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Association of the Cytopathic Effect of Sindbis Virus with Increased Fatty Acid Saturation

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SUMMARY

The effect of Sindbis virus on cell leakiness, as measured by chromium release, and on the fatty acids in chick embryo fibroblasts and Vero cells was studied. The appearance of the cytopathic effect in both cell types coincided with virus replication, increased cell leakiness and relative increase in saturated eighteen carbon fatty acid (C18:0, stearic acid) compared to the eighteen carbon unsaturated fatty acids (C18UFA). It is postulated that the increase in cell leakiness and the appearance of cytopathic effect could be the result of a physical change in the lipids of the cell membrane brought about by the increase in the saturation of the eighteen carbon fatty acids.

The two general types of cytopathic effect produced by viruses are the rounding and detachment of cells, and the production of syncytia (polykaryons, fusion from within). The appearance of both coincides with replication of the respective viruses, and also with increased cell permeability (Katzman & Wilson, 1974). Although there is an extensive literature on all the aspects of virus–cell interactions, the mechanism of the production of cytopathic effect is poorly understood (Bablanian, 1975).

Recently, it was found that viruses can induce a change in the degree of saturation of the fatty acids (FA) in infected cells. The virulent strains of Newcastle disease virus (NDV) produced a marked and progressive increase in the unsaturated fatty acids (UFA), while the avirulent strains failed to do so. It was postulated that the greater capacity of the virulent strains for production of polykaryons (syncytia) in vitro was due to the greater fluidity of the cell membranes resulting from the increase in the UFA (Blenkharn & Apostolov, 1981). In a separate study, it was also shown that the fusogenic properties of NDV and Sendai viruses (capacity for cell fusion, haemolysis and cell entry) can be inhibited after treatment of their virions with free iodine (Apostolov, 1980). There was good correlation between the degree of saturation of the FA with iodine and the degree of inhibition of haemolysis. It was postulated that the saturation of the double bond in the UFA by iodine, with the resultant increase in their melting points, led to membrane immobilization (Blenkharn & Apostolov, 1980).

In an effort to study the effect of a lytic virus on the FA, we chose Sindbis virus and chick embryo fibroblast (CEF) cells. The strain of Sindbis designated AR339 (obtained from Mr J. Selway, Wellcome Research Laboratories, Beckenham, Kent, U.K.) was passaged four times in CEF. The cells, prepared by a standard method, were seeded and grown at the second passage in 25 cm² plastic bottles to 70% confluence or about 0.5 × 10⁶ cells/bottle. The cultures were infected at an m.o.i. of 50 and after 1 h adsorption were washed and maintained in minimal essential medium (MEM) until harvested. At timed intervals these cultures were frozen and thawed three times, extracted and analysed by gas–liquid chromatography (GLC) using a method described previously (Apostolov & Barker, 1981). Radioactive chromium labelling was applied to the cells while in suspension before seeding them into tubes (10⁵ cells/tube) (Koschel, 1971; Polos & Gallaher, 1981). The chromium was applied at 37 °C for 30 min with 100 μCi Na₂[⁵¹Cr]O₄ (sp. act. 250 μCi/μg Cr). The ⁵¹Cr was obtained from Amersham International. After washing, the cells were exposed to virus at an m.o.i. of 50 for
Short communications

Cytopathic effect

Fig. 1. Kinetics of virus replication, cell permeability and C18 sat./C18 unsat. ratio in CEF. The cells were infected with 50 p.f.u./cell and yields of virus were determined at the indicated intervals (▲). 51Cr release counts (●) were obtained by subtracting the counts of the control cells from the infected ones. The saturation index (C18 sat./C18 unsat.) was obtained by gas-liquid chromatography and represents the ratio of the respective peak areas (●, control; □, infected). Cytopathic effect is represented by crosses, e.g. 5+, 100% cytopathic effect.

1 h. After three washings the cells were suspended in growth medium supplemented with 5% foetal calf serum (FCS) and distributed into tubes. At timed intervals the supernatants were harvested and counts made in a Wallac gamma counter GTL 300–500. The plaque assay was done on supernatants of bottles subsequently processed for GLC. Plastic Petri dishes (8 cm²) with CEF grown to confluence overlaid with 0.5% serum maintenance medium containing 0.5% agarose were used. After 3 days incubation and removal of the overlay, the cell sheet was stained with 20% (w/v) crystal violet solution in 50% ethanol containing 10% formalin.

The combined results of two separate experiments are shown in Fig. 1. We used the ratio of peak area of saturated eighteen carbon FA (C18 sat.) over the combined peaks of the eighteen carbon unsaturated FA (C18 unsat.) because in the programme for GLC used, the peaks of the three UFA (C18:1, C18:2; C18:3) coincided, but more importantly because there was little change in the other major group of FA – the sixteen carbon ones (Apostolov & Barker, 1981). Under the conditions of a single-step cycle the exponential rise in virus replication (p.f.u. counts), 51Cr release and saturation index (the ratio of C18 sat. over C18 unsat.) coincide with the appearance of the cytopathic effect.

In Fig. 2 we present the combined results of two separate experiments with the same virus, but in Vero cells (the seed cells were obtained from Flow Laboratories). The methodology was the same as in the experiment reported in Fig. 1. In spite of more than five passages of the virus in Vero cells, the virus was less cytopathic than in CEF and even at very high input multiplicities (m.o.i. 100) produced a late cytopathic effect in about 60% of the cells. Again the cytopathic effect coincided with the rise in p.f.u., 51Cr release and the saturation index.

The main question raised by these results is whether the reversible increase in the saturation index is involved in the causation of the cytopathic effect. The physical and
thermodynamic properties of the lipids depend predominantly on the degree of saturation of the constituent FA (Seelig & Seelig, 1977). Although changes in tissue-culture medium can induce changes in the saturation of the FA (Rothblat & Kritchevsky, 1967), it is only recently that it has been shown that significant changes in the saturation of the FA can be induced by viruses (Blenkharn & Apostolov, 1981). It is interesting that the changes in saturation are limited to the C18 FA for Sindbis virus (Fig. 1 and 2) as well as interferon (Apostolov & Barker, 1981). As the C18 FA constitute more than 60% of all the FA of the cell, the changes in these should affect the physical properties of the lipids most. There is a big difference in the melting points of the stearic acid (C18:0, m.p. 69.4 °C), oleic acid (C18:1, m.p. 13.4 °C), linoleic acid (C18:2, m.p. –9 °C) and linolenic acid (C18:3, m.p. –16 °C). It is reasonable to postulate that the increase in saturation of the C18 FA leads to physical change in the lipids of the cell membranes, resulting in increased cell leakiness and the appearance of cytopathic effect. In a recent paper it was shown that the changes in cell permeability in poliovirus-infected cells for the monovalent cations were unselective and the rounding of the cells (cytopathic effect) was probably not an osmotic pressure phenomenon (Nair, 1981).

The reversible nature of the change in the saturation of the C18 fatty acids is interesting. Further work to study this problem is in progress.

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