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RELATION OF INCUBATION TEMPERATURE TO INTERFERON PRODUCTION IN MOUSE PERITONEAL CELLS INFECTED WITH NEWCASTLE DISEASE VIRUS

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Summary. — "Freshly" cultivated mouse peritoneal cells produce less interferon at 26° C than at 36° C during a 6-hour period after infection with Newcastle disease virus (NDV). However, the precultivation of cells at 36° C for 5 hours before infection with NDV raised the subsequent 6-hour interferon yield at 26° C to titres comparable to those obtained at 36° C. The capacity of virus-induced inteferon production at 26° C decreased in the course of cell precultivation at 26° C to the levels found in cells which were infected without preincubation at 36° C and subsequently transferred to 26° C for additional cultivation for 6 hours. These results are discussed in view of the known ability of peritoneal cells to release a supposedly preformed interferon after non-viral stimulation in vitro. Preformed interferon, the antiviral protein induced by this interferon and/or presence of a "blocker" might be involved in the phenomena observed.

Introduction

Mouse peritoneal cells produce interferon after infection with myxoviruses and they also release this or a related substance after stimulation with non-viral agents in vitro (Lackovič *et al.*, 1967). In both cases different temperature optima are required for maximal stimulation. When myxoviruses were used as interferon inducers, the best temperature conditions were about 39° C, whereas 26° C were optimal when endotoxin was employed (Lackovič *et al.*, 1967). During cultivation, peritoneal cells loose their ability to release interferon after non-viral stimulation. This process is temperature-dependent, too. At low temperatures it proceeds slowly, but becomes more rapid at higher temperatures, for example at 36° C (Borecký and Lackovič, 1968). There seems to be a relation between the temperature optimum and the temperature-dependent loss of capability for non-viral stimulated interferon release by mouse peritoneal cells (Waschke *et al.*, 1969).

In this work we studied the influence of various cultivation periods on interferon production in mouse peritoneal cells after infection with NDV at

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different temperatures. The capacity of interferon production in vitro at suboptimal temperatures in relation to the process of "aging" of these cells by cultivation at different temperatures was investigated.

Materials and Methods

The preparation and cultivation of the mouse peritoneal cells, the origin of the Hertfordshire strain of NDV used as interferon inducer, and the method of interferon titration in mouse fibroblasts (L cells) using encephalomyocarditis (EMC) virus as challenge have been described previously (Borecký and Lackovič, 1964; Lackovič and Borecký, 1965; Lackovič *et al.*, 1967). The interferon titres are given as the reciprocals of the highest dilution of an interferon sample which completely protected the L cells against the cytopathic effect of EMC virus under the conditions of the titration used.

Results

Mouse peritoneal cells ($2.5-4.4 \times 10^6$ cells per tube) in medium 199 with 10% foetal calf serum, penicillin and streptomycin were incubated at 36° C after distribution into tubes for different periods. They were then inoculated

Table 1. The influence of preincubation of mouse peritoneal cells at 36° C on subsequent NDV-induced interferon production at 26° C (6 hours' yield)

Exp. No.	Control 26° C	Interferon titre ($\log_2$) after preincubation of cells at 36° C before infection with NDV for					Control 36° C
		30 min	60 min	120 min	240 min	360 min	
1	6.5	6.5	ND	8.0	9.5	ND	10.0
2	5.5	ND	5.0	5.5	8.0	ND	9.0
3	4.0	4.0	ND	ND	5.0	6.0	6.0
4	5.0	ND	ND	ND	ND	8.0	8.0

ND — not done.
Control 26° C: Interferon titres in NDV-infected cells kept for 6 hours at 26° C.
Control 36° C: Interferon titres in NDV-infected cells kept for 6 hours at 36° C.

