STUDY OF THE CONDITIONS OF FORMATION OF INTERFERON IN A TISSUE CULTURE OF CHICK EMBRYO FIBROBLASTS INFECTED WITH JAPANESE ENCEPHALITIS VIRUS

(UDC 576.858.25.095.38)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 2, pp. 75-78, February, 1966
Original article submitted July 24, 1964

It has been established that interferon regularly is formed in the most varied types of cells during the multiplication of various viruses in them. At the same time, the conditions of the appearance of interferon may depend upon the nature of the virus and the type of cell [2,3,4,7,9,11,13].

The purpose of this work was to study the conditions of formation of interferon in a tissue culture of chick embryo fibroblasts, infected with Japanese encephalitis virus.

EXPERIMENTAL PROCEDURE

In the experiments, we used Japanese encephalitis virus, strain 47 (JE), in the form of a 10% marrow suspension of infected white mice, and vesicular stomatitis virus, strain Rew Jersey (VS), enriched on a primary chick fibroblast tissue culture.

JE virus was titered with a factor of 10 by intracerebral infection of white mice. Three mice were taken for each dilution. VS virus was titered in test tubes with a chick fibroblast tissue culture (3 to 4 test tubes in each group), into which 0.3 ml portions of the corresponding 10-fold dilutions of the virus were introduced.

The results were determined two to three days after infection according to the cytopathogenic effect induced by this virus in the tissue culture. LD_{50} and TCD_{50} were determined according to Reed and Munch.

JE virus was inactivated by heating in the following way. A suspension (10%) of mouse brain was clarified by 10 min centrifuging at 1000 rpm; six parts of the virus suspension was combined with two parts of a 2% sodium citrate solution and one part borate buffer solution with pH 8.5; the mixture was heated at 56° for an h [8].

A primary one layer chick embryo fibroblast culture was used as the tissue culture. The medium for the tissue cultures was Porterfield's medium [10], consisting of Gey's salt solution, 5% inactivated calf serum, and 0.5% lactalbumin hydrolyzate.

Interferon was titered according to the following procedure. The fluids taken from the tissue cultures at various periods after infection with JE virus were heated for an h at 60°, diluted 1:2 in Porterfield's medium, and their inhibiting activity with respect to VS virus was verified. For this purpose, 1 ml portions of each dilution were introduced into three to four test tubes with chick embryo fibroblast tissue culture, having preliminarily removed the original medium. After 24 h, 1.0-2.0 log TCD_{50} of the VS virus was added. The control was made up of the culture fluid taken from uninfected cultures and heated at 60° for an h. The results were determined two to three days after infection, taking into consideration the absence of cytopathogenic changes in cultures containing interferon, and the cellular degeneration in tissue cultures infected with VS virus in the presence of the normal culture fluid. The interferon titer was determined according to Reed and Munch.

TABLE 1. Quantitative Accumulation of Interferon in a Tissue Culture of Chick Embryo Fibroblasts Infected with Various Doses of JE Virus

Experiment No.	Dose of the virus (in log LD_{50}/ml)	Interferon titer after infection			Virus concentration (in log LD_{50}/ml) after infection		
		1st day	3rd day	5th day	1st day	3rd day	5th day
1	6,8	0	1:19	1:13	4,0	7,8	7,2
	4,8	0	1:22	1:22	4,8	7,5	6,2
	2,8	0	1:19	1:22	3,0	7,5	7,0
	0,8	0	1:19	1:19	0	5,8	7,2
2	5,0	0	1:3	1:3	4,2	5,2	4,2
	3,0	0	1:6	1:6	4,8	5,0	3,2
	1,0	0	1:6	1:3	0	4,2	4,8

TABLE 2. Inhibiting Activity of Culture Fluid and Cellular Extract from Cells of Chick Fibroblasts Infected with JE Virus

Experiment No.	Dose of virus (in log DL_{50}/ml)	Titer of interferon during observation period (in days)							
		Culture fluid				Cells			
		1st day	2nd day	3rd day	5th day	1st day	2nd day	3rd day	5th day
1	5,2	1:6	1:22	1:25	1:22	0	0	0	0
2	4,2	0	1:22	1:37	1:44	0	1:2	1:2	1:2

