Semliki Forest Virus-Chick Embryo Cell Interactions

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(Accepted 25 January 1973)

SUMMARY

There is no correlation between virulence in experimental animals and such physical properties of Semliki Forest virus (SFV) as rates of inactivation or electrophoretic and chromatographic distribution patterns. Changes in the surface properties of the virus can be induced in most strains of SFV by addition of polysaccharide derivatives during virus growth in chick embryo cells; these changes cause a variation in the pattern of infection in both chick embryo cells and mice.

Glucosamine and DEAE-dextran both inhibit a function involved in the release of virus from chick embryo cells. It is suggested that one or more of at least five sites may be used during the ‘budding’ of virus from the cell depending on the strain of SFV.

In sugar-free media, mannosamine inhibits transfer of infective material across the cell membrane; both glucose and mannose relieve this inhibition, but relief by galactose is variable, indicating that more than one mechanism may be used. Transfer of infective material across the cell membrane is not always a corollary of attachment of virus to the cell.

INTRODUCTION

Arboviruses mature by ‘budding’ through cell membranes. During this process certain host-imposed characteristics are conferred on the virus particle; as a result the virus population may be reduced in virulence (Hearn & Soper, 1961), and its susceptibility to neutralization by antiserum altered. Attempts have been made to correlate such changes of biological properties with easily determined marker properties. For instance, Hruskova, Danes & Kliment (1970) found a variation in the degree of virulence of Venezuelan equine encephalomyelitis (VEE) virus after passage in mice, guinea-pigs, chick embryos or chick embryo cells (CEC), but were unable to correlate this variation with either plaque size, inactivation at 54 °C or sensitivity to sodium dextran sulphate in plaque assay or CEC culture. Differences in the lipid content of the VEE virus particle have been found after passage in various host systems (Heydrick, Wachter & Hearn, 1966), but again there was no correlation with change of virulence.

The work of Bradiš, Allner & Maber (1971) in defining original and derived strains of SFV in terms of grades of virulence ranging from high (L-strain), through intermediate (V- and K-strains) to low (A-strains), has provided the opportunity for a study of potential virulence markers of another arbovirus closely related to VEE virus. This paper presents these results. Emphasis is placed on the interaction of substituted polysaccharides, hexosamines and related sugar derivatives, and multiplication of SFV in tissue culture, and the influence of polysaccharides on plaque formation in agar suspensions of CEC. The effects
of these macromolecules vary widely from one virus-cell system to another (Balazs & Jacobson, 1966); elucidation of their mode of action should therefore be particularly rewarding. Data on purely physical properties are restricted to results that aid the interpretation of these interactions.

**METHODS**

*Viruses.* The derivation of five strains of SFV from the three original isolations has been described by Bradish et al. (1971). Following their notation, passes in mice (no cipher), CEC(c), mouse L-cells (L), and hamsters (H) are indicated after the strain designation: thus V14.C2 indicates 14 mouse passes followed by two CEC passes since the first isolation of strain V. The sample of the V-strain from L-cells (V14.L1) was that used in previous studies (Fleming, 1971a, b). Stock viruses were stored at −75 °C.

*Virus production.* Production in cultures of L-cells has been described (Fleming, 1971a). CEC monolayers in Carrel flasks were formed from $2 \times 10^7$ cells in Parker’s medium 199 containing 5% calf serum (199,5CS) and used after 24 h incubation at 34 °C under air with 5% carbon dioxide. Details of virus growth media are given in the text; unless otherwise stated, additives were included in the virus inoculum.

*Plaque assay.* The standard plaque assay suspension contained $10^8$ CEC in 10 ml of 0.75% Special Agar-Noble (Difco laboratories, Detroit) in 199,5CS (Bradish et al. 1971); agar was routinely autoclaved for 20 min at 15 lb/in² before mixing with the tissue culture medium. Monolayers for plaque assay were formed as above from $5 \times 10^7$ CEC per 7 cm glass Petri dish.