with 10^6 EID_{50} of NDV and kept at 26° C for an additional 6 hours. The interferon titres of these samples were compared with controls inoculated with the same dose of NDV at the beginning of the cell cultivation, and kept for 6 hours at 26° or 36° C. The results of four experiments are summarized in Table 1. As expected, the "fresh" cells of the control samples produced less interferon at 26° C during a 6-hour period than they did in the same period of time at 36° C. However, becoming "aged" in vitro, the cells started producing more interferon at 26° C (during 6 hours) until the interferon yield reached the level found in the 36° C-controls. Fig. 1 illustrates the kinetics of this event. The absolute values of the corresponding experimental titrations differed as a result of different cell concentrations used in the various experiments. For summarizing the results, the absolute values were converted into relative ones. The interferon yields of the 26° C-controls were taken as $\log_2 2 = 1.0$, and the differences of the experimental values to

those of the 26° C-controls in each experiment were calculated as the corresponding $\log_2$-values and added to 1.0. Thereby the interferon titres of "fresh" cells infected with NDV determined after 6 hours' incubation at 36° C were, on the average, 3 $\log_2$ steps higher than those found at 26° C. As demonstrated in Fig. 1, the cells "aged" two hours at 36° C started to enhance their interferon production and after a precultivation for 5 to

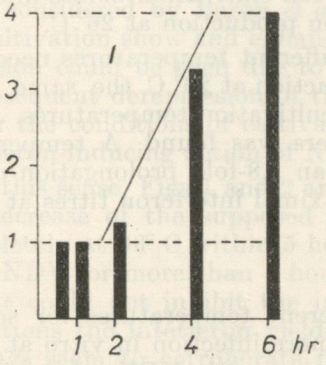

Fig. 1.

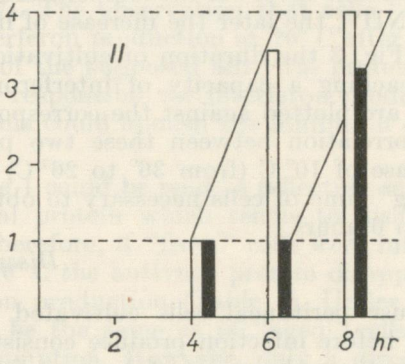

Fig. 2.

Fig. 1. The influence of preincubation of mouse peritoneal cells at 36° C on interferon production after stimulation with NDV at 26° C
For explanation see Table 1.
Abscissa: hours of preincubation; ordinate: relative interferon titres (see text)
Black columns: Cells preincubated at 36° C, then infected and transferred to 26° C
——— Interferon titre in NDV-infected cells incubated for 6 hours at 36° C only (36° C-control)
— · — · Interferon titre in NDV-infected cells incubated for 6 hours at 26° C only (26° C-control)

Fig. 2. The influence of preincubation of mouse peritoneal cells at 30 or 26° C on subsequent interferon production after stimulation with NDV at 26° C
Empty columns: Cells preincubated at 30° C, then infected and transferred to 26° C
Black columns: Cells preincubated at 26° C, then infected and further incubated at 26° C
Other explanations as in Fig. 1.

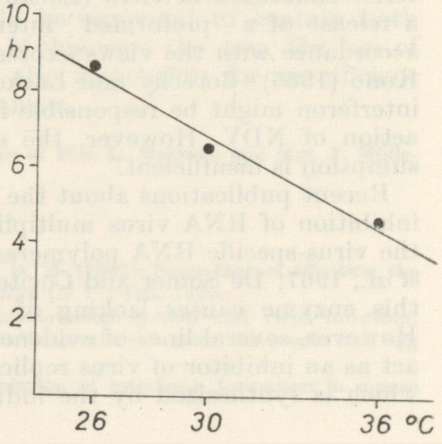

Fig. 3.

Relation of preincubation of mouse peritoneal cells for different times at different temperatures to subsequent maximal interferon production at 26° C after stimulation with NDV

Abscissa: temperature of preincubation.
Ordinate: hours of preincubation before infection with NDV. The "maximal interferon production" relates to the titres obtained 6 hours after infection with NDV at 26° C.

6 hours at 36° C these cells produced the same amounts of interferon within the following 6 hours at 26° C as the "fresh" cells in the 36° C-controls.

Fig. 2 illustrates the results of experiments in which we determined the influence of cell precultivation at 30 and 26° C, respectively, on the subsequent interferon production at 26° C during a 6-hour period after infection with NDV. The experiments were performed as described above. They showed that the lower the temperature during cell cultivation before infection with NDV, the later the increase of interferon production at 26° C.

