Effect of Ultraviolet Irradiation and Heating on the Interferon-inducing Capacity of Human Adenoviruses

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SUMMARY

Heating adenovirus types 8 and 12 for 2 to 3 min. at 56° simultaneously reduced both interferon-inducing capacity and infectivity. In contrast, virus inactivated by u.v.-irradiation effectively stimulated interferon production in chick cells.

Chick cells infected with adenovirus type 12 and incubated at 25°, 35° or 40° produced about the same amount of interferon but the kinetics of interferon production differed, i.e. at the higher temperatures interferon formation commenced earlier.

The possibility that the penton antigen may be responsible for interferon induction by human adenoviruses in chick cells is discussed.

INTRODUCTION

Burke & Isaacs (1958a) found that inactivated influenza virus was a more efficient interferon inducer in chick chorioallantoic membranes than the infective virus. Other inactivated myxoviruses and inactivated vaccinia virus have also been reported to be effective inducers (Burke & Isaacs, 1958b; Henle et al. 1959; German, Panouse-Perrin & Quero, 1968; Glasgow & Habel, 1962), whereas inactivated arboviruses fail to induce interferon (Vilček, 1961, 1963; Ho & Breinig, 1962; Lockart, 1963).

The formation of interferon by several types of human adenoviruses in primary chick embryo fibroblast cells has been observed in this laboratory (Beládi & Pusztai, 1967). Adenovirus purified by CsCl-gradient centrifugation also induces interferon synthesis in chick cells (Beládi & Pusztai, 1967; Ho & Köhler, 1967). It was observed however that adenovirus heated at 56° failed to stimulate interferon production (Beládi & Pusztai, 1967) and this prompted us to study the effect of u.v.-irradiation on the interferon-inducing capacity of adenoviruses. We compare here the effect of heat inactivation and u.v.-irradiation upon the ability of adenoviruses to induce interferon.

METHODS

Viruses. Adenovirus type 6 was kindly provided by Dr H. G. Pereira, National Institute for Medical Research, London. Type 8 was isolated in HeLa cells from a patient suffering from kerato-conjunctivitis during an epidemic in Szeged (Beládi et al. 1963), type 12 was kindly supplied by Dr K. Köhler, Max Planck Institute, Tübingen. Each type was propagated in HEp2 cells cultured in Eagle's basal medium containing...
10% tryptose phosphate broth and 10% calf serum (Macpherson & Stoker, 1962). After infection the cells were maintained in Gey's medium enriched with 5% rabbit serum and 0.25% lactalbumin hydrolysate. Sindbis virus used for challenge was grown in primary chick cells cultured in Gey's medium supplemented with 5% calf serum and 0.25% lactalbumin hydrolysate.

*Infectivity titrations.* Infectivity assays were made in test-tube cultures of HEP2 cells. Serial tenfold dilutions of the virus were prepared in Hanks's balanced salt solution and three cultures were used for each dilution. The medium was renewed every 4 or 5 days and the cultures were examined for cytopathic effect for 4 weeks. Virus infectivity was calculated as TCD50 using the method of Reed & Muench (1938). Infectivity of Sindbis virus was determined by the plaque assay of Porterfield (1959), somewhat modified (Béládi, 1962).

*Heat inactivation at 56°.* One ml. samples of the undiluted virus sealed in 5 ml. ampoules were completely immersed in a water-bath maintained at 56° ± 0.1°. After appropriate heating the samples were rapidly removed and chilled in ice-cold water.

*Ultraviolet irradiation.* The source of u.v. light was a Hanau germicidal lamp. The u.v. dose was measured directly with a Laterjet dosimeter. Undiluted virus samples of 1 ml. were put in 60 mm. Petri dishes and irradiated with a u.v. dose of 15.5 ergs/mm²/sec. The Petri dishes were shaken constantly during the irradiation.

