Studies on the Pathogenesis of Heart Lesions in Dogs Infected with Pseudorabies Virus

G.R. Olson and L.D. Miller*

ABSTRACT

Pseudorabies virus was inoculated by various routes into dogs to determine the relationship of pseudorabies virus to the development of heart lesions. Electrocardiograms and serum samples for lactate dehydrogenase isoenzymes were taken twice daily. Transitory and persistent arrhythmias were a consistent finding. Heart lesions were noted within each of the inoculated groups. These changes varied from severe multifocal areas of hemorrhage and myocardial degeneration to small zones of myolysis. A ganglioneuritis of the stellate ganglia and autonomic ganglia within the heart were also consistent findings. Herpes-like viral particles were found by electron microscopy in various autonomic ganglia and in myocardial endothelial cells. No viral particles were found in myocardial cells. Significant increases in lactate dehydrogenase-1 were noted. It was concluded that fatal arrhythmias resulted from pseudorabies virus infections in the dog after the occurrence of myocardial and ganglionic lesions.

Key words: Pseudorabies, Aujeszky's disease, *Herpesvirus suis*, arrhythmia, stellate ganglioneuritis, myocarditis.

RÉSUMÉ

Cette expérience consistait à inoculer le virus de la pseudo-rage à des chiens, par diverses routes, afin de déterminer la relation possible entre ce virus et le développement de lésions cardiaques. On procéda à des électrocardiogrammes et on préleva des échantillons de sang, pour la recherche des isoenzymes de la lactate-déshydrogénase, deux fois par jour. On observa constamment des arythmies transitoires et persistantes. On nota aussi des lésions cardiaques, indépendamment de la route d'inoculation. Ces lésions se traduisaient par de nombreux foyers d'hémorragie et de dégénérescence du myocarde, ou par de petites zone de myolyse. Une inflammation des ganglions stellaires et des ganglions autonomes du myocarde s'avéra aussi constante. La microscopie électronique permit de mettre en évidence des particules virales, semblables à un herpèsvirus, dans divers ganglions autonomes et dans les cellules endothéliales du mycarde. Les cellules musculaires du myocarde ne recelaient toutefois pas de particules virales. On enregistra aussi une élévation appréciable de la lactate-déshydrogénase #1. Les auteurs conclurent que les arythmies fatales résultaient de l'infection des chiens par le virus de la pseudo-rage et qu'elles survenaient après la développement des lésions du myocarde et des ganglions nerveux.

Mots clés: pseudo-rage, maladie d'Aujeszky, herpèsvirus porcin, arythmie, inflammation des ganglions stellaires, myocardite.

INTRODUCTION

Pseudorabies (Aujeszky's disease) is caused by a herpesvirus that is infectious to many domestic and wild animals. Dogs can be infected by a variety of routes experimentally. The disease has been initiated by feeding infectious material and by subcutaneous, intramuscular, intracranial and intraocular routes (1,2,3). Under natural conditions a number of reports indicate the source of virus to be virus-contaminated flesh from swine and sometimes of cattle or rats that was consumed by the dog (4,5,6).

Most reports about canine infections have focused mainly on lesions of the gastrointestinal tract, central nervous system or lesions associated with self-trauma ("mad itch"). Several investigators have alluded to a possible cardiomyotropism because acute myocardial inflammation has been noted (1,2). Also, the occurrence of degenerative lesions in the cardiac autonomic ganglia of pseudorabies-infected swine has led to questions about a possible cardioneurogenic pathogenesis of death due to a fatal syncope of the heart (7). The purpose of this study was to determine if pseudorabies virus (PRV) has a cardiomyotropism in the dog and to evaluate electrocardiographic tracings in dogs inoculated with PRV.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Twenty dogs of various breeds and sizes were obtained through the Laboratory Animal Resources (LAR) section at Iowa State University. These animals ranged from six months to four years of age. Upon arrival at the LAR farm, the dogs were administered vaccines for rabies (Rabguard-TC — Norden Laboratories, Lincoln, Nebraska); canine parvovirus, canine distemper, canine parainfluenza, canine hepatitis and leptospirosis (Sentrypar DHP/L — Beecham Laboratories, Bristol, Tennessee).

*Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50010.

Funds furnished by the USDA in cooperation with Iowa State University through General Cooperative Agreement No. 58-519B-9-821.

Submitted January 14, 1985.