*Mouse inoculation.* Random-bred Porton mice were inoculated with 0.025 ml of virus solution by intraperitoneal (i.p.) or intracerebral (i.c.) route. When assessing response patterns, mice surviving primary inoculation were challenged at 14 days by i.p. administration of 100 LD₅₀ units of the virulent strain L10.H6.C1. Each LD₅₀ was determined by interpolation of a response–log dose graph.

*Materials.* Sodium dextran sulphate and DEAE-dextran, both prepared from dextran of mol. wt. $2 \times 10^6$, were obtained from Pharmacia Ltd., Sweden.

Sugars and related compounds were obtained as follows: D-glucosamine HCl, D-mannosamine HCl, D-galactosamine HCl, 2-deoxy-D-glucose, 2-deoxy-D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, N-acetyl-D-mannosamine. H₂O, d-glucose-1-phosphate, d-glucose-6-phosphate, d-fructose-1-phosphate, d-fructose-6-phosphate, glucosamine-6-phosphate, uridine-5'-diphosphoglucose, uridine-5'-diphospho-N-acetylglucosamine from Sigma Chemical Co., U.S.A.; d-arabinose, 6-deoxy-D-glucose, 6-deoxy-D-galactose from Koch–Light Laboratories, England; D-galactose, 6-deoxy-D-mannose from Light and Co. Ltd., England; fructose from G. T. Gurr, England; D-glucose from May and Baker Ltd., England; and D-mannose from B.D.H. Chemicals Ltd., England. N-idoacetyl-α-D-glucosamine and N-fluoroacetyl-α-D-glucosamine were prepared by Dr P. W. Kent.

*Chromatography.* Hydroxylapatite was prepared by Dr J. A. Parry for chromatography on columns 60 cm by 1.6 cm diameter. Virus samples were adsorbed from 0.4 M, and eluted with a linear 0.4 to 0.8 M gradient of di-ammonium hydrogen orthophosphate (B.D.H. Chemicals), pH 8.0 to 8.2. Concentrations of ammonium phosphate in the eluate were determined refractometrically. Samples were diluted at least 1000-fold before assay of infective virus.

*Virus inactivation.* Methods have been described for the thermal inactivation of virus (Fleming, 1971a) and for its treatment with guanidine hydrochloride (Fleming, 1971b). For u.v. inactivation, samples of virus solutions 0.5 mm deep were irradiated 50 cm from a
Fig. 1. Influence of DEAE-dextran on plaque formation in agar suspensions of CEC containing 5% calf serum. Agar autoclaved for 20 min at 15 lb/in². Plaques counted after 48 h at 34 °C.

Phillips 30 W u.v. tube. Results for virus inactivation are presented in terms of the original concentration of infective virus, $V_0$, and the concentration of surviving infective virus, $V_s$.

Extraction of agar. One hundred g of Special Agar-Noble in 1500 ml of water was stirred for 22 h at 37 °C, centrifuged for 30 min at 1600 g and the gel discarded. The supernatant fluid was further centrifuged for 1 h at 44000 g and the clear yellow solution obtained was freeze-dried to give 3.4 g of agar heteropolysaccharide (agar HPS). This material was readily soluble in water, remained in solution on adding an equal vol. of double strength saline, but did not completely dissolve in saline. Agar HPS flocculated from saline on adding ethyl alcohol to 75% (v/v), but remained in solution in the absence of salts. Agar HPS was negatively charged at pH 7.5 and was thus precipitated by DEAE-dextran; experiments on growth of SFV in CEC monolayers showed that 9 μg DEAE-dextran neutralized 100 μg agar HPS. Paper chromatography of acid hydrolysates of agar HPS separated mainly galactose with about 10% glucose and mannose residues. Although the original agar contained 0.3% sulphur, sulphate could not be detected in either agar HPS or its acid hydrolysates; uronic acid residues were also absent as shown by a negative reaction in the Dische test. The source of its negative charge has not been determined.

