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学位論文

題　目　Virological and pathological studies on reactivation of canine herpesvirus

学位論文の基礎となる学術論文

題　目　Virus reactivation in bitches with a medical history of herpesvirus infection

著　者　名　Okuda,Y., Ishida,K., Hashimoto,A., Yamaguchi,T., Fukushi,H. Hirai,K. and Carmichael, L.E.

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著　者　名　Okuda,Y., Hashimoto,A., Yamaguchi,T., Fukushi,H., Mori,S., Tani,M., Hirai,K. and Carmichael, L.E.

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題　目　愛玩鳥の大腸菌症について
著　者　名　島倉省吾、葛谷光隆、鎌数真美恵、吉田徹也、奥田恭之、津久見清、福士秀人、平井克哉
学術雑誌名　岐阜大学農学部研究報告に発表
(巻・号・頁)　(49・209～216)
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題　目　輸入愛玩鳥からサルモネラの分離について
著　者　名　島倉省吾、葛谷光隆、森腰俊亨、福士秀人、奥田恭之、平井克哉
学術雑誌名　岐阜大学農学部研究報告に発表
(巻・号・頁)　(50・251～257)
発行年月　1985年12月

題　目　Chlamydial complement fixing antibodies in cows, horses and pigs from 1980 to1983
著　者　名　Fukushi, H., Ogawa, H., Morikoshi, T., Okuda, Y., Shimakura, S. and Hirai, K.
学術雑誌名　Research Bulletin of the Faculty of Agriculture, Gifu Universityに発表
(巻・号・頁)　(50・259～263)
発行年月　1985年12月

既発表学術論文

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| 題　目 | An enzyme-linked immnosorbent assay for the detection of antibodies to Chlamydia psittaci in animal sera |
| 著　者　名 | Fukushi, H., Hayashi, Y., Okuda, Y., Shimakura, S., and Hirai, K. |
| 学術雑誌名 | Research Bulletin of the Faculty of Agriculture, Gifu Universityに発表 |
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| 題　目 | 犬・猫・兎及ラット胎児の胃の超音波画像所見について |
| 著　者　名 | 橋本　晃、工藤忠明、奥田恭之 |
| 学術雑誌名 | 獣医画像診断に発表 |
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| 著　者　名 | 橋本　晃、工藤忠明、奥田恭之 |
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# Virological and Pathological Studies on Reactivation of Canine Herpesvirus

(犬ヘルペスウイルスの再活性化に関する
ウイルス学的および病理学的研究)

1993

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# Virological and Pathological Studies on Reactivation of Canine Herpesvirus

（犬ヘルペスウイルスの再活性化に関する
ウイルス学的および病理学的研究）

Okuda Yasuyuki

CONTENTS

# PREFACE

High mortality in pups during the first few weeks of life, particularly among kennel populations in breeding and laboratory establishments, is one of the most important problems encountered in canine medicine. Mortality in young pups between birth to weaning is approximately 30%, and more than 80% of deaths occur within the first week after birth (1). Reported causes of death include birth trauma, accidents, congenital anomalies, abandonment, cannibalism, lactation failure, environmental conditions and recognized infectious diseases such as infectious canine hepatitis, canine distemper, toxoplasmosis, and various bacterial infections (1, 18). In the large majority of cases, however, the specific cause remains unclear. This fact is a major cause of concern for veterinary practitioners, dog breeders and laboratory establishments.

A significant advance in the clarification of the cause of neonatal deaths was made by the isolation of a herpesvirus from septicemic and dead infant pups (10, 12). Thereafter, canine herpesvirus (CHV) has been considered a major cause of neonatal pup death and a newly recognized serious infectious disease (4, 6, 61).

CHV infection in neonatal pups is a fatal acute infectious disease characterized by generalized focal necrosis and hemorrhages (11, 13). It was first described as a specific disease entity by Carmichael et al. (11) in 1965. Following the initial report in the United States of America, the disease has been

reported in England in1966 (47), France in 1967 (48), Australia in 1970 (26), the Netherlands in 1971 (56), Norway in 1973 (8), South Africa in 1974 (3), Germany in 1977 (5), New Zealand in 1977 (17), Switzerland in 1978 (50), Ireland in 1978 (53) and Italy in1980 (55), suggesting a worldwide distribution. Under both natural and experimental conditions, CHV causes fatal infection at less than 3 weeks of age (11, 13, 28, 51, 57, 61), whereas in adult dogs the virus causes non-fatal infection of the respiratory and genital tracts (2, 7, 9, 29, 45). In Japan, Motohashi and Tajima (35) isolated CHV from an adult dog with a severe respiratory disease. Hashimoto et al. (21) reported a neonatal fatal disease with characteristic hemorrhage and necrosis caused by CHV in 1978.

The main clinical features in neonatal pups with CHV fatal infection were anorexia, diarrhea, dyspnea, continual crying (3, 11, 13, 32, 50, 51). Pathologically, lesions associated with either naturally acquired or experimentally produced CHV infections are essentially similar (10, 11, 28, 60). The characteristic gross lesions seen at necropsy are ecchymoses foci of necrosis in the liver, kidneys, lungs, intestines and diffuse pulmonary congestion, splenomegaly and lymphadenitis (3, 11, 14, 28, 50, 60). Histopathological lesions consist principally of disseminated focal necrosis and hemorrhages with or without intranuclear inclusion bodies in degenerated cells. The lesions are present in all the major organs of the body including the liver, kidneys, spleen, heart, lungs, adrenal glands, intestines and brain (3, 11, 13, 28, 50, 57, 60).

On the other hand, herpesviruses usually present inapparent symptoms after cease of virus shedding in primary infection. Previous studies (27, 30, 33, 38-40, 58) showed that some herpesviruses latently infecting the nerve system could be reactivated by stimuli or treatment with immunosuppressive drugs. Latent infection and its reactivation in hosts or laboratory animals were reported in HS (herpes simplex) virus (23, 24, 31, 33), AD (Aujeszky's disease) virus (36, 40, 58), IBR (infectious bovine rhinotracheitis) virus (37, 39), and VZ (varicella and zoster) virus (27). Some evidences have also indicated that latent sites of these viruses located around sensory and autonomic nerve ganglia. However, there is no information available on CHV latency to date.

The purpose of this treatise is first to present the initial description of CHV reactivation with a medical history and second to investigate the possibility of repeated reactivation of CHV in not only adult dogs but also pups infected experimentally.

Chapter 1

# Virus reactivation in bitches with a medical history of canine herpesvirus infection

## INTRODUCTION

Death of neonatal pups caused by infection with CHV has been well recognized since this disease was described by Carmichael et al. in 1965 (11). Its clinical, virologic, and pathologic features have been reported by several investigators (11, 13, 20-22, 28, 44). Moreover, Stewart et al. (54) postulated that CHV remains latent in the fetus after transplacental infection. Cornwell and Wright(13) reported that pups could become virus carriers after recovery from CHV infection. Hashimoto et al. also has reported the pathologic lesions caused by CHV in a bitch with naturally developing, possibly recurrent, CHV infection (20). It is well known that human (19, 42, 46), bovine (37-39) and porcine (36, 40, 58) herpesviruses could be in the latent state in their hosts after primary infection and reactivated by treatment with various pharmacologic agents under natural and experimental conditions. However, little is known about the biologic behavior of CHV in adult bitches after an abortion, and to the author's knowledge, there has been no description of the virus reactivation in bitches with a medical history of CHV infection. The purpose of the study reported here was to demonstrate reactivation of the virus by administrating prednisolone (PD) to bitches naturally infected with CHV.

## MATERIALS AND METHODS

Bitches with a medical history of CHV infection: During the summer of 1990, abortions in Beagles kept in a breeding kennel

were determined to be caused by CHV infection. Twenty of 300 bitches failed to give birth to pups. CHV was isolated from various organs of the dead fetuses and pups, and it also was observed by electron microscopy in liver and kidney preparations of the fetuses. Seven of the Beagle bitches known to have aborted fetuses or pups infected with CHV were supplied for the present study. They remained healthy before the experiment, and results of virus isolation assays were negative throughout the 6-month observation period before the study was begun. Before the experimental treatment was initiated, serum neutralizing (SN) antibody titers of the bitches to CHV ranged from 1:16 to 1:320.

Experimental procedure: Five bitches (Nos. 1, 2, 3, 4 and 5) were given 600 mg of PD every 24 h to induce immunosuppression. The other 2 bitches (Nos. 6 and 7) were allocated as nontreated controls. In addition, 2 noninfected bitches (Nos. 8 and 9; free of CHV antibody) were treated 5 times with 600 mg of PD to serve as treated controls. To examine effects of PD on the hematologic profiles, total leukocyte and lymphocyte numbers were counted before and during the treatment period.

Virus isolation and titration: Nasal, oral, ocular, and vaginal secretions were collected on sterile cotton swabs and used for virus isolation. Tissues (nasal mucosa, tonsils, lungs, mandibular lymph nodes, trigeminal ganglia, and lumbosacral ganglia) also were obtained from each dog at necropsy for virus isolation attempts. All samples were homogenized in phosphate buffered saline solution (PBSS) and then inoculated onto monolayers of dog

kidney (DK) cells. The isolates were titrated as described previously (21).

Identification of isolates: Inoculated cell monolayers with cytopathologic effects (CPE) were examined for the presence of CHV by the direct fluorescent antibody (FA) method. A specific conjugate for CHV was prepared from the pooled sera of rabbits, which had been inoculated with the virus, by procedures described previously (25).

Determination of SN titers: SN antibody titers were determined as described previously (21). Sera were collected from each dog once a week after the first dose of PD.

