Interaction between Viruses and Platelets: Requirement of Haemolytic Activity for Platelet Aggregation Induced by Paramyxoviruses

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SUMMARY

Newcastle disease virus (NDV) or Sendai virus added to washed suspensions of rabbit or human platelets induced a brisk platelet aggregation (P.A.) response concomitant with release of biologically active components from storage granules (platelet release reaction, P.R.). NDV which had been rendered non-infectious by u.v. irradiation for 5 min retained its capacity to induce P.A. and P.R. and to agglutinate or lyse erythrocytes. Treatments of paramyxoviruses (heating at 56 °C; disruption by Tween 80/ether) which resulted in loss of haemolytic activity also eliminated P.A. and P.R. reactions but had little or no effect on virus haemagglutinating properties. A non-haemolytic virus, influenza A2, had no detectable effect on platelets when used at a concentration having a haemagglutination titre equivalent to that of platelet-aggregating doses of NDV or Sendai virus.

It was concluded that the properties of paramyxoviruses responsible for causing lysis of erythrocytes were required for induction of P.A. and P.R. reactions. In this respect, the virus-induced platelet responses may be similar to other cellular reactions to paramyxoviruses such as fusion of cell membranes and development of cytotoxic or cytopathic changes.

INTRODUCTION

Although thrombocytopenia is a prominent feature of many virus infections, there is relatively little evidence to substantiate the various mechanisms proposed to explain this host response to a virus (McKay & Margarettten, 1967). Experimental studies indicate that viruses may react directly or indirectly with platelets leading to several phenomena, some of which may be interrelated: (1) phagocytosis of virus particles by platelets (Mustard & Packham, 1968); (2) stabilization of infectivity or accelerated inactivation of arboviruses incubated at 37 °C in washed suspensions of human or rabbit platelets, respectively (Larke & Wheelock, 1970; Larke, Chernesky & Turpie, 1972); (3) agglutination or clumping of platelets by myxoviruses and paramyxoviruses, with or without platelet lysis (Jerushalmy, Kohn & de Vries, 1961; Terada et al. 1966); (4) alteration of the clotting and clot-retracting activities of platelets by Newcastle disease virus (NDV) and influenza virus (Jerushalmy et al. 1962; Cohen et al. 1966); (5) aggregation of platelets by soluble complexes formed by
viruses and their specific antibodies (Myllyla et al. 1969); and (6) induction of platelet release (P.R.) and platelet aggregation (P.A.) reactions by paramyxoviruses in the absence of gammaglobulin or plasma co-factors (Turpie et al. 1973).

The P.R. and P.A. reactions, collectively termed viscous metamorphosis of platelets in the earlier literature, refer to the striking biochemical and morphological alterations which platelets undergo in response to a wide variety of agents (perhaps the best studied of these being the enzyme thrombin): the platelets become adherent to each other, in association with the release from storage granules of adenine nucleotides and biologically active amines such as serotonin (Mustard & Packham, 1970). In a detailed study reported previously, we concluded that P.A. induced by NDV and Sendai virus was mediated by adenosine diphosphate (ADP) lost from platelets through the release reaction and by platelet lysis induced by these viruses (Turpie et al. 1973). This communication presents evidence that P.A. induced by paramyxoviruses is mediated through the virus component(s) also capable of causing lysis of erythrocytes.

**METHODS**

*Preparation of rabbit platelets.* Rabbits were anaesthetized with sodium pentobarbital (30 mg/kg body weight), the carotid artery cannulated and the blood collected into acid-citrate-dextrose (1 part to 6 parts blood). Washed platelet suspensions were prepared by the method of Ardlie, Packham & Mustard (1970) and adjusted to 10^9 platelets/ml.

*Preparation of human platelets.* Blood was collected by venepuncture from normal subjects who were on no medications. Washed platelet suspensions were prepared by the method of Mustard et al. (1972) and adjusted to 10^9 platelets/ml.

*Radioactive labelling of platelets.* Platelets were labelled by incorporating 5-hydroxytryptamine creatinine sulphate ([3H]-serotonin; Amersham/Searle Corporation, Des Plaines, Ill.) into the first washing fluid (3 μCi/10^10 platelets) and incubating for 30 min at room temperature.