RESULTS

The influence of DEAE-dextran on plaque assay

The ability of many viruses to form plaques in a cell suspension in agar, or in a monolayer under agar, is determined in part by particular properties of the agar (Wasserman, 1968). Inhibitions of plaque assays have often been attributed to sulphated polysaccharides that are either present in the agar preparation or released during autoclaving (Sokolov & Germanov...
The presence of such substances can lead to complete inhibition of plaque formation or enhancement of plaque size and number (Styk & Rada, 1964). When inhibition is caused by negatively charged polyions, addition of a polycation such as DEAE-dextran may allow plaque production (Liebhaber & Takemoto, 1961).

The manner in which DEAE-dextran influenced the plaque assay of SFV depended on the cell from which the virus was derived; results obtained by adding various concentrations of DEAE-dextran to CEC suspensions in 0.75% agar in 199, 5CS are shown in Fig. 1 for SFV from L-cells (V14.L1) and CEC (V14.C2). The concentration of DEAE-dextran at the maximum plaque count (80 μg/ml in Fig. 1) was proportional to the agar concentration, but varied from one agar batch to another. The ratio of the plaque count at this optimal concentration to that without DEAE-dextran is referred to in this paper as the plaque enhancement ratio; this ratio may be less than one when agars with low inhibitor content are used because DEAE-dextran is itself inhibitory.

When the calf serum concentration in the assay suspension was reduced to less than 0.1% a second maximum appeared at 15 μg/ml of DEAE-dextran (Fig. 2); this second maximum was eliminated by aqueous extraction of the agar. Prolonged autoclaving of the agar released a further component that was not neutralized by DEAE-dextran (Fig. 2). The plaque size also varied with concentration of DEAE-dextran, showing a single maximum at 80 μg/ml for all calf serum concentrations tested.

Results presented later will show that DEAE-dextran can inhibit both attachment of virus to cells and a later stage of multiplication. If anionic components were not present in the agar the plaque count would drop sharply on addition of DEAE-dextran; thus absence of enhancement as shown in the lower curve of Fig. 1 does not indicate that the virus is not susceptible to agar inhibitors merely that it is less sensitive.

The demonstration of plaque enhancement by DEAE-dextran requires selection of an
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Fig. 3. Chromatography of SFV on hydroxylapatite. (a) V14.LI at 20 °C. (b) V14.c2 at 20 °C. (c) V14.c2 at 0 °C. Infectivity (●); plaque enhancement ratio (○). The arrows indicate the plaque enhancement ratio of the sample applied to the column.

Fig. 4. Thermal inactivation at 50 °C in 199.5CS of several strains of SFV grown in L-cells. Strains in order of increasing virulence: A7[74].c2.LI (▲); A7.c2.LI (○); V14.c2.LI (●); K3.c2.LI (□); L10.H6.c2.LI (△).
agar with appropriate inhibitor content and the incorporation of calf serum; substitution of bovine plasma albumin (0.1 to 1%, w/v) for calf serum reduced the ability of DEAE-dextran to neutralize agar inhibitors.

**Population heterogeneity**

Results for the separation of virus subpopulations of the V-strain by chromatography on hydroxylapatite are shown in Fig. 3. The L-cell derived virus (V14.L1) provided a conveniently homogeneous reference sample (Fig. 3 a); profiles of the CEC derived virus prepared for these studies are shown for elution at 20 °C (Fig. 3 b) and 0 °C (Fig. 3 c). The CEC-derived virus is heterogeneous and the separation of virus fractions with different plaque enhancement ratios depended on the temperature of the experiment. It is also interesting to note that although homogeneous (V14.L1) and heterogeneous (V14.c2) profiles were obtained by zonal electrophoresis in sucrose density gradients, this technique did not separate fractions with different plaque enhancement ratios.

The properties of the virus that determine plaque enhancement ratios are thus surface characteristics that are not associated with charge.

