THE PRODUCTION AND EFFECT OF INTERFERON IN ORGAN CULTURES OF CALF TRACHEA

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Received for publication April 25, 1968

INTERFERON is found in the lungs of animals which have been infected with influenza virus (Isaacs and Hitchcock, 1960) and it is widely believed that it plays a part in the resistance of the respiratory tract to infection with this and other viruses. However, at the time these studies were begun it was not known whether infection of the ciliated epithelium of the respiratory tract was followed by the release of interferon or whether exogenous interferon would prevent infection of these specialised cells. As it had been shown that respiratory viruses could be grown in calf trachea cultures (Hoorn, 1964; Reed unpublished) studies were therefore undertaken to investigate the questions using this model system.

MATERIALS AND METHODS

The viruses used as interferon inducers were parainfluenza type 1, Sendai passed in chick embryos; parainfluenza type 3, strain 33 (Bakos and Dinter, 1960) passed in calf kidney cells; rhinovirus SD-1 strain, passed in calf kidney tissue cultures. The same viruses were used to challenge organ cultures treated with interferon.

Organ cultures were prepared from tracheal epithelium of young calves as described previously by Hoorn (1964); Hoorn and Tyrrell (1965). Usually 3 tissue fragments were planted with the ciliated surface uppermost in each 6 cm. plastic petri dish and 5·0 ml. of Eagle's medium with antibiotics was added. The dishes were incubated at 33° in a humidified plastic box in an atmosphere of 5 per cent CO_2 in air.

For inoculation the medium was removed and 0·3 ml. of virus suspension was dripped onto the fragments. After 1 hr. of adsorption at room temperature 5·0 ml. of Eagle's medium was added to each dish. In some experiments 0·1 ml. samples were taken on appropriate days for virus and interferon assay. In other experiments all the medium was collected and replaced with fresh Eagle's solution.

For virus infectivity assays the samples were stored at — 35° and then thawed and inoculated simultaneously into primary calf kidney tissue cultures.

For the interferon assay the virus samples were inactivated by adding 1·0 N HCl until pH 2·0 was reached. After 24 hr. at 4° the pH was brought to 7·0 using 1·0 N NaOH. Calf kidney tissue cultures and tubes were inoculated with 2-fold dilutions of each sample and after 24-hr. incubation at 37° $10^7 TCID_{50}$ of Sendai virus was added. Using the quantitative haemadsorption method (Finter, 1964) the interferon titre was taken as the reciprocal of the dilution at which Sendai virus growth was reduced by 50 per cent.

RESULTS

The production of interferon in organ cultures

In the first experiments Sendai virus which had been propagated in eggs was inoculated in large amounts into culture dishes from which small samples were removed each day and titrated for the presence of interferon and infectious virus. The results, shown in Fig. 1, indicate quite clearly that the virus multiplied

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and that interferon was produced. It is of interest that the interferon seemed to appear a little before the maximum titre of virus.

In other systems interferon is produced by infection with several different viruses and so organ cultures were infected with about $10^3 TCD_{50}$ of 3 different viruses all of which were known to multiply well in these cultures. They were Sendai virus, parainfluenza virus type 3 and the bovine rhinovirus SD-1. In this case the medium was changed completely after 2, 4 and 7 days. Fig. 2 shows that in each case interferon was produced. Sendai virus seemed to be the best inducer and the rhinovirus induced interferon rather late. By prolonging the experiment it was shown this time that interferon production eventually ceased.

TABLE.—*Interferon Production in Organ Cultures Inoculated with Different Respiratory Viruses*

Virus	Expt. No.	Dose of virus ($TCID_{50}$)	Interferon titres in medium collected from organ cultures on indicated day after virus inoculation						
			1	2	3	4	5	6	7
Sendai	1	10^3	128		128		8		*
	2	10^4	16	256			0		
	3	10^5	1024				0		
SD-1	1	$5 \cdot 10^1$	128				2		
	2	$5 \cdot 10^2$	8		32		0		
	3	10^3	4	64		8		0	
Parainfluenza 3	1	10^3	32		0		0		
	2	10^4	512				8		

* All the medium was collected on the last day shown within the " box " and presumably contained interferon which had accumulated throughout the period from the previous collection.

In further experiments on the same pattern (Table) these results were confirmed and it was also shown that both the Sendai and type 3 parainfluenza viruses seemed to produce more interferon after larger inocula were given. There was no such difference in the experiments with rhinovirus. The effect may have occurred because non-infectious parainfluenza virus particles in the inoculum induced interferon, whereas rhinovirus particles did not. This would be consistent with the appearance of interferon before infectious virus when large doses were given (Fig. 1).