Pathologic examinations: Pathologic examinations were conducted on samples of nasal mucosa, tonsils, lungs, mandibular lymph nodes, central nervous system (CNS) and selected ganglia. After macroscopic examinations, specimens from each dog were fixed in neutral buffered 10% formalin solution and embedded in paraffin. Sections were stained with hematoxylin and eosin (H-E).

## RESULTS

Total leukocyte numbers increased about 2 to 2.3 times at 1 or 2 weeks after initiation of treatment, whereas the mean number of lymphocytes decreased markedly (about one-fifth) at 1 week after treatment (data not shown). These changes continued until euthanasia; however, clinical signs of CHV infection were not detected in the bitches.

In bitches Nos. 1, 2, 3 and 4, the virus was isolated from

various secretions (Table 1). Nasal swab samples were the most frequent source of CHV between 5 and 21 days after initiation of treatment. The maximal titer in the nasal secretions : ($10^{5.25}$, $10^{1.5}$, $10^{10.5}$ and $10^{6.0}$ $TCID_{50}$/0.1ml for bitches Nos. 1, 2, 3 and 4, respectively) were higher than in the other secretions. The virus was recovered from the vaginal secretion of bitch No 1, and oral, and ocular secretions of bitch No. 3. In the latter bitch, the virus was isolated on day 5 after initiation of treatment, earlier than from bitches Nos. 1, 2 and 4, the virus was detected at days 10, 13 and 14, respectively. The virus was also isolated from nasal mucosa and tonsil samples from bitch No. 1, which was euthanatized on the 14th day after initiation of treatment with PD. The titers in those tissues were $10^{3.25}$ and $10^{5.5}$ $TCID_{50}$/0.1ml, respectively. All of the isolates were identified as CHV by direct fluorescent antibody examination. In bitches Nos. 2, 3, 4 and 5 virus was not detected from tissue specimens. Virus was not detected in any secretion or organ of the control bitches.

The pretreatment titers of 4 bitches from which CHV was reactivated were low, ranged between 1:16 and 1:32 (Table 2). The SN titer of bitch No. 5 was 1:320. The titers of bitches Nos. 3 and 4, increased steadily to 1:320 at 1 week after the initiation of reexcretion. Furthermore, the titer of bitch No. 4 continued to increase and reached 1:2048 by the 6th post treatment week. However, the antibody titers remained essentially constant in the other bitches, including the control bitches.

At necropsy, atrophy or disappearance of lymph follicles

with remarkable depletion of lymphocytes were seen in the mandibular lymph nodes and the tonsil of most bitches, including bitches Nos. 8 and 9. Small necrotizing lesions and monocyte infiltration were observed in the tonsil epithelium and turbine submucosa of bitch No. 3 from which CHV was isolated from oral and ocular secretions. Perivascular cell infiltration in the medulla oblongata (Fig. 1) and small nodes of glial cells in the obex (Fig. 2) were observed in bitches Nos. 1 and 2. The distribution of those lesions corresponded with the trigeminal nerve nuclei and bundle regions. Also, small focal necrosis in area adjacent to the nucleus trigenimi in the the medulla oblongata in bitch No. 2 (Fig. 3). Eosinophilic intranuclear inclusion bodies, typical of CHV infection, were not found in any lesion, and lesions were not observed in the CNS of other bitches, including the controls.

Small foci of nerve cell, degeneration and loss, associated with focal infiltration of mononuclear cells were found in the trigeminal ganglion of bitches Nos. 1 (Figs. 4 and 5) and 2, and in the lumbosacral ganglia of bitch No. 1. Neuronophagia also was observed in those lesions; however, no changes appeared in the ganglion of the 4 control dogs. Control bitches (Nos. 6 and 7) were normal histologically.

## DISCUSSION

Evidence obtained from this study indicated that CHV reactivation was induced in bitches by a 5-day course of treatment with PD 6 months after natural infection in a breeding kennel. The

absence of detectable shedding of infectious virus in the previously infected bitches during the 6-month period prior to immunosuppressive treatment, and the determination of CHV in secretions within 1-2 weeks following PD treatment suggested that herpesvirus persists and may involve establishment of a latent infection. The continued circulation of SN antibody over a period of several months after abortion, and an increase in SN antibody titers in some dogs following PD treatment, shortly after detecting viral recrudescence in secretions, may be considered additional evidence of persistent viral expression.

It is recognized that corticosteroids depress cell-mediated immunity (34). In the present study, the mean number of lymphocytes decreased to one-fifth the original number on the 7th day after the initiation of PD treatment. CHV was recovered from various secretions and organs from the 5th to 21st day after initiation of PD treatment. In animals with experimentally induced infections with IBR virus (37-39) or AD virus (40, 58), the viruses were excreted from their hosts within a week after initiation of PD treatment, and excretion continued for several days. The reactivation patterns in the present study resembled those of IBR virus and AD virus.

CHV was isolated from nasal secretions from 4 of 5 infected bitches treated with PD. Moreover, the titers in nasal secretions were higher than in other secretions. Although the nasal CHV titer of bitch No. 3 was unexpectedly high ($10^{10.5}$ $TCID_{50}$/0.1ml), titers of the reactivated virus in the other dogs were similar to those

reported for experimental IBR virus and AD virus infection. The author's results strongly indicated that nasal exudates may be epizootiologically the most important routes of CHV transmission.

CHV could not be isolated from any tissue or secretion from bitch No. 5. The SN titer of that bitch was higher (1:320) than the others before treatment. Failure to isolate the virus from this bitch may be attributed to differences in the amount of virus to which the dogs were initially exposed, or the time of primary infection.

Pathologic examinations revealed necrotizing lesions in the tonsil epithelium of one bitch No. 3, that also excreted CHV in nasal secretions. Small necrotic and mononuclear cell infiltrations were also observed in the nasal mucosa of bitch No. 3. In bitch No. 2, the virus was isolated from the nasal mucosa and tonsil, but lesions were absent. From these results, only mild lesions in tonsil and nasal mucosa were induced by CHV infection and intranuclear inclusion bodies were not found.

Nerve cell degeneration and loss, mononuclear cell infiltration, and neuronophagia were seen in the trigeminal ganglia of bitches Nos. 1 and 2. These findings resembled, qualitatively, those of our earlier report of CHV infection (21). It is suggested, therefore, that the aforementioned findings might be related to CHV. It seems likely that the lesion sites represent proliferating or recrudescent sites of CHV, although inclusion bodies were not found in the lesions. Additional studies, using in situ hybridization methods, would be necessary to confirm the presence of CHV in the

CNS of latently infected dogs.

The ability of CHV to establish latent infection has been suggested in previous studies. Stewart et al. (54) and Cornwell and Wright (13) suggested that pups known to be infected with CHV that recovered might become carriers of CHV. Earlier report of the author's Department (20) gave additional support to the idea that CHV may establish a latent infection in the nervous system of a bitch previously infected with CHV, however, the biological behavior of CHV in healthy bitches after an abortion is not clear.

The present results indicate that CHV may induce latent infections that can be reactivated by immunosuppression, as is the case with several other herpesviruses.

## SUMMARY

Virologic and pathologic investigations were done on PD-treated bitches with a history of CHV infection. Reactivation of CHV was demonstrated in 5 Beagle bitches after daily administration of 600 mg of PD for 5 days. The reactivation was confirmed in 4 of 5 bitches. CHV was recovered from nasal, oral, vaginal, and ocular secretions on the 5th to 21st day after initiation of treatment with PD, and also from nasal mucosa and tonsil tissues. Results indicated that latent CHV infections develop and that the virus may be reactivated, without clinical signs, in dogs with a history of CHV infection.

Table 1 Recovery of canine herpesvirus in mucosal secretions

| Bitch No. | Swabbed Sites | Days after initiaton of treatment with prednisolone | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5[a] | 6 | 7 | 8 | 9 | 10 | 13 | 14 | 16 | 18 | 20 | 21 | 24 |
| Treated infected bitches | | | | | | | | | | | | | | |
| 1 | Nasal | - | - | - | - | - | 0.5[b] | 3.25 | 5.25[c] | | | | | |
| | Oral | - | - | - | - | - | - | - | - | | | | | |
| | Ocular | - | - | - | - | - | - | - | - | | | | | |
| | Vaginal | - | - | - | - | - | - | - | 0.25 | | | | | |
| 2 | Nasal | - | - | - | - | - | - | 0.75 | 1.5[c] | | | | | |
| | Oral | - | - | - | - | - | - | - | - | | | | | |
| | Ocular | - | - | - | - | - | - | - | - | | | | | |
| | Vaginal | - | - | - | - | - | - | - | - | | | | | |
| 3 | Nasal | 5.5 | 10.5 | 8.5 | 12.5 | 8.5 | 7.5 | - | - | - | -[c] | | | |
| | Oral | - | 1.5 | 3.5 | - | - | - | - | - | - | - | | | |
| | Ocular | - | - | - | 1.5 | 1.0 | 1.5 | 1.5 | 1.5 | - | - | | | |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | | | |
| 4 | Nasal | - | - | - | - | - | - | - | 1.5 | 6.0 | 2.5 | 1.5 | 1.5 | -[d] |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Ocular | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | -[c] |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Ocular | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Nontreated infected bitches (control) | | | | | | | | | | | | | | |
| 6 & 7 | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | -[c] |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Ocular | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Treated noninfected bitches (control) | | | | | | | | | | | | | | |
| 8 & 9 | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | -[c] |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Ocular | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | - | - | - |

a: Last day of prednisolone treatment.
b: Data are expressed as $\log_{10}TCID_{50}/0.1ml$.
c: Nos. 1 and 2 were euthanatized at day 14, No. 3 at day 19, Nos. 5, 6, 7, 8 and 9 at day 24.
d: No. 4 was euthanatized at 6th week.
-: Negative

Table 2 Serum neutralizing titers of bitches

| Bitch No. | Before treatment | Weeks after initiation of treatment with prednisolone | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Treated infected bitches | | | | | | | |
| 1 | 16 | 32 | 40[a] | | | | |
| 2 | 32 | 20 | 28[a] | | | | |
| 3 | 32 | 20 | 28 | 320[a,b] | | | |
| 4 | 16 | 32 | 28 | 28 | 320 | 538 | 2048[a] |
| 5 | 320 | 320 | 160 | 160[a] | | | |
| Nontreated infected bitches (control) | | | | | | | |
| 6 | 40 | 28 | 40 | 32[a] | | | |
| 7 | 28 | 14 | 28 | 28[a] | | | |
| Treated noninfected bitches (control) | | | | | | | |
| 8 | <2 | <2 | <2 | <2[a] | | | |
| 9 | <2 | <2 | <2 | <2[a] | | | |

a: Euthanatized. See Table 1 for information on d of euthanasia.
b: Data were determined at 19th day.