**Inactivation properties**

Between 20 and 50 °C SFV (V-strain) is thermally inactivated by two processes, one of which predominates below 41 °C and the other above this temperature (Fleming, 1971 a); in this higher temperature range the rate of inactivation depends partly on the cell of origin of the virus. Fig. 4 shows the results for inactivation at 50 °C of the five strains of SFV after parallel passage in L-cells to produce particularly uniform virus populations with a common cell of origin. The V-strain is markedly susceptible to heterogeneity imposed by the host cell
Fig. 6. Effects of anionic polysaccharides on multiplication of SFV. Monolayers of CEC in Carrel flasks were inoculated with 9 ml of virus (A7[74], c2.1.t) at $3 \times 10^2$ p.f.u./ml in 199,5CS containing polysaccharide(s). The infectivity of the supernatant medium was assayed after 16 h at 34 °C. Sodium dextran sulphate (●); agar HPS (▲); sodium dextran sulphate with 200 μg/ml agar HPS (○).
Fig. 7. Multiplication of SFV (V14.c2) in CEC. Monolayers in Carrel flasks were inoculated with 10 ml of virus in 199,5CS. The infectivity of the supernatant medium was assayed after incubation at 34 °C. Infectivity (○); plaque enhancement ratio (▲).

**Influence of polyions on virus multiplication in CEC monolayers**

As shown in Fig. 5(a), attachment of SFV to CEC monolayers at 37 °C took place by two processes; the first was complete in less than 5 min, the second was either reversible for 30 min after inoculation or was due to an alteration of the equilibrium between adsorbed and free virus particles at this time. The influence on plaque count of DEAE-dextran, sodium dextran sulphate, and agar HPS, when present throughout the adsorption phase only, is shown in Fig. 5(b); only agar HPS caused a simple response. Agar HPS and sodium dextran sulphate were complementary in their enhancement of adsorption (Fig. 6); the presence of an optimal concentration of agar HPS had little effect on the inhibiting action of sodium dextran sulphate when both substances were present throughout the growth cycle (Fig. 6).

Release of SFV from CEC monolayers at 34 °C began 4-5 h after inoculation and, at multiplicities of inoculation less than 10^-3 p.f.u./cell, continued for a further 10 h. The plaque enhancement ratio of the released virus was limited to discrete values which, for the V-strain, increased with successive virus growth cycles (Fig. 7).

When present throughout the growth cycle, DEAE-dextran and agar HPS caused variations of the yields of virus from CEC monolayers that depended on the strain of SFV. At the extremes, yields of strain A[74] were enhanced by agar HPS and depressed tenfold by DEAE-dextran, while those of strain V were depressed 50-fold by agar HPS and 10000-fold by DEAE-dextran (Figs. 8a, 9a). Lower yields in the presence of DEAE-dextran were due to the release of fewer infective particles not to slower multiplication. In general, these effects were opposite to those seen when the polysaccharides were present only during virus adsorption (Fig. 5), showing that they can influence both initial and later stages of infection.

The plaque enhancement ratio of the progeny virus of the cultures described in Figs. 8(a) and 9(a) are shown in Figs. 8(b) and 9(b). For virus of the V-strain, increased concentrations
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Fig. 8. Multiplication of SFV (A\textsuperscript{74}, CI. 1.1) in CEC in presence of polysaccharides and plaque enhancement ratio of progeny virus. Monolayers in Carrel flasks inoculated with 9 ml of virus at 3 × 10\textsuperscript{6} p.f.u./ml in 199,5CS containing DEAE-dextran (○); agar HPS (▲). The infectivity and plaque enhancement ratio of the supernatant medium were assayed after 16 h at 34 °C.

of DEAE-dextran or agar HPS led to lower values of the plaque enhancement ratio; but again only discrete values were found with sharp changes at reproducible concentrations of polysaccharide. By contrast, the plaque enhancement ratio of strain A\textsuperscript{74} did not increase on passage in CEC, and was not affected by the presence of DEAE-dextran or agar HPS during the growth cycle. The incorporation of DEAE-dextran or agar HPS in the culture medium thus altered the properties of strain V: high values of the plaque enhancement ratio were only obtained by repeated passage in CEC (Fig. 7); no matter how high the enhancement ratio of the inoculum, lower values resulted after passage in the presence of agar HPS. It is concluded that the plaque enhancement ratio is a characteristic of the virus population that is not directly genotypically determined; chromatography on hydroxylapatite has already indicated that it is associated with a surface property of the virus (Fig. 3) and further studies of thermal inactivation at 50 °C supported this view. Modification of phenotype can thus be induced by passage in different cell lines (Fig. 1), or with different growth media during virus synthesis in CEC.