*Platelet aggregation (P.A.).* Samples of a platelet suspension (1 ml) were warmed at 37 °C for 5 min and P.A. was recorded after the addition of 0.1 ml of virus or control stimulus, using a turbidimetric device (platelet aggregometer) (Mustard et al. 1964). P.A., as measured by changes in light transmission, was expressed as a percentage of the aggregation response induced by the lowest dilution of untreated, control virus employed in a specific experiment. Comparative P.A. readings were taken 1 min following the addition of stimulus.

*Platelet release (P.R.) – release of platelet [3H]-serotonin.* Samples were removed from the aggregometer 1 min after the addition of virus or control stimulus and centrifuged immediately for 1 min in an Eppendorf centrifuge (Brinkmann Instruments, Rexdale, Ont.). Platelet-free supernatant fluids (0.1 ml) were added to scintillation vials containing 2 ml of absolute ethanol and 10 ml scintillator solution prepared by dissolving 5 g of 2,5-diphenyloxazole and 0.3 g of 2,2′-phenylenebis (5 phenyl-oxazole) in 1 l of toluene. Radioactivity was counted in a Philips liquid scintillation counter. Release of [3H]-serotonin was expressed as a percentage of that released in response to the lowest dilution of untreated, control virus employed in a specific experiment.

*Viruses.* Sendai virus and the Hickman strain of NDV were used in these experiments. A virulent strain of NDV isolated from fowl dying from Newcastle disease will be referred to as the Ontario isolate. One million 50% egg infectious doses (EID50) of virus were inoculated into the allantoic cavity of 11-day-old embryonated hens’ eggs which were incubated at 36 °C for 48 to 72 h. Infectious allantoic fluids were harvested, pooled and centrifuged at 9500g for 15 min in a Beckman L3-50 ultracentrifuge. The supernatant fractions were
Platelet aggregation by paramyxoviruses

then centrifuged at 15000g for 45 min, following which the pellets were resuspended in phosphate-buffered saline (PBS) containing 0.001 M-CaCl₂, 0.5 ml per 9.0 ml of original allantoic fluid. Each resuspended pellet was disrupted for 2 min in an Artek Sonic 300 dismembrator (12 Hz), employing a sonicating cup with a cold-water jacket. The preparations were then pooled and centrifuged at 9500g for 15 min and the supernatant fluids used as virus seed. The infectivity titre of NDV was 10⁶.5 to 10⁷.5 EID₅₀/ml and that of Sendai virus was 10⁶.0 to 10⁷.0 EID₅₀/ml. Samples of virus seeds were stored in polystyrene tubes at −70 °C until required for experimental use. Influenza A viruses (PR8/34 and A₂/HK/1/68) and uninfected control allantoic fluids were processed in a similar manner. Influenza viruses were obtained from the Virus Laboratories, Laboratory Centre for Disease Control, Ottawa, and possessed infectivity titres between 10⁶.5 and 10⁷.5 EID₅₀/ml.

Titration of virus haemagglutination (H.A.). Virus H.A. was assayed using the Cooke Engineering microtitre system (Sever, 1962). Serial twofold dilutions of virus seeds were made in PBS (beginning with initial dilutions of 1:10 and 1:100) and mixed with equal vol. of 0.5% washed guinea-pig erythrocytes. H.A. end-points were determined following incubation for 45 min at room temperature and expressed as H.A.U per 0.05 ml.

Titration of virus haemolysis (H.L.). Employing a method adapted from Neurath & Sokol (1962), 0.4 ml of a 5% washed suspension of human group O erythrocytes was added to 0.2 ml of a virus preparation. The resulting mixture was incubated at 38 °C in a shaking water bath for 2 h (60 strokes/min). The samples were then cooled immediately in a 4 °C water bath. PBS (0.4 ml) was added to each sample which was then centrifuged at 20000g for 15 min at 4 °C. The haemoglobin content of the supernatant fluids was measured as E₅₄₀ in a Coleman spectrophotometer. For each experiment, maximum H.L. (100%) was represented by the haemoglobin content in the supernatant fraction of the erythrocyte suspension exposed to undiluted, untreated virus seed.

