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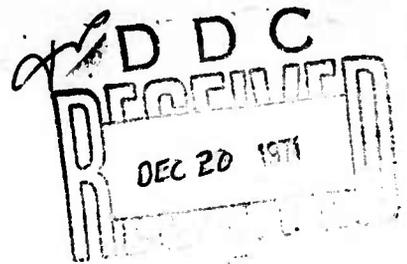
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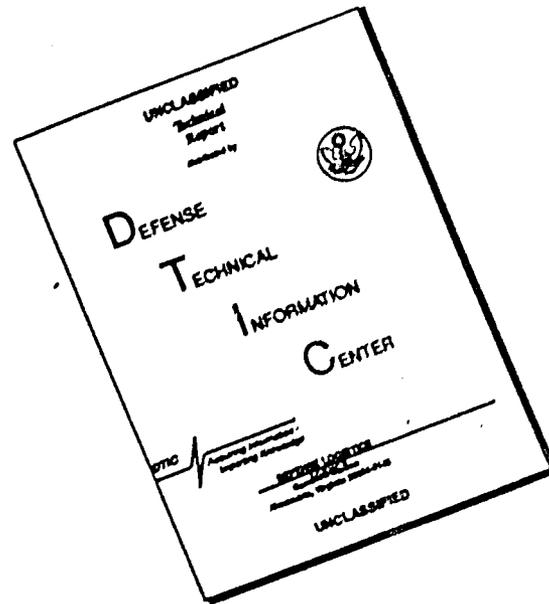
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DEVELOPMENT AND SUBSTANTIATION OF THE PRINCIPLES OF OBTAINING
LARGE AMOUNTS OF INTERFERON.

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The optimum conditions for the formation of interferon, induced by Group A arboviruses (VEE and Sindbis viruses), in primary trypsinized chick embryo fibroblast cultures, using various methods of cultivation (roller, suspension and monolayer stationary methods), were studied. The viability and concentration of cells, (in terms of 1 ml of the medium), are the most important factors determining the level of interferon production. Multiplicity of infection is important, mainly when suspension is used, and is optimally 1 - 10 PFU. A rise in the incubation temperature brings about an increase in the production of interferon, induced by the Sindbis virus.

The level of interferon production depends on a number of factors, among which the type of the inductor-virus, the type of cells and the method of cultivation occupy the principal place. We set ourselves the task of developing a simple and accessible method of obtaining large amounts of highly active interferon.

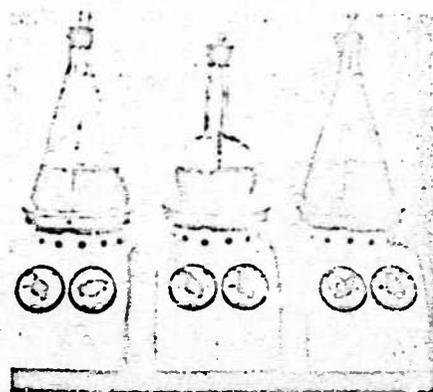
As a working model we used Group A arboviruses and chick embryo fibroblasts (CEF), because previously we had shown that the viruses of this group have a short reproductive cycle (6 - 8 hours) and were good interferon-producing agents, and CEF cells had the maximum sensitivity to these viruses and were very simple and convenient for work (References 2,3)

Material and Methods

As inductors we used Venezuelan equine encephalomyelitis (VEE) viruses and the Sindbis virus (SV), which underwent a series of passages in CEF cultures. As a rule, the vesicular stomatitis virus (VSV) and the VEE viruses were test viruses.

We conducted the investigations on fibroblasts (CEF), which we prepared by the ordinary method (Reference 1).

NOT REPRODUCIBLE



Spinner-Cultures

The basic details of the cultivation methods are set forth at length in our other reports (References 2,5,6). In this study we also made wide use of the spinner culture method, in which we mixed the cellular suspension with a magnet suspended on a chain (viz. Figure).

We determined the interferon activity by the method of inhibiting the plaque formation of VEE or VSV viruses under agar. We expressed the interferon titer in IE_{50} /ml.

We determined the infectious activity of the model viruses by the method of titration by plaques under agar, and the hemagglutinating activity, in accordance with the principles established by Clark and Casals. We described both methods previously (References 2,4)

Results

At first we studied the accumulation of interferon in monolayer stationary, roller (grown in revolving vessels) and suspension (grown in a freely suspended state) cultures. The results of the study made of the activity of interferon, induced by the VEE virus under various conditions of cultivation and with a multiplicity of infection, are summarized in Table 1.

As is seen from this Table, the interferon titers in roller and suspension cultures are 2 - 5 - 10 times higher than the titers obtained in monolayer stationary cultures. In the CEF cellular suspension, high interferon titers (up to $4000 IE_{50}$ /ml) are revealed when the cells are infected with massive virus doses; in other words, in the suspension, as well as in the monolayer stationary cultures, the interferon production was directly proportional to the multiplicity of infection. In contrast to other methods of cultivation, in the roller cultures the interferon titers reached the maximum value, even after infection with limiting virus cultures. After 24 hours of incubation the interferon titers were equally high when infected with both a large and a small multiplicity of infection.