The concentrations of DEAE-dextran or agar HPS in CEC cultures at which changes occurred in the phenotype of the progeny virus are presented in Table 1 for five strains of SFV.

Effects of aminosugars and related compounds on virus multiplication in CEC monolayers

Kaluza, Scholtissek & Rott (1972) have shown that glucosamine and 2-deoxyglucose inhibit replication of SFV in CEC. Similar observations have been made in the present study, but it was of particular interest to note that, in the absence of glucose, mannosamine was also a potent inhibitor of SFV multiplication. Experiments on inhibition by hexosamines have therefore been restricted to primary CEC monolayers which do not require added...
hexose to support virus growth (Zwartouw & Algar, 1968). Inhibition by glucosamine was not relieved by glucose, mannose or galactose; nor by glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate, UDP glucose or UDP N-acetylglucosamine, substances that are intermediates in cellular aminosugar metabolism. Inhibition by mannosamine was not relieved by fructose, glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate, fructose-6-phosphate, glucosamine-6-phosphate, UDP glucose or UDP N-acetylglucosamine, but was relieved by glucose, mannose and, to a variable extent, by galactose. These effects are summarized in Table 2. They are not entirely strain-determined. For instance, in additional experiments, samples of the V-strain with high (3500) and low (1.6) plaque enhancement ratios were inhibited to 0.01 and 1 % of the normal yields respectively by mannosamine (1 mg/ml) and these values were raised to 0.03 and 6 % by addition of 2 mg/ml of galactose.

Of the other sugars listed under Materials, only 2-deoxyglucose and N-iodoacetyl glucosamine possessed measurable inhibitory properties. However, washing CEC monolayers after exposure to these compounds did not allow virus multiplication, whereas mannosamine and glucosamine were readily and reversibly removed. Fig. 10 shows the results of draining monolayers infected in the presence of these hexosamines, and replacing with inhibitor-free virus solution at a time when control monolayers were producing virus at maximum rate. Virus solutions that had been incubated with the cultures were preferred for replacement because of the very low (less than 1 %) adsorption of virus from the super-
Table 1. Concentrations of DEAE-dextran or agar HPS in monolayers of chick embryo cells at which changes of phenotype of progeny SFV occur

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Concentration (µg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEAE-dextran</td>
</tr>
<tr>
<td>L</td>
<td>10</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
</tr>
<tr>
<td>K</td>
<td>30</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>A[74]</td>
<td></td>
</tr>
</tbody>
</table>

Results were obtained from curves such as those shown in Figs. 8(b) and 9(b).

Table 2. Effect of hexosamines on the yield of SFV from monolayers of chick embryo cells

<table>
<thead>
<tr>
<th>Added sugar:</th>
<th>L</th>
<th>V</th>
<th>K</th>
<th>A[74]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque enhancement ratio...</td>
<td>2</td>
<td>49</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Ratio of yield to inoculum in sugar-free medium</td>
<td>28,000</td>
<td>8,000</td>
<td>1,000</td>
<td>4,000</td>
</tr>
<tr>
<td>Galactosamine, 1 mg/ml</td>
<td>55*</td>
<td>77</td>
<td>65</td>
<td>68</td>
</tr>
<tr>
<td>Glucosamine, 1 mg/ml</td>
<td>1</td>
<td>0.1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mannosamine, 1 mg/ml</td>
<td>2</td>
<td>0.1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Mannosamine, 1 mg/ml + glucose, 2 mg/ml</td>
<td>61</td>
<td>42</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td>Mannosamine, 1 mg/ml + mannose, 2 mg/ml</td>
<td>34</td>
<td>29</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Mannosamine, 1 mg/ml + galactose, 2 mg/ml</td>
<td>9</td>
<td>0.4</td>
<td>8</td>
<td>49</td>
</tr>
</tbody>
</table>

Monolayers of CEC in glucose-free Earle’s salt solution containing 5% calf serum were inoculated with approximately 10^-9 p.f.u./cell; virus in the supernatant medium was assayed after 16 to 18 h at 34 °C.

