PATHOGENESIS OF PIGEON HERPESVIRUS INFECTION

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INTRODUCTION

Classical signs of pigeon herpesvirus (*Pigeon herpesvirus 1*, PHV) infection are conjunctivitis, rhinitis and focal necrosis in mouth, pharynx and larynx (Cornwell and Wright, 1970; Vindevogel, Pastoret, Burtonboy, Gouffaux and Duchatel, 1975; Vetési and Tanyi, 1975; Vindevogel and Duchatel, 1978, 1979; Callinan, Kefford, Borland and Garrett, 1979). Moreover, during natural outbreaks, lesions may be observed in the trachea, liver, spleen, kidney and pancreas (Cornwell and Wright, 1970; Boyle and Binnington, 1973; Vetési and Tanyi, 1975; Vindevogel and Duchatel, 1979; Callinan *et al.*, 1979). In the natural disease, primary infection of the upper digestive and respiratory tracts can thus be followed by viral dissemination throughout the body.

Numerous workers have reported that the disease was difficult to reproduce in pigeons (Surman, Purcell, Tham, Wilson and Schultz, 1975; Callinan *et al.*, 1979). If the virus is injected intraperitoneally, the main lesions to be found are pancreatitis, peritonitis and, in some cases, hepatic necrosis (Cornwell, Wright and McCusker, 1970), while pigeons infected by the pharyngeal or laryngeal route develop local foci of necrosis and ulcers as in the natural disease (Cornwell *et al.*, 1970; Vindevogel *et al.*, 1975; Vindevogel, Pastoret and Burtonboy, 1980). After recovery, pigeons become asymptomatic carriers and episodes of recurrence may occur spontaneously or after cyclophosphamide (Cy)-treatment (Vindevogel *et al.*, 1980; Vindevogel and Pastoret, 1980).

The aims of the present investigations were to determine whether viraemia and infection of several organs occur during primary infections or during episodes of recurrence, and whether virus can multiply in pigeon embryos. In an attempt to ascertain the mode of viral spread in tissues we also wanted to determine if PHV can be freely transmitted from cell to cell in the presence of high titres of antibodies.

MATERIALS AND METHODS

Pigeons. Twenty-three squabs (baby pigeons) were divided into 4 groups (A, B, C and D) of 10, 5, 4 and 4 birds, respectively, and maintained in isolation. They were 5- to 6-week-old and were from parents free of the infection.

Virus inoculation. All 23 squabs were infected by painting the pharynx with 10^5 plaque-forming units (pfu) of the PHV/B/Cu$_1$ strain (Vindevogel *et al.*, 1975) (The day of inoculation was considered to be day 0).

Groups A and B. The pharyngeal mucous membrane of each squab of groups A and B was swabbed with sterile cotton-tips on days 1 to 6 inclusive. On day 6, pigeons of both groups were killed. Representative samples of pharynx, larynx, trachea, liver, spleen, kidney, pancreas, ovary or testicle and brain of pigeons of group A were harvested for histological examination; the same organs from pigeons of group B and livers from pigeons of group A were assayed for viral infectivity.

Histology of groups A and B. The organs, fixed in Bouin, were processed to paraffin, sections were cut 6 μm thick and stained with haematoxylin and eosin (HE).

Assays for viral infectivity for groups A and B. Pharyngeal swabs were suspended in 1 ml of minimum essential medium with 5 per cent dimethylsulfoxide (DMSO) and penicillin (10 000I U) and streptomycin (10 mg per ml) (MEM).

A 10 per cent suspension of triturated tissues was homogenized in the same medium, and then centrifuged (1000 g for 10 min).

Infectious particles were titrated in the supernatants and in swabs as previously described (Vindevogel *et al.*, 1980).

Groups C and D. Squabs of group C were treated with Cy for 4 days as previously described (Coignoul and Vindevogel, 1980). Seven days after the first injection of Cy, squabs of groups C and D were infected (day 0).

On days 40 to 43, and 80 to 83, squabs in both groups were treated with Cy as before. On days 39, 79 and 90, antibodies were titrated in pooled sera of each group as previously described (Hoskins, 1967; Vindevogel *et al.*, 1980).

On days 0 to 10, 39 to 50 and 79 to 90, the pharyngeal mucous membrane of each squab was swabbed daily and 2 ml of blood was taken in heparinized tubes (Lithium-heparin 10 ml monovets, Walter Sarstedt laboratory, West Germany) from all the birds.

