I and Con A after Infection with Influenza or Vesicular Stomatitis Viruses.

Time after Infection	Concn of Lectin Required for 4 + Agglutination (μg/ml)	
	RCA_I	Con A
Uninfected Cells	>15	>800
Influenza Virus		
0.5 hr	2.5	250
2 hr	2.5	250
4 hr	2.5	125
8 hr	1.2	31
VSV		
1 hr	10	400
4 hr	5	100
7 hr	5	100

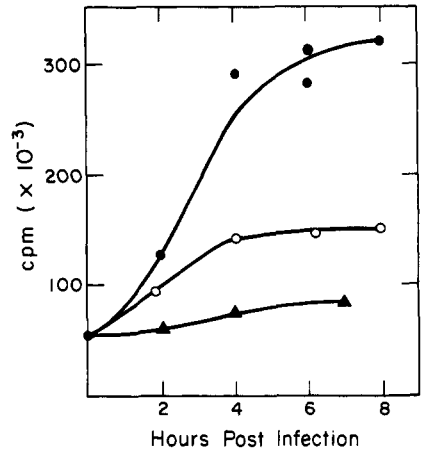

FIGURE 3: BHK or PyBHK cells were infected with either influenza or vesicular stomatitis virus. At various times after infection specific [^{125}I]RCA_I binding to the cells was measured at 4° and calculated as indicated in Materials and Methods: (●) influenza virus-infected BHK cells; (○) influenza-virus infected PyBHK cells; (▲) vesicular stomatitis virus-infected BHK cells. Cells (2×10^6 per ml) were incubated for 10 min at 4° with 100 μg/ml of [^{125}I]RCA_I (specific activity 1×10^5 cpm/μg) in a total volume of 0.3 ml. After the incubation the cells were washed twice by centrifugation. Controls contained 0.1 M β-lactose in incubation and wash media and averaged 5–10% of the samples without saccharide inhibitors present. Standard deviations for the data of five different experiments averaged <15%.

tein synthesis. Cycloheximide-treated cells bound the same number of [^{125}I]RCA_I molecules as the untreated controls at 2 hr post-infection, but further increases in RCA_I binding sites did not occur. When polyoma-transformed BHK cells were infected with influenza virus under identical conditions, the increase in RCA_I-binding sites during infection was much less than the corresponding increase in BHK cells (Figure 3). Experiments with [^{125}I]Con A showed little or no increase in Con A-binding sites on BHK cells infected with either virus, similar to recent reports on Newcastle virus-infected BHK cells (Reeve and Poste, 1974; Reeve *et al.*, 1974).

Discussion

The present study demonstrates that both influenza and vesicular stomatitis viruses have saccharide residues on their surfaces which are capable of interacting with Con A, RCA_I, and WGA. This interaction can result in agglutination of the viruses by these lectins, while certain other lectins with different saccharide-binding specificities are inactive (see also Oram *et al.*, 1971; Calafat and Hageman, 1972; Okada and Kim, 1972; Becht *et al.*, 1971, 1972). Quantification of Con A and RCA_I molecules bound to influenza particles indicates that the number of lectin receptors closely approximates the number of spike glycoproteins present on the surface of the viral particles. This correspondence and the localization of ferritin–lectin conjugates to the viral membrane surface suggest that most, if not all, of the binding sites detected in our experiments are located on the carbohydrate portions of viral coat glycoprotein molecules. Similar to Klein and Adams (1972), it was noticed that the ferritin–lectin molecules bind far enough off the viral membrane surface to implicate the spike structures as the lectin receptors. This has also been demonstrated using stripped ("spikeless") virus particles. Klenk *et al.* (1972) showed that spikeless fowl plague virus particles lose their reactivity to Con A, but gain reactivity toward *D. biflorus* agglutinin.