* Figures are quoted as percentages of the yields in sugar-free medium.

Responses in mice

Responses in mice following i.p. inoculation of SFV are presented in Table 3. The effect of injecting virus in agar HPS solution was to reduce lethality by a factor of 20 to 50. This was almost entirely due to a lower incidence of infection; there was no significant change in numbers of mice protected by primary inoculation. Experiments in CEC have shown that agar HPS can reduce the yield of SFV without a concomitant inhibition of interferon synthesis; this probably also occurred in vivo to produce the reductions in lethality noted, but it should be noted that agar HPS increased the yield of strain A[74] in CEC monolayers (Fig. 8a).

SFV (V-strain) grown in CEC monolayers without additive to give virus with high plaque enhancement ratio, or with agar HPS to give low plaque enhancement ratio, caused different responses following i.p. inoculation of 35-day-old mice. Parallel assay of these two virus populations by plating in CEC suspensions and by i.c. inoculation of suckling mice.
showed that the plaque assay system was five times more sensitive to virus of high plaque enhancement ratio. This tends to reduce the difference in LD₅₀ between the two populations, but the ratio killed to protected is unaltered.

DISCUSSION

Rational virulence markers must ultimately depend on some physical or chemical property of the virus particle; the recognition that differences of virus populations exist can, however, be achieved without the need for understanding the particular property involved. The results presented in this paper indicate that the carbohydrate moiety of the virus glycoprotein can play an important part in determining the outcome of infections in experimental animals; differences in the carbohydrate moiety can be demonstrated by the influence of mannosamine on virus infection in vitro, or, more easily, by variation of the plaque enhancement ratio. The correspondence of low plaque enhancement ratio with higher virulence holds only for a given strain, however, and a more general correlation must await selection of an appropriate cell population.

The carbohydrate content of the virus glycoprotein can thus exert a unique influence on
Table 3. Response of mice as numbers dead or protected following i.p. inoculation of Semliki Forest virus

<table>
<thead>
<tr>
<th>Age of mice (days)</th>
<th>13</th>
<th>13*</th>
<th>27</th>
<th>27*</th>
<th>35</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque enhancement ratio</td>
<td>1</td>
<td>1</td>
<td>320</td>
<td>320</td>
<td>1800</td>
<td>2</td>
</tr>
<tr>
<td>N.p.f.u. inoculated and response</td>
<td>4</td>
<td>4*</td>
<td>12</td>
<td>12*</td>
<td>80</td>
<td>66</td>
</tr>
<tr>
<td>N/1000 p.f.u. inoculated and response</td>
<td>0:2:8</td>
<td>0:0:10</td>
<td>—</td>
<td>—</td>
<td>0:0:10</td>
<td>0:2:8</td>
</tr>
<tr>
<td>N/100 p.f.u. inoculated and response</td>
<td>0:0:10</td>
<td>0:1:9</td>
<td>1:4:4</td>
<td>0:3:6</td>
<td>0:1:9</td>
<td>0:1:9</td>
</tr>
<tr>
<td>N/10 p.f.u. inoculated and response</td>
<td>4:1:5</td>
<td>0:1:8</td>
<td>0:5:4</td>
<td>0:2:7</td>
<td>0:1:9</td>
<td>4:1:5</td>
</tr>
<tr>
<td>N p.f.u. inoculated and response</td>
<td>9:1:0</td>
<td>0:4:6</td>
<td>1:3:4</td>
<td>0:0:8</td>
<td>0:3:7</td>
<td>8:1:1</td>
</tr>
<tr>
<td>N/1000N p.f.u. inoculated and response</td>
<td>—</td>
<td>—</td>
<td>7:0:1</td>
<td>2:5:1</td>
<td>7:3:0</td>
<td>10:0:0</td>
</tr>
<tr>
<td>p.f.u./LD50</td>
<td>0:6</td>
<td>13</td>
<td>100</td>
<td>5500</td>
<td>2500</td>
<td>11</td>
</tr>
</tbody>
</table>