Infectious particles were titrated in pharyngeal swabs. Samples of blood were diluted 1/2 in MEM, frozen and thawed once to disrupt cells, then centrifuged (1000 g for 10 min); supernatants, diluted 1/10, were assayed for virus isolation on chicken embryo fibroblasts (CEF), and infectious particles were then titrated in all positive samples. Adsorption was made at room temperature to avoid coagulation of the inocula.

As a harmful effect of heparin has been described for other herpesviruses (Choi, Swack and Hsiung, 1978), samples of PHV/B/Cu$_1$ were treated in the same way as blood in heparinized tubes and viral titres compared with that of untreated virus. As previously described for groups A and B, organs were harvested for virus isolation and titration from all dead squabs and from all surviving pigeons killed on day 90.

On days 0 to 10, 39 to 50 and 79 to 90, clinical signs were scored once daily. The score record ranged from 0 to +++ as follows: 0, no signs; +, congestion of buccal and pharyngeal mucosa; ++, swelling of soft palate and orifice of larynx with a few foci of necrosis in mouth and pharynx; +++, mucosal necrosis and ulcerated lesions.

Virus identification. In all groups, virus isolated on CEF from pharyngeal swabs on days 4, 44 or 49, 84 or 89, from samples of blood, and from organs, was identified as PHV by indirect immunofluorescence staining as previously described (Vindevogel, Duchatel and Gouffaux, 1977; Vindevogel and Duchatel, 1978).

Pathogenicity of PHV for pigeon embryos. PHV/B/Cu$_1$ strain was inoculated into the allantoic cavity of 15 9- to 11-day-old pigeon embryos obtained from parents free of the infection; 8 were infected with 50 pfu and 7 with 500 pfu. Liver from all embryos either dead or killed at the 17th incubation day was triturated in MEM medium (1/2) and after storage at −70 °C was inoculated on CEF for virus isolation and titration.

Plaque-formation under antiserum. A rabbit was immunized by 3 series of 40 intra-dermal injections at 4-week intervals of PHV pellets supplemented with Freund's complete adjuvant. Pellets were prepared by the method of Aguilar-Setién, Pastoret, Burtonboy and Schoenaers (1978) from the PHV/B/Cu$_1$ strain. The neutralizing titre of the rabbit serum was determined by the 50 per cent plaque-reduction test under 1 per cent agarose (Hoskins, 1967); 50 pfu of PHV were allowed to react

with different dilutions of serum, then inoculated on CEF grown in Petri dishes, and after 1 h of adsorption at 37 °C, cultures were incubated for 4 days.

Control cultures were inoculated with 50 pfu and after 1, 2, 3 and 4 h of adsorption at 37 °C were also incubated for 4 days, either under the rabbit anti-PHV serum diluted 1/10 in maintenance medium or under medium with added 1 per cent agarose. Numbers of pfu were compared.

RESULTS

Groups A and B

Results are summarized in Tables 1 and 2. Following infection, squabs excreted PHV as previously described (Vindevogel et al., 1980), except for pigeons 11 and 13 (group B) which presented a very short and low rate of viral excretion.

Pigeons of group A developed lesions similar to those encountered during natural infections. Multiple foci of necrosis were detectable in the pharyngeal stratified squamous epithelium of all pigeons and in the salivary glands. The foci were numerous in the tunica propria and the submucosa. Foci contained cells at different stages of degeneration and necrosis, and intra-nuclear inclusions were present in adjacent epithelial cells. Large foci extended through the epidermis and ulcers were formed. Similar foci of necrosis were observed in the laryngeal epithelium.

Five pigeons showed a moderate tracheitis; in the upper part of the trachea some necrotic foci of epithelial cells were associated with loss of cilia, destruction of mucous glands, and lymphoid cells infiltrated the lamina propria. Inclusion bodies were also seen in epithelial cells around the foci of necrosis. No lesions were observed in lungs, spleen, kidneys, pancreas, brain and genital organs. Pigeon Number 4 was the only one with hepatitis: intra-nuclear inclusions bodies were found in many hepatic cells widely spread throughout the organ. Most infected cells showed chromatin margination and inclusions surrounded by a clear "halo". Virus was isolated from the liver (1×10^4 pfu per ml).