TABLE 3. Influence of Preliminary 24-h Contact of Interferon with Chick Fibroblast Tissue Culture on the Multiplication of JE Virus and the Formation of New Interferon

Experiment No.	Dose of virus (in log LD_{50}/ml) taken for infection	Amount of virus (in log LD_{50}/ml) after influence		Titer of interferon in tissue culture	
		With interferon	With normal culture fluid	After treatment with interferon	After treatment with normal culture fluid
1	5,0	3,8	6,8	1:3	1:37
2	4,8	4,0	7,0	1:2	1:27
3	5,0	4,0	6,2	1:3	1:37

EXPERIMENTAL RESULTS

In studying the dynamics of the accumulation of interferon on a single layer culture of chick fibroblasts infected with various doses of the virus, we confirmed the data of [12] on the absence of any relationship between the dose of JE virus and the amount of interferon formed (Table 1).

Since interferon is formed in cells, we attempted to detect its presence and the dynamics of its accumulation directly in homogenates of the infected cell layer.

Four flasks with tissue culture of chick embryo fibroblasts were infected with 4-5 log LD_{50}/ml of JE virus. After 1, 2, 3, and 5 days, the virus-infected medium was collected (one flask was used for each period of observation), and after repeated washings with phosphate buffer solution, the cells were removed with versene and homogenized by 10 min shaking with glass weighing bottles on a shaker.

After 10 min centrifuging at 1000 rpm, the precipitate was resuspended in the original volume of Porterfield's medium. In the culture fluid and cellular extracts obtained at all periods of observation, we determined the inhibiting activity of interferon by titering in test tubes with a tissue culture of chick fibroblasts (Table 2).

From the data presented in Table 2, it is evident that interferon was not accumulated in the cells, but rapidly passed into the fluid medium surrounding them. Only rarely was a small inhibiting activity noted in the extracts of the cells, if interferon was already contained in the medium in a sufficiently high titer.

There are indications in the literature of the possibility of producing interferon with the aid of viruses inactivated by heating or ultraviolet radiation [4-6,8]. We studied interferon production in small flasks with a single layer tissue culture, into which 5-6 ml of inactivated JE virus, containing 7-8.0 log $LD_{50/0.03}$ ml of the virus before inactivation, was introduced. After 3-h contact [8], the virus was removed by 2 washings with a phosphate buffer solution, and fresh medium was introduced into the flask. After 24 and 72 h, the inhibiting activity with respect to VS virus was determined in the native culture fluid taken from the flasks, by titering on test tube cultures. To monitor the completeness of inactivation of the JE virus, the material obtained from the flasks after 24 and 72 h was injected without dilution into the brains of 5 white mice. No interfering activity could be detected in the 7 experiments, even in whole culture fluid. The interferon titer was equal to zero.

The infected mice remained healthy, which indicated the absence of active virus in the culture fluid. Thus, under the influence of JE virus inactivated by heating upon the cells, no interferon was formed. Our results agree with the data of Vilcek [12].

A greater amount of interferon was detected [9] in cells that were preliminarily treated with interferon and infected with live or inactivated influenza virus. The difference in the yield of interferon in comparison with untreated cells was explained by the authors by adjustment of the cell mechanism to manufacture of interferon.

We verified the possibility of increasing the interferon production under the indicated conditions. Flasks with a 24-h single-layer chick fibroblast tissue culture were treated with interferon in a 1:2 dilution (the titer of the interferon used was equal to 1:44, as against 30 $CTD_{50/0.03}$ ml of the VS virus). Before infection of the flasks with the JE virus, interferon was removed by 2 washings with phosphate buffer solution. In the control, the tissue cultures were treated with normal culture fluid 24 h before their infection with JE virus. The inhibiting activity of the newly formed interferon was determined 96 h after infection in the culture fluid of the infected flasks, by titering on a chick fibroblast tissue culture, and the virus concentration was determined by intracellular titering on white mice.

From Table 3, it is evident that preliminary treatment of the cells with interferon led to a sharp reduction of its manufacture, which does not confirm the previously published results [9].

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