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Unsaturated Free Fatty Acids Inactivate Animal Enveloped Viruses

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With 2 Figures

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Summary

Unsaturated free fatty acids such as oleic, arachidonic or linoleic at concentrations of 5—25 μg/ml inactivate enveloped viruses such as herpes, influenza, Sendai, Sindbis within minutes of contact. At these concentrations the fatty acids are inocuous to animal host cells *in vitro*. Naked viruses, such as polio, SV40 or EMC are not affected by these acids. Saturated stearic acid does not inactivate any viruses at concentrations tested. Though the mode of action of unsaturated fatty acids is not understood, electronmicrographs of enveloped viruses treated by them indicate that the inactivation is associated with disintegration of the virus envelope.

Introduction

It is widely accepted that the lipids of the viral membrane in enveloped animal viruses are mainly derived from the host cell plasma membrane during the maturation and "budding out" of the virions (1). Moreover, since all viral lipids originate from the host cell, and since their majority exists there prior to viral infection (2) it was possible to study virus-specific lipid selectivity by comparing the lipid composition of the viral envelope with that of the host cell plasma membrane. Such studies have established that virtually all the different groups of lipids that are found in the host plasma-membrane are also present in the viral membrane envelope. However, quantitatively, the lipid composition of viral membranes differs from plasma membranes of host cells in the following parameters: The ratio of sphingomyelin to phosphatidylcholine, the ratio of saturated to unsaturated fatty acids and the ratio of cholesterol to phospholipids are significantly higher in the lipid core of the virus envelopes as compared to the lipid bilayer of the host cell plasma membrane (1, 3, 4). These lipids alterations are

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also associated with a marked increase in the relative amount of "rigid" lipids (5) and therefore it is possible to assume that the overall "membrane rigidity" is higher in the viral envelope. Indeed with the aid of recently developed new techniques of electron spin resonance (6) and fluorescence polarization (7) it has been demonstrated that the degree of "membrane rigidity" is significantly higher in the viral membrane than in the host cell plasma membrane (8—12).

The chemical specificity and the dynamic organization of lipids in the viral membrane, as well as the mechanism which controls selective incorporation of rigid lipids into the virion's envelope during viral maturation are not yet fully understood. However, the question arises as to whether a "rigid membrane" envelope is required for an effective viral infection by enveloped viruses. In order to achieve a better insight into this problem we have studied the effect of incorporation of unsaturated free fatty acids into the viral envelope on viral infectivity *in vitro*. The rational behind these experiments is based on previous findings indicating that the presence of unsaturated phospholipid acyl chains (13) and unsaturated free fatty acids (14) in a membrane is associated with a marked increase in "membrane fuidity".

Materials and Methods

Viruses, Cells and Media

The following viruses were used: Myxoviruses (Influenza A, Mel.), Paramyxoviruses (Sendai and Newcastle disease viruses), Arboviruses (Sindbis and West Nile), Picorna (Poliovirus strain MEF2 and Encephalomyocarditis), Papova (SV40) and Herpes viruses 1 and 2.

Myxo- and paramyxoviruses were grown and titrated in 10-day old embryonated eggs; arboviruses in BHK-21 cells (Eagle's medium). EMC virus was titrated in L-929 cells (Eagle's medium), Poliovirus in monkey line BGM (M-199 medium), Herpes virus in Vero cells (M-199 medium) and SV40 in monkey BSC line.

Fatty Acids

Oleic acid (cis-9-octadecenoic acid), Linoleic acid (cis-9—12-octadecadienoic acid), Arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid from porcine liver)—(Sigma) were used at concentrations of 5—100 μg/ml. Stock solutions of 1 mg/ml were prepared in ethanol, and from this solution dilutions were made in buffer. For studies on incorporation of linoleic acid, 1-^{14}C Linoleic acid (51 mCi/mmol)-(Amersham) was used.

Electron Microscopy

Na-phosphotungstate (pH = 7.3) stained virus suspensions were examined in JEM-100B transmission electronmicroscope.

Fluidity Measurements

Suspensions of control and of cells with unsaturated fatty acids were labeled with DPH and their fluorescence polarization was determined in an MV-1 Elscint Microviscosimeter (5).

Results

Our present results indicate that a short (10—60 minutes) *in vitro* incubation of enveloped viruses (Myxovirus, Paramyxovirus, Arbovirus or (Herpesvirus) with low (10—100 μg/ml) concentration of linoleic acid (C18:2) results in a marked

loss of viral infectivity (Table 1). Linoleic acid is an essential fatty acid which is readily taken up by the cells and metabolized to phospholipids (15). Linoleic acid, up to the concentration of 25 μg/10^6 cells/ml, does not affect the growth of BHK cells. The cells in monolayer multiply to the same extent as control cells (Fig. 1) and incorporate ^{3}H-TdR equally well (data not shown).

