PIGEON HERPES INFECTION: EXCRETION AND RE-EXCRETION OF VIRUS AFTER EXPERIMENTAL INFECTION

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INTRODUCTION

Herpesvirus (*Pigeon Herpesvirus 1*, PHV) has been isolated from diseased pigeons in the United Kingdom (Cornwell, Weir and Follett, 1967), Czechoslovakia (Krupička, Šmid, Valiček and Pleva, 1970), Australia (Boyle and Binnington, 1973), Belgium (Vindevogel, Pastoret, Burtonboy, Gouffaux and Duchatel, 1975), Hungary (Vetési and Tanyi, 1975) and Iraq (Mohammed, Sokkar and Tantawi, 1978).

This agent is important in the etiology of the respiratory tract illness of the pigeon (Vindevogel and Duchatel, 1978). Clinical experience shows that debilitating factors may precipitate the disease in apparently normal pigeons (Vindevogel and Duchatel, 1979).

This paper describes viral excretion after experimental infection of normal and immunodepressed pigeons, and investigations to determine if the virus can be re-excreted by the birds, spontaneously or experimentally.

MATERIALS AND METHODS

Pigeons. The squabs (baby pigeons) used were divided into two groups (A and B) of 6 and 5, respectively. They were 5-week-old from parents free of the infection and were maintained in isolation.

Experimental schedule. Squabs belonging to group A were treated with cyclophosphamide (Cy) for 4 days as previously described (Coignoul and Vindevogel, 1980). Seven days after the first injection of Cy, squabs of both groups were infected by pharyngeal painting with 10^5 plaque-forming units (pfu) of the PHV_1 strain (Vindevogel *et al.*, 1975).

After experimental infection, the pharyngeal mucous membrane of each squab was swabbed with sterile cotton-tips daily for the first 8 days, on days 10, 12, 13 and 15, and after that, every 5th day up to day 90. Blood was taken from all the

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birds, at 5-day intervals, for 1 month and then at 15-day intervals up to day 90. On the 90th day, and for 4 consecutive days, the squabs in both groups were treated with the same doses of Cy as before. The birds were swabbed daily from the beginning of this treatment until day 101, on days 103 and 105 and on every 5th day up to day 120. Blood was taken every 7th day from day 90 up to day 120.

Virus titration. Swabs were suspended in minimum essential medium with added 5 per cent dimethylsulphoxide and antibiotics (penicillin 10 000 U and streptomycin 10 mg per ml), and frozen at −70 °C.

The suspended infectious particles were titrated on chicken embryo fibroblasts (CEF) by the agar overlay technique (Vindevogel, Duchatel and Gouffaux, 1977).

The suspended viral particles from swabs taken on days 4, 91 and 95 after infection were examined, after negative staining, under the electron microscope (Phillips EM 300).

Characterization of the excreted virus. Virus excreted on days 4 and 95 was identified as PHV by indirect immunofluorescence staining as previously described (Vindevogel *et al.*, 1977; Vindevogel and Duchatel, 1978).

Sero-neutralization. Sera of each group sampled at the same day were pooled and inactivated by heating for 30 min at 56 °C. Specific antibodies were titrated according to the technique of 50 per cent plaque-reduction (Hoskins, 1967; Vindevogel and Duchatel, 1978).

RESULTS

The results are summarized in Figs 1, 2 and 3.

Viral Excretion after Experimental Infection and Spontaneous Re-excretion

Titration of virus in pharyngeal swabs Daily titration of infectious particles showed a similar viral excretion in both groups All the squabs excreted infectious particles 24 h after infection, with a maximum of excretion between the 2nd and the 4th day. Excretion persisted for as little as 5 days in one bird. The highest titre observed in the pharyngeal swabs was $1{\cdot}5 \times 10^4$ pfu.

Electronmicroscopic examination of the viral suspensions failed to demonstrate virus.

One squab of each group died from the disease but the clinical signs and lesions observed were far more intense in group A than in group B.

After the first period of continuous excretion following the primary infection, spontaneous re-excretion without clinical signs was shown by several pigeons of both groups, although some in each group showed no re-excretion.

Neutralizing antibodies. Neutralizing antibodies appeared in the squabs of group B from the end of the first week following infection. The highest titre was observed on day 20 (145) and then decreased slowly until day 90 (64). The serological response of the squabs belonging to group A showed a similar pattern but was weaker. The highest titre was 26 on day 20; the titres then slowly decreased and became non-specific after 2 months (< 8).

Experimental Viral Re-excretion

Titration of virus in pharyngeal swabs. All the pigeons re-excreted virus after Cy-treatment, which was carried out on day 90 after infection.

Animals belonging to group A re-excreted infectious particles 2 to 5 days after the first injection of Cy. This time varied from 1 to 10 days in group B.