## EXPLANATION OF FIGURES

Fig. 1. Perivascular cuffing of mononuclear cells in the area adjacent to the nucleus trigemini of the medulla oblongata. Bitch No. 1, H-E stain, X210.

Fig. 2 Small node of glial cells in the area of tractus trigemini in Bitch No. 2, H-E stain, x430.

Fig. 3 Small focal necrosis in the area adjacent to the nucleus trigemini of the medulla oblongata Bitch No.2, H-E stain, x210.

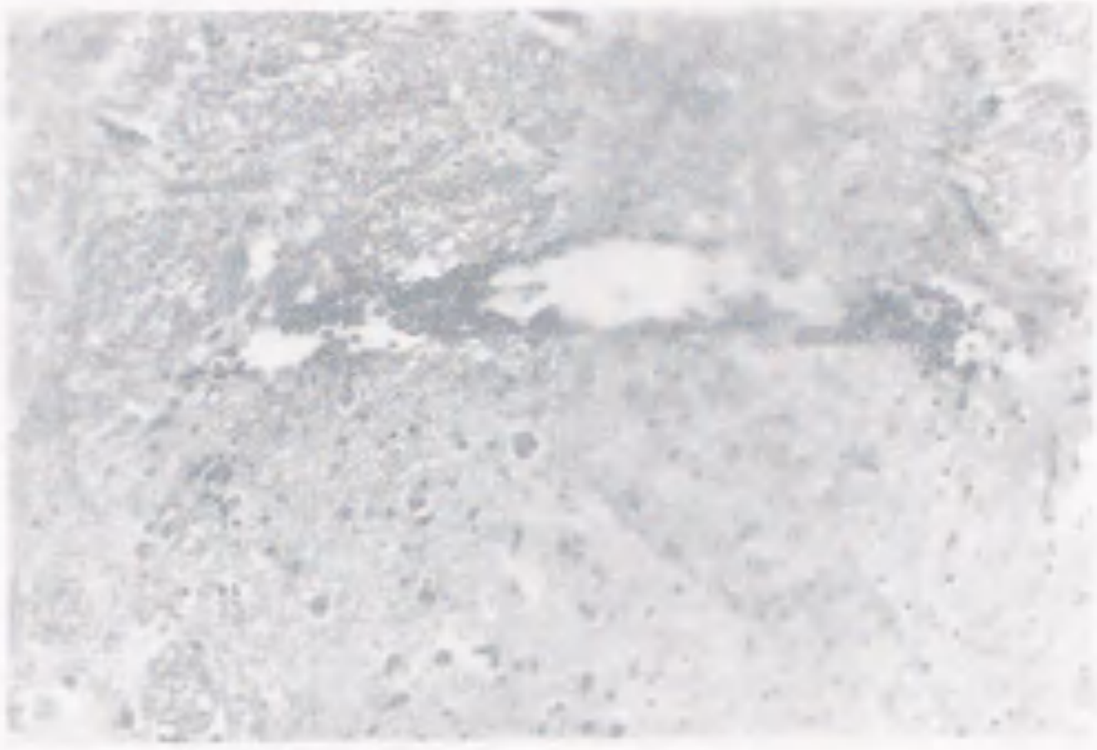

Fig. 1.

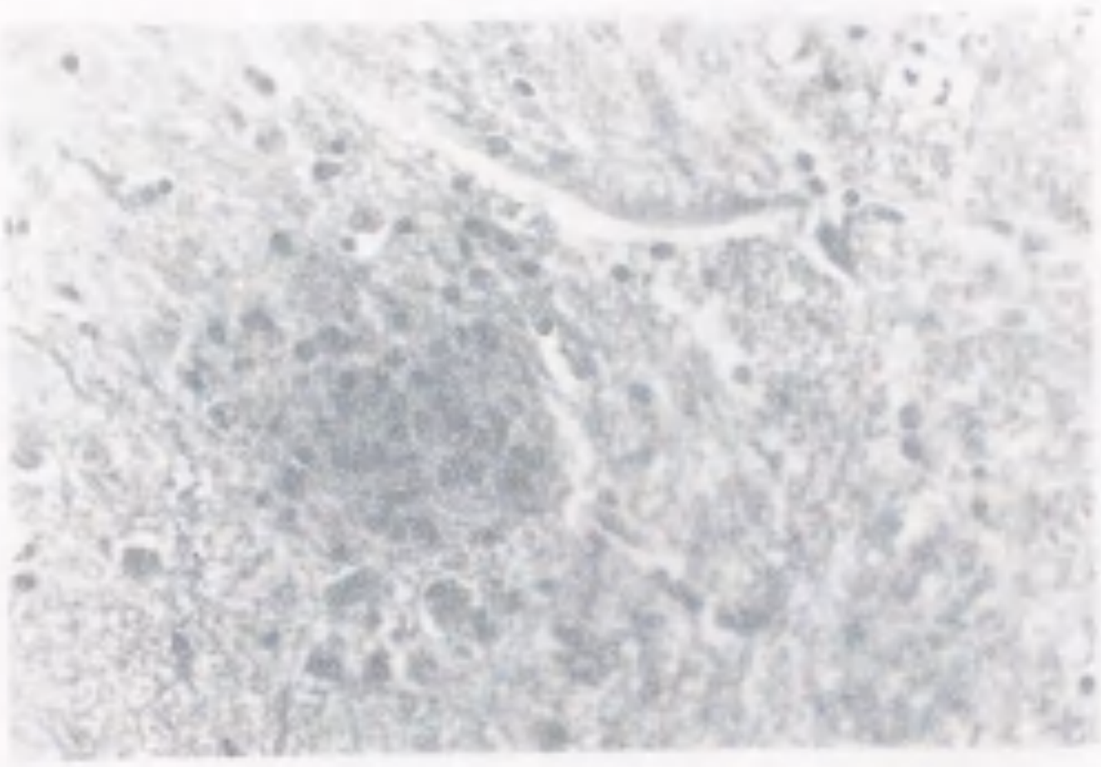

Fig. 2.

Fig. 3.

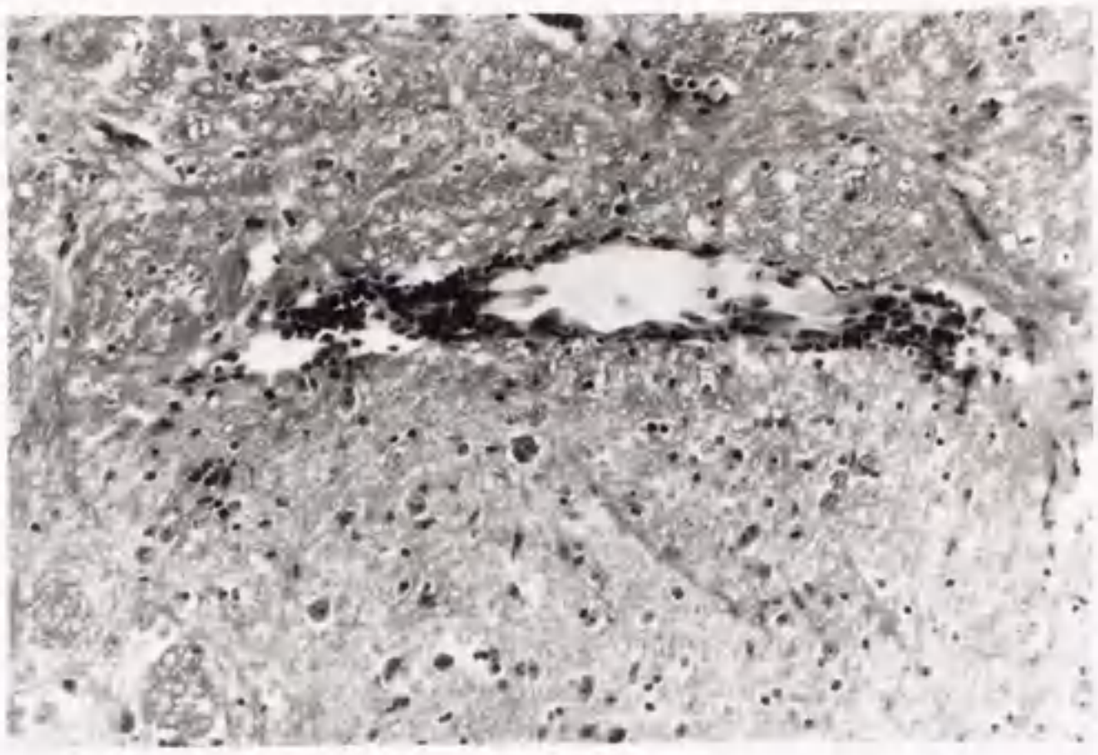

Fig. 1.