The virus inhibitory activity detected has not been characterized in every sample; however from the method of assay it must be acid stable. Some high titre samples were studied further and shown to be inactivated by trypsin and to be active in calf kidney cells and not in human cells. Semliki Forest virus and Sindbis virus were susceptible to inhibition in calf kidney cells. Activity was still observed if the medium was removed from the tissue cultures and they were washed before challenge. Finally the molecular weight of the active material

was shown to be 33,200–38,000 by chromatography on a porous polyacrilamide gel column (Bio-Gel P-150). The inhibitory activity therefore seems to be due to a protein with the biological properties and molecular weight typical of interferons induced by viruses.

The effect of calf kidney interferon on virus multiplication in calf trachea

In these experiments calf kidney interferon with a titre about $\frac{1}{8}$ was used. Five ml. was added to each dish after the growth medium was removed. Control cultures received 5 ml. of medium only. The cultures were incubated overnight and next day the medium was changed and the challenge virus was added. The medium was replaced at intervals of 2 or 3 days and the old medium was titrated

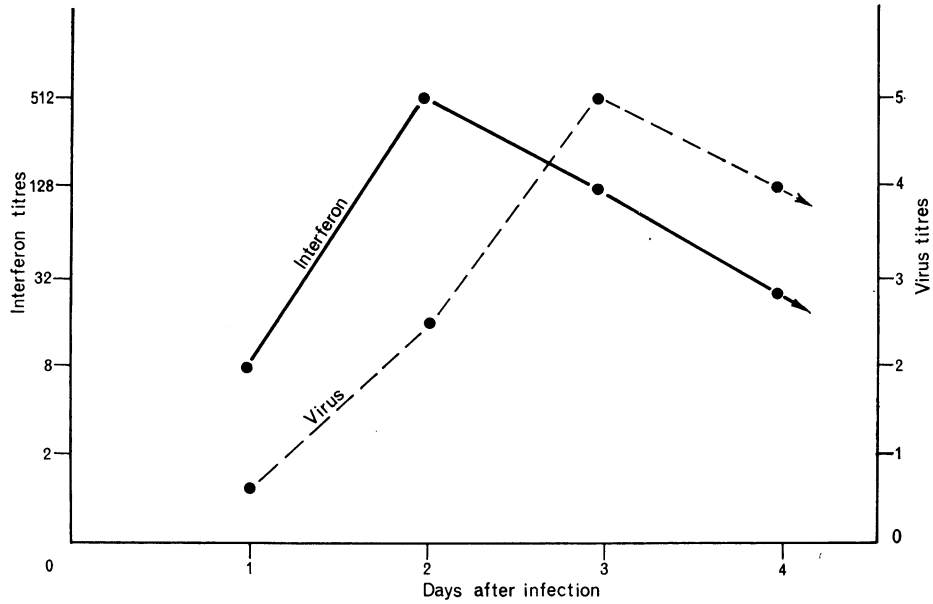

FIG. 1.—Accumulation of interferon in the medium of organ cultures inoculated with Sendai virus (10^5 TCD_{50}). At each date small amounts of medium were removed from the plates.

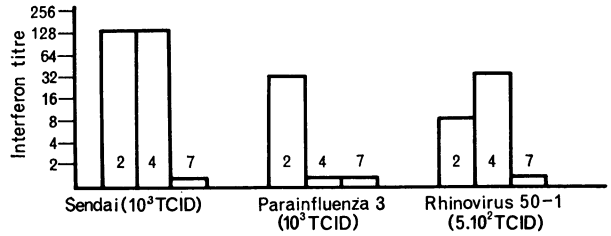

FIG. 2.—The production of interferon in organ cultures inoculated with 3 respiratory viruses. Three experiments are shown. The inoculum used is given under the columns and the number of days after inoculation on which the medium was changed and saved for assay is shown above the baseline. The figures in the columns—the days after organ cultures inoculation at which whole medium was decanted for interferon assay and replaced with the fresh maintenance medium.

for infectious virus. The results are shown in Figs. 3 and 4. The difference in virus titres between treated and control organ cultures was usually 1–2 $\log_{10}$ units, implying that virus production was reduced 90–99 per cent. The ciliary activity of the treated cultures continued to the end of the experiment whereas that in the controls ceased; this meant that cell damage, as well as virus production, was prevented by interferon. It was also noted that the interferon effect persisted for at least two weeks although it was removed before the virus was added. The experiment was repeated 6 times using 3 different batches of interferon and similar results were obtained.

