INHIBITION OF INTERFERON ACTION BY p-FLUOROPHENYLALANINE

By DR. ROBERT M. FRIEDMAN* and DR. JOSEPH A. SONNABEND
National Institute for Medical Research, Mill Hill, London, N.W.7

THE mechanism of action of the antiviral protein interferon[1] is unknown. Incubation for some hours at 37° C is necessary for interferon action since cells treated with interferon and kept at 2° C showed much less resistance to virus infection than cells similarly treated but kept at 37° C (ref. 2), suggesting that an active metabolic process is necessary for development of full antiviral activity. The action of interferon also requires DNA-dependent RNA synthesis on the part of the cell since interferon action was blocked in cells incubated with actinomycin *D* before the addition of interferon[3].

Since RNA synthesis is required for interferon action, it was of interest to determine whether protein synthesis is also necessary. The results of the work recorded here indicate that protein synthesis is required, as interferon action was inhibited in cells incubated with both interferon and the amino-acid analogue *p*-fluorophenylalanine (FPA).

The origin and preparation of the Semliki Forest virus (SFV) pools used in these investigations and the preparation of chick embryo fibroblast (CEF) cultures have been previously described[3]. The virus growth medium consisted of *tris* (0·002 M) buffered Gey's salts with 0·25 per cent lactalbumin hydrolysate, 0·1 per cent peptone, and 2·5 per cent calf serum. During treatment with FPA, buffered Gey's salts only was used for both experimental and control plates. In experiments using FPA the virus growth medium was supplemented with DL-phenylalanine, 100 µg/ml. SFV was assayed and interferon titrated by previously described methods[4]. DL-FPA was obtained from Calbiochem. In all experiments it was used at a concentration of 100 µg/ml.

In order to estimate interferon action in the experiments to be described, CEF's were infected with SFV at a virus plaque-forming unit to cell multiplicity of 20 : 1. This multiplicity of infection was shown in preliminary examinations to yield maximum virus growth at 8 h after infection; in addition, endogenous interferon production had not yet started at that time. After 1 h at 37° for virus adsorption the cells were washed, and the virus growth medium added. After an additional 7 h at 37° the plates were frozen and thawed and the fluids assayed for SFV.

CEF plates were incubated with buffered Gey's salts, or FPA, or interferon, or FPA with interferon. After 5 h, the cells were washed three times and infected with SFV. The results of assays for SFV growth are shown in Table 1. In this experiment pretreatment with FPA only slightly inhibited the growth of SFV. Interferon 1·2 or 0·6 units †, respectively, inhibited virus growth to 5 and 11 per cent of virus controls. On plates incubated with both FPA and interferon, growth of the virus was inhibited, respectively, to 35 and 49 per cent of virus controls. The figures in parentheses in Table 1 and the other tables are the percentage of virus growth in the plates treated with FPA and interferon if the plates treated with FPA alone are taken as controls.

At both concentrations used in this and other similarly conducted experiments, the antiviral action of interferon was inhibited by FPA. Preliminary investigations utilizing tritiated adenosine or leucine labelled with carbon-14 showed that chick cells treated with 100 µg/ml. of FPA had rates of RNA and protein synthesis equal to those of untreated controls confirming the findings of other workers[5].

* U.S. Public Health Service, at present attached to the National Institute for Medical Research.

† One unit of interferon reduced by 50 per cent the number of vaccinia virus plaques formed on CEF.

Table 1. INHIBITION OF INTERFERON ACTION BY *p*-FLUOROPHENYLALANINE (FPA)

Pretreatment of cells*	Yield of SFV at 8 h (PFU × 10⁶/ml.)	Virus growth as % of virus control
None (virus control)	215	100
FPA only	210	98 (100)†
Interferon (1·2 units)	11	5
FPA + Interferon (1·2 units)	70	33 (33)
Interferon (0·6 units)	23	11
FPA + Interferon (0·6 units)	105	49 (50)

* Cells were pretreated with buffer, interferon, FPA (100 µg/ml.) or both interferon and FPA (100 µg/ml.) for 5 h, then washed 3 × and infected with Semliki Forest virus (SFV).

† Figures in parentheses represent virus growth in plates treated with FPA and interferon as a percentage of the growth of virus in plates treated with only FPA.

It was of interest to determine whether the inhibition of interferon action by FPA waned with time. CEF's were therefore treated in the same manner as in the previously described experiment. Immediately after the FPA and interferon had been washed off, one set of plates was infected with SFV (Table 2*A*). Another set was allowed to incubate at 37° C for 20 h with virus growth medium and then infected with SFV (Table 2*A*). On the plates infected immediately after the removal of FPA and interferon the inhibitory effect of the FPA was not completely reversed by washing and addition of phenylalanine. The virus growth in plates treated with interferon was 5 per cent of that of the virus control. In plates treated with both interferon and FPA, the virus growth was 13 per cent of the controls, 25 per cent if the inhibition due to FPA was considered.

On plates kept for 20 h before being infected, the antiviral action of the FPA had been almost completely reversed. The effect of the interferon was somewhat less than in the plates infected immediately. The action of FPA to inhibit the antiviral activity of interferon was not reversed by the prolonged incubation between the removal of FPA and interferon and the infection with SFV, since virus growth was increased to 34 per cent of controls in these samples. These results indicated that FPA inhibition of interferon action was persistent.