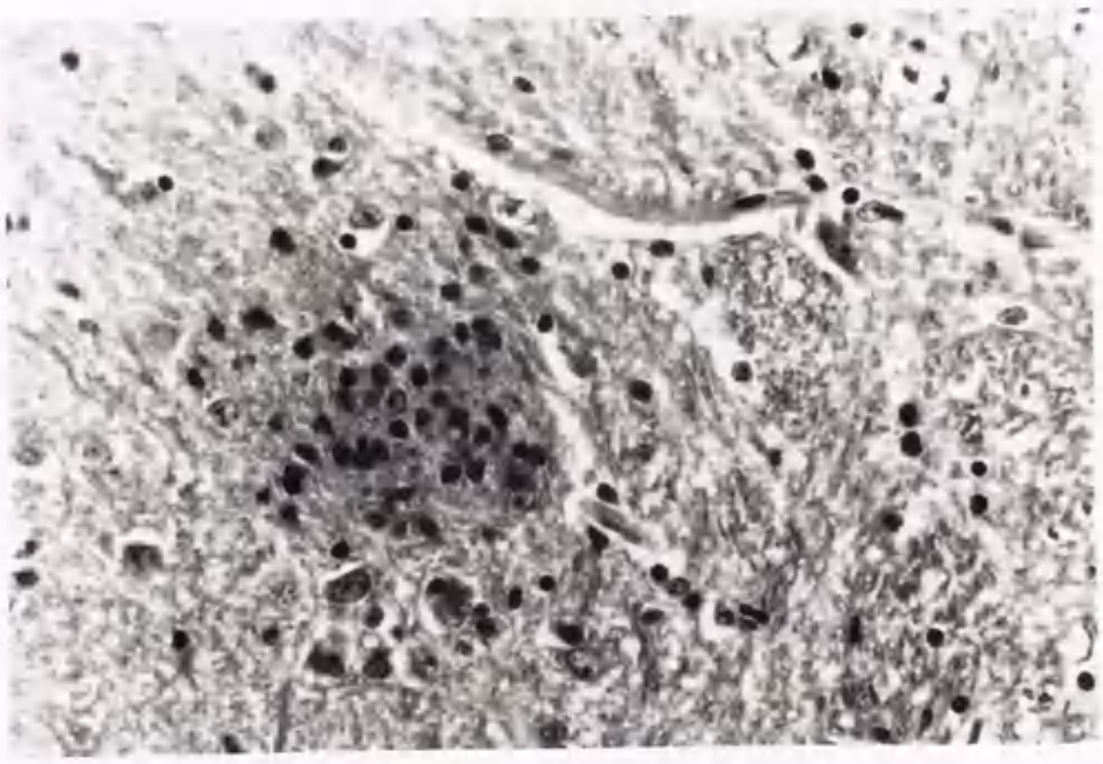

Fig. 2.

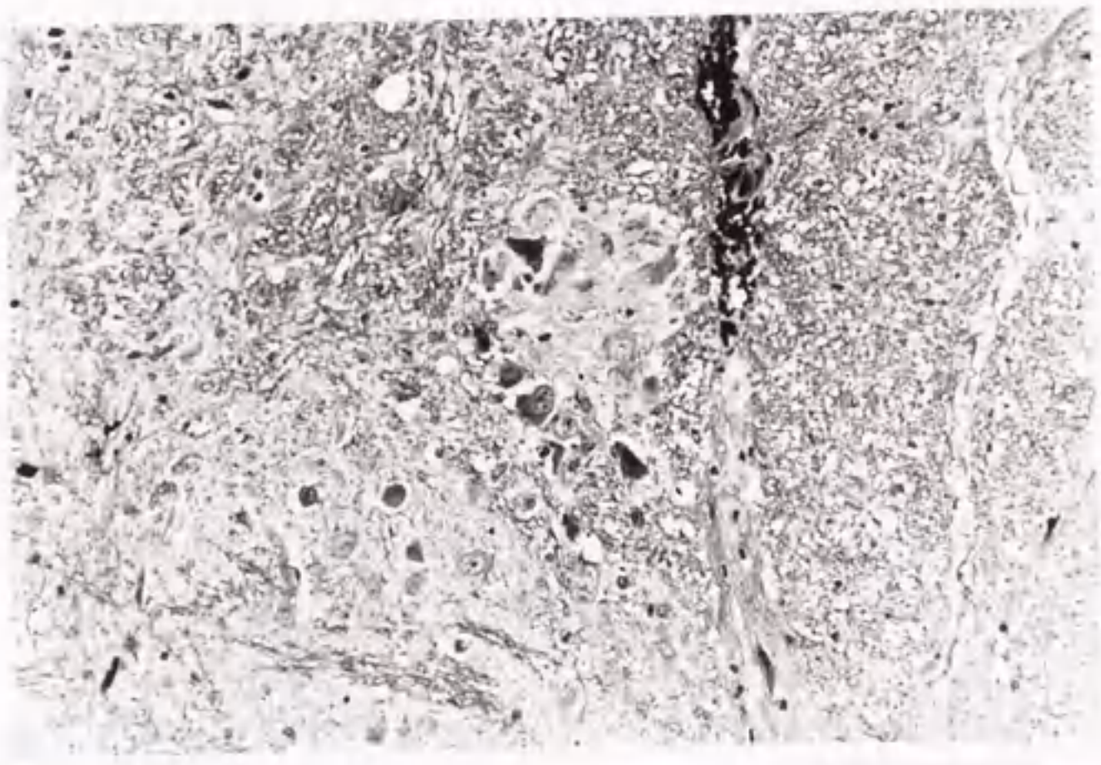

Fig. 3.

# EXPLANATION OF FIGURES

Fig. 4 Nerve cell degeneration and loss associated with focal infiltration of mononuclear cells in the trigeminal ganglion. Bitch No. 1, H-E stain, x430.

Fig. 5 Higher magnification of the same lesion as shown Fig. 4. Nerve cell degeneration and loss associated with neuronophagia in the trigeminal ganglion. Bitch No. 1, H-E stain, x210.

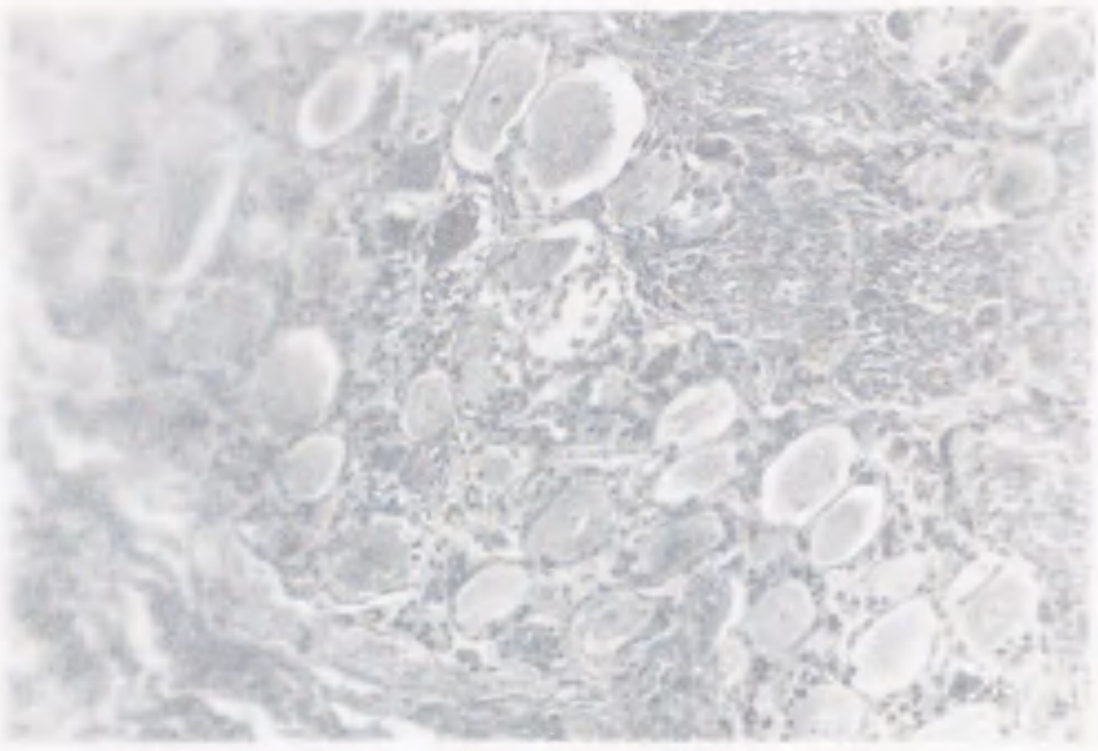

Fig. 4.

