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EFFECT OF INTERFERON AS A FUNCTION OF ITS DOSE AND ADDITION TIME

F I YERSHOV IE B TAZULAKHOVA

D I Ivanovskiy Institute of Virology, AMN SSSR, Moscow (Institut virusologii)

The result of interferon on the infection of a cell by viruses depends as much on the peculiarities of each separate component of the system as on the quantity of and type of interrelationships. So far as the process of formation and development of cellular resistance during addition of homological interferon is concerned, the level of resistance is significantly affected by the time period during which interferon is introduced and the quantity of interferon. Interest in the influence of these factors has provoked study of these two factors and their relationship to the dynamics and final results in the infective process.

In the present work virological methods and time-lapse photography are used in combination with a phase-contrast microscope. These methods represent more objective and improved attempts to record the dynamics of the various biological processes involved. The possibility of following multiple reproduction of the studied processes on a screen allows slight but highly significant details to be exposed so that the exact time of the appearance of these details during the behavior of the cell can be ascertained. We believed that the combination of these methods would allow a correlation to be developed between the peculiarities of viral reproduction during the introduction of interferon into the culture fluid and the cellular reactions under these conditions.

Materials and Methods

Viruses. Venezuelan Horse Encephalytis (VEE) and Eastern Northamerican Horse Encephalomyelitis were received from the Museum of Viruses of the D. I. Ivanovskiy Institute of Virology, AMN SSSR. For the experiment the viruses were implanted in chicken embryo fibroblasts. The modified method of Porterfield was used for determination of the infectious titer.

Cells. The original tryptic fibroblasts of chicken embryo was used, as well as transplantable A-1 and KEM cells.

For microphotographic filming of cells before the experimental phase, they were cultivated in V I Milyutin's glass chamber and after interferon was added, microcamera MKU-1 was used under phase contrast with a speed of one frame every 20 to 30 seconds, which was sped up 500 to 600 times during projection.

Interferons. For extraction and titration of interferons the methods we described earlier were used. We determined the titer of interferon in the culture fluid of A-1 and KEM according to the suppression of the cytopathologic effect (CPE).

Investigative Results

Dynamics of the development of cellular resistance. For determination of the dynamics of the development of cellular resistance, the cells were treated with various concentrations of interferon. Subsequently interferon was carefully washed off each hour, after which the cell was instantly infected with a massive dose of virus (10 - 20 DOE per cell). Eighteen hours after the infection of the culture fluid, it was collected and the viral titer was identified.

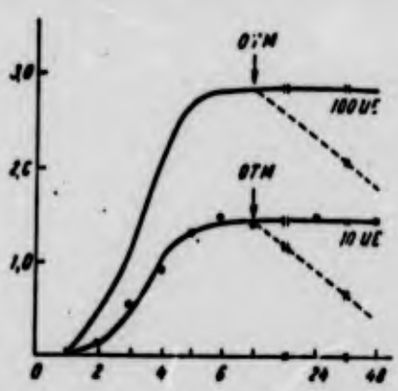
As follows from dia. 1, the growth of resistance can be noticed 90 minutes after introduction of interferon, maximum resistance being reached after 5-6 hours. At the farthest level of resistance changes take an indefinitely long time according to the presence of interferon in the culture fluid. Removal of interferon leads to gradual decrease in cellular immunity, which is registered over a period of six to seven hours.

It must be especially emphasized that the level of cellular resistance is in direct dependence on the concentration of interferon. In other words, a greater or lesser interferon dose can regulate the extent of cellular immunity.

The effect of interferon in relation to its time of dosage. The goal of the following series of experiments was to determine the influence on reproduction of viruses of the time of adding interferon.

Previous experiments established that basic laws of the formation and growth of cellular resistance is not changed in relation to the period of interferon injection with respect to the moment of infection. In this regard, in the case of injection of interferon immediately after infection of the cell or during infection the growth of cellular resistance is generally superimposed upon the reproductive cycle of the virus, which consists of 6 to 8 hours for VEE and EEE.

As is evident from Dia 2, suppression of viral reproduction is noted under these conditions. The later interferon is added, the less the suppression is expressed. The most noticeable suppression of viral reproduction, naturally, was observed during the adding of interferon several hours before infection, in which case the characteristic cytopathological influence of the virus resulting in acute destruction and total death of the cell does not develop.



