Egg Fluids Contain a Stimulating Substance for Growth of Viruses

An enhancing factor for replication of viruses was found to be present in embryonated eggs infected with viruses belonging to the myxo and paramyxovirus groups[1-3]. It was suggested that the enhancing ability of this agent was due to its inhibitory effect on interferon. The following report demonstrates the presence of a stimulating agent for growth of viruses in fluids of non-infected fertilized eggs.

Materials and methods. The enhancing factor. Allantoic or yolk sac fluids were harvested from fertilized Leghorn or White-Roc chicken eggs.

Cell cultures. Mouse L_{929} cells were grown in Eagle's minimum essential medium supplemented with 10% calf serum. Primary African green monkey (Vervet) kidney cells were grown in M_{199} medium containing 3% calf serum. Primary chick embryo fibroblasts (CF) were prepared from 10-day-old chick embryos and grown in M_{199} medium enriched with 10% calf serum. An established cell-line of hamster embryo cells-Clone B[4], was grown in Eagle's modified medium containing 10% calf serum. Cells reaching a confluent monolayer were further grown in maintenance medium supplemented with 1% calf serum.

Viruses. Semliki forest virus (SFV), strain B 26146, originally obtained from the American Virus Registry, was kindly supplied by Prof. R. GOLDWASSER, Israel Institute for Biological Research, Ness-Ziona. Stock of virus was prepared in L_{929} cells. Vesicular stomatitis virus (VSV) was grown in clone B cells. Virus yields were determined by the plaque assay on clone B cell monolayers in petri dishes overlaid by maintenance medium containing 1.5% carboxymethylcellulose.

Enhancement assay. Cells were treated with allantoic or yolk sac fluids diluted 1:10 in maintenance medium. After 24 h incubation at 37 °C, the supernatants were discarded and cells infected with viruses tested for stimulation. Following 1 h of absorption at 37 °C, the virus inoculum was removed, the unabsorbed virus particles washed away, and maintenance medium added. After 24 h of incubation at 37 °C, tissue culture fluids were harvested and virus yields were titrated by the plaque assay on clone B cells. The enhancement rate was determined by computing the logarithmic difference between virus titer in cultures treated with the enhancing factor and control titer in non-treated cultures.

Interferon assay. Titer of mouse interferon was determined in L_{929} cells by plaque inhibition assay[5] using VSV as indicator virus. One Plaque-depressing-dose (PDD_{50}) is defined as that dose of interferon which reduces plaque number to 50% of the control count.

Results and discussion. The first step was to determine the virus-cell system most susceptible to enhancement. The following viruses were tested for sensitivity to the henancing effect: Chikungunya virus, Western equine encephalitis virus, Semliki forest virus, vesicular stomatitis virus, Sindbis virus, Turkey meningoencephalitis virus, encephalomyocarditis virus, Newcastle disease virus, Sendai virus, influenza virus, *Herpes simplex* virus, and *Vaccinia* virus.

The enhancement assays were conducted in 3 types of tissue cultures: L_{929} cells, Vervet-kidney cells and chick fibroblasts. Of all the viruses tested, the Semliki forest virus was found to be the most susceptible to enhancement. The multiplication of this virus was stimulated in chick fibroblasts and monkey-kidney cell cultures, but was most prominent in L_{929} mouse cell-line. Therefore, the L_{929}-SFV system was chosen as a model for further investigation of this phenomenon.

Two strains of SFV were isolated by 3 successive steps of purification from single plaques. The most sensitive was the strain producing large plaques (8–9 mm in diameter) under agarose overlay, or large elongated plaques (4×15 mm) under carboxymethylcellulose overlay. It was found that as multiplicity of infection decreased (from 1 PFU to 10^{-5} PFU per cell), a higher rate of enhancement was achieved.

An experiment was designed to establish whether cell sensitivity to the enhancing factor is influenced by the age of cells in culture. It was found (Table) that while 2-day-old cells did not respond to the enhancing factor, a stimulation of 5 logarithms was obtained by using 5-day-old cultures. These data also indicate a marked depression of virus growth in aged cells; however, treatment with egg fluids reverted the capacity of aged cells to produce a high yield of virus, similar to the yield obtained in young cells.

Having determined the optimal age of the cell monolayer and the number of virus particles for infection, experiments were performed to establish the rates of enhancement during growth cycles of SFV in 5-day-old L_{929} cells, infected at a very low multiplicity (10^{-5} PFU/cell). Figure 1 illustrates a 10 times stimulation in virus multiplication already within the first viral growth cycle (5–10 h). The rate of stimulation increases up to 10^5 times in subsequent cycles.