Titration of virus infectivity. The EID₅₀ was measured by inoculating 0.1 ml of serial 0.5 log₁₀ dilutions of virus samples into the allantoic cavity of embryonated hens’ eggs. Allantoic fluid harvested after 2 days’ incubation at 37 °C was then assayed for H.A. as an index of virus replication.

Treatment of virus with u.v. irradiation. Samples of NDV (2 ml) were placed in shallow cups (2 cm diam.) at a distance of 14 cm from a General Electric G30T8, 30 W germicidal lamp and exposed to u.v. irradiation for varying intervals.

Treatment of virus with heat. NDV was heated in a 56 °C water bath for 5, 15 or 40 min intervals. In all cases, the action of heat was stopped by placing the samples in an ice bath.

Treatment of virus with Tween 80/ether. A vol. of 0.25 ml of 1.25% Tween 80 (Fisher Scientific Co., Toronto, Canada) was added to 2.5 ml of NDV seed. The mixture was stirred at 4 °C for 10 min, after which 2.5 ml of ether was added and mixed for 20 min at 4 °C. The resulting material was centrifuged at 900g for 20 min. The bottom fraction was collected and residual ether evaporated under air. Saline was treated similarly to serve as a control for the presence of residual Tween 80 or ether. As a further control, saline was substituted for the vol. of Tween 80 and ether added to a virus sample.

RESULTS

P.A. and P.R. induced by viruses

Addition of 10⁶.5 EID₅₀ of NDV to a washed suspension of rabbit platelets initiated the response shown in Fig. 1. The preliminary depression in the aggregometer tracing immediately following the addition of a stimulus has been shown to be due to a change in
Fig. 1. Aggregation of rabbit platelets in response to $10^{+2}$ EID₉₀ of NDV (Hickman strain).

Table 1. *Haemagglutination (H.A.), haemolysis (H.L.), platelet aggregation (P.A.) and release of platelet $[^3H]$-serotonin (P.R.) in response to viruses and egg material*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Dilution</th>
<th>H.A.</th>
<th>% H.L.</th>
<th>% P.A.</th>
<th>% P.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newcastle disease virus (Hickman strain)</td>
<td>Undiluted</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:10</td>
<td>8192*</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>—</td>
<td>99·0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>2048</td>
<td>60·2</td>
<td>24·1</td>
<td>10·5</td>
</tr>
<tr>
<td></td>
<td>1:80</td>
<td>—</td>
<td>43·1</td>
<td>3·8</td>
<td>3·8</td>
</tr>
<tr>
<td></td>
<td>1:160</td>
<td>512</td>
<td>8·9</td>
<td>0</td>
<td>1·9</td>
</tr>
<tr>
<td>Sendai virus</td>
<td>1:20</td>
<td>2048</td>
<td>72·3</td>
<td>42·7</td>
<td>38·4</td>
</tr>
<tr>
<td>Influenza virus (A₂/HK/1/68)</td>
<td>1:8</td>
<td>2048</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Allantoic fluid</td>
<td>Undiluted</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chorioallantoic membrane</td>
<td>1:2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0·7</td>
</tr>
</tbody>
</table>

* Titre expressed as the reciprocal of the highest dilution of virus causing H.A.

platelet shape from discoid to spherical (Mustard & Packham, 1970). This was followed by a rapid increase in light transmission as the platelets became aggregated.

Table 1 summarizes interactions of NDV, Sendai virus and influenza virus with erythrocytes and rabbit platelets. A comparison of equivalent haemagglutinating doses (2048 H.A.U.) of the three viruses showed that only those which caused lysis of erythrocytes (NDV and Sendai virus) induced P.A. and P.R. reactions. P.A., P.R. and H.L. were dose-dependent, as shown when NDV was serially diluted through 1:160. Similar observations of dose-
Table 2. Effect of u.v. irradiation on NDV-induced haemagglutination (H.A.), haemolysis (H.L.), platelet aggregation (P.A.), release of platelet [3H]-serotonin (P.R.) and on virus infectivity (Hickman strain)

<table>
<thead>
<tr>
<th>Time of u.v. exposure (min)</th>
<th>H.A.</th>
<th>% H.L.</th>
<th>% P.A.</th>
<th>% P.R.</th>
<th>Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8192*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>8192</td>
<td>100</td>
<td>99:3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>8192</td>
<td>100</td>
<td>86:9</td>
<td>86:1</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>8192</td>
<td>100</td>
<td>73:6</td>
<td>68:4</td>
<td>0</td>
</tr>
<tr>
<td>65</td>
<td>8192</td>
<td>68:9</td>
<td>52:4</td>
<td>35:0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Titre expressed as the reciprocal of the highest dilution of virus causing H.A.