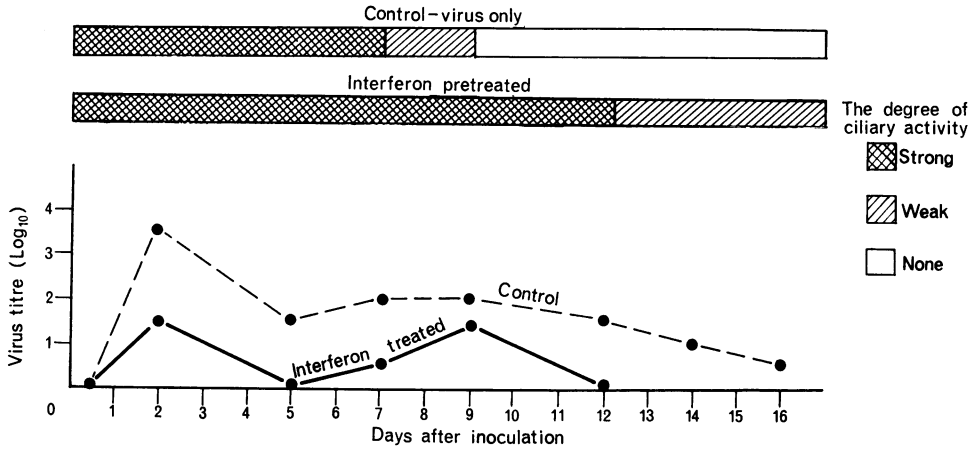

FIG. 3.—The multiplication of SD-I rhinovirus in calf organ cultures treated with calf interferon for 1 day before inoculation. The " box " shows the degree of ciliary activity.

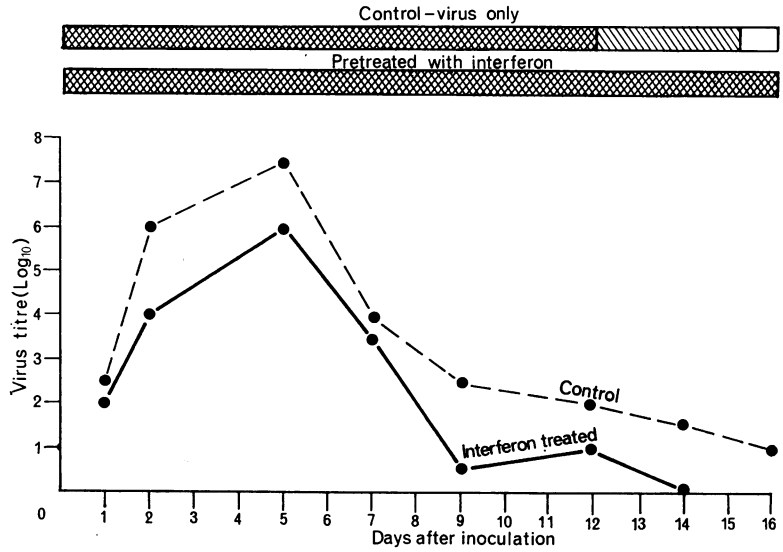

FIG 4.—The multiplication of Sendai virus in calf organ cultures treated with calf interferon for one day before inoculation.

DISCUSSION

Pathological studies (Hoorn and Tyrrell, 1966) and immunofluorescent staining (Shroyer and Easterday, 1968; Hers, personal communication) all show that influenza viruses infect only the epithelium of organ cultures of respiratory epithelium. Rhinoviruses and parainfluenza viruses also produce superficial degenerative changes only (Hoorn and Tyrrell, 1965; Tyrrell and Horn, 1965; Reed, personal communication). It is believed that the substance found in our cultures was interferon and it is concluded therefore that it was produced in the tracheal epithelium.

Since these epithelial cells can also be protected by interferon it is concluded that previous failures to prevent respiratory disease by the use of exogenous interferon in volunteers was due to failure to get enough interferon to enough cells. Recent studies in mice have, in fact, shown that organ cultures of their tracheas may be protected with interferon and that if intact animals inhale enough aerosolised interferon they also become relatively resistant to influenza virus infections (Finter, personal communication).

Finally, the long period of resistance to virus infection might be due to some complex phenomenon, whereby virus induced further interferon production by interferon-treated cells. On the other hand it may reflect a prolonged specific effect of interferon. It is of interest that interferon may be found in the respiratory secretions of subjects with a number of respiratory virus infections and this may be related to the 6 week period of relative resistance to infection which follows exposure to common cold viruses (see Lidwell and Williams, 1961; Cate, Couch and Johnson, 1964).

SUMMARY

An inhibitor of virus multiplication, apparently an interferon with a molecular weight of about 30,000 has been found in the medium of organ cultures of calf trachea infected with parainfluenza 1 (Sendai) and bovine parainfluenaa 3 viruses and bovine rhinovirus (strain SD-1).

Similar organ cultures were protected against infection with parainfluenza 1 and rhinovirus by overnight exposure to low concentrations of calf kidney interferon.

I wish to thank Dr. N. Finter for the supply of calf kidney interferon, Dr. A. Phillips and Dr. H. Zola for help with chromatography, Mrs. B. Head and Miss N. Frost for technical assistance and Dr. D. A. J. Tyrrell for discussions and assistance in preparing the manuscript.

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