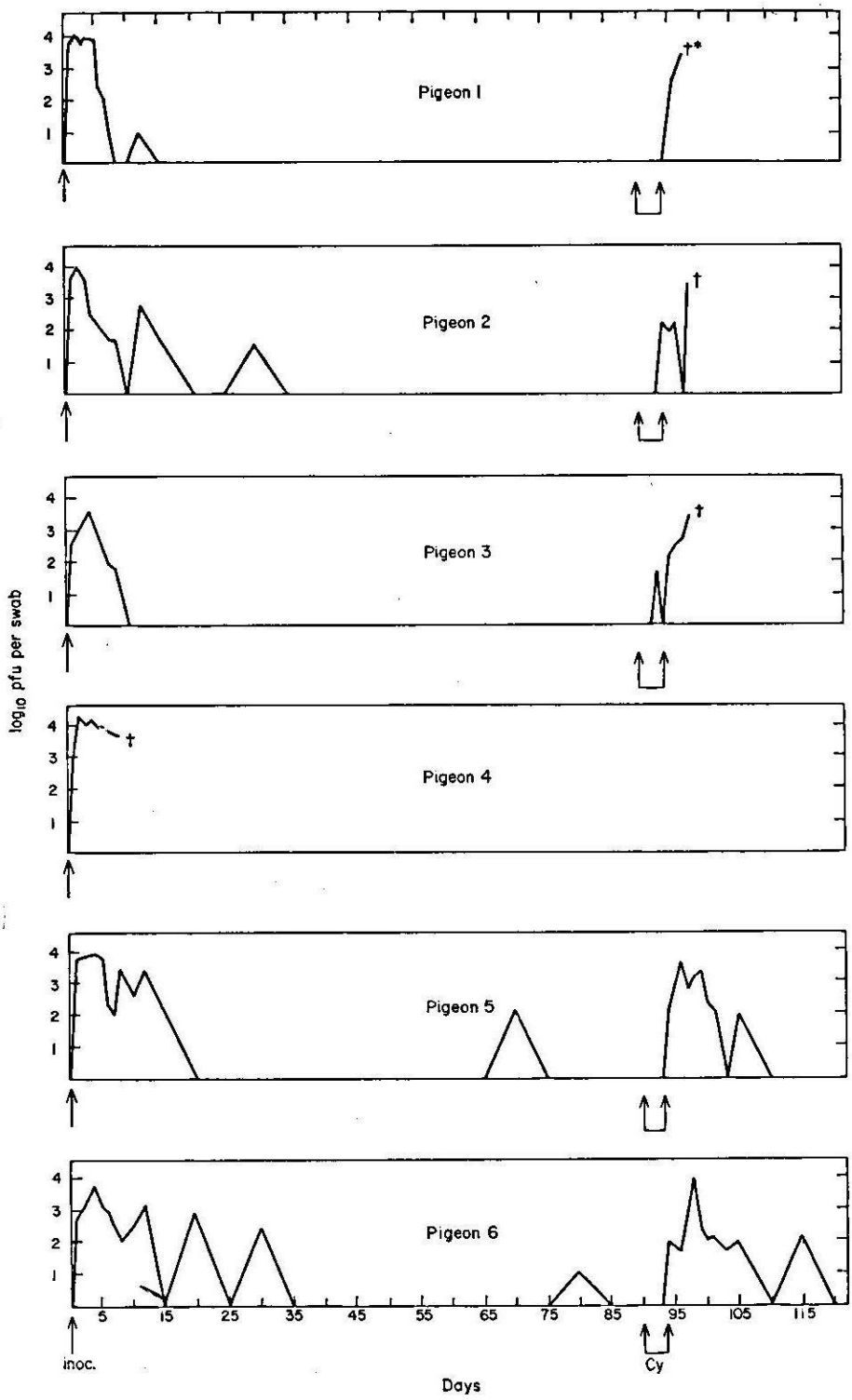

Fig. 1. The excretion of PHV in the pigeons of group A. * death.