Table 3. Effect of heat on NDV-induced haemagglutination (H.A.), haemolysis (H.L.), platelet aggregation (P.A.), and release of platelet [3H]-serotonin (P.R.). Comparison of Hickman strain and Ontario isolate

<table>
<thead>
<tr>
<th>Time of exposure at 56 °C (min)</th>
<th>H.A.</th>
<th>% H.L.</th>
<th>% P.A.</th>
<th>% P.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hickman</td>
<td>Ontario</td>
<td>Hickman</td>
<td>Ontario</td>
</tr>
<tr>
<td>0</td>
<td>10240*</td>
<td>5120</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>2560</td>
<td>5120</td>
<td>8:2</td>
<td>8:2</td>
</tr>
<tr>
<td>15</td>
<td>80</td>
<td>5120</td>
<td>7:0</td>
<td>5:7</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>5120</td>
<td>0:8</td>
<td>0:9</td>
</tr>
</tbody>
</table>

* Titre expressed as the reciprocal of the highest dilution of virus causing H.A.

dependency were made using Sendai virus. Uninfected allantoic fluid, either crude or processed similarly to virus seeds, and saline extracts of uninfected chorioallantoic membranes did not agglutinate or lyse erythrocytes and did not elicit P.A. and P.R. reactions.

Data comparable to that summarized in Table 1 were obtained when washed suspensions of human platelets were used in place of rabbit platelets.

Effect of u.v. irradiation on NDV-induced P.A. and P.R.

Exposure of NDV to u.v. irradiation for 5 min (Table 2) did not affect H.A., H.L., P.A. or P.R. but did destroy virus infectivity, indicating that virus-induced P.A. and P.R. did not require infectious particles. With more prolonged irradiation, there was a gradual diminution of P.A. and P.R., no effect on H.A. but moderate reduction in H.L.

Effect of heat on NDV-induced P.A. and P.R.

After exposing either the Hickman strain or Ontario isolate of NDV to heat (56 °C) for 5 min (Table 3), the P.A. response was eliminated while the P.R. reaction and haemolytic activity were reduced by approx. 90%. The H.A. activity of the Ontario isolate was stable at 56 °C for as long as 40 min, whereas that of the Hickman strain was reduced more than 100-fold after only 15 min of heating. These data indicated that by exposing viruses to heat the haemolytic and H.A. activities of NDV could be separated to a variable extent using the Ontario isolate; furthermore, the capacity of NDV to induce P.A. and P.R. responses was related closely to virus haemolytic activity and appeared to be independent of H.A. activity.
Table 4. **Effect of Tween 80/ether treatment of NDV (Hickman strain) on virus-induced haemagglutination (H.A.), haemolysis (H.L.), platelet aggregation (P.A.), and release of platelet $[^{3}H]$-serotonin (P.R.)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H.A.</th>
<th>% H.L.</th>
<th>% P.A.</th>
<th>% P.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDV + saline</td>
<td>5120*</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NDV + Tween 80/ether</td>
<td>20480</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Titre expressed as the reciprocal of the highest dilution of virus causing H.A.

**Effect of Tween 80/ether treatment on NDV-induced P.A. and P.R.**

The data presented in Tables 1 to 3 suggested that virus-induced P.A. and P.R. responses might be mediated through the haemolytic component of the virus rather than through its haemagglutinin. Treatment of paramyxoviruses with Tween 80/ether has been shown to release haemagglutinin from other virus components (Norrby, 1964). A control preparation of NDV (Hickman strain) treated with saline had an H.A. titre of 1:5120 and elicited maximal H.L., P.A. and P.R. Following treatment of the same virus seed with Tween 80/ether, the H.A. titre was 1:20480 but the preparation had no demonstrable P.A., P.R. or haemolytic activity (Table 4).

Platelets pre-incubated with saline which had been treated with Tween 80/ether showed P.A. and P.R. responses following the addition of untreated NDV, eliminating the possibility that residual Tween 80 or ether might have rendered the platelets refractory to stimulation.

