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PRODUCTION OF INTERFERON BY SOME ARBOR
VIRUSES OF GROUP A.

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Prior to performing any investigation involving the antiviral inhibitory agent interferon the latter has first to be obtained in highly purified state. Among the best producers of interferon are the group A of arbor viruses. Previously studied viruses of this group, namely Sindbis, Chikungunya and KEE (1-4), are also distinguished by high sensitivity to the action of interferon. These properties, coupled with the ability of arbor viruses of group A to cause rapid disintegration of the usual cell cultures and their accurate enumerability using the plaque method, render these viruses extremely useful prototypes for investigation of the production and action of interferon.

It was sought to ascertain the optimal conditions for maximal production of interferon by chick embryo fibroblasts which had been infected with the eastern equine encephalomyelitis (EEE), Venezuelan equine encephalomyelitis (VEE) and Semliki forests (SF) viruses.

Material and methods

VIRUSES. The arbor viruses KEE, VEE and SF were obtained from

the virus museum of the "Ivanovskii" Institute of Virusology. The viruses were serially transferred on chick embryo fibroblasts. At the time of the investigation EEE had gone through 5, VEE through 24 and SF through four serial transfers. Culture fluid collected 24 hr after infection of the cells was used as the virus-containing material.

The infectious titer of the viruses ranged 10^8 - 10^9 plaque-forming units (PFU) per ml.

Cells. In most of the experiments use was made of a culture of chick fibroblasts which had been prepared by trypsinization of 10-day embryos. The cells were suspended in lactalbumin hydrolysate containing 10% heated ox serum and antibiotics at a density of $1-1\frac{1}{2}$ million cells per ml. Aliquots of 10 ml of the suspension were poured into Carrel's dishes. After formation of a cell layer (after 24-28 hr) the dishes were used for production or titration of interferon.

Production of interferon. To produce interferon use was made of various amounts of native viruses and of viruses whose infectious properties had been attenuated or eliminated. In the latter case the viruses were heated at 56° C for 60 min or at 65° for 30 min. In another series of tests the viruses were inactivated by ultraviolet rays UV-applied for 2-16 min from a BUF-15 mercury quartz lamp set at a distance of 25 cm from a Petri dish which contained 5 ml of virus suspension.

We also tested our modification of the method of double infection of cells (5): first the culture was treated with 2 ml of UV-inactivated virus, and 1-5 hr later the cells were reinfected with intact virus.

Chick embryo fibroblasts which had been infected in various ways were incubated for 24 hr, and then the culture fluids were collected and heated at 65° for 30 min. Special experiments showed that this treatment completely inactivated the viruses without decreasing the titers of interferon.

Titration of interferon. Activity of the interferons obtained was tested against virus EEE. Twice-diluted specimens of interferon were placed in Carrel's dishes for 24 hr and then the cells were washed with Hank's solution and infected with 100-150 PFU of EEE virus per dish. Contact of virus and cells lasted for 1 hr and then the virus was drawn off with a pipet, and the culture was washed with Hank's solution and covered over with agar.

Composition of the covering layer was as follows: 0.8 ml Hay's solution A, 0.2 ml of Hay's solution B, 0.2 ml of lactalbumin hydrolysate, 0.3 ml of ox serum, and 0.2 ml of chick embryo extract. The pH of the medium was adjusted to 7.4 with sodium bicarbonate, and 0.3 ml Tris buffer and 2 ml 2% Difco agar were added. The dishes were incubated at 37° C for 2 hr, and then the cells were stained with neutral red. Activity of interferon was determined from the degree of inhibition of plaque formation by the EEE virus.

Results

Determination of the dynamics of increase in interferon during the course of the viral infection following massive infection of cells showed that interferon first appeared in the culture fluid 6-8 hr

after cessation of contact of virus with cells and coincided temporally with the end of the cycle of virus multiplication. The titer of interferon steadily increased and reached a maximum at the 24th hr. when one usually noted total destruction of the cells of the infected culture. To study the connection between the production of interferon and the density of infection use was made of the same doses of the selected viruses, viz. 10^7 , 10^5 and 10^3 PFU per dish which were equal to between 1 and 0.0001 PFU per cell. The results of these experiments are given in the Table.

The interferon yield is in direct relationship to the amount of virus introduced. For instance, doses equal to 0.0001 PFU per cell induced virtually no formation of interferon. However, with a dose of 1 PFU per cell its titers were low. It is noteworthy that the ability of the three tested viruses to produce interferon was approximately identical. The same pattern was observed on using partially inactivated (by ultraviolet or by heating) virus: with increase of the grade of inactivation of the virus its interferon-producing activity declined. Heating at 65°C for 30 min not only rendered the virus completely non-infective but also abolished its capacity to produce interferon.

