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INTERFERON INDUCTION BY GAL VIRUS IN CHICK EMBRYO CELLS

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Summary. — It was found that GAL (gallus adeno-like) virus, an avian adenovirus, induces the production of interferon in chick embryo cells (CEC). The physicochemical and biological properties of the interferon formed are similar to those of other known interferons.

Adenoviruses were considered to be incapable of inducing interferon production (Larke, 1966). However in 1967 Béládi and Pusztai (1967) found that the human adenoviruses are good inducers of interferon formation in CEC. Later, Pusztai *et al.* (1969*a*) described the presence of interferon in sera of chicks inoculated with human adenoviruses; the effect of heating and ultraviolet irradiation upon the ability of the adenoviruses to induce interferon was also studied (Pusztai *et al.*, 1969*b*). The present communication deals with the production of interferon by CEC infected with GAL virus.

GAL virus, kindly supplied by Dr. T. Szent Iványi, Institute of Epizootiology, Veterinary University, Budapest, was grown in chicken kidney cell cultures derived from 3—7 days old chickens. For production of interferon, monolayers of CEC prepared from 11-day-old embryos were grown in 60 mm diameter Petri dishes. After 24 hours of incubation the cultures were inoculated with 10^3—10^5 TCD_{50} of GAL virus which was allowed to adsorb for 2 hours at 35° C. Then the cells were washed twice with Hanks' solution and fed with 5 ml of medium consisting of Gey's solution enriched with 0.25% lactalbumin hydrolysate, and 5% calf serum. Culture fluids were harvested after 48 hours and centrifuged at 2500 rev/min for 15 minutes. Supernatants were heated for 2 hours at 56° C to eliminate the infective virus and then tested for interferon activity. Samples were taken for infectivity titration before heating. Virus infectivity was tested in tube cultures of chicken kidney cells and after 7 days of incubation the titre was expressed as TCD_{50} determined by the formula of Reed and Muench. Interferon was assayed by plaque reduction method using Sindbis virus as challenge; the titres were expressed as reciprocals of the dilution causing 50% plaque reduction.

In CEC inoculated with GAL virus the titre of interferon was related to the multiplicity of infection (Table 1).

To prove that GAL virus is responsible for the interferon induction, the virus was mixed with homologous immune serum and then inoculated into CEC cultures; the resulting interferon titre was < 4. In the control with normal rabbit serum, the interferon titre was 64. The results showed that GAL virus after treatment with homologous immune serum was incapable of inducing interferon formation.

The kinetics of interferon formation was then investigated. CEC monolayers were infected with GAL virus and the culture fluids were collected and tested for interferon activity as well as for infectivity at intervals. Interferon was first observed at 6 hours after infection. The amount of

Table 1. Interferon induction in CEC by GAL virus at different multiplicity

Dose of virus inoculated (log TCD_{50})	Titre of interferon
5.5	256
4.5	64
3.5	4
2.5	< 4

interferon increased gradually and the maximum level was reached at 48 hours (Fig. 1). The virus also multiplied in CEC, though no visible cytopathic effect was observed on examination of the unstained cells by light microscopy.

Some properties of the antiviral substance produced were investigated. It was found to be sensitive to trypsin (200 μg/ml; 37° C; 1 hour), was not sedimented at 105 000 × g for 2 hours, and was stable at pH 2 for 24 hours and at 60° C for 1 hour. Heating at 80° C caused about 50% reduction in its

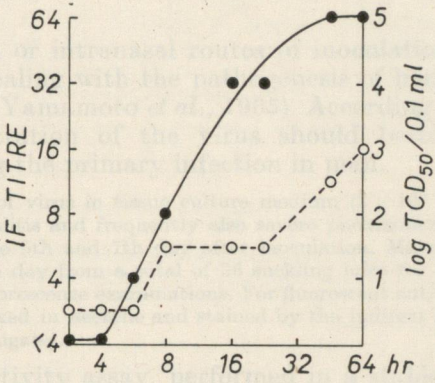

Fig. 1.

Production of interferon and virus by chick fibroblast cells inoculated with GAL virus Abscissa: hours after infection; left ordinate: titre of interferon (●); right ordinate: titre of virus (○)

activity. It had no direct effect on the challenge virus and prevented the multiplication of viruses of different groups, i.e. Sindbis, vesicular stomatitis and vaccinia viruses. Actinomycin D inhibited its antiviral action, GAL immune serum had no effect on it. Furthermore it showed species specificity, was active only in chick cells, but not in hamster embryo cells. These findings suggest that the viral inhibitor induced by GAL virus in CEC is a protein of low molecular weight, inhibits the growth of viruses in cells through some intracellular action involving DNA-dependent synthesis of RNA and is active against unrelated viruses. Thus in all properties investigated so far this antiviral substance fulfils the criteria for acceptance of it as an interferon (Burke and Skehel, 1967).

GAL virus, like human adenoviruses, induces interferon formation in CEC. Whereas human adenoviruses do not replicate in these cells, in GAL virus-infected CEC both virus reproduction and interferon formation occurs. It may be assumed that the lack of the cytopathic effect of GAL virus in CEC is due to the interferon formation observed.

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