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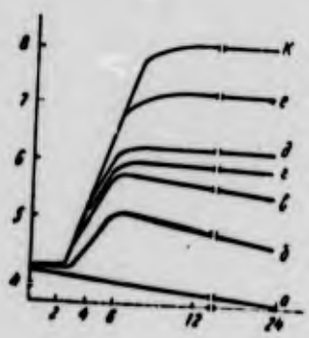
Dia 1. Dynamics of the development of cellular resistance and its dependence on the dose of interferon.

On the x-axis - time of treating cells with 10 and 100 immunizing units (IU);

on the y-axis - lg of inhibition of VEE in comparison with the control. Dotted line designates the dynamics of the reduction of cellular resistance during washing out of interferon after 7 hours.



Dia 2. Influence of interferon on viral reproduction in relation to time of addition with respect to infection.

Interferon added in a dose of 100 IU/ml: a--after 24 hours; b--simultaneously with the infection; c--after 1 hour; d--after 2 and 3 hours; e--after 4 hours; f--6 hours after infection of cell by virus; g--control, quantity of infection 5-10 BOE/cell

The meaning of the quantity of infection. In special experiments the intensivity of viral reproduction was determined by ascertaining the overall medium yield of viral particles in one infected cell in the presence of a constant dose of interferon, using, to a great extent, a quantitative relationship between cells and viruses.

As follows from the data in the table, the level of resistance is connected not only with the time at which interferon is added but also with the initial rate of infection. The fewer the number of virus used to infect the cell, the more effectively the influence of interferon will be expressed. This, as it appears to us, is connected with the fact that during minor rate of infection, interferon has time to defend the surrounding originally uninfected cells and avert the cycle of viral reproduction.

DEPENDENCE OF CELLULAR RESISTANCE ON THE TIME OF ADDING INTERFERON AND THE QUANTITY OF INFECTION

Time of Adding Interferon[1]	Infective Quantity			
	0.01 BOE/cell	0.1 BOE/cell	1 BOE/cell	10 BOE/cell
Immediately after infection	3.4[2]	2.4	2.0	0
2 Hours after infection	2.0	1.0	2.0	0
4 Hours after infection	0.7	0.4	0	0
6 Hours after infection	0.2	0.4	0	0

1 Interferon was added in all experiments in the quantity of 100 IU/ml.
2 The level of suppression of reproduction of VEE virus (in lg) in comparison with the control.

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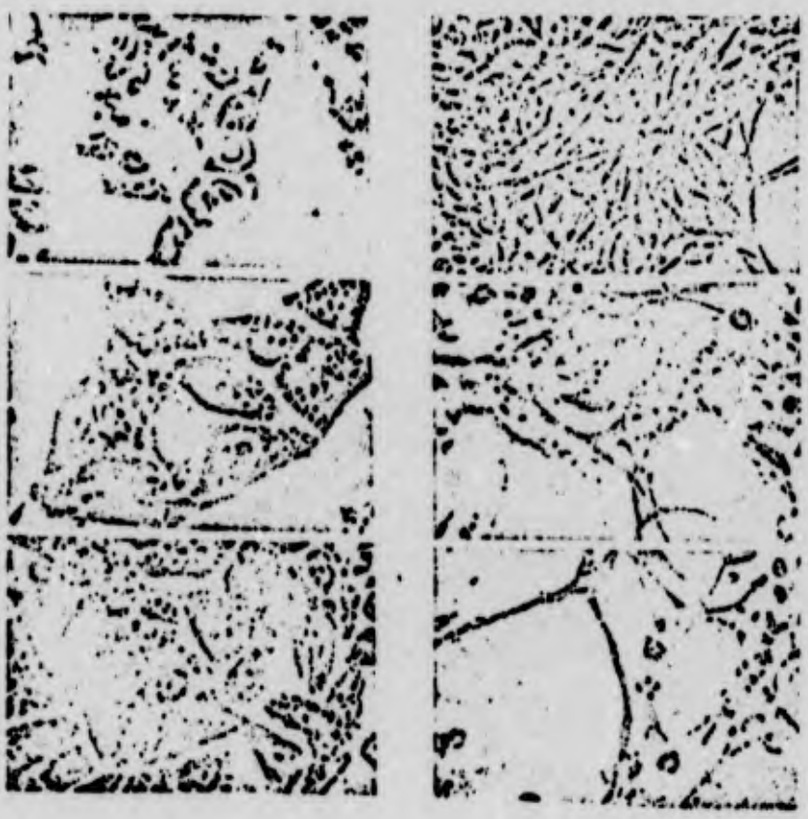
The behavior of infected cells after addition of interferon. Cells A-1 and KEM or fibroblasts of chicken embryo cultivated in microchambers and infected by VEE and EEE virus. Multiple infection consisted of 5-10 ID50/cell. Two to 3 hours before infection, simultaneously with the infection, or 2-3 hours after cellular contact with the virus in culture fluid, a large quantity of homological interferon was added. The period of observation was 24 to 72 hours.