In Fig. 3 the duration of cultivation at different temperatures necessary for reaching a capacity of interferon production at 26° C the same as at 36° C are plotted against the corresponding cultivation temperatures. A linear correlation between these two parameters was found. A temperature decrease of 10° C (from 36° to 26° C) led to an 1.8-fold prolongation of the "aging" time of cells necessary to obtain maximal interferon titres at 26° C within 6 hours.

Discussion

Mouse peritoneal cells cultivated at different temperatures for several hours before infection produce consistently more infection in vitro at 26° C during the subsequent 6 hours (after infection with NDV) than freshly cultivated cells. How to explain this event?

Actinomycin D, mitomycin, or puromycin can block the interferon production in peritoneal cells after infection with NDV (Borecký and Lackovič 1968) in accordance with results obtained in other systems (Heller, 1963; Wagner, 1965, etc). Therefore, this type of production can be assumed a de novo interferon synthesis. Shekel and Burke (1967, 1968*a*, *b*) demonstrated the requirement of some early steps of the virus multiplication for virus-induced interferon synthesis. They suggested that such an early step could be the formation of a double-stranded RNA during the multiplication of RNA viruses. A gradual inhibition of this step would cause a gradual inhibition of the interferon induction by the RNA virus.

Mouse peritoneal cells release interferon also after treatment with bacterial endotoxin in vitro (Lackovič *et al.*, 1967). This was suggested to be a release of a "preformed" interferon rather than a de novo synthesis in accordance with the views expressed by Youngner *et al.* (1965) and Ho and Kono (1965) (Borecký and Lackovič, 1968). The presence of the preformed interferon might be responsible for an inhibition of the interferon-inducing action of NDV. However, the experimental evidence supporting this assumption is insufficient.

Recent publications about the mode of interferon action suggest that the inhibition of RNA virus multiplication by interferon is due to blocking of the virus-specific RNA polymerase synthesis (Miner *et al.*, 1966; Sonnabend *et al.*, 1967; De Somer and Cocito, 1968; Levy and Carter, 1968). A lack of this enzyme causes lacking of synthesis of viral double—stranded RNA. However, several lines of evidence suggest that the interferon itself does not act as an inhibitor of virus replication. Instead, a so-called antiviral protein which is synthesized by the inducing action of interferon was assumed to

exert the protective effect in interferon-treated cells (Taylor, 1964; Levine, 1964; Friedman and Sonnabend, 1964; Lockart, 1964; Taylor, 1965).

If both assumptions hold true for peritoneal cells, a balanced coexistence of preformed interferon with an antiviral substance stimulated by interferon itself in the cell can be suggested. A similar role of interferon in maintaining the antiviral state in cells via the continually induced antiviral protein has been considered by Baron *et al.* (1967). The observation that cells "aged" by cultivation show and enhanced interferon production at 26° C after NDV infection could be then due to a loss of the supposed antiviral protein and a subsequent derepression of the gene responsible for interferon production under the conditions of cultivation. This could abolish the inhibition of the interferon-inducing action of NDV.

In this sense, Figs 1 and 2 and Table 1 could be read as reflecting reciprocal decrease of the supposed antiviral protein which seems to disappear completely at 36° C within 5 hours. Therefore, if "fresh" cells were infected with NDV for more than 5 hours at 36° C the antiviral protein disappeared and it could not inhibit the interferon production (Table 1). Under these conditions the interferon yield would be the same as in "aged" cells. Our findings seem to corroborate this assumption. However, only a direct determination of the supposed antiviral protein could confirm definitely the assumption made above. Unfortunately, until now there are no suitable methods available for this purpose.

Another possible explanation of the phenomena observed is given by the findings of Isaacs and Rotem (1966). These authors reported on a protein which inhibits interferon production in the allantoic fluid of eggs infected with several myxoviruses, including NDV. The inhibitor was called "blocker" and was found to differ from interferon in some properties which could differentiate it from the latter. It was suggested that "blocker" might be a repressor of the interferon synthesis. This could mean that the synthesis of the "blocker" protein is coded for by the cell genome, and it might be present also in other cell species capable of interferon production. So far, there are no direct proofs of this assumption. However, taking it into consideration the mouse peritoneal cells could be supposed to contain both "preformed" interferon and its "blocker". If this were the case, the loss of "blocker" during precultivation might be also responsible for an efficient derepression of interferon-producing mechanism.

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