PHV was isolated from the tracheas of 3 pigeons of group B (Nos. 12, 14 and 15) and from the liver and spleen of one pigeon (No. 14). All other attempts to isolate virus from internal organs were unsuccessful.

Groups C and D

Results are summarized in Figs 1, 2. Typical excretion of virus (Vindevogel et al., 1980) followed primary infection in both groups. As previously described (Vindevogel et al., 1980), signs and lesions were far more intense in Cy-treated squabs (group C) than in untreated ones, excepted for No. 23 (group D). One pigeon of group C died (No 17), but no infectious particles were isolated from its internal organs. Virus was isolated from blood of 2 pigeons in group C on day 6; at a low titre from Number 18, and on days 4, 5, 6 at a higher titre from Number 19. On day 39, neutralizing antibody titres in pooled sera were 64 and 160, respectively, for groups C and D.

TABLE 1

EXCRETION OF PHV BY PIGEONS OF GROUP A AND HISTOLOGICAL LESIONS IN MAIN ORGANS

| Pigeons | pfu per pharyngeal swab on day | | | | | | Histological lesions on day 6 | | | |
	1	2	3	4	5	6	Pharynx	Trachea	Liver	Other organs
1	$6{\cdot}8 \times 10^3$	$1{\cdot}1 \times 10^4$	$5{\cdot}4 \times 10^3$	$9{\cdot}3 \times 10^3$	$9{\cdot}0 \times 10^3$	$2{\cdot}5 \times 10^2$	+	+	—	—
2	$5{\cdot}2 \times 10^3$	$9{\cdot}0 \times 10^3$	$4{\cdot}0 \times 10^3$	$2{\cdot}8 \times 10^2$	$1{\cdot}5 \times 10^2$	$9{\cdot}0 \times 10^1$	+	—	—	—
3	$4{\cdot}0 \times 10^2$	$8{\cdot}0 \times 10^2$	$2{\cdot}0 \times 10^3$	$4{\cdot}0 \times 10^3$	$1{\cdot}0 \times 10^3$	$2{\cdot}8 \times 10^2$	+	+	—	—
4	$1{\cdot}0 \times 10^3$	$2{\cdot}0 \times 10^4$	$1{\cdot}1 \times 10^4$	$1{\cdot}5 \times 10^4$	$9{\cdot}5 \times 10^3$	$6{\cdot}0 \times 10^3$	+	+	+	—
5	$6{\cdot}6 \times 10^3$	$6{\cdot}8 \times 10^3$	$8{\cdot}0 \times 10^3$	$9{\cdot}0 \times 10^3$	$6{\cdot}0 \times 10^3$	$2{\cdot}0 \times 10^2$	+	—	—	—
6	$5{\cdot}0 \times 10^2$	$9{\cdot}0 \times 10^2$	$2{\cdot}2 \times 10^3$	$6{\cdot}0 \times 10^3$	$1{\cdot}2 \times 10^3$	$9{\cdot}0 \times 10^2$	+	—	—	—
7	$7{\cdot}4 \times 10^3$	$9{\cdot}0 \times 10^3$	$9{\cdot}5 \times 10^3$	$1{\cdot}0 \times 10^4$	$6{\cdot}0 \times 10^3$	$8{\cdot}5 \times 10^2$	+	+	—	—
8	$3{\cdot}0 \times 10^3$	$1{\cdot}6 \times 10^3$	$2{\cdot}0 \times 10^3$	$3{\cdot}8 \times 10^3$	$1{\cdot}3 \times 10^3$	$1{\cdot}2 \times 10^2$	+	—	—	—
9	$5{\cdot}0 \times 10^3$	$3{\cdot}0 \times 10^3$	$4{\cdot}5 \times 10^3$	$6{\cdot}0 \times 10^3$	$9{\cdot}5 \times 10^2$	$3{\cdot}0 \times 10^2$	+	+	—	—
10	$2{\cdot}0 \times 10^3$	$6{\cdot}8 \times 10^3$	$9{\cdot}0 \times 10^2$	$1{\cdot}0 \times 10^3$	$2{\cdot}5 \times 10^3$	$7{\cdot}0 \times 10^2$	+	—	—	—