Infection of BHK cells with influenza or VSV results in a dramatic increase in cell agglutination by Con A. Although earlier studies had reported that Con A-mediated agglutination of mammalian cells increases during nononcogenic viral infection (Zarling and Tevethia, 1971; Poste, 1972, Poste and Reeve, 1972; Becht *et al.*, 1972; Tevethia *et al.*, 1972), the present study shows that this increase is not accompanied by an increase in Con A-binding sites on the surfaces of infected cells. Reeve *et al.* (1974) have recently reported similar findings on the quantitative binding of Con A to Newcastle's disease virus-infected and uninfected cells. They have found that increased agglutinability of the virus-infected cells may be due to an increased mobility of Con A receptors after infection (Reeve and Poste, 1974), similar to the increased Con A receptor mobility after transformation (Nicolson, 1973b; Inbar and Sachs, 1973; Rosenblith *et al.*, 1973). Our results also show a large increase in agglutinability of BHK cells by RCA_I following infection by either virus. In every case examined, most of the increase in lectin-mediated agglutinability took place before significant levels of viral glycoprotein synthesis occurred, indicating that other early events in the infectious cycle such as virus-cellular membrane fusion or virus-induced release of lysosomal constituents (Poste, 1972) must be responsible for altering existing cellular components, increasing susceptibility to agglutination. During influenza virus infection, the increased cell agglutination is accompanied by a large (5–7-fold) increase in the number of RCA_I binding sites. Part of this increase (approximately 25%) occurs early, within 0.5–2 hr post-infection. Significant synthesis of viral proteins does not occur at these early times (Etchison *et al.*, 1971); thus, the increase in RCA_I receptors must be due to an alteration of preexisting cellular or membrane components similar to the Con A receptor modification early after infection. Consistent with this proposal is the finding that concentrations of cycloheximide that almost completely inhibit cell protein synthesis during viral infection have no effect on the early increase in agglutinability and [^{125}I]RCA_I binding. It is likely that the neuraminidase activity of the infecting viruses may be, in part, responsible for the increase in RCA_I receptors (Nicolson, 1973a), since we have shown that treatment of BHK cells with soluble neuraminidase from either *Clostridium perfringens* or *Vibrio cholerae* results in increased RCA_I agglutination of BHK cells and a two- to threefold increase in the number of RCA_I-binding sites on the cell surface (G. L. Nicolson, M. Lacorbiere, and W. Eckhart, in preparation). The bulk of the increase in RCA_I-binding sites after influenza virus infection occurred after the early events in viral replication were completed and viral glycopro-

tein synthesis had reached maximal levels. Because virions bind large quantities of RCA_I and the kinetics of appearance of these sites on the surface of infected cells is similar to the kinetics of viral glycoprotein synthesis in infected cells, we feel that the late increase in RCA_I sites represents binding of the lectin to viral glycoproteins inserted into the cell membrane. This conclusion is supported by the fact that the late increase in RCA_I sites can be blocked by addition of cycloheximide to inhibit viral glycoprotein synthesis; however, these experiments must be interpreted with caution since little is known about the turnover of cellular membrane components under these conditions.

Influenza infected polyoma-transformed BHK cells were also examined for changes in agglutination and binding of $[^{125}I]RCA_I$. RCA_I agglutinability of PyBHK cells was high prior to infection (Nicolson and Blaustein, 1972) and did not dramatically change during infection, and the increase in RCA_I-binding sites on PyBHK cells during infection lagged well behind similar increases in normal BHK cells. Since these experiments were performed under identical conditions with the BHK cell experiments using the same multiplicity and preparation of virus, the differences must be due to cell alterations produced by transformation. Possibilities are that the infection process is not as efficient as in PyBHK cells, the synthesis of viral components is impaired or the insertion of viral components into cellular membranes is affected. In this regard Mallucci and Skehel (1971) observed that infection of polyoma-transformed cells with fowl plague virus results in synthesis and release into the media of viral hemagglutinin but does not result in production of infectious virus. Similarly, influenza infection of PyBHK cells may result in cellular synthesis of RCA_I-binding viral components that are not ultimately inserted into the cell membrane.

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