*Production of interferon.* The method for preparing chick cell cultures and the medium used were described by Béládi (1962). Cultures in 60 mm. Petri dishes were seeded with 2.5 x 10⁷ primary chick fibroblast cells. Two days later the medium was removed and 0.1 ml. of the appropriate virus inoculum was introduced into two cultures. After 1 hr adsorption the inoculum was removed and the cell sheets were washed twice with 2 ml. of Hanks's solution, then 5 ml. of fresh medium was added to each culture. After incubation at 35° for 48 hr—except where indicated otherwise—the fluids were harvested and pooled, then centrifuged at 3000 rev./min. for 15 min. The supernatant fluids after heating at 56° for 2 hr were kept at 4° until tested for interferon activity.

*Interferon assays* were performed by the plaque-reduction method. Primary chick embryo fibroblast monolayers in 60 mm. Petri dishes were exposed for 4 hr to 1.5 ml. of twofold serial dilutions of interferon samples, then the fluids were removed and the cultures challenged with 100 to 150 p.f.u. of Sindbis virus. After 120 min. the cultures were overlaid with agar medium. Two cultures were used for each dilution. Plaques were counted after 2 days. The interferon titre was expressed as the reciprocal of the dilution which reduced the plaque count to 50% of that of the controls.

**RESULTS**

*Interferon induction by adenovirus heated at 56°*

After heating adenovirus type 12 for different times both the infectivity and the interferon-inducing capacity of the virus were reduced (Fig. 1). Treatment for 2 min. completely abolished the interferon-inducing capacity and caused 4 log. reduction in the infectivity.
**Effect of u.v. and heating on adenoviruses**

**Interferon induction by ultraviolet irradiated adenovirus**

Infectivity and interferon-inducing capacity were tested before and after u.v. irradiation of adenovirus type 12. Interferon induction by the virus was much less sensitive to irradiation than infectivity (Fig. 2). Indeed at u.v. doses completely abolishing infectivity considerable inductive capacity remained.

Interferon formation by chick cells inoculated with both u.v. irradiated and heated adenovirus type 8 was also studied. The data obtained were similar to those observed with type 12 (Fig. 3).

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![Fig. 1. Effect of heating at 56° on the infectivity and interferon-inducing ability of adenovirus type 12.](image)

*Fig. 1. Effect of heating at 56° on the infectivity and interferon-inducing ability of adenovirus type 12. **—**, Infectivity; **—**, interferon production.*

**Interferon induction by adenovirus type 8**

We have observed previously using types 3, 6 and 16 of human adenoviruses that high multiplicity is needed for efficient interferon production (unpublished data). Since Hanna & Jawetz (1962) reported that type 8, in contrast to other adenoviruses, has a low infective titre we were interested to determine the effect of multiplicity upon interferon induction by adenoviruses. Chick cells were accordingly inoculated with
type 8 and, for comparison, with type 6, at different multiplicities (Table 1). Although $10^{6.5}$ TCD50 of type 6 did not stimulate interferon production $10^{5.5}$ TCD50 of type 8 was sufficient to induce a good yield of interferon.

![Fig. 2. Effect of u.v.-irradiation on the infectivity and interferon-inducing ability of adenovirus type 12.](image)

![Fig. 3. Effect of heat at 56°C and u.v.-irradiation on the infectivity and interferon-inducing ability of adenovirus type 8.](image)
**Effect of u.v. and heating on adenoviruses**

Table 1. *Interferon induction by adenovirus types 6 and 8 at different multiplicities*

<table>
<thead>
<tr>
<th>Type</th>
<th>Log.TCD 50</th>
<th>Multiplicity</th>
<th>Interferon titre</th>
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<tr>
<td>6</td>
<td>7.5</td>
<td>2.1</td>
<td>128</td>
</tr>
<tr>
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<td>6.5</td>
<td>0.21</td>
<td>4</td>
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<tr>
<td></td>
<td>5.5</td>
<td>0.021</td>
<td>&lt;4</td>
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<tr>
<td></td>
<td>4.5</td>
<td>0.0021</td>
<td>&lt;4</td>
</tr>
<tr>
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<td>2.5</td>
<td>0.000021</td>
<td>256</td>
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<tr>
<td></td>
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<td>0.0000021</td>
<td>32</td>
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<td>0.5</td>
<td>0.00000021</td>
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</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.000000021</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

![Graph showing interferon production](image)

Fig. 4. Effect of temperature on interferon production in chick cells inoculated with adenovirus type 12. \( \triangle \) Interferon titre at 25°; \( \bigcirc \) at 35°; \( \square \) at 40°.