TABLE 2

EXCRETION OF PHV BY PIGEONS OF GROUP B AND VIRAL INFECTIVITY OF MAIN ORGANS

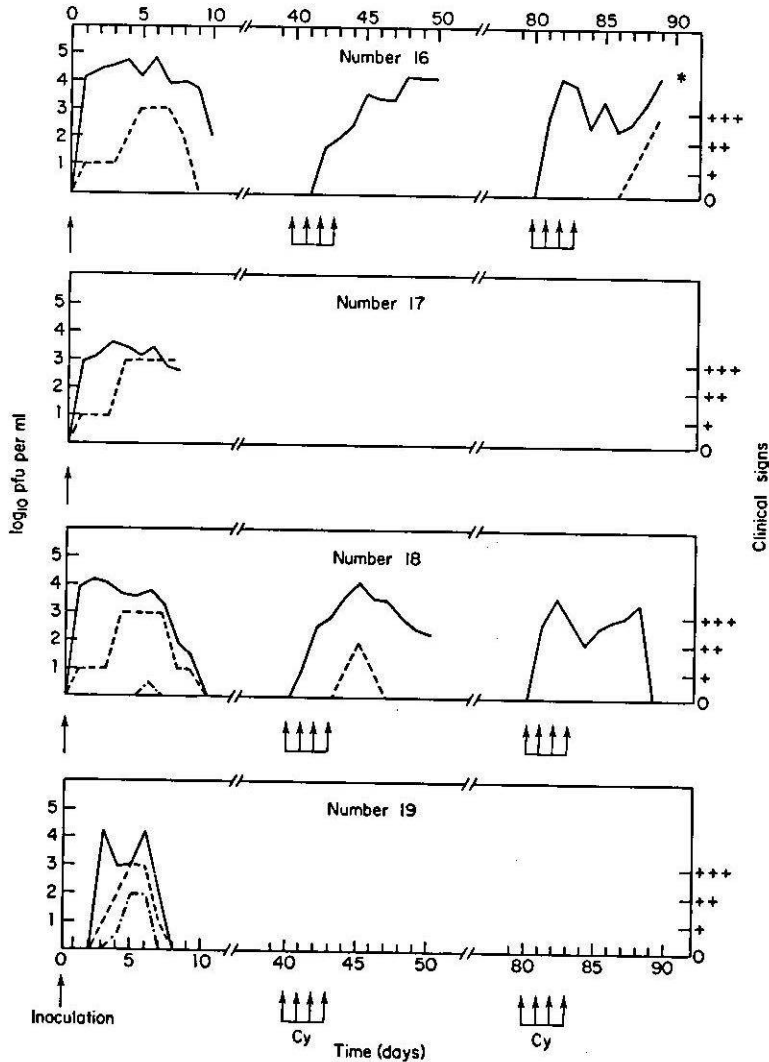| Pigeons | pfu per pharyngeal swab on day | | | | | | pfu per ml of tissue suspensions on day 6 | | | |
	1	2	3	4	5	6	Trachea	Liver	Spleen	Other organs
11	4.0×10^2	1.0×10^2	0	0	0	0	0	0	0	0
12	2.2×10^3	1.9×10^3	2.1×10^3	2.1×10^3	1.3×10^3	1.5×10^3	0.5×10^1	0	0	0
13	1.0×10^2	0	0	0	0	0	0	0	0	0
14	0	3.4×10^3	2.0×10^3	1.9×10^4	1.2×10^3	2.0×10^3	5.0×10^2	1.5×10^4	0.3×10^1	0
15	5.7×10^3	2.3×10^4	2.3×10^4	9.3×10^3	2.3×10^3	1.1×10^3	1.0×10^2	0	0	0

Fig. 1. Excretion of PHV (——), viraemia (— · —) and clinical signs (— —) in group C. *death.

