PIGEON HERPES VIRUS

II. Susceptibility of Avian and Mammalian Cell Cultures to Infection with Pigeon Herpes Virus

By

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INTRODUCTION

The growth of pigeon herpes virus (HVP) in chicken embryo fibroblast (CEF) cultures was described by Vindevogel, Duchatel and Gouffaux (1977), but little is known about the host specificity of HVP. Cornwell and Weir (1970) reported the non-susceptibility of HeLa cells, strain L cells and primary renal cultures of calf and dog to HVP. The purpose of the present work was to determine whether avian and mammalian cells were susceptible to HVP.

MATERIALS AND METHODS

Cell cultures. Primary chick embryo fibroblast and primary duck embryo fibroblast (DEF) cultures were prepared by conventional procedures, CEF from 9-day-old and DEF from 12- to 14-day-old embryos. Secondary CEF and DEF were obtained by trypsinization of primary cells. Chicken kidney cells (CK) were prepared from 1- to 2-week-old chickens by one round of trypsinization of 12 h at 4 °C. Chicken embryo liver (CEL) cultures were prepared from 14-day-old embryos. Trypsinization was carried out at 37 °C by a multiple extraction process comprising 6 rounds of 10 min each.

Viable cells were counted and diluted in growth medium as follows:

CEF and DEF: $7{\cdot}5 \times 10^5$ cells/ml, CK and CEL: $1{\cdot}5 \times 10^6$ cells/ml.

Seven cell lines of mammalian origin (Table 1) were derived from stocks maintained at the Faculty of Veterinary Medicine.

The growth medium was previously described (Vindevogel, Pastoret, Burtonboy, Gouffaux and Duchatel, 1975). For cultivation of CEL, CK and mammalian cell lines 10 per cent foetal bovine serum was used. The maintenance medium was changed at 3-day intervals. The cultures were incubated at 37 °C and examined daily.

Virus and inoculation procedure. The HVP strain has been described (Vindevogel *et al.*, 1975). Cultures on coverslips were inoculated with 0·2 ml of medium containing 10^5 pfu and cultures in Falcon plastic flasks (25 cm^2–50 ml) with 1 ml of medium containing 5×10^5 pfu. The inoculation procedure was described by Vindevogel *et al.* (1977).

Changes in cell cultures. Development of lesions and presence of viral antigen were

studied as described previously (Vindevogel *et al.*, 1977). Coverslips of avian cell cultures were harvested 24, 48, 72 and 96 h after inoculation, coverslips of mammalian cell cultures on the 4th and the 7th day. Control cells inoculated with phosphate-buffered saline solution (PBS) were used for comparison.

TABLE 1

MAMMALIAN CELL LINES TESTED FOR SUSCEPTIBILITY TO INFECTION WITH HVP

Culture code	*Tissue of origin*
MDBK	Bovine kidney
PK 15	Pig kidney
MDCK	Dog kidney
CRFK	Cat kidney
VERO	Green monkey kidney
BHK	Baby hamster kidney
HELA	Human epithelioid carcinoma

Virus content in the cell cultures. (1) *Viral replication in avian cell cultures.* The virus content of primary DEF, CK, CEL and secondary CEF and DEF cultures was calculated 24, 48, 72 and 96 h after inoculation. The procedure for the determination of the virus contents was described by Vindevogel *et al.* (1977).

(2) *Assay for infectivity of HVP-inoculated culture lines.* Mammalian cell cultures were harvested 7, 14 and 21 days after inoculation, and after 3 blind passages at weekly intervals. They were further assayed for viral infectivity by inoculation on primary CEF.

RESULTS

The results are summarized in Table 2.

TABLE 2

AVIAN AND MAMMALIAN CELL CULTURES SUSCEPTIBLE TO INFECTION WITH HVP

Cell cultures	*Days after inoculation*	*Cytopathic effect*	*Viral antigen*	*Infectivity assay in CEF Titre pfu/ml*
Secondary chicken embryo fibroblast	1	+	+	NT
	2	+	+	$6{\cdot}8\times10^{5}$
Primary duck embryo fibroblast	1	+	+	$1{\cdot}4\times10^{5}$
	2	+	+	$3{\cdot}5\times10^{5}$
Secondary duck embryo fibroblast	1	+	+	$1{\cdot}3\times10^{5}$
	2	+	+	$3{\cdot}6\times10^{5}$
Primary chicken embryo liver	1	+	+	$1{\cdot}3\times10^{4}$
	2	+	+	$1{\cdot}2\times10^{5}$
Primary chicken kidney	1	+	+	$5{\cdot}8\times10^{2}$
	2	+	+	$1{\cdot}0\times10^{3}$
	3	+	+	$5{\cdot}0\times10^{4}$
	4	+	+	$8{\cdot}8\times10^{4}$
Baby hamster kidney cell line	4	+	+	NT
	7	+	+	$2{\cdot}5\times10^{2}$
	14	+	NT	$3{\cdot}0\times10^{3}$
	21	+	NT	$1{\cdot}0\times10^{4}$

NT, not tested.