* Inoculum contained 225 μg of agar HPS.
^* Medium in third CEC passage contained 200 μg/ml of agar HPS.
† Responses are shown in the table as numbers of mice dying within 14 days of primary inoculation: number surviving challenge: number dying within 14 days of challenge.

virus-cell interactions. Studies on the structure of SFV have shown that apparently only the protein of the virus envelope contains carbohydrate (Kennedy & Burke, 1972). Sindbis virus is structurally similar to SFV and is identical in this respect; more detailed analysis showed the existence of two glycoproteins (Schlesinger, Schlesinger & Burge, 1972) the carbohydrate content of which depended on the cell of origin of the virus (Strauss, Burge & Darnell, 1970). The association of carbohydrate with only envelope protein is not surprising since the specific transferases responsible for glycosylation of the peptide chains are located in the cell membranes (Hallinan, Murty & Grant, 1968; Schenkein & Uhr, 1970; Zagury et al. 1970), where virus ‘buds’ in the final stage of maturation. Glycosyl transferases have specificities both for the activated sugar and the protein or glycoprotein receptor (Roseman, 1970), and, since only enzymes already present in the cell are likely to be used in virus protein glycosylation (Burge & Huang, 1970), virus maturation can only occur in cells with these necessary enzyme systems. The most likely linkage to the nascent protein is of N-acetylglucosamine (Strauss et al. 1970) by a β-glycosidic bond to the amido group of asparagine (Marshall & Neuberger, 1970; Spiro, 1970) and blocking of the essential enzyme catalysing this addition is the most probable site of glucosamine inhibition (Klenk, Scholtissek & Rott, 1972); this also serves to explain the failure of mannosamine and galactosamine to inhibit this step in the growth cycle. Subsequent addition of carbohydrates to the new glycoprotein is possibly less critical and the heterogeneity of some preparations of SFV may be due to variations of these carbohydrate moieties. The alterations of surface properties of the virus induced by incorporation of DEAE-dextran or agar HPS in virus growth media may then be ascribed to the progressive and successive inhibition of different glycoprotein-glycoside transferases. The differences noted in Table 1 may thus indicate differential blocking of permitted sites of maturation for the five strains of SFV.

It has been proposed (Winterburn & Phelps, 1972) that the carbohydrate moiety of glycoproteins determines their destination, possibly by interaction with glycosyl transferases.
in the receptor cells. The stepwise rise in the plaque enhancement ratio of SFV with successive growth cycles in CEC can only be explained by the preferential uptake of virus by cells with some similar properties to those from which the infecting virus derived, thus implicating some similar selection mechanism. The unusual reactivation of SFV following exposure to urea or guanidine hydrochloride, which is accompanied by a change in the plaque enhancement ratio (Fleming, 1971b), may thus be due to modification of the carbohydrate structure of the glycoproteins in the virus envelope; the extent of the reported reactivation of up to a 1000-fold suggests that the integrity of the carbohydrate is essential for the successful infection of the cell. The experiments described here show that adsorption of virus does not guarantee infection of the cell; selection can therefore occur at a later stage, presumably in the penetration of the cell membrane.

Transfer through the cell membrane both during the early and final stages of infection is only one of the functions of the cell essential to virus growth; the uniqueness of transport phenomena is one way in which cells could accept or reject infective material and by which infection may be directed to particular regions of the cell. Since cellular enzyme systems change or develop during growth of an animal, this factor may contribute to the animal host susceptibility range. The lack of correlation between virulence in mice and markers involving growth of virus in CEC may therefore be expected; it seems essential for markers to be determined using primary cells, or organs, of the relevant host. It must, however, be realized that synthesis of complete virus is not always necessary for pathogenicity – abortive infections may be fatal in some cases (Walder, 1971).

The author is grateful to Miss A. Beeson for skilled technical assistance; Dr P. W. Kent for the preparation and gift of N-iodoacetyl-α-D-glucosamine and N-fluoroacetyl-α-D-glucosamine; Dr D. C. Ellwood for the analysis of agar HPS; K. Allner and K. Gerdes for performing the animal experiments.

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(Received 13 December 1972)