After Cy-treatment on days 40 to 43, 2 pigeons of group C (Numbers 16 and 18) re-excreted infectious particles 1 day after the first Cy-injection, while a third one (Number 19) did not. Only one pigeon (Number 18) presented lesions typical of the disease. All pigeons of group D re-excreted the virus, but latency time varied from 1 to 9 days. Three squabs of that group developed intense pharyngeal lesions. Number 20 died on day 47, but all its internal organs remained free from viral localization. Number 22 died on day 55 after showing leg paralysis and tremor of the neck and head; virus was isolated from its blood on days 49 and 50, and from its brain after death (5×10^2 pfu per ml), but all other organs remained free of virus. On day 79, titres of pooled sera were 178 for group C and 200 for group D.
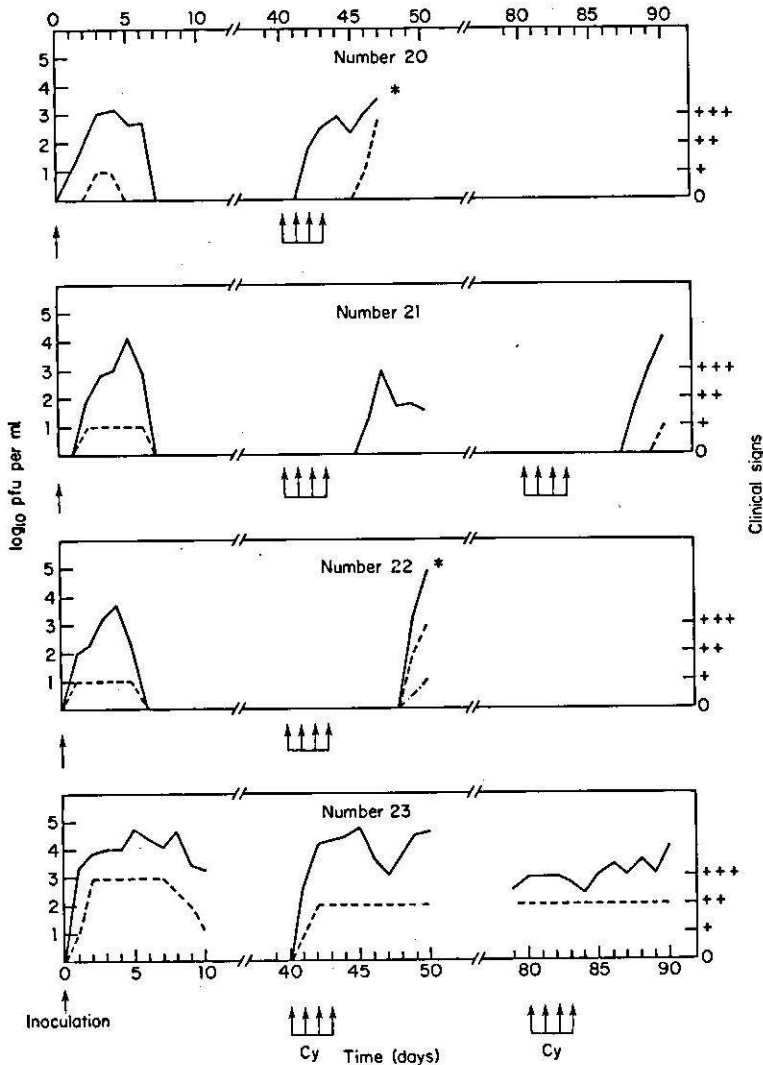
Fig. 2. Excretion of PHV (———), viraemia (— · —) and clinical signs (— —) in group D. *death.

The second Cy-treatment carried out on days 80 to 83, was followed in 3 out of 5 surviving pigeons (Numbers 16, 18 and 21) by a new virus re-excretion as marked as the previous ones, with lesions in 2 pigeons (16 and 21). Again, Number 19 did not re-excrete virus. Number 23 was still showing clinical signs and shedding virus before the beginning of Cy-treatment. No viraemia was observed. One pigeon (Number 16) died on day 89. Virus was isolated from the trachea of Numbers 16, 21 and 23. All other organs from all pigeons were devoid of virus. On day 90, titres of antibodies were 98 for group C and 110 for group D.

The titre of stock virus treated in heparinized tubes was 1.0×10^5 pfu per ml, while titre of untreated virus was 1.4×10^5 pfu per ml.

Pathogenicity of Virus for Pigeon Embryos

Data are given in Table 3. All embryos survived infection by 50 pfu, and 3 out of 7 died after infection by 500 pfu. Virus was isolated from the liver of 3 embryos infected with 50 pfu and from 6 infected with 500 pfu.