The preceding experiments suggested that protein synthesis was required for full interferon action. The following experiment was performed to test whether FPA could inhibit the antiviral activity of interferon after protein synthesis had been allowed to proceed for some time following the addition of interferon. CEF's were treated with interferon for 5 h and then with FPA for 5 h. The results (Table 2*B*) showed again that interferon and FPA together had less inhibitory action than interferon alone. When cells were first incubated with interferon and then with FPA, however, the inhibitory action of interferon was the same as in cells treated with interferon and then with *tris* buffered Gey's salts. These results indicated that once protein synthesis had been allowed to proceed after the

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Table 2. FPA INHIBITION OF INTERFERON ACTION

(*A*) *Persistence of FPA inhibition of interferon action*

Pretreatment of cells*	Yield of SFV at 8 h (PFU × 10⁶/ml.)	Virus growth as % of virus control
SFV infection immediately after removing FPA and interferon		
None (virus control)	39	100
FPA only	20	50 (100)†
Interferon (0·6 units)	2·1	5
Interferon (0·6 units) + FPA	5·0	13 (25)
SFV infection 20 h after removing FPA and interferon		
None (virus control)	32	100
FPA only	29	90 (100)
Interferon (0·6 units)	3·9	12
Interferon (0·6 units) + FPA	11	34 (38)
(*B*) *Lack of inhibition of interferon action by FPA added after interferon*		
FPA and interferon added simultaneously		
None (virus control)	52	100
FPA only	45	87 (100)
Interferon (0·6 units)	8	15
Interferon (0·6 units) + FPA	16	32 (36)
Interferon added before FPA		
None (virus control)‡	54	100
FPA, then buffer	33	61 (100)
Interferon, then buffer	6·5	12
Interferon, then FPA	4·3	8 (13)

*, †, See footnotes for Table 1.

‡ Cells were pretreated with FPA (100 μg/ml.) or interferon (0·6 units) for 5 h, FPA or interferon washed off, then buffer or FPA (100 μg/ml.) added for 5 h and washed off. Plates were then infected with SFV.

addition of interferon, the antiviral activity of interferon could not be inhibited by FPA.

FPA is incorporated into proteins which in some instances investigated have reduced biological activity[6]. Since no inhibition of RNA synthesis was found at the concentration of FPA used in this work, the inhibition of interferon action observed indicates that synthesis of protein is required for interferon action.

The findings that interferon did not directly inactivate viruses[2], that a period of incubation at 37° was necessary for interferon action[2], and that protein and RNA synthesis[3] are required for interferon action all indicate that metabolic activity is necessary for interferon action. This suggests that either interferon itself is altered intracellularly to express its antiviral action, or the antiviral action is due to an induced change within the cell. As protein synthesis is required for interferon action, the latter would appear to be the simpler explanation of interferon action, though the former is possible. Interferon could be an inducer of a specific RNA which in turn acts as a messenger for the production of an antiviral protein. Interferon does not stimulate interferon production[7] so that interferon itself could not be the antiviral protein. Since interferon inhibits both RNA and DNA viruses, the proposed antiviral protein might act on RNA synthesis. There is indeed some indication that both crude and purified interferon inhibit total cellular RNA synthesis[8].

After the experiments described in this work had been completed, a paper by Lockart was published with conclusions generally in agreement with those reached by us[9]. The experimental evidence presented by Lockart was, however, open to other interpretations than that protein synthesis was necessary for interferon action.

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PROTEIN SYNTHESIS AND TURN-OVER IN CULTURED PLANT TISSUE: SOURCES OF CARBON FOR SYNTHESIS AND THE FATE OF THE PROTEIN BREAKDOWN PRODUCTS*

By DR. R. G. S. BIDWELL
Department of Botany, University of Toronto
AND
DR. R. A. BARR and PROF. F. C. STEWARD, F.R.S.
Laboratory of Cell Physiology and Growth, Cornell University, Ithaca, New York

A PREVIOUS investigation of metabolism and protein synthesis in highly organized plant cells directed particular attention to the contrast between actively growing carrot cells and those in a more quiescent state[1,2]. It was then found necessary to recognize the complexity of the cells, that is, the existence of distinct compartments in which the same metabolite might undergo different reactions. It was also necessary to distinguish between different sources of carbon for protein synthesis and to involve the carbon of protein after turn-over in the general metabolism of the cell to a greater extent than is usually done. A diagrammatic scheme to express these general ideas was formulated (ref. 1, *loc. cit.* Fig. 1).

Further experiments along these general lines have been made from several points of view. (*a*) To bring new evidence to bear on the sources of carbon for protein synthesis; (*b*) to provide new evidence on protein turnover in these cells and to show the metabolic fate of the carbon so released; (*c*) to contrast the metabolism of asparagine and glutamine in cultured plant cells.

Because *in toto* the experimental data are extensive, they will be presented here in summary form only. The purpose of this summary is to bring the results obtained into the context of present-day thinking on cellular metabolism. This is of particular interest because of present views on the mechanism of protein synthesis, its genetic

* This work represents a collaboration between the Cornell and Toronto Laboratories. The work at Cornell was supported by grants from the National Institutes of Health, U.S. Department of Health, Education and Welfare, to one of us (F. C. S.). This made possible the participation of one of us (R. G. S. B.) as a visiting professor at Cornell University. This also furnished the valuable assistance of Mrs. M. O. Mapes with the aseptic culture methods and provided for a large amount of analytical work under the immediate supervision of one of us (R. A. B.). Part of the work was carried out in the Toronto Laboratories with the assistance of grants from the National Research Council of Canada and the Ontario Research Foundation to one of us (R. G. S. B.).

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