Table 1. *Effects of linoleic acid on the infectivity of viruses*

Virus			Time incubation min. at 25° C	Test cells	Virus infectivity	
Envelope	Nucleic acid	Name			Control (log)	Decrease (log)
+	RNA	Sendai	60	Eggs	8.3	4.8
		NDV	15	Eggs	8.0	2.5
		Influenza A_1 (MEL)	30	Eggs	6.0	3.0
		Sindbis	45	BHK	7.3	3.0
		West Nile	30	BHK	7.1	3.8
	DNA	Herpes 1 (VR)	30	Vero	6.5	4.2
−	RNA	Polio	45	BGM	8.5	0
		EMC	45	L929	8.6	0
	DNA	SV40	30	BSC	8.0	0

Viruses listed in this table were incubated with 10 μg/ml of linoleic acid then titrated in eggs or cell cultures. Results are expressed as logs of EID_{50} (eggs) PFU (cell cultures)

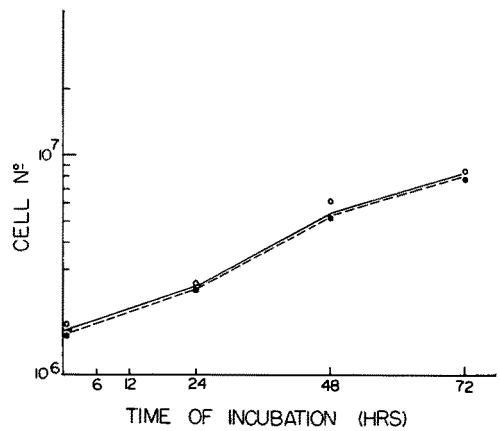

Fig. 1. Effect of linoleic acid on the growth of BHK cells. BHK cells were incubated in the presence (• — — — •) or absence (o———o) of linoleic acid (10 μg/ml) in parallel dishes and at time indicated cultures were washed, cells removed by trypsin, and the suspensions were counted in a hemocytometer

Sindbis virus, taken as a model, was inactivated not only by linoleic acid but also by oleic acid (C18:1) and arachidonic acid (C20:4) (Table 2); stearic acid (C18:0) however was without effect. It may thus be concluded that the inhibition of viral infectivity is associated with unsaturated free fatty acids, presumably due

to their incorporation into the viral membrane lipids. Additional evidence in support of this assumption was obtained from similar experiments carried out with naked (non-enveloped) viruses. An *in vitro* incubation (up to 45 minutes) of poliovirus strain MEF_2, encephalomyocarditis, and SV40 viruses with linoleic acid (10 μg/ml) did not affect the infectivity of these viruses (Table 1). It is there-