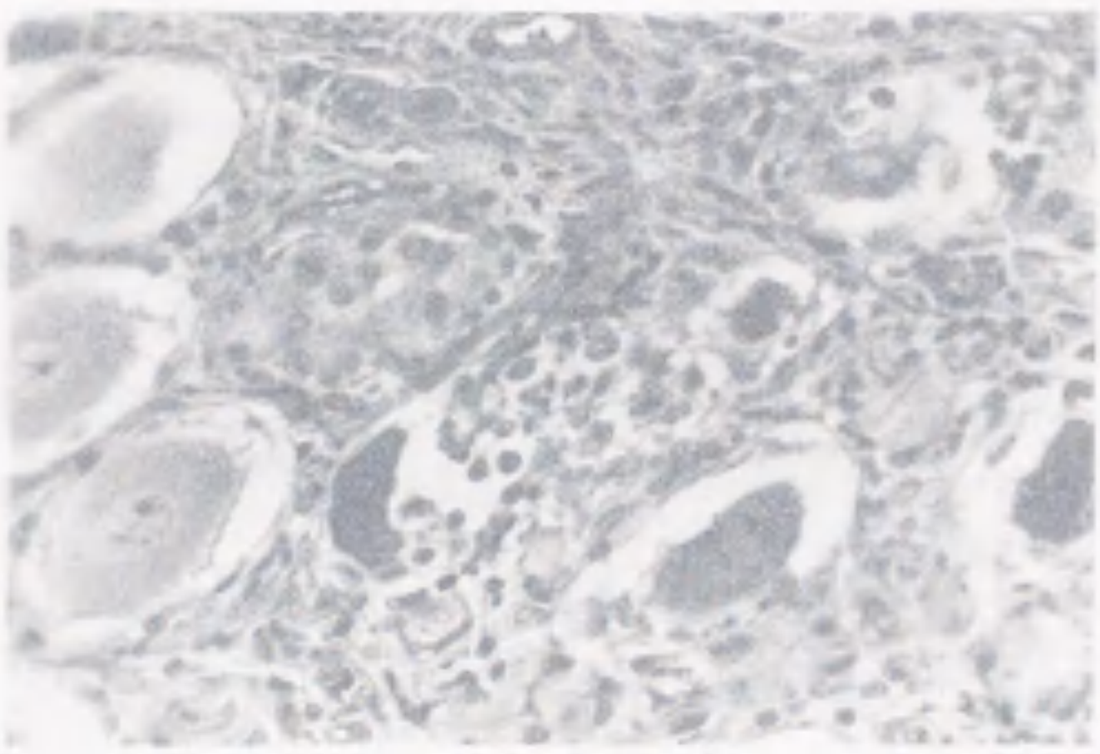

Fig. 5.

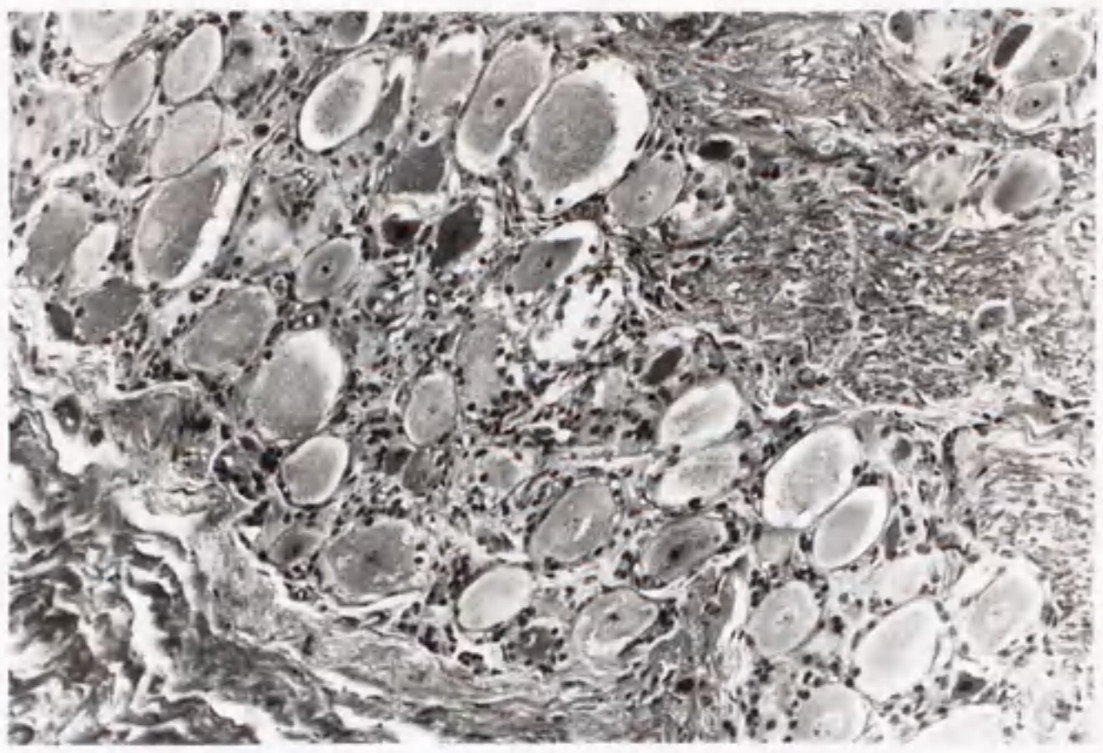

Fig. 4.

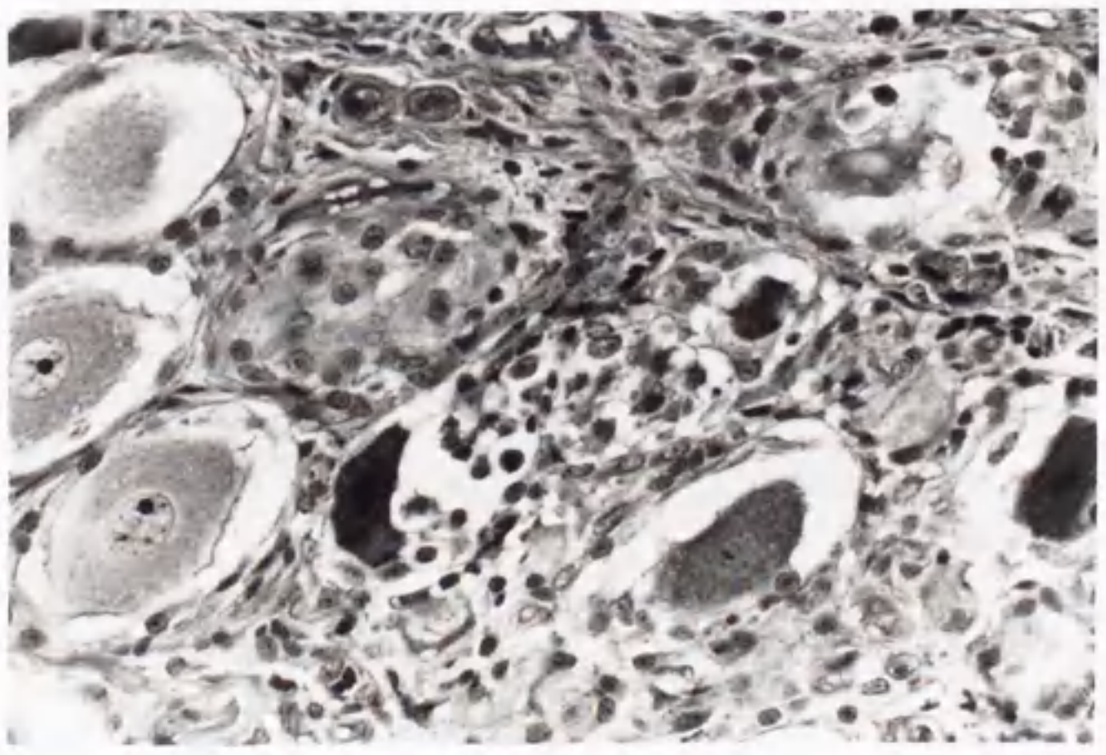

Fig. 5.

Chapter 2

# Repeated canine herpesvirus reactivation in experimentally infected pups and adult dogs by an immunosuppressive drug

## INTRODUCTION

Fatal disease in pups caused by CHV was first described by Carmichael et al. (11). Clinical, pathologic and virological features of CHV in pups, fetuses or pregnant dams have been reported by several investigators (13, 21, 22, 28, 44, 54). On the other hand, CHV infection in nonpregnant adult dogs has received little study, probably because clinical signs are usually inapparent or mild in animals older than a few weeks. In previous study, we reported that dams with a history of abortions subsequently had latent infections which could be reactivated by treatment with prednisolone (PD) (41). In human beings, repeated recurrences of herpesviruses are well known, e.g., HS virus (16, 49) and VZ virus (27), and the general mechanisms have been clarified (40, 49). Also, reactivation of several herpesviruses in domestic animals, e.g., AD virus (15) and IBR virus (15), has been demonstrated in both natural and experimental infections. There are only a few reports, however, which describe repeated episodes of reactivation of animal herpesviruses (43).

The author has observed only 1 occurrence of a repeated abortion due to CHV in a naturally infected dam (20). Although repeated abortions appear infrequent, recurrent episodes of viral shedding from latently infected dogs may be common. To the author's knowledge, however, repeated reactivation of experimental CHV has not been reported. Demonstration of repeated viral activation and excretion following latent CHV infection, which possibly persists throughout life, would be

important to an understanding of the epizootiology of the disease. Although the mechanism of CHV reactivation is not known, it is likely due to stress-induced immunosuppression since cortisone compounds are known to have immunosuppressive properties and, as mentioned above (41), PD has been shown to provoke CHV reactivation following natural or artificial infections.

The purpose of this study was to determine whether administration of high doses of PD to pups or adult dogs at intervals of 1-3 months after experimental CHV infections would provoke repeated episodes of viral reactivation and shedding. In these studies, both virologic and serologic responses to CHV were examined.

## MATERIALS AND METHODS

Virus: The GCH-1 strain of CHV was serially passaged 3 times in DK cell cultures as described (21, 44). The supernatant fluid from frozen and thawed infective cell cultures was used for dog inoculations and for SN tests. The infectivity titer of the stock CHV preparation was $10^{7.0}$ $TCID_{50}$/0.1ml inoculum.

Animals: Five 2-year old beagle dogs (3 males and 2 females) weighing 2.8-3.6 kg and five 2-3 months old mongrel pups were used in this study. They were all seronegative for SN antibodies at the time of virus inoculations.