The best method of producing highly active interferon was the method of successive infection of cells with partially inactivated and intact virus (see Table).

In the light of the foregoing data, the first infection was done with viruses inactivated by 2-min irradiation, and the second one with intact undiluted virus (10^7 - 10^8 PFU per dish). The optical interval between the two infections was 3 hr. By observing

the stated conditions, one obtained highly active interferon which was capable in the case of VEE virus of inhibiting 73-98% of the plaques at a dilution of 1:640. Increase or decrease of the interval between the infections led to a decrease of the titers of the interferon produced. In cases where the first infection was performed with viruses irradiated for longer than a min the amount of interferon produced was likewise lower. A similar result was obtained on using diluted virus for the second infection.

When the KEE and VEE viruses were used for infecting established cell lines of human and murine origin (cells A-1 and KEM*) the yield of interferon was negligible. For example, even after using the method of double infection the titers of the interferon produced did not exceed 1:4 - 1:8¹.

Discussion of the results

While all three arbor viruses tested (KEE, VEE and SF) produced interferon, the best producer was the VEE virus, which with various modes of infection regularly evoked formation of interferon at rather higher titers than the other viruses (see Table). The best reference virus is probably the KEE virus, which is highly sensitive interferon and produces easily countable large plaques on a monolayer of chick embryo fibroblasts.

The decrease of infectivity resulting from simple dilution or inactivation of viruses regularly led to decrease in the interferon titers. This rule does not, however, extend to briefly (2 min)

*KEM = cells of mouse embryo - Translator

irradiated viruses, which are actually somewhat better producers of interferon than unirradiated ones (see Table). It maybe that this result is due to the fact that even in the case of 99.9 - 99.99% inactivation of viruses that is observed in these cases, the residual fraction of active virus is reasonably high ($2 \times 10^4 - 2 \times 10^5$ PFU per dish), so that in combination with a large amount (approx. 2×10^8 PFU per dish) of the inactivated virus conditions are favorable for production of interferon.

A direct link between production of interferon and density of infection with arbor viruses was discovered some time ago (6) for the KEE-cell system. This phenomenon is apparently connected with the fact that with small doses of infection the interferon which is produced, on the one hand, successfully protects uninfected cells and on the other hand inhibits the production of new interferon in the infected cells (7).

With viruses KEE, VEE and SF which had been completely inactivated by heating, there was no production of interferon, as was found also in respect of other arbor viruses of group A (8-11). The maximal yield of interferon was observed following double infection of cells.

According to a report (5), the preliminary contact with inactivated virus would, so to speak, prepare the cells for subsequent intensive elaboration of interferon after infection of them with untreated virus. There is an undoubted synergism between the action of active and inactive forms of the virus, though the details of the mechanisms of this process are unknown.

Summary

1. The KEE, VEE and SF viruses rapidly produced interferon in chick embryo fibroblasts.

2. Maximal titers of interferon in the culture fluid were obtained by using the method of double infection of the cells with the partially inactivated followed by the intact virus.

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Footnote of p.34

¹ Activity of interferon in these cases was assessed from cytopathic effect on homologous cells.

Activity of interferons obtained following various modes of infection¹

VIRUS PRODUCING INTERFERON	VIRUS USED FOR INFECTION	TITER OF INTERFERON
KEE	10 ⁷ PFU/ml ²	
	10 ⁵ PFU/ml	
	10 ³ PFU/ml	
VEE	10 ⁷ PFU/ml	
	10 ⁵ PFU/ml	
	10 ³ PFU/ml	
SF	10 ⁷ PFU/ml	
	10 ⁵ PFU/ml	
	10 ³ PFU/ml	
KEE	HEATED	
	at 50° for 60 min.	
	at 65° for 30 min.	
VEE	at 65° for 30 min.	
	at 65° for 30 min.	
KEE	UV-irradiated for	
	2 min.	
	4 min.	
	8 min.	
	16 min.	

TABLE (Contd.)

VIRUS FROM WHICH INTERFERON	VIRUS USED FOR INFECTION	TITER OF INTER- FERON
VEE	UV-irradiated for 2 min 10 min	
KEE	Irradiated for 2 min	
VEE	Irrad. for 2 min Irradiated continuously for: 3 hrs. 4 " 5 "	

Connotations:

++++ denotes inhibition of 90-100% of plaques

+++	"	"	"	61-90%	"	"
++	"	"	"	41-60%	"	"
+	"	"	"	21-40%	"	"
-	"	"	"	0-20%	"	"

¹ Mean result of three identical experiments.

² Untreated virus was used in this series of experiments.

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