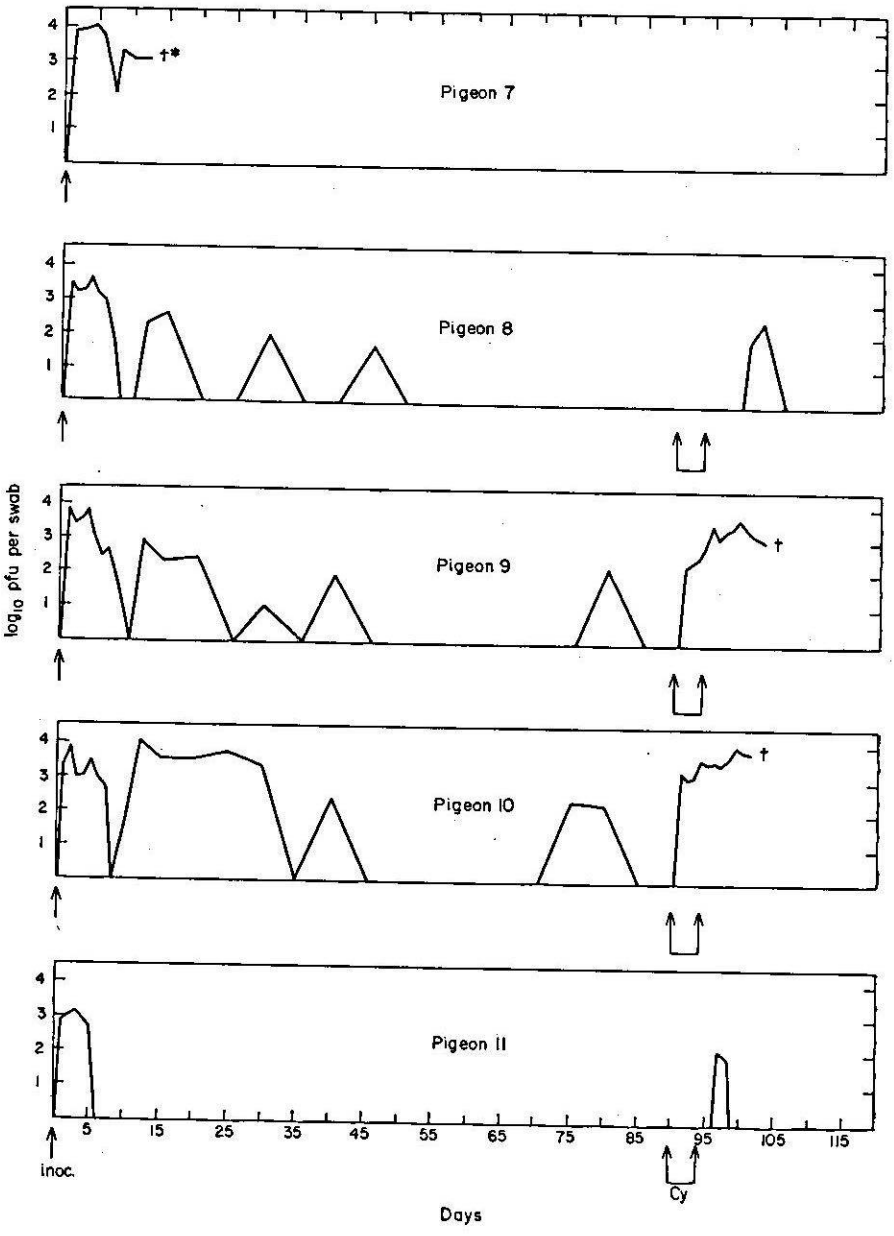

Fig. 2. The excretion of PHV in the pigeons of group B. * death.

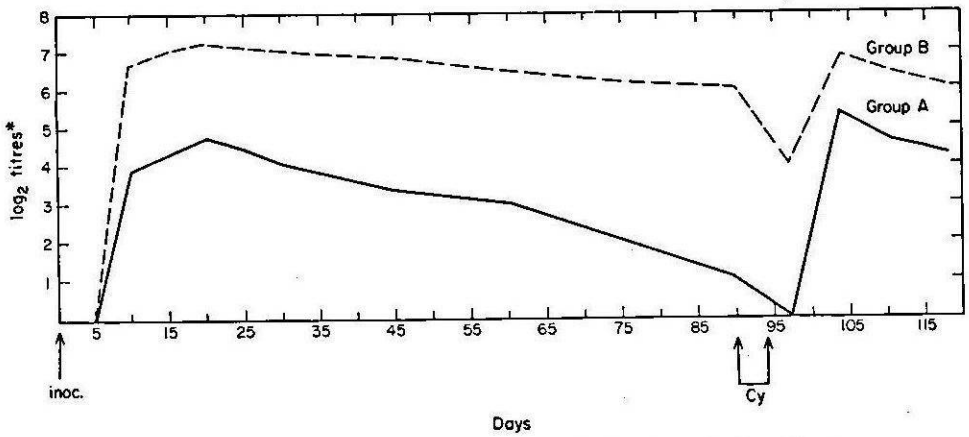

Fig. 3. The pattern of antibodies in groups A and B. * reciprocal of the dilutions.

Electron microscopic examination of the suspended swabs failed to reveal virus particles.

Three pigeons belonging to group A and two belonging to group B died within the period of re-excretion with signs and lesions of the natural disease. The two other pigeons of group A were asymptomatic despite a significant excretion of infectious particles; in contrast, the two surviving birds in group B developed pharyngeal lesions associated with a less degree of viral excretion.