Dia 3. Interferon in 8IU/ml dose added 3 hours before infection of cell KEM by EEE virus.

a--view of cell 5 hours after putting in chamber, attachment and flattening; b--cellular layer 15 hours after interferon injection; c--single layer without indications of destruction after 48 hours. Phase contrast. Mag 320X

Dia 4. Microphotos of fibroblasts of chicken embryo (1-day culture) infected by a massive dose of EEE (5 ID50/cell). Two hours after infection added homological interferon (final concentration 64IU/ml).

a--fibroblasts of chicken embryo right after adding interferon; b--after 12 hours; c--characteristic acute destruction of cell 24 hours after injection of interferon. Phase contrast. Mag 200X

In controlled experimental conditions it was detected that during the absense of interferon in the medium the process we described earlier of acute destruction of the cells develops. Such a picture was observed during injection even of a con-

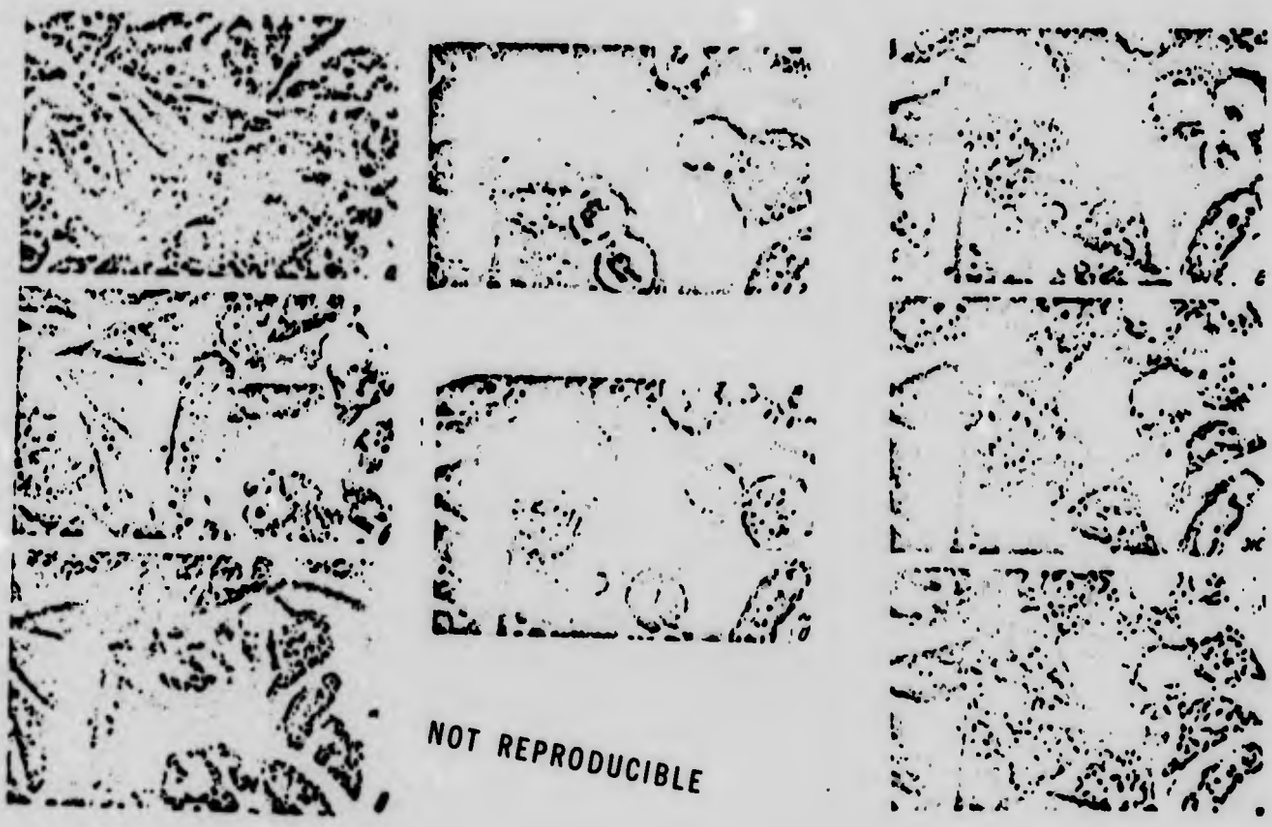
siderable quantity of interferon (500 to 1000IU/ml) [illegible] for given tissue and subsequent cellular infection. Addition of homological and heterological interferon to uninfected cells did not cause any noticeable influence over their morphology or speed of layer formation.