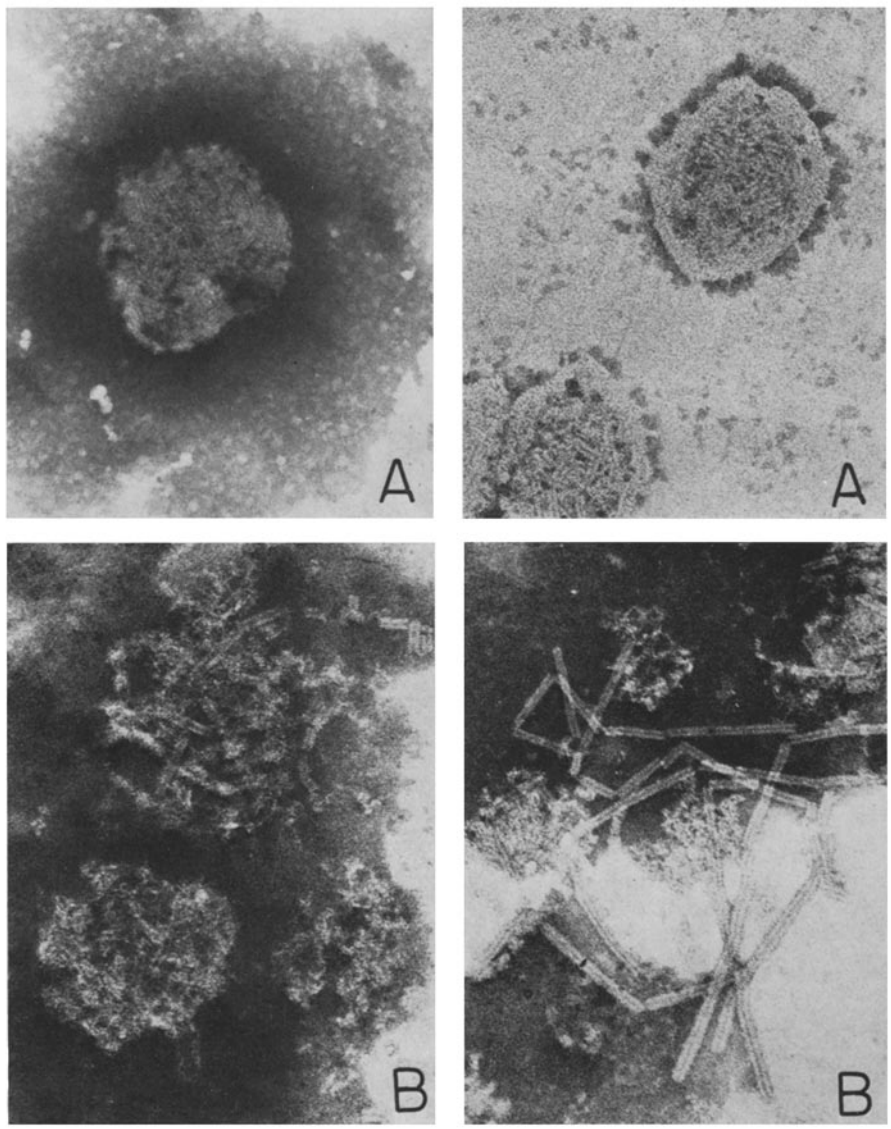

Fig. 2. Electronmicrographs of Sendai virus before *(A)* and after *(B)* treatment with linoleic acid (10 μg/ml) for 8 minutes. Control samples of Sendai virus are shown in *(A)*, Sendai virus treated with linoleic acid is shown in *(B)*. These samples were stained with phosphotungstate and examined with a JEM-100B transmission electronmicroscope. The results show a structural damage of the virions after treatment with linoleic acid. Magnification (×85,000)

fore suggested that only viruses that contain a lipid membrane are susceptible to inhibition by unsaturated free fatty acids. Furthermore, the suppression of infectivity of enveloped viruses by unsaturated free fatty acids is probably due to changes in the dynamic organization of the viral envelope which causes structural damage to the virions. Electronmicroscopical studies support this hypothesis. As it is shown in Fig. 2, the envelope of Sendai virus treated with free unsaturated fatty acids was destroyed so as to release its nucleocapsids. Similar disruption was observed in Sindbis virus treated with linoleic acid (not shown).

Table 2. *Effect of polyunsaturated fatty acids on Sindbis virus*

Concentration of fatty acids μg/ml	Virus infectivity after exposure to			
	Linoleic	Arachidonic	Oleic	Stearic
Control 0	7.3	7.6	7.8	8.0
10	3.6	4.0	6.0	8.0
50	3.3	3.8	3.6	8.0
100	1.0	2.1	ND	ND

To a suspension of linoleic, arachidonic, oleic and stearic acid in one ml of PBS, 2—6×10^7 PFU of Sindbis virus were added with vigorous shaking. After 15 minutes incubation at room temperature, the suspensions were titrated on BHK cells

Discussion

Our observations are in good agreement with recent studies carried out with the lipid containing bacteriophage PR4 (16), which also suggested that a short *in vitro* treatment with PR4 with low concentrations of unsaturated free fatty acids or their derivatives inhibited the entry of the phage DNA into *Escherichia coli* and thus caused a marked loss of infectivity of PR4 bacteriophage.

SANDS *et al.* (17), also demonstrated that treatment of enveloped PM2 phage with monopalmitolein resulted in complete disassembly of the phage, and that unsaturated monoglycerides and alcohols were active against enveloped phages and herpes virus (18).

In our experiments we have demonstrated a direct effect of unsaturated fatty acids on the lipid envelope of a variety of animal viruses. Their envelopes disintegrated under the influence of unsaturated fatty acids such as linoleic, oleic or arachidonic, but were not affected by stearic acid. The loss of protective envelope in these viruses accounts for the loss of their infectivity.

The molecular details of the interaction between unsaturated free fatty acids and the membrane bilayers of enveloped virus are still obscure. There are two possible mechanisms which can account for the inactivation of enveloped viruses by the unsaturated fatty acids: 1. the fatty acids form micelles which act as a detergent; 2. monomers of fatty acids are incorporated into the lipids of the envelope of the virions and change its dynamic equilibrium. Since the effective working solution of fatty acids was about one third of their critical micellar concentration (16, 19), its detergent activity is unlikely. One can therefore conclude that the disruption of the virions was indeed due to the incorporation of monomers or oligomers of the unsaturated fatty acids into the lipids of the virion envelope.

In any case, inhibition of enveloped virus infectivity induced by treatment with "fluid" free fatty acids presents one model system convenient for study of the relation between the lipid composition of the viral envelope and virus infectivity. Moreover, these present findings may also be useful for the possible development of new antiviral drugs against clinically important human viruses such as Herpes virus and Influenza virus.

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