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Enhancement of SFV replication in 2- and 5-day-old cell cultures

Treatment of cells	2-day-old cultures		5-day-old cultures	
	Yield of virus PFU/ml	Rate of enhancement log	Yield of virus PFU/ml	Rate of enhancement log
Control-medium	1.5×10^9	–	2×10^4	–
Yolk sac fluid	$3.\times10^9$	0.15	1×10^9	4.9
Allantoic fluid	2×10^9	0.05	9×10^8	4.8

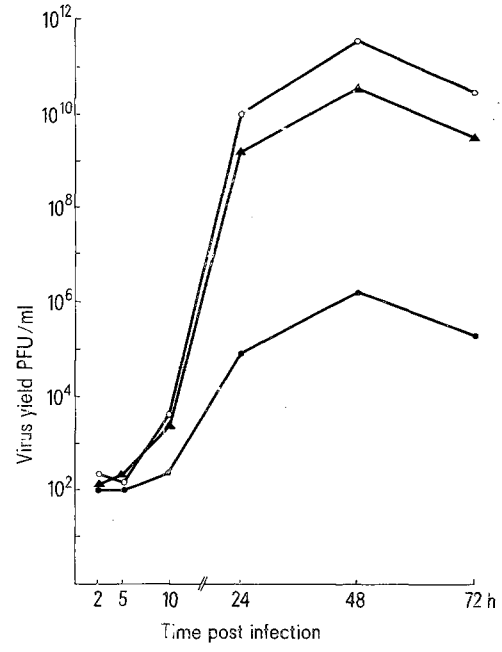

Fig. 1. Growth cycle of SFV in treated and untreated L_{929} cells. Control untreated cells (●); cells treated with yolk sac fluid (○); cells treated with allantoic fluid (▲).

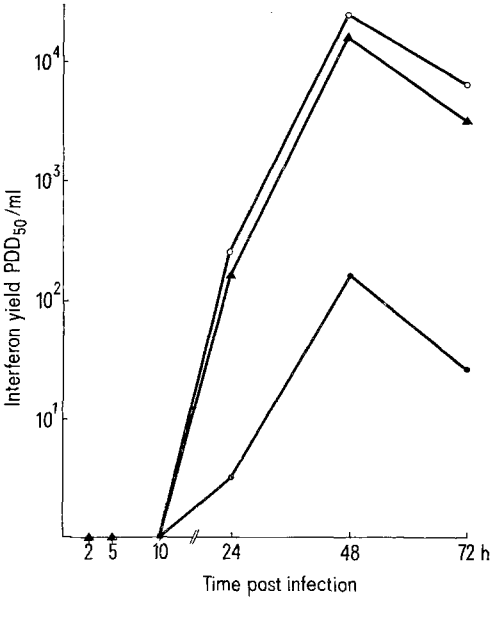

Fig. 2. Interferon production in treated and untreated L_{929} cells during SFV growth. Control untreated cells (●); cells treated with yolk sac fluid (○); cells treated with allantoic fluid (▲).

Reports in the literature[6,7] reveal that egg fluids have an inhibitory effect on interferon. It was thus desirable to clarify whether a blocking effect on the endogenic interferon, formed during viral replication, was responsible for the stimulation of SFV.

The effect of egg fluids on interferon synthesis was the first parameter to be explored. The data obtained (Figure 2) indicate that egg fluids, far from having a suppressive effect on interferon production, actually increased its yield, possibly as a consequence of enhanced virus titer.

An inhibitory effect on the antiviral action of interferon might be the alternative mechanism for the stimulatory phenomenon observed. Therefore, interferon, obtained from West Nile virus-infected mice brain, was titrated in L_{929} cells treated by the enhancing factor and in untreated control cells, using VSV as a challenge virus. Only a slight inhibition (about 2-fold) of interferon activity was observed after egg fluids treatment, a result which cannot explain the 10^5-fold enhancement in virus production.

These experiments indicate that the mechanism of the enhancement phenomenon is not based mainly on an inhibitory effect of the endogenic interferon. It is possible that the enhancing factor acts as a stimulator in one of the stages of viral multiplication.

The precise chemical nature of the enhancing factor, present in egg fluids, has not yet been determined. However, investigation of some of its physical and chemical properties have eliminated the possibility of its being a protein, lipid or nucleic acid. This conclusion is based on the fact that the enhancing factor preserved its activity after heating at 100 °C for 60 min, and even after exposure to 120 °C at a pressure of 1.5 atmospheres for 20 min. This factor is neither dialyzable nor can it be sedimented at 123,000 × *g* for 4 h, and retains its activity after treatment at different pH values over a range from pH 1 to pH 10 for 72 h. Exposure to proteolytic enzymes, such as trypsin or pronase, and to lipase, RNase or DNase, did not lead to any decrease in the enhancing activity.

Résumé. Un agent stimulant la reproduction des virus a été trouvé dans les liquides des œufs fécondés, non-infectés. Cet agent a stimulé la multiplication des virus Semliki Forest jusqu'à 10^5 fois dans les cellules L_{929}. Nous avons constaté que le méchanisme de ce phénomène stimulant n'est pas basé principalement sur l'effet suppressif de l'interféron endogénique.

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Lateral Hypothalamic Self-Stimulation and Post-Stimulation Eating

We have shown that eating can be elicited by electrical stimulation of the neocortex and hippocampus, as well as by KCl-induced single waves of spreading depression in these structures[1–3]. In rats the eating in all cases occurs reliably with a latency of 2–6 min and is usually preceded by short bouts of shaking (especially upon hippocampal stimulation). We have suggested that propagated steady potential change is the common underlying event that