Viral Infection and PD Treatment:

Adult dogs- Four adult dogs were inoculated by intranasal (2 ml) and intravenous (4 ml) routes with the stock CHV. Two weeks

later, all inoculated dogs had ceased primary viral excretion since swab samples of the nasal cavity, oral pharynx, vaginal and penis were negative on repeated weekly samplings. Then 9 weeks after primary CHV infection, 3 inoculated and 1 noninfected adults were each given daily doses (600 mg) of PD for a total of 5 days. Three months after the first series of 5-day treatment, a second 5-day treatment course of PD was performed. For controls, one infected male was not treated with PD and one noninfected female was treated with PD. To examine effects of PD on the hematologic profiles, total leukocytes and lymphocytes were enumerated daily during the treatment period and at weekly intervals of thereafter.

Pups- Four pups also were each inoculated by the intranasal (2 ml) and intravenous (2 ml) routes with stock CHV. Ten days later, all inoculated pups had ceased viral excretion since swab samples of nasal cavity, oral pharynx, vaginal and penis were negative on biweekly samplings. One month after the initial inoculations, 3 infected pups and 1 noninfected pups were given 200 mg of PD for 3 consecutive days. Then 7 weeks after the first series of treatments with PD, a second 3-day treatment course was performed in the same manner. As with the adult dogs, 1 infected male was not treated with PD and 1 noninfected female was treated with PD. Total leukocytes and lymphocytes of each pup were enumerated as described above.

Dose and frequency of PD treatments were similar to those done in a previous study (41).

Virus isolation and titration: The infectivity titers of swab

samples taken from several sites, noted above, were monitored for 2 weeks following inoculation with CHV. Oral, nasal, vaginal and penile secretions from each dog were collected on sterile cotton swabs for virus isolation attempts. All swabs were placed in chilled PBSS, pH 7.2 and mixed vigorously. Serial 10-fold dilutions were then prepared in PBSS and inoculated in 0.1 ml amounts onto monolayers of DK cells. The titration method used has been described in detail previously (21). Titers are expressed as $\log_{10}TCID_{50}$/0.1ml.

Identification of isolates: Inoculated DK cell monolayers with CPE were examined for the presence of CHV by the direct FA method. A specific CHV conjugate was prepared from hyperimmune rabbit serum and used as described previously (25).

Serology: SN antibody titers were determined as described previously (21). Sera were collected from each dog before inoculation, and at weekly intervals for periods of 14 weeks (pups) or 28 weeks (adults).

## RESULTS

Adult dog responses. After initiation of the first course of PD treatments, the total leukocyte numbers increased approximately twofold within 7 days (data not shown). A similar increase in total leukocyte numbers also was seen after initiation of the second course of treatment. In contrast, the mean number of lymphocytes decreased markedly to about 15% of the preinoculation number by post inoculation (PI) week 1. Lymphocyte

numbers did not return to pretreatment values during the remainder of the experimental period.

Following CHV infection, there was continuous excretion of CHV in nasal secretions in all 3 dogs for approximately 2 weeks. Excretion then ceased and was not detected until 5 to 9 days after treatment with PD, which was initiated at PI week 9 (data not shown). SN antibodies were first detected at PI week 2 and titers increased steadily between PI week 2 and 4. Antibody titers then declined after PI week 4 and reached a 'steady state' (titers of 1:32-1:64).

After administration of the first course of PD, CHV was detected in all 3 infected dogs (Nos. 1, 2 and 3) between days 5 and 32 (Table 3). Virus was not recovered from the infected, but untreated, or the noninfected controls. The site from which CHV was isolated for the longest time, in each case, was the nasal cavity. Much lower infectivity titers, and shorter periods of viral excretion, were found in swab samples of the oral pharynx, vagina or penis. Maximum viral infectivity titers of the nasal samples ranged from $10^{4.0}$ through $10^{8.5}$. Nasal secretion of CHV commenced 5-9 days after the start of PD treatment and continued for 16-24 days.

The second course of PD treatment was given 3 months after the start of the first course and resulted in reactivation of CHV in 2/3 dogs (Nos. 1 and 2). In both instances, viral excretion was first detected on post treatment days 7-9 and it continued for 12 days. Nasal secretion infectivity titers of the 2 dogs ranged from $10^{1.25}$

to $10^{5.0}$ and oral titers were $10^{0.25}$ to $10^{3.0}$ (Table 3). In both animals, the duration of excretion was shorter than that observed following the first period of steroid-induced reactivation.

There was a significant (>4-fold) increase in SN antibody titers after the first course of PD treatment (Table 5); followed by a slow decline. Titers were not affected by the second PD treatment. The level of antibody did not appear to affect viral reactivation in dogs (Nos. 1 and 2) that had high titers prior to treatment. Antibody titers remained at 1:16-1:32 in the nontreated, infected (control) dog. Clinical signs were not observed in any dog during the study.

Pup responses. Total leukocyte responses of pups after infection and following PD treatments were virtually the same as observed in the adult dogs (data not shown).

Viral excretion was confirmed from each of the three sites sampled between PI days 4 and 7 (data not shown). The nasal secretion infectivity titers ranged from $10^{4.0}$ to $10^{8.0}$ $TCID_{50}$/0.1ml inoculum. Virus could no longer be isolated, however, after PI day 14. Clinical signs were mild, consisting only of serous ocular and nasal discharged and anorexia for 3 to 5 days after the infection. Serum neutralizing antibody levels of the infected pups increased by PI week 2, reaching maximal titers of 1:40-1:160 by PI week 3-4. The noninfected control pup remained seronegative.

Reactivation of CHV following PD treatment was confirmed in two pups (Nos. 6 and 7) after the first series of treatment (Table 4). The highest titers ($10^{7.5}$ and $10^{2.0}$, respectively) were

demonstrated in the nasal secretions, where viral excretion continued for 3 to 8 days and then ceased. In 1 pup (No. 6), virus also was recovered in low titers from oral-pharyngeal samples.

After the second 3-day series of PD treatments, started 50 days after the first course, CHV was recovered, variably, from all 3 pups between post treatment days 4 to 12. Virus was isolated only from nasal swab samples, where infectivity titer $10^{8.0}$ was found in 1 pup (No. 8) that also shed virus for the longest period (9 days) after PD treatment.

Serum neutralizing antibody titers before PD treatment reached maximal values 2-3 weeks after virus inoculation in all infected pups (Table 6). Titers remained constant for 1-2 months and then declined about 2-fold. In contrast to the results following PD treatment of infected adult dogs, pup titers did not differ from those of the non-treated, infected control.

## DISCUSSION

Recrudescent herpesvirus infections in humans and animals are common and have received extensive study (15, 16, 27, 40, 41, 49), but there has been only a limited study (43) of experimental herpesvirus infections of animals which focus on recurrent episodes of viral shedding. In the present study the author demonstrated, for the first time, repeated reactivation of CHV in experimentally infected pups and adult dogs which had received two courses of an immunosuppressive corticosteroid drug (prednisolone) separated by periods of 6 (pups) or 12 (adults)

weeks. PD was selected to attempt reactivation of CHV in this study because of our previous success with this drug in provoking CHV recrudescence following natural infection (41). Reactivation was provoked by 3 or 5-day courses of PD, respectively. Viral excretion patterns in both adults and pups were similar to those reported by us in a previous study (41), where viral recrudescence occurred in bitches which had aborted as a consequence of natural CHV infection following treatment with PD. One difference observed in the patterns of viral shedding was the tendency for infectivity titers to be lower following the second course of PD; also, the duration of viral excretion was shorter than that which followed the first PD treatment. These results suggest that CHV remains latent following infection and that the virus may be provoked repeatedly by corticosteroid drugs to active replication and excretion. Such episodes of asymptomatic viral shedding may ensure the survival of the virus in the dog population and constitute a source of infection for susceptible contacts during periods of shedding.

A difference noted between the antibody responses of adults and pups following steroid treatment was the failure of pups to have increases in SN titers, even though recrudescence was demonstrated. The author does not have a satisfactory explanation for this phenomenon, however, it would not seem likely to be a consequence of the quantity of reactivated virus, for the amounts excreted by two pups (Nos. 7 and 8) were similar to those of the adults, where evident increases (>4-fold) in SN antibodies were

observed. Differences in SN titer fluctuations were observed after the first and second courses of treatment, but the number of animals was too few for determination of the statistical significance of those observations. Nevertheless, our results suggest that circulating antibody to CHV does not appear to contribute to the establishment or maintenance of latency, which is consistent with findings concerning human HSV (52).

Although it appeared that the titers of CHV in nasal secretions and the duration of shedding were lower after the second PD treatment, the number of dogs studied was not sufficient to allow a definitive conclusion.

It is noted that even the large doses of PD used in this study failed, in some instances, to provoke viral reexcretion. This was not unexpected, since studies with HSV have shown that immunosuppressive steroid drugs do not affect all control mechanisms of HSV latency (59). Also, Carmichael in 1972 (Unpublished data) observed that treatment with antithymocyte serum (ATS) or ATS + PD provoked CHV recrudescence in some dogs that failed to respond to PD alone.

The present results provide a plausible explanation for the persistence of CHV infection in kennels or congregated dogs in the absence of clinical signs. Recrudescent virus, especially in nasal secretions from latently infected dogs, may serve as a source of infection for long, but undetermined, periods. Abortions, fetal or neonatal infections occur sporadically, especially when susceptible dams are introduced into a kennel for breeding.