Viral Replication in Avian Cell Cultures

Cytopathic changes appeared 24 h after inoculation in DEF and in CEL cultures, whereas CK cells showed gross lesions on the 48th h. Changes in DEF

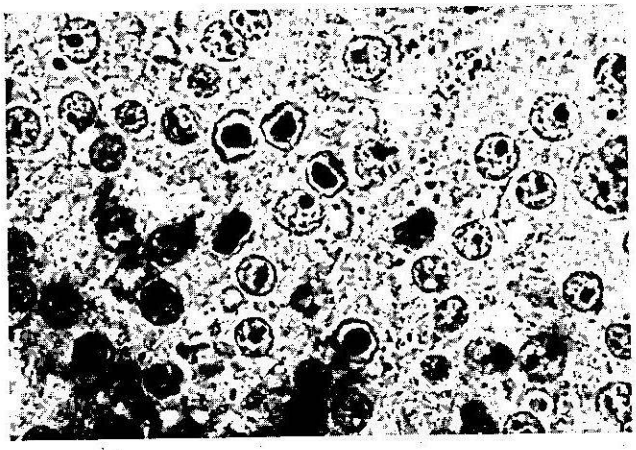

Fig. 1. CEL cell culture 48 h after inoculation showing type A nuclear inclusions. Haemalum eosin. ×400.

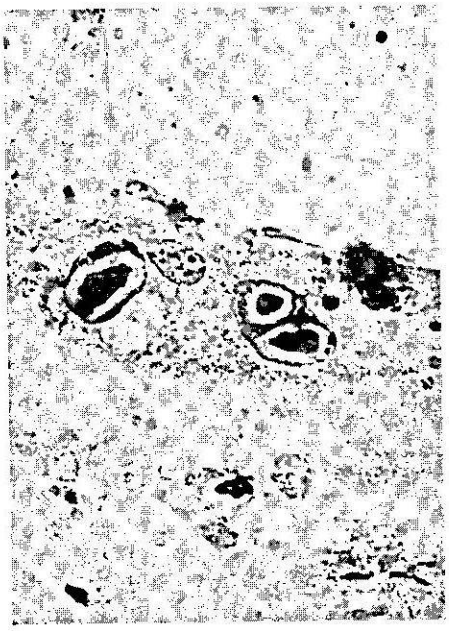

Fig. 2. CK cell culture 72 h after inoculation showing type A nuclear inclusions. Haemalum eosin. ×400.

consisted of the development of round refractile cells and large stellate syncitia were observed after the inoculation; the granular debris usually remained adherent to the plastic as in the CEF cultures. Changes in CEL and CK

cultures consisted of rounding of cells; affected cells became rapidly detached from the monolayer leaving a clear central area.

Cowdry type A intranuclear inclusions bodies were found in cells around the cytopathic areas from 24 h after inoculation in DEF, CEL and CK cultures (Figs 1 and 2); specific cytoplasmic and nuclear HVP-immunofluorescent antigen was demonstrated in these 3 avian cell types.

Generalization of the CPE in DEF and CEL cultures was complete 48 h after inoculation and on the 96th h in the CK cultures.

The chronological development of lesions in secondary CEF and DEF was similar to the primary cultures.

Virus titres of the embryo fibroblast and liver cell cultures were maximal 48 h after inoculation and were between 10^5 and 10^6 pfu/ml. The maximum virus content of the CK cell cultures was reached after 4 days and remained inferior to 10^5 pfu/ml.

Viral Replication in Mammalian Cell Cultures

Except for the BHK cell line, the appearance of inoculated mammalian cells remained normal throughout the 3 weeks of the observation period. In stained preparations, no inclusions and no immunofluorescent antigen were observed; all attempts to re-isolate HVP on susceptible CEF failed.