Adding of interferon 2 hours before infection of cellular culture lowers the complete cytopathological effect (dia 3). The cell [illegible] (dia 3,a). The cellular layer is gradually grown, generating a single layer (dia 3 b-d). It follows that in cultures treated beforehand with interferon an atypical multiple polemytosis is sometimes observed. In some immune to destruction by interferon of cells KEM a process of cytoplasmic "boiling" (cytoammilationkinesis) was observed.

Adding even greater amounts of interferon 2-3 hours after infection did not protect infected cells from death. In all experiments of this series the development of typical acute cellular destruction was noted. In dia 4 a-e is presented a series of photographs with microphotography in which the homological interferon was injected into the culture fluid of infected fluid 2 hours after infection. The process of destruction characterised by surrounding and quickly shriveling of cells with formation of amorphous detritus that leads to appearance of "windows" and gradual layer destruction developed. The process ended 20-24 hours after contact of virus with the cell.

It is important to emphasize that later addition of interferon does not extend the period of culture life.

Interesting results were achieved during addition of interferon immediately after contact of virus with cells. In the first hours after infection changes in the culture were not apparent (dia 5 a). Then the cells died.



Dia 5. Microphotography of A-1 cell (two day culture) after adding interferon

(CPE/ml) at the same time as the infection (5 BOE/cell).

The interval between a and b consists of 4 hours, b and c - 30 minutes; g and h - 5 hours. Explained in the text. Phase contrast. Mag: a,b - 400X, c - h -720X.

Consideration of the results

The experiment that was conducted permitted the establishment of immediate connections between the intensity of inhibition of reproduction of viruses due to the influence of interferon and the reaction of cells to viral infection.

In relation to the period of adding interferon, its concentration, and the quantity of infection of the cell, various results of viral infection can be observed, varying from complete protection of the culture during pre-infection treatment to total cell destruction in the case when interferon is added after infection with a large dose of viruses. Original "curing" influence of interferon is observed during adding of it immediately after infection and is characterized by partial death of the cell in the first hours after viral entry. However in the case that the basic mass of the cells is not affected and retains the ability to attain fission, a gradual restoration of a single layer is later observed.

Similar results follow analysis as an intermediate stage. It can be explained that antiviral albumin (AVA) produced by the cell accumulates in the cell only after an interval which, according to our data, is about 5-6 hours. Therefore, in the first hours after the infective process begins and during it destruction continues for 2-4 hours until cells of the culture can accumulate enough AVA to stop reproduction of the virus and development of TsPE. Here, however, it is important to underline that such "convalescence of the cell has a conditional character, in that the cell is a carrier of latent infection so that during removal of interferon from the culture fluid the characteristic acute cellular destruction develops. In a word, interferon does not possess a sterilizing influence and does not have the ability to eliminate viral nucleic acid or its infecting activity.