## SUMMARY

To examine the possibility of repeated reactivation of CHV, 2 serial treatments with the corticosteroid drug (PD) were given at different periods following oral-nasal infection of pups and adult dogs. CHV was not recovered from infected, nontreated dogs or from uninfected, treated controls. Viral reactivation of CHV, without clinical signs, was induced twice in 2/3 adults and in 2/3 pups treated at intervals that ranged from 1 to 3 months following the initial infections. Highest vital titers were obtained from nasal swab samples, with lower titers found in the oral pharynx, penis or vagina. In some, but not all dogs, the infectivity titers of the nasal secretion samples were higher after the initial PD treatments than after the second treatments. The duration of viral shedding after the second series of steroid treatments also was shorter than the shedding period following the initial reactivations. The results presented here suggest that latent CHV occurs in both pups and adults dogs following infection and that active infections, with viral shedding, may occur repeatedly for prolonged, but undetermined periods.

Table 3 Repeated recovery of canine herpesvirus in mucosal secretions of adult dogs

| Animal No. | Swabbed Sites | Days after initiation of first course of treatment: 5[a] | 7 | 9 | 12 | 14 | 16 | 19 | 21 | 24 | 28 | 32 | 35 | 92 | 98 | 101 | 105 | 109 | 112 | 117 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Days after initiation of 2nd course of treatment: 1[b] | 7 | 10 | 14 | 18 | 21 | 26 |
| Treated infected dogs | | | | | | | | | | | | | | | | | | | | |
| 1(M) | Nasal | - | - | 0.25[c] | 0.5 | 0.5 | 0.5 | 1.5 | 1.75 | 4.0 | 2.75 | 0.75 | - | - | 2.5 | 4.0 | 2.25 | 1.75 | - | - |
| | Oral | - | - | - | - | 0.5 | 1.5 | 1.5 | - | - | - | - | - | - | 0.25 | 1.5 | - | - | - | - |
| | Penile | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2(M) | Nasal | 0.5 | 2.75 | 5.0 | 8.25 | 8.5 | 6.75 | 2.75 | 2.0 | - | - | - | - | - | 1.5 | 2.25 | 5.0 | 1.25 | - | - |
| | Oral | 1.75 | 1.5 | 3.25 | 3.5 | 3.75 | 2.0 | - | - | - | - | - | - | - | - | 1.5 | 3.0 | 0.25 | - | - |
| | Penile | - | - | - | - | 0.25 | 0.5 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3(F) | Nsal | - | - | 2.5 | 4.0 | 6.25 | 7.75 | 7.0 | 1.75 | 0.25 | - | - | - | - | - | - | - | - | - | - |
| | Oral | - | - | - | 0.5 | 1.25 | 2.5 | 1.75 | 0.25 | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | 0.75 | 0.75 | - | - | - | - | - | - | - | - | - | - | - | - |
| Nontreated infected dogs (control) | | | | | | | | | | | | | | | | | | | | |
| 4(M) | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Penile | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Treated noninfected dogs (control) | | | | | | | | | | | | | | | | | | | | |
| 5(F) | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

a: Last day of the first course of prednisolone treatment.
b: First day of the second course of prednisolone treatment.
c: Data are expressed as $\log_{10}TCID_{50}/0.1ml$.
-: Negative
(M),(F): Male, Female

Table 4 Repeated recovery of canine herpesvirus in mucosal secretions of pups

| | | Days after initiation of first course of treatment | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 6 | 8 | 10 | 12 | 15 | 17 | 50 | 53 | 55 | 57 | 59 | 61 | 64 |
| | | Days after initiation of 2nd course of treatment | | | | | | | | | | | | | |
| | | 1 | 4 | 6 | 8 | 10 | 12 | 15 | | | | | | | |
| Treated infected dogs | | | | | | | | | | | | | | | |
| 6 (M) | Nasal | 1.0[a] | 6.0 | 7.5 | 7.0 | 2.5 | - | - | - | - | 2.0 | 3.0 | 1.5 | - | - |
| | Oral | - | 1.5 | 2.0 | 1.0 | - | - | - | - | - | - | - | - | - | - |
| | Penile | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 (M) | Nasal | - | - | 2.0 | 1.0 | - | - | - | - | - | 1.0 | - | - | - | - |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Penile | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 (F) | Nasal | - | - | - | - | - | - | - | - | 1.0 | 7.5 | 7.5 | 8.0 | 4.0 | - |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaginal | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Nontreated infected dog (control) | | | | | | | | | | | | | | | |
| 9 (M) | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Penile | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Treated noninfected dog (control) | | | | | | | | | | | | | | | |
| 10 (F) | Nasal | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Oral | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vaiginal | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

a: Data are expressed as $\log_{10}TCID_{50}/0.1ml$.
-: Negative
(M),(F): Male, Female

Table 5 Serum neutralizing titers of adult dogs after canine herpesvirus infection and prednisolone treatment

| Animal No. | Weeks after virus inoculation | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9[a] | 10 | 11 | 12 | 13 |
| | | | | | | | | | | Weeks after initiation of first PD treatment | | | | |
| | | | | | | | | | | 0 | 1 | 2 | 3 | 4 |
| Treated infected dogs | | | | | | | | | | | | | | |
| 1 | <2 | <2 | 11 | 128 | 181 | 91 | 64 | 64 | 45 | 32 | 32 | 45 | 64 | 91 |
| 2 | <2 | <2 | 45 | 724 | 1448 | 91 | 91 | 91 | 64 | 64 | 64 | 91 | 64 | 1448 |
| 3 | <2 | <2 | 8 | 91 | 128 | 91 | 91 | 64 | 64 | 64 | 45 | 45 | 128 | 362 |
| Nontreated infected dog (control) | | | | | | | | | | | | | | |
| 4 | <2 | <2 | 32 | 128 | 512 | 128 | 64 | 64 | 32 | 32 | 32 | 32 | 32 | 16 |
| Treated noninfected dog (control) | | | | | | | | | | | | | | |
| 5 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |

| Animal No. | Weeks after virus inoculation | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22[b] | 23 | 24 | 25 | 26 | 27 | 28 |
| | | | | | | | | | Weeks after initiation of first PD treatment | | | | | | |
| | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| | | | | | | | | | Weeks after initiation of 2nd PD treatment | | | | | | |
| | | | | | | | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Treated infected dogs | | | | | | | | | | | | | | | |
| 1 | 363 | 1024 | 724 | 512 | 362 | 362 | 512 | 362 | 181 | 91 | 64 | 64 | 64 | 32 | 32 |
| 2 | 2896 | 1448 | 1448 | 512 | 724 | 362 | 256 | 256 | 362 | 362 | 362 | 362 | 724 | 512 | 362 |
| 3 | 256 | 128 | 181 | 181 | 362 | 362 | 256 | 181 | 181 | 91 | 91 | 91 | 64 | 64 | 64 |
| Nontreated infected dog (control) | | | | | | | | | | | | | | | |
| 4 | 32 | 16 | 32 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| Treated noninfected dog (control) | | | | | | | | | | | | | | | |
| 5 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |

a: First course of treatment with prednisolone.
b: Second course of treatment with prednisolone.

Table 6 Serum neutralizing titers of pups after canine herpesvirus infection and prednisolone treatment

| Animal No. | Weeks after virus inoculation: 0 | 1 | 2 | 3 | 4[a] | 5 | 6 | 7 | 8 | 9 | 10[b] | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Weeks after initiation of first PD treatment: 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| | Weeks after initiation of 2nd PD treatment: 0 | 1 | 2 | 3 | 4 | | | | | | | | | | |
| Treated infected dogs | | | | | | | | | | | | | | | |
| 6 | <2 | 10 | 160 | 160 | 160 | 160 | 320 | 320 | 160 | 160 | 80 | 80 | 80 | 80 | 80 |
| 7 | <2 | 10 | 80 | 160 | 80 | 20 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| 8 | <2 | 10 | 20 | 40 | 40 | 20 | 20 | 20 | 20 | 40 | 80 | 80 | 40 | 80 | 160 |
| Nontreated infected dog (control) | | | | | | | | | | | | | | | |
| 9 | <2 | 10 | 20 | 40 | 40 | 20 | 20 | 20 | 20 | 40 | 80 | 80 | 40 | 80 | 160 |
| Treated noninfected dog (control) | | | | | | | | | | | | | | | |
| 10 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 |

a: First course of treatment with prednisolone.
b: Second course of treatment with prednisolone.

# CONCLUSION

The author theorized that CHV has potential for latent infection as with other herpesviruses, on the bases of a following series of studies performed in the Department of Microbiology of Gifu University: 1) natural recrudescent stillbirth suspected by CHV; 2) vaginal lesions in bitches with a medical history of CHV; and 3) experimentally proved vertical infections in pregnant bitches.

In author's initial studies, attempts were made to experimentally induce the reactivation of latent CHV, and resolve the route of reexcretion and the latent sites of virus. Moreover, the second series of experiments were undertaken to clarify the possibility of repeated reactivation of CHV.

1. Virus reactivation in bitches with a medical history of abortion

In a breeding kennel, frequent abortions were observed during a short period. After abortions had occurred, however, these bitches did not have any further symptoms. CHV was not isolated from those bitches for about 6 months after abortions until the start of the experiment. Five of 7 supplied bitches, which had aborted were given dairy dose of 600 mg of PD subcutaneously for 5 consecutive days. During and after PD treatment, virus isolation attempt was made. No bitches developed any symptoms caused by CHV infection. However, CHV was isolated from nasal, oral, ocular or vaginal secretions in 4 of 5 bitches for several days during 5 to

21 days after the initiation of PD treatment. Histopathologically, mononuclear cell infiltration was in nasal mucosa and tonsil suggested that those areas are considered as the virus excreting regions. Mild perivascular cell infiltration also was observed in medulla oblongata and small glial cell nodes were noted in obex. In trigeminal and lumbosacral ganglia, nerve cell degeneration and loss, along with neuronophagia, also were observed. These regions were therefore considered as the latent regions of latent viral persistence.