**DISCUSSION**

A brisk P.A. response, as illustrated by the aggregometer tracing in Fig. 1, was induced by NDV and Sendai virus. It was not possible to evoke P.A. or P.R. reactions using concentrations of influenza virus type A (Hong Kong variant) which had H.A. titres equivalent to those of platelet-aggregating doses of NDV or Sendai virus (Table 1).

In other experiments, we attempted unsuccessfully to induce P.A. with the PR8 strain of influenza A virus using H.A. concentrations equivalent to those employed by Terada et al. (1966) in their comparative studies of virus interactions with platelets and erythrocytes. These authors reported that both live and heat-inactivated influenza virus adsorbed rapidly to human platelets and that during this process, prominent platelet clumping occurred. Under their experimental conditions, platelets which had lost normal metabolic activity following storage for as long as 22 days still adsorbed live virus as actively as in the fresh state. In contrast, we have shown that virus-induced P.A. and P.R. reactions were impaired when the platelets were deprived of a source of metabolic energy (Turpie et al. 1973).

A previous report by Jerushalmy et al. (1961) indicated that the time-curves of adsorption of NDV and influenza virus (strain FM1) to human platelets at various temperatures resembled the time-curves of adsorption to erythrocytes.

It would appear that Terada et al. (1966) and Jerushalmy et al. (1961) were measuring agglutination or clumping of platelets by viruses with an affinity for sialic acid receptor sites, a virus-cell interaction resembling more closely the agglutination of erythrocytes (H.A. reaction). However, certain aspects of their reports are difficult to explain on the basis of an H.A. type of response: (1) platelets which adsorbed live or heat-inactivated influenza virus remained agglutinated and underwent swelling, ballooning and fragmentation, regardless of whether subsequent elution of virus occurred (Terada et al. 1966) and (2) less virus eluted from platelets than from erythrocytes and the process of elution from platelets took
Platelet aggregation by paramyxoviruses

a much longer time – observations which were attributed to possible incorporation of virus into platelets (Jerushalmy et al. 1961).

The term ‘viscous metamorphosis-like’ was applied by Cohen et al. (1966) to morphological and biochemical alterations of platelets in the presence of NDV, suggesting that they were studying responses related to the P.A. and P.R. reactions reported here. However, it is difficult to compare closely our own findings with data reported from other laboratories. Although some virus-platelet reactions, such as the acceleration of thromboplastin formation by NDV, occurred within a few minutes, most other data had been recorded following the incubation of viruses with platelets at 37 °C for 90 min without stirring (Jerushalmy et al. 1962; Cohen et al. 1966). P.A. and P.R. reactions reported in our studies occurred within 1 to 2 min after the addition of NDV or Sendai virus to stirred suspensions of platelets at 37 °C. Thus, by using sensitive turbidimetric techniques to record P.A. and radioisotopes to measure serotonin release from the secretory granules, our observations resembled most closely the platelet responses reported by other investigators using stimuli such as ADP, thrombin or collagen (Mustard & Packham, 1970).

Various properties of NDV were studied to determine the component(s) responsible for inducing P.A. and P.R. reactions. Virus infectivity could be destroyed by brief exposure of NDV to u.v. light but this irradiation had no appreciable effect on virus interaction with either platelets or erythrocytes (Table 2). Data from several of our experiments indicated that virus haemagglutinin was not the active component inducing P.A. and P.R. reactions: (1) a concentration of influenza virus having an H.A. titre equivalent to that of platelet-aggregating doses of NDV and Sendai virus had no detectable effect on platelets (Table 1); (2) Ontario isolate of NDV retained full H.A. activity after heating at 56 °C while undergoing a striking reduction in its capacity to induce P.A. and P.R. responses (Table 3); and (3) high concentrations of partially purified haemagglutinin released by treating NDV with Tween 80/ether did not induce P.A. or P.R. reactions (Table 4).