TABLE 3

VIRUS ISOLATIONS FROM DEAD AND LIVE EMBRYOS INOCULATED WITH PHV

Virus dose : pfu per embryo	Number of dead embryos	Virus isolations	
		Number positive	pfu per ml of liver
50	0/8	3/8	$2 \cdot 1 \times 10^2$
			$1 \cdot 2 \times 10^2$
			$4 \cdot 3 \times 10^2$
500	3/7	6/7	$1 \cdot 0 \times 10^5$ (a)
			$4 \cdot 5 \times 10^3$ (a)
			$9 \cdot 0 \times 10^4$ (a)
			$1 \cdot 3 \times 10^3$ (b)
			$1 \cdot 7 \times 10^3$ (b)
			$2 \cdot 4 \times 10^3$ (b)

(a) dead embryos
(b) live embryos

Plaque Formation under Antiserum

Results are given in Table 4. The rabbit antiserum neutralizing titre was 128. Plaque formation was observed in cells under the corresponding antiserum. After standard adsorption of the virus for 1 h at 37 °C, the plaque number was smaller than in cultures maintained under agarose, but increased with the time of adsorption and reached the same number as under agarose after 4 h of adsorption. Plaques produced under specific antibodies were almost perfectly circular, with a clear circumference.

TABLE 4

PLAQUE FORMATION UNDER ANTISERUM

Plaque reduction test under 1 per cent agarose		Hours of adsorption	Mean plaque number in control dilutions	
Rabbit antiserum dilutions	Mean plaque number		under 1 per cent agarose	Under 10 per cent rabbit antiserum
8	0	1	49	14
16	0	2	52	28
32	7	3	50	36
64	18	4	49	48
128	25			
256	36			
512	43			

DISCUSSION

The Cy-treatments carried out 40 and 80 days after experimental infection were followed, in all birds except Number 19, by a period of virus re-excretion nearly as high as that following the initial infection. Several birds not treated with Cy before infection may re-excrete virus later than treated ones as was

previously described by Vindevogel *et al.*, 1980. It may be due to the fact that they present a better immune response as shown by their sero-conversion. Indeed, in Cy-treated pigeons, the B-cell population virtually disappears in the bursa of Fabricius (Coignoul and Vindevogel, 1980), and immunoglobulin synthesis is altered (Vindevogel *et al.*, 1980). The mechanism whereby Cy-treatment provokes re-excretion of PHV is still unknown, but it may be a cytotoxic effect in cells latently infected as has been suggested by Openshaw (1980) working with mice infected with *Human herpesvirus 1*.

After experimental infection, virus usually remains confined near the site of inoculation. Tracheal contamination occurs most probably by tissue contiguity as a result of laryngeal infection. This route of contamination cannot however explain the viral localization in such organs as liver and spleen as was observed in Numbers 4 and 14 (groups A and B); such localizations are, moreover, far less frequent. In those cases, virus seems to be disseminated by blood. Indeed, a transient viraemia was observed especially in squabs treated with Cy. Viraemia may thus occur, but mainly in pigeons weakened, as in natural outbreaks, by debilitating factors such as parasitic disorders or secondary bacterial invaders (Vindevogel and Duchatel, 1978; Vindevogel and Duchatel, 1979). Cy-treatment simply mimics the natural conditions. No viraemia was observed during the second episode of re-excretion, notwithstanding the fact that heparin does not exert a significant influence on the titration of PHV, and when the remaining pigeons were killed on day 90 all the internal organs remained free of virus.

One pigeon died with encephalitic symptoms after the first episode of recurrence; virus was isolated from its brain, and viraemia was observed before the occurrence of nervous signs. Natural outbreaks of encephalomyelitis have also been associated with herpesvirus infection in pigeons (Mohammed, Sokkar and Tantawi, 1978; Al Falluji, Al Sheikhly and Tantawi, 1979; Tantawi, Al Falluji and Sheikhly, 1979). Encephalitis can thus occur after pigeon herpesvirus infection, but infection of the brain may be due to viral dissemination by viraemia rather than viral contamination by the nervous pathway as with infectious bovine rhinotracheitis virus (*Bovid herpesvirus 1*, IBRV) (Hall, Simmons, French, Snowdon and Asdell, 1966; Narita, Inui, Namba and Shimizu, 1976, 1978a, b).

No genital form of the disease could be detected since genital organs of all squabs, either dead or killed, remained free of virus. This agrees with the failure to demonstrate the presence of the virus or its antigen in cell cultures derived from embryos from infected parents (Vindevogel and Pastoret, 1980). Thus egg transmission of the virus seems unlikely, but cannot be definitely excluded during the acute phase of the disease since viraemia may exist and sublethal infection of embryos may occur if the viral dose is low.