Table 1 Effect of the Method of Cultivation and the Multiplicity of Infection on Interferon Production

Multiplicity of Infection (in PFU/cell)	No of Experiment	Method of Cultivation		
		Stationary Monolayer	Roller	Suspension
		Interferon		Titer (in IE ₅₀ /ml)
1 - 10	1	320	1280	4000
	2	640	900	1400
	3	560	640	1000
10 ⁻⁵ - 10 ⁻⁶	1	128	640	360
	2	32	640	400
	3	8	1280	-

Table 2 The Production of Infectious Virus, Hemagglutinins and Interferon in a Roller Culture.

No of Experiment	Titer		
	PFU/ ml	GAE/ ml	IE ₅₀ /ml
1	5 x 10 ⁹	2048	1280
2	2 x 10 ⁹	1024	640
3	9.1 x 10 ⁸	256	640
4	8.4 x 10 ⁸	128	640
5	5 x 10 ⁸	64	640
6	1.7 x 10 ⁷	64	320
7	2 x 10 ⁷	0	320
8	10 ⁶	0	40

Remark: GAE are Hemagglutinating units.

Table 3 Production of Infectious Virus, Interferon and Hemagglutinins in Suspension

Virus	Titer		
	1 g PFE/ml	CAE/ml	IE ₅₀ /ml
VEE	9.0	2048	330
	8.8	1024	1000
	8.5	-	1000
	8.3	1024	330
	6.6	0	320
SV	8.6		3000
	8.5		320
	8.4		320
	8.2		100
	7.7		320

Table 4 Production of CEF Interferon at Various Temperatures

Inductor-Virus	Incubation Temperature			
	37°	39°	41°	43°
	Titer (in IE ₅₀ /ml)			
SV	400	1600	3200	3200
VEE	400	200	100	-

The numerous subsequent experiments served as a basis for our conclusion that, all things being equal, the level of interferon production is determined mainly by the condition of the cells and their viability. In fact, if we sum up the results of the same types of experiments (constant multiplicity of infection, the same culture medium, method of cultivation, e.t.c), we can observe a rather clear correlation between the level of virus production (infectious and hemagglutinating activity) and interferon production (Table 2). This pattern is especially well expressed when roller cultures are used. When the cells are grown and the viruses are cultivated in suspension, the relationships among the various indicators of cellular culture productivity (virus productivity, interferon titers) is not so obvious (Table 3).

To obtain maximally active interferon preparations, we attempted to apply, for suspension cultures, the parameters recommended by Marcus (Reference 7) with regard to the Sindbis virus: the use of "old" (5 - 7 days) cells with a multiplicity of infection of 5 PFU/cell and an incubation temperature of 39°.

The first breeding position, in the case of suspended cultures, is not advisable, because a considerable part of the cellular population dies during such prolonged mixing. For example, the interferon titer was equal to 3000 IE₅₀/ml when the cells were infected without preliminary incubation, 1000 IE₅₀/ml during a 48 hour preliminary incubation, and 330 IE₅₀/ml during a preliminary 5 day monolayer cellular cultivation. These data clearly demonstrate the reduction in interferon titers in "old" suspended cultures.

The results of the investigations showed that, when the incubation temperature rises, an increase in interferon production is actually observed. For example, at 39° and higher, the interferon titers, after 24 hours, constantly reached 1600 IE₅₀/ml, whereas on incubation with the thermostat at 37°, the production of interferon per 1 ml of stored medium did not exceed 400 IE₅₀/ml. Here, however, we must stress that this statement is correct only with regard to the Sindbis virus, and when the VEE virus is used, interferon production progressively declines as the temperature rises (Table 4). This result, obtained when viruses of one group are used, show the great individual differences in the interference-inducing capacity of viruses.

Discussion

When selecting the object of the investigation and the conditions that can affect the level of interferon production, we proceeded from the assumption that cell concentration (in terms of 1 ml of the medium) and viability and the rate of virus reproduction and interferon production play the most important part in this process. In summing up the above-stated data, it seems appropriate to determine certain optimum cultivation conditions necessary for ensuring the production of highly active interferon.

Special attention should be given to preserving a high viability of the cells in the condition of their considerable concentration ($3 \times 10^6 - 6 \times 10^6$ cells/ml). This can be attained by the use of roller and suspension cultivation methods, which are efficient and require small expenditures of material and the work time. This study has established that these methods are very convenient for the production of considerable amounts of interferon.

The multiplicity of infection is mainly important when suspension cultures are used. At the same time, its optimum values vary in the limits 1 - 10 PFU/cell. The optimum cultivation temperature is a factor requiring an experimental check for every virus used. For example, the optimum temperature

value, when the Sindbis virus is used as an interferon inductor, is 39 - 43°, and for VEE 37°. The use of a non-serum media is preferable. Enrichment of medium # 199 with lactalbumin hydrolysate (0.1% of the dry preparation) makes it possible to increase interferon production 2 to 4 times. The time recommended for collecting the interferon-containing culture liquid is 24 hours.

As shown in this study, the conditions presented are optimal for ensuring a good production of interferon induced by a group arboviruses.

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