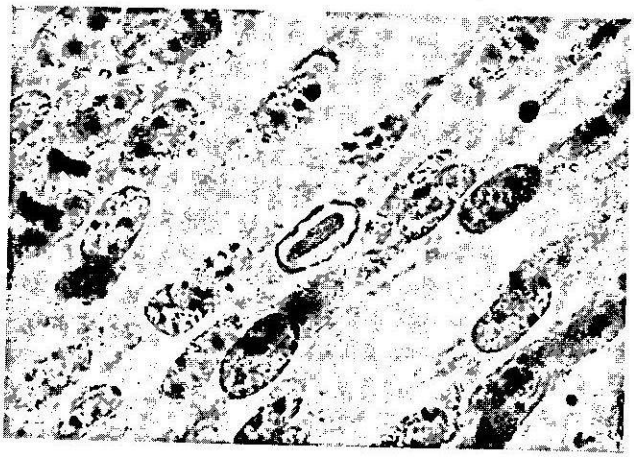

Fig. 3. BHK cell line 4 days after inoculation showing type A nuclear inclusion. Haemalum eosin. × 400.

The HVP-infected BHK cultures showed rounding, clumping and retraction of cells 3 days after inoculation. The foci slowly enlarged and increased in number until the end of the examination period. In stained preparations harvested 4 and 7 days after inoculation, Cowdry type A inclusions were observed in cells of normal shape in the expanding rim of the lesions (Fig. 3), whereas no inclusions were seen in uninfected BHK control cultures. Cytoplasmic fluorescence was observed in the foci of infected cultures stained by the fluorescent-antibody technique (Fig. 4); uninfected cultures similarly treated

were all negative. HVP was re-isolated on CEF from all harvests of infected BHK and specific antigen was demonstrated in these re-isolates by immunofluorescence. Virus content in infected BHK increased slowly: 10^4 pfu/ml was reached 3 weeks after inoculation and $1{\cdot}1 \times 10^3$ pfu/ml after 3 blind passages at weekly intervals. Attempts to re-isolate a virus from the BHK control cultures were unsuccessful.

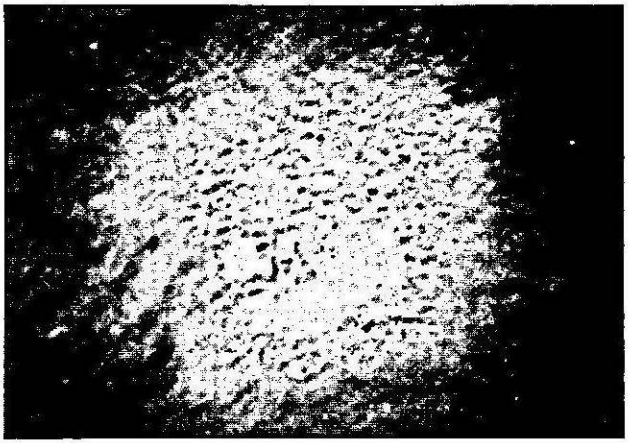

Fig. 4. BHK cell line 4 days after inoculation in which viral antigen is indicated by fluorescence in a focus. Fluorescent antibody stain. × 40.

DISCUSSION

HVP was cytopathic in all the avian cultures examined and the lesions observed in CEL and in CK cultures were similar to those reported by Cornwell and Weir (1970). Changes in DEF cells consisted of the development of large stellate syncitia. Similar variations in the cytopathic response depending on the type of cells were mentioned by Purchase, Burmester and Cunningham (1971) and by Meulemans, Halen, Schyns and Vindevogel (1973) with turkey herpes virus and Marek's disease virus. The data indicate that the BHK cell line is susceptible to infection with HVP. The morphological modifications of the infected BHK cell cultures which included the presence of refractile round cells and type A intranuclear inclusions, were supported by the demonstration of HVP-specific immunofluorescent antigen. Further evidence of the replication of HVP in BHK cell cultures was given by the increase in virus titre of HVP-infected cell cultures on CEF. These morphological and antigenic changes were not due to a latent virus since the BHK control cultures showed no viral antigen and assays for infectivity on CEF remained negative. All the other mammalian cell lines examined were refractory to infection.

SUMMARY

Duck embryo fibroblasts, chicken embryo liver cell cultures, chick kidney cell cultures and 7 mammalian cell lines were tested for susceptibility to pigeon

herpes virus (HVP). All avian cell cultures were susceptible to HVP but there were variations in the cytophatic response. The baby hamster kidney cell line was susceptible to infection with HVP: morphological modifications were supported by the demonstration of HVP-specific antigen and the increase in virus titre. All other mammalian cell lines were refractory to infection with HVP.

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