It has also been shown that, after cell infection, PHV can be transmitted in vitro from cell to cell in the presence of high titres of specific antibodies as described for infectious laryngotracheitis virus of fowls (*Phasianid herpesvirus 1*, ILTV) (Atherton and Anderson, 1957). This is also probably true for the in vivo situation. It may therefore be expected that, after primary infection of epithelia and appearance of the immune response, PHV can spread from cell

to cell without being directly affected by circulating antibodies. Indeed, it has already been observed that high titres of specific antibodies do not prevent mild and delayed episodes of recurrence and, conversely, that recurrent episodes are not more frequent when the animals are devoid of specific antibodies (Vindevogel *et al.*, 1980). This suggests that humoral immunity does not play a major role in the control of PHV infection.

Cy-treatment failed to provoke viral re-excretion by Pigeon 19. It is possible that detection of viral excretion was unsuccessful, even if reactivation occurred, because of the high immune status of the animal provoked by intense viral multiplication and marked viraemia as shown for IBRV infection in cattle (Pastoret, Aguilar-Setién, Burtonboy, Mager, Jetteur and Schoenaers, 1979; Pastoret, Babiuk, Misra and Griebel, 1980).

SUMMARY

Experiments were designed to investigate the pathogenesis of pigeon herpesvirus (*Pigeon herpesvirus 1*, PHV) infection.

Experimental pharyngeal infection of susceptible pigeons may be followed by viral localization and development of lesions outside the upper digestive and respiratory tracts. Viraemia was detected during the primary infection in some pigeons previously treated with cyclophosphamide (Cy).

After primary infection, viral re-excretion was twice provoked by Cy-treatment in all pigeons except one, and was as heavy as that following the initial infection.

During the first episode of re-excretion one pigeon presented viraemia, died a few days later with signs of encephalitis, and PHV was isolated from the brain.

No viraemia could be detected during the second episode of re-excretion and virus was only isolated from the pharynx and trachea, probably by contamination via the larynx.

Virus was never isolated from genital organs taken from either dead or killed animals, but sublethal infection of pigeon embryos could be produced experimentally.

It was also shown that, after cell infection, PHV can be transmitted in vitro from cell to cell in the presence of high titres of specific antibodies.

PHV can thus be spread either by tissue contiguity, even in the presence of specific antibodies, or by viraemia especially when the pigeons are immunosuppressed.

REFERENCES

Aguilar-Setién, A., Pastoret, P. P., Burtonboy, G., and Schoenaers, F. (1978). Test d'hypersensibilité retardée au virus de la rhinotrachéite infectieuse bovine (*Bovid herpesvirus* 1) avec du virus purifié. *Annales de Médecine Vétérinaire*, **122,** 193–199.

Al Falluji, N. M., Al Sheikhly, F., and Tantawi, H. H. (1979). Viral encephalomyelitis of pigeons: pathology and virus isolation. *Avian Diseases*, **23**, 777–784.

Atherton, J. G., and Anderson, W. (1957). Propagation in tissue culture of the virus of infectious laryngotracheitis of fowls. *Australian Journal of Experimental Biology*, **35**, 335–346.

Boyle, D. B., and Binnington, J. A. (1973). Isolation of a herpesvirus from a pigeon. *Australian Veterinary Journal*, **49**, 54.

Callinan, R. B., Kefford, B., Borland, R., and Garrett, R. (1979). An outbreak of disease in pigeons associated with a herpesvirus. *Australian Veterinary Journal*, **55**, 339–341.

Choi, Y. C., Swack, N. S., and Hsiung, G. D. (1978). Effect of heparin on cytomegalovirus replication (40098). *Proceedings of the Society for Experimental Biology and Medicine*, **157**, 569–571.

Coignoul, F., and Vindevogel, H. (1980). Cellular changes in the bursa of Fabricius and thymus of cyclophosphamide-treated pigeons. *Journal of Comparative Pathology*, **90**, 395–400.

Cornwell, H. J. C., and Wright, N. G. (1970). Herpesvirus infection of pigeons. I. Pathology and virus isolation. *Journal of Comparative Pathology*, **80**, 221–227.