Virus neuraminidase was considered as a possible inducer of the platelet responses observed in our system in view of a report by Hovig (1965) that P.A. was induced by the selective removal of sialic acid from the platelet membrane. However, studies by Dr M.A. Packham (personal communication) have indicated that highly purified neuraminidase was capable of removing all available sialic acid from the membrane of washed rabbit platelets without causing P.A. or P.R.; neuraminidase-treated platelets were capable of aggregation by subsequent stimulation with thrombin or ADP. The neuraminidase preparation used by Hovig (1965) may not have been completely free of contaminating proteolytic enzymes since impure preparations of neuraminidase have caused small P.A. and P.R. responses in our studies. Platelets treated with chromatographically purified neuraminidase were unresponsive to a subsequent inoculum of NDV, thus indicating that attachment of virus to receptor sites on the platelet membrane was a necessary step in NDV-induced platelet reactions (Chernesky, Larke & Turpie, 1973). Influenza viruses, although reputedly high in neuraminidase content, did not induce platelet reactions or lyse erythrocytes in our experiments. Fenner (1968) has suggested that the high neuraminidase activity and consequent rapid elution of myxoviruses from erythrocytes may account for the usual lack of haemolytic activity demonstrated by influenza viruses.

Thus, we have observed that viruses (NDV and Sendai virus) known to cause lysis of erythrocytes are also capable of inducing P.A. and P.R. reactions. Treatments of these viruses which reduced their haemolytic activity similarly decreased their ability to react with platelets in our experiments, leading to the conclusion that the haemolytic component of paramyxoviruses caused the observed P.A. and P.R. reactions.
Stronger support for the concept that the haemolytic activity of NDV and Sendai virus was responsible for P.A. and P.R. reactions induced in our test system would have come from experiments using haemolysin completely free of other virus components. Despite repeated attempts, we were unable to repeat the experiments of Neurath (1964) in which he had separated fractions relatively high in virus haemolytic activity from haemagglutinin by disruption of virus with sodium deoxycholate and subsequent column chromatography. Our inability to separate an active haemotytic component from disrupted virus particles may be explained by the recent observations of Apostolov & Almeida (1972). In an elegant morphological study of haemolysis and cell fusion induced by Sendai virus, these authors demonstrated by electron microscopy the rapid production of holes in erythrocyte membranes and emphasized the importance of an integral virus envelope and innermost (nanogranular) layer for the process to occur. Presumably, intact NDV or Sendai virus with haemolytic activity may create breaks in the platelet membrane, leading to release of adenine nucleotides including ADP which then causes platelet aggregation. In addition to the rapid release of platelet granule contents induced by NDV there is a slower loss of cytoplasmic constituents indicating platelet lysis (Turpie et al. 1973) and this lysis has been confirmed by electron microscopy of aggregated platelets (Larke, Chernesky & Turpie, 1971).

Cell lysis and fusion of cell membranes by paramyxoviruses are closely related phenomena, if not both part of a single response. Bratt and co-workers, in detailed quantitative studies, have shown that strains of NDV with high haemolytic activity were also the most potent inducers of ‘fusion from without’ in cultures of chick embryo cells (Bratt & Clavell, 1972; Clavell & Bratt, 1972a, b). These authors describe this type of fusion as the direct result of the interaction between cell membranes and virus particles in the infecting inoculum, without the requirement of productive infection.

The phenomenon of cell fusion may also occur as a cytotoxic or cytopathic response to infection by paramyxoviruses (Bratt & Gallaher, 1969). There is a marked similarity between our findings in a study of virus-induced platelet reactions and those reported by Mason & Kaufman (1961) in an investigation of factors affecting the cytotoxic reaction between NDV and cells in vitro. In both studies, virus-cell interaction took place rapidly: P.A. occurred within 1 min following addition of NDV, while the cytotoxic reactions described by Mason & Kaufman (1961) became irreversible to neutralization if immune serum was added to cell monolayers 3 min or more after inoculation of NDV. Virus infectivity was not required for the induction of platelet responses nor cytotoxic reactions induced by NDV. Another similarity between the two studies was the observed heat destruction of the P.A. factor or the cytotoxic activity of NDV without loss of virus haemagglutinin. Mason & Kaufman (1961) drew an analogy between the cytotoxic reaction and haemolysis caused by NDV; likewise, we have shown, albeit indirectly, that the component(s) of paramyxoviruses capable of causing lysis of erythrocytes are also responsible for inducing P.A. and P.R. reactions in vitro.

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REFERENCES

Platelet aggregation by paramyxoviruses 

213


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