2. Repeated virus reactivation in experimentally infected adult dogs and pups with CHV

Five normal adult Beagle dogs and 5 normal mongrel pups were supplied for this study. All dogs, except the controls, were exposed by two or three routes to CHV. One to three months after inoculation, the dogs were confirmed to have latent infection, and three of each group were treated with PD for 5 or 3 consecutive days, respectively. In some of them, CHV was isolated after PD treatment. Moreover, after further confirmation of latent infection, PD was administered again to determine whether or not reactivation of latent CHV could be repeated. Both the first and second treatments provoked reactivation of CHV in 2 or 3 of the dogs from the two groups. Repeated reactivation of CHV was thus confirmed in the present study. High titers of virus were detected from nasal swabs. After the first series of treatments the virus titers tended to be higher than after the second series of

treatments. Also, the periods of excretion in the second series were shorter than found after the first series of treatments.

Herpesviruses are known to establish latent infections of the nervous system and to be excreted again after various stimili. In animals, this is true of AD virus, IBR virus, feline herpesvirus and equine rhinopneumonitis virus; in humans, it is recognized in HS virus and VZ virus. However, it is not possibly known if CHV reactivates occurs in dogs with a medical history of infection. In the present studies, the author showed for the first time that the latent infection by CHV was reactivated by PD treatment. The histopathological findings suggested that tonsil or nasal mucosa may be the primary sites of excretions and that trigeminal or lumbosacral ganglia may be sites of latency. These experiments also revealed the reactivation can be provoked in both adult dogs and pups. Moreover, the reactivation may be provoked repeatedly by treatment with an immunosuppressive drug.

CHV infections in young dogs usually do not cause clinical illness; only mild respiratory signs have been noted in the experimental cases. However, CHV infection of neonates or pregnant bitches could result in neonatal deaths, abortions or stillbirths, leading to huge loss of pet or laboratory dogs in breeding kennels. The present basic knowledge in the biological studies of CHV infection will be useful to understand prevention, therapy and control of the virus infection in dogs, and also elucidate the mechanism of latent infection in this disease by the

use of molecular biological methods.

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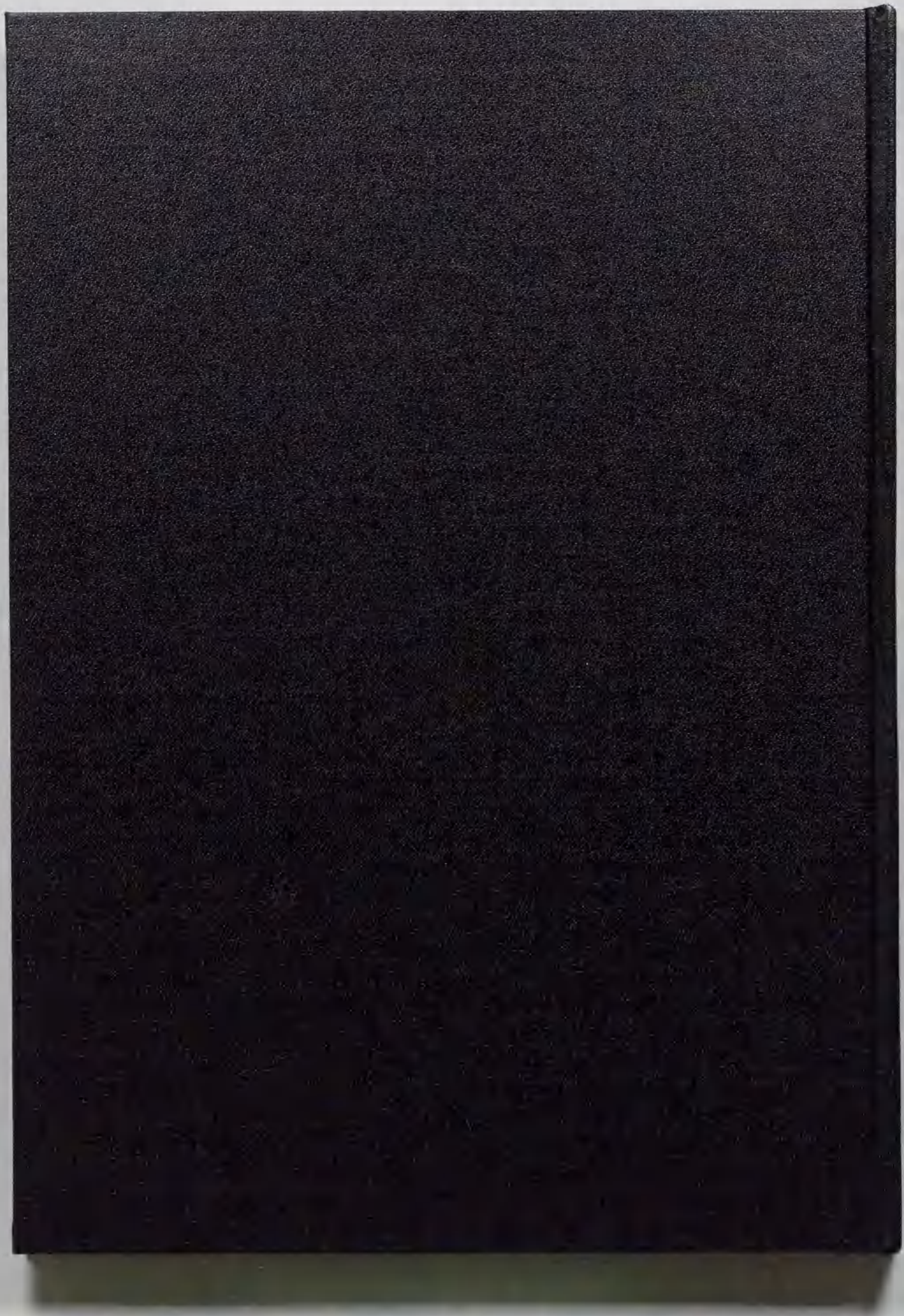

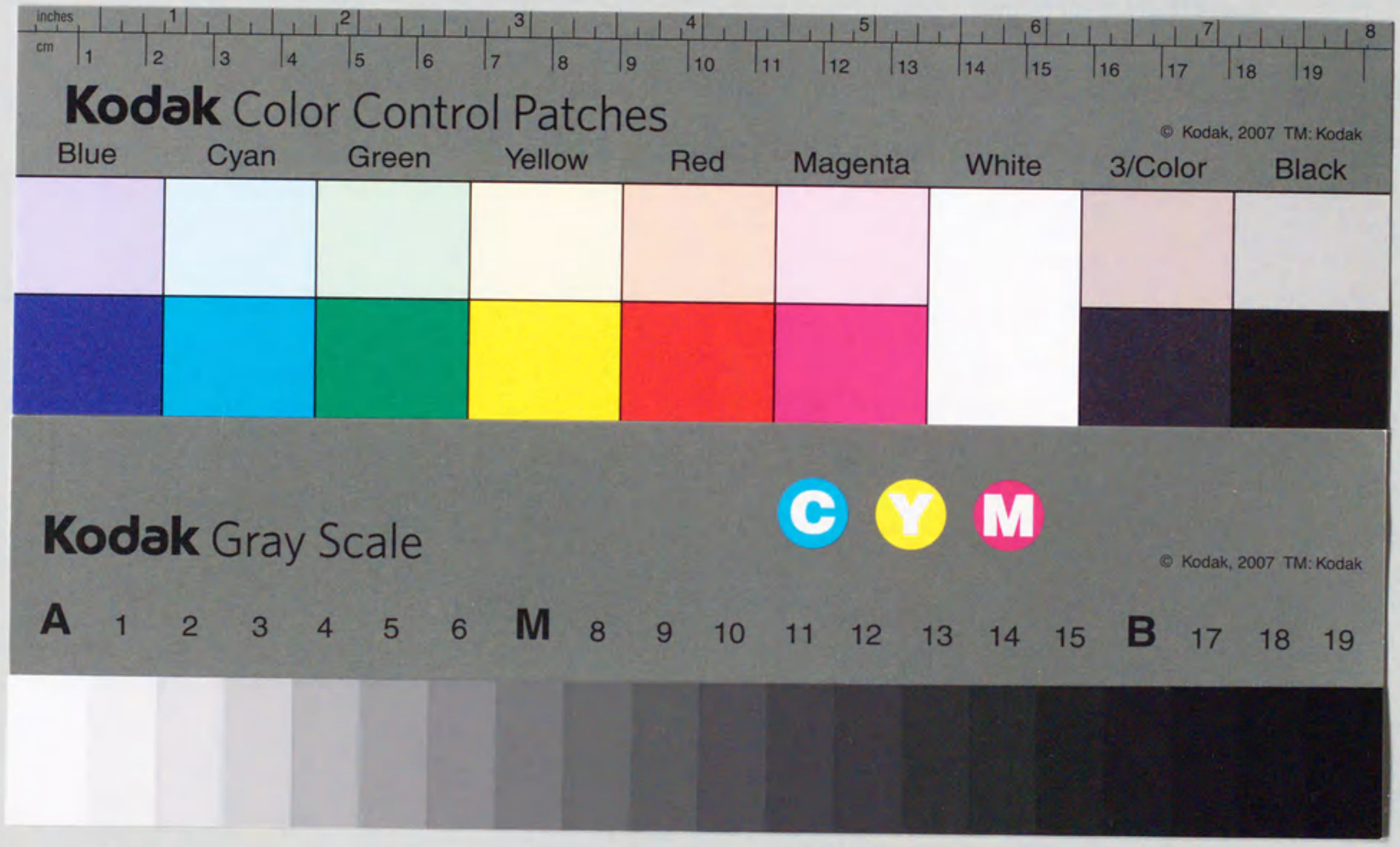
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