Cornwell, H. J. C., Wright, N. G., and McCusker, H. B. (1970). Herpesvirus infection of pigeons. II. Experimental infection of pigeons and chicks. *Journal of Comparative Pathology*, **80**, 229–232.

Hall, W. T. K., Simmons, G. C., French, E. L., Snowdon, W. A., and Asdell, M. (1966). The pathogenesis of encephalitis caused by the infectious bovine rhinotracheitis virus. *Australian Veterinary Journal*, **42**, 229–237.

Hoskins, J. M. (1967) *Virological Procedures*, pp 258–260 Butterworths, London.

Hough, V., and Robinson, T. W. E. (1975). Exacerbation and reactivation of herpesvirus hominis infection in mice by cyclophosphamide. *Archives of Virology*, **48**, 75–83.

Mohammed, M. A., Sokkar, S. M., and Tantawi, H. H. (1978). Contagious paralysis of pigeons. *Avian Pathology*, **7**, 637–643.

Narita, M., Inui, S., Namba, K., and Shimizu, Y. (1976). Trigeminal ganglionitis and encephalitis in calves intranasally inoculated with infectious bovine rhinotracheitis virus. *Journal of Comparative Pathology*, **86**, 93–100.

Narita, M., Inui, S., Namba, K., and Shimizu, Y. (1978a). Neural changes in calves intravaginally inoculated with infectious bovine rhinotracheitis virus. *Journal of Comparative Pathology*, **88**, 381–386.

Narita, M., Inui, S., Namba, K., and Shimizu, Y. (1978b). Neural changes in calves after intraconjunctival inoculation with infectious bovine rhinotracheitis virus. *Journal of Comparative Pathology*, **88**, 387–394.

Openshaw, H. (1980). Communication at the "*International Conference on Human Herpesviruses*". Atlanta, GA., U.S.A.

Pastoret, P. P., Aguilar-Setién, A. Burtonboy, G., Mager, J., Jetteur, P., and Schoenaers, F. (1979). Effect of repeated treatment with dexamethasone on the reexcretion pattern of infectious bovine rhinotracheitis virus and humoral immune response. *Veterinary Microbiology*, **4**, 149–155.

Pastoret, P. P., Babiuk, L. A., Misra, V., and Griebel, P. (1980). Reactivation of ts and non-ts infectious bovine rhinotracheitis vaccine virus with dexamethasone. *Infection and Immunity*, **29**, 483–488.

Surman, P. G., Purcell, D. A., Tham, V. L., Wilson, A. J., and Schultz, D. J. (1975). The isolation of a herpesvirus from a pigeon and experimental infection in psittacine birds. *Australian Veterinary Journal*, **51**, 537–538.

Tantawi, H. H., Al Falluji, M. M., and Sheikhly, F. (1979). Viral encephalomyelitis of pigeons: identification and characterization of the virus. *Avian Diseases*, **23**, 785–793.

Vetési, F., and Tanyi, J. (1975). Occurrence of a pigeon disease in Hungary caused by a herpesvirus. *Magyar Allatorvosok Lapja*, 193–197.

Vindevogel, H., and Duchatel, J. P. (1978). Contribution à l'étude de l'étiologie du coryza infectieux du pigeon. *Annales de Médecine Vétérinaire*, **122,** 507–513.

Vindevogel, H., and Duchatel, J. P. (1979). Les principales maladies infectieuses du pigeon. *Annales de Médecine Vétérinaire*, **123,** 17–27.

Vindevogel, H., Duchatel, J. P., and Gouffaux, M. (1977). Pigeon herpesvirus. I. Pathogenesis of pigeon herpesvirus in chicken embryo fibroblasts. *Journal of Comparative Pathology*, **87,** 597–603.

Vindevogel, H., and Pastoret, P. P. (1980). Pigeon herpes infection: natural transmission of the disease. *Journal of Comparative Pathology*, **90,** 409–413.

Vindevogel, H., Pastoret, P. P., and Burtonboy, G. (1980). Pigeon herpes infection: excretion and re-excretion of virus after experimental infection. *Journal of Comparative Pathology*, **90,** 401–408.

Vindevogel, H., Pastoret, P. P., Burtonboy, G., Gouffaux, M., and Duchatel, J. P. (1975). Isolement d'un virus herpès dans un élevage de pigeons de chair. *Annales de Recherches Vétérinaires*, **6,** 431–436.