Neutralizing antibodies. Ninety days after infection, the Cy-treatment led to a decrease in the titre of specific antibodies 3 days after the last injection; this was followed by an increase 7 days later (group A: 40; group B: 112); then the titres slowly decreased.

DISCUSSION

All the squabs excreted infectious particles of PHV 24 h after experimental infection, and the excretion often persisted, at a high titre, for 7 to 10 days. A similar pattern has been described for the excretion of infectious bovine rhinotracheitis virus (*Bovid Herpesvirus 1*, IBRV) by cattle (Pastoret, Burtonboy, Aguilar-Sétien and Schoenaers, 1978b). Hitchner, Fabricant and Bagust (1977) have also shown that avian infectious laryngotracheitis virus (*Phasianid Herpesvirus 1*, ILTV) multiplies at the sites of initial exposure where viral antigen can be detected from the 2nd to the 7th day.

Pastoret *et al.* (1978b) described a ratio of 1 infectious particle for 100 to 1000 physical ones when IBRV is excreted by cattle. In pigeon herpes infection, the titre of excretion of physical particles must be less than 10^6 per swab since the electronmicroscopic examinations of swab suspensions were negative, 10^6 particles being the threshold of sensitivity for this technique (Pastoret *et al.*, 1978b).

The typical lesions appeared 3 to 4 days after infection of the pigeons, when the viral excretion reached its higher titre. The Cy-treated squabs did not excrete virus at a higher titre but showed a more severe clinical disease with

lesions of a more acute nature. Hough and Robinson (1975) obtained similar results with mice infected with herpes simplex virus (*Human Herpesvirus 1*, HSV) and submitted to Cy-treatment.

The development of neutralizing antibodies during pigeon herpes infection is similar to that described for IBR (Pastoret *et al.*, 1978b), and their appearance is inhibited by previous treatment with Cy, as has been shown with ILT (Robertson, 1977).

Viral persistence and intermittent recovery in the host after primary infection with some herpesviruses such as IBRV and ILTV has been demonstrated (Komarov and Beaudette, 1932; Gibbs, 1933; McKercher, Wada and Straub, 1963; Snowdon, 1965). PHV seems to follow the same pattern. Indeed, mild episodes of recurrence, without clinical signs, occurred spontaneously in both groups. High titres of specific antibody did not prevent these recurrences, and, conversely, recurrent episodes were not more frequent when the animals were nearly devoid of specific antibody. Moreover, despite the episodes of re-excretion, specific antibody decreased gradually. Pastoret, Aguilar-Sétien, Burtonboy, Mager, Jetteur and Schoenaers (1979) have also observed that the titre of specific neutralizing antibodies against IBRV in cattle remain unchanged when a mild re-excretion occurs, and Griffiths, Stagno, Reynolds and Alford (1978) did not observe any fluctuation in the humoral immunity of some women who re-excreted human cytomegalovirus.

The Cy-treatment carried out 90 days after the experimental infection was followed, in all the birds, by a period of viral excretion nearly as great as that following the initial infection. This period of re-excretion was accompanied by lesions in 7 birds and 5 died. Specific neutralizing antibodies first decreased and then reached a high titre in both groups. This booster effect is probably due to the intense re-excretion of the virus.

The reactivation of several herpesviruses is often attributed to the immuno-suppressive effect of drugs or to the transient failure of the immune defence mechanisms of the host (Wilton, Ivanyi and Lehner, 1972; Olding, Jensen and Oldstone, 1975). Several theories on the role of immunological mechanisms in the prevention of recurrent episodes have been postulated, some emphasizing the humoral and others the cell-mediated system (Davies and Carmichael, 1973; Stevens and Cook, 1974; Shillitoe, Wilton and Lehner, 1977; Pastoret, Aguilar-Sétien, Burtonboy and Schoenaers, 1978a; Pastoret *et al.*, 1979).

Further work is needed to understand the mechanism by which the excretion of PHV is reactivated by Cy. Cy-treatment produces a transient but profound deficiency of both B and T-cells in pigeons (Coignoul and Vindevogel, 1980). Payne, Rennie, Powell and Rowell (1978) have suggested that Cy interferes with immunity after vaccination against Marek's disease by altering thymus and cell-mediated immunity, and not by an effect on the bursa and humoral immunity.

SUMMARY

The excretion of pigeon herpesvirus and the development of neutralizing antibodies were studied after experimental infection of two groups of pigeons, one of which had previously been treated with cyclophosphamide (Cy).

Natural excretion of the virus occurred in both groups during a 90-day period without influencing the titre of antibodies, the pigeons becoming asymptomatic carriers of the virus after the initial infection.

Birds previously treated with Cy developed lower titres of neutralizing antibodies. The Cy-treatment of all the animals 90 days after the experimental infection provoked re-excretion of the virus.

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