**Effect of temperature on interferon production by chick cells inoculated with type 12**

Chick cells inoculated with \( 10^4 \) TCD 50 of type 12 were incubated at 25°, 35° or 40°. The media of two cultures at each temperature were pooled at intervals as indicated in Fig. 4 and the interferon content was tested. Interferon production clearly occurred at each temperature, but at the higher temperatures the interferon appeared sooner and reached maximum value more rapidly.
Viruses, whether infective or inactivated, may induce interferon formation. Myxoviruses inactivated at 56° or by u.v. irradiation are more effective inducers than in the infective state (Burke & Isaacs, 1958a, b). Arboviruses when treated similarly cannot induce interferon, although it has been reported that arboviruses inactivated under certain conditions may also be capable of inducing interferon formation (Gifford & Heller, 1963; Le Clerc & Cogniaux-Le Clerc 1965). Infective Newcastle disease virus was found to be a poor interferon inducer in chick cells and certain doses of u.v. irradiation actually enhanced the interferon-inducing ability of the virus (Ho, 1964; Youngner et al. 1966). The u.v. dose used to promote this effect was, however, limited to a very narrow dose range. Youngner et al. (1966) emphasized the decisive importance of the host cell system in determining the efficacy of Newcastle disease virus to induce interferon. These workers found that interferon induction by infective or u.v. irradiated Newcastle disease virus occurred in L-cells. Inactivation of Newcastle disease virus at 56° destroyed its interferon-inducing ability in both L-cells and chick cells.

We have shown that inactivation of adenovirus at 56° destroys its interferon-inducing capacity, whilst both u.v. inactivated and infective adenovirus induce interferon production. In this respect the human adenovirus chick cell system is similar to the Newcastle disease virus L-cell system (Youngner et al. 1966).

Our data suggest that the interferon-inducing ability of adenoviruses in chick cells is not related to the multiplicity of infection employed since type 8 induced about the same amount of interferon as type 6, although 100,000-fold fewer TCD50 of type 8 were used.

Jawetz et al. (1958) described a low infective titre as a peculiar property of type 8 strains. The finding that type 8 virus has the same titre of complement-fixing antigen as other types with high infective titre raises the possibility that adenovirus type 8 may be associated with additional non-infective virus or soluble antigens (Jawetz et al. 1958; Hanna & Jawetz 1962). Since type 8 appears to be an efficient interferon inducer, it is most likely that the infectivity of adenoviruses is not of critical importance in the induction of interferon synthesis in chick cells. This fact corresponds to our previous finding that trypsin-treated adenoviruses, though infective, do not stimulate production of interferon (Bélaïdi & Pusztai, 1967).

Studies of the penton antigen of adenoviruses have shown that it is sensitive to trypsin (Rowe et al. 1958; Pereira, 1958) but resistant to u.v. irradiation (Rowe et al. 1958). Our previous studies (Bélaïdi & Pusztai 1967) and the present data therefore support the hypothesis that the penton antigen of the virus is in some way responsible for interferon production. Although the penton antigen had been reported as stable at 56° in respect of its cell-detaching capacity (Rowe et al. 1958; Everett & Ginsberg, 1958), Russell, Valentine & Pereira (1967) recently found that heating adenovirus type 5 at 56° resulted in specific disintegration of the penton base followed by the liberation of the surrounding five hexons. The finding that the adenovirus heated at 56° is incapable of stimulating chick cells to form interferon is thus presumably due to the loss of the penton bases as a result of heating. Studies to resolve the role of the penton antigen in the production of interferon in chick cells are currently in progress.

The finding that in the adenovirus + chick cells system interferon production occurs at 25° is of particular interest, since in the Eastern equine encephalomyelitis virus +
chick cells system no interferon formation could be demonstrated even at 30° (Wagner, 1963). In the tick-borne encephalitis virus + L-cells system interferon production varies markedly according to the incubation temperature. Thus the highest titres of interferon were found at 39° and little or no interferon was produced at 32° (Stanček, 1965). In contrast to this, adenovirus type 12 is an effective inducer of interferon production in chick cells incubated at 25°.

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REFERENCES


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