Also, they were treated for intestinal parasites (Canopar — Burroughs Wellcome Co., Research Triangle Park, North Carolina) and blood was examined for microfilariae of *Dirofilaria immitis*. All dogs were serologically negative for pseudorabies antibodies using the microtitration serum neutralization test. The dogs were housed in individual cages in an isolation room.

Four dogs were randomly assigned to each of five groups (Table I).

Each of the inoculated animals was administered 2.3×10^6 plaque-forming units of PRV in 1 mL of Earle's balanced salt solution by the following routes: intravenous, intramuscular, subcutaneous and oral-nasal. Half of the inoculum was given by each route in the oral-nasal group using a sterile cannula.

TABLE I. Treatment Groups

Group		Dog Identification No.
I	Control (uninoculated)	7,9,16,20
II	Intravenous inoculation	1,8,11,14
III	Intramuscular inoculation	3,5,10,18
IV	Subcutaneous inoculation	2,6,13,17
V	Oral-nasal inoculation	4,12,15,19

ELECTROCARDIOGRAPHY

An initial tracing was procured on the day before inoculation. Subsequently, electrocardiograms were obtained twice daily from all dogs as they progressed through the experiment. A Burdick EK/SA electrocardiograph (Burdick Corp., Milton, Wisconsin) was used in making all tracings. The dogs were positioned in right lateral recumbency during the recordings, and a standard three-lead system was employed on all occasions. A paper speed of 50 mm/sec and a standard of 1 mV = 1 cm were used throughout the study.

LACTATE DEHYDROGENASE ANALYSIS

Blood specimens were collected twice daily by cephalic venipuncture. Collection of specimens started before viral inoculation and continued until death or euthanasia. Serum was obtained and held at room temperature. Total lactate dehydrogenase (LDH) and LDH isoenzyme analyses were performed within 48 hours.

Lactate dehydrogenase isoenzyme values were ascertained according to the prodecure as described in Gelman's LDH Isoenzyme Substrate Set, product number 51233 (Gelman Sciences Inc., Ann Arbor, Michigan). Total lactate dehydrogenase values were determined according to the procedure as described in Worthington's Statzyme LDH (L-P), product number 27601 (Worthington Diagnostic Systems Inc., Freehold, New Jersey).

VIRAL INOCULUM

A field isolate of a virulent Iowa strain of PRV was propagated on porcine kidney cells, and a pool of virus from the fourth tissue culture passage was frozen at -70° C. The titer was determined to be 2.3×10^6 plaque-forming units per mL.

LIGHT MICROSCOPY

Tissues sampled for light microscopy were: right and left atria; right and left ventricles; interventricular septum; right and left stellate (cervicothoracic) ganglia; right and left vagal nerves; right and left sympathetic trunks; lung and liver. These tissues were fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 6 μm. All sections were stained with hematoxylin and eosin.

ELECTRON MICROSCOPY

Tissues sampled for electron microscopy were: right and left atria; right and left ventricles; right and left stellate ganglia; right and left vagal nerves; and the right and left sympathetic nerve trunks. Fixed tissues were cut into 1 mm^3 pieces, postfixed with 1% buffered osmium tetroxide,

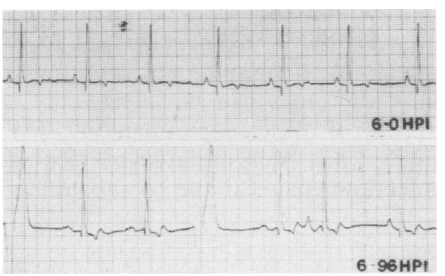

Fig. 1. The electrocardiogram (Lead II) of dog 6 recorded at 96 HPI features several premature ventricular complexes.

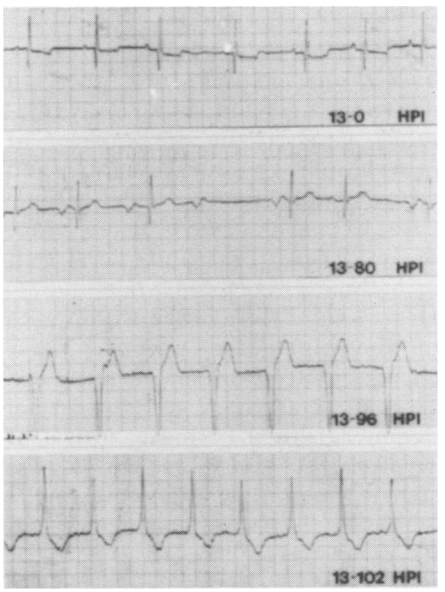

Fig. 2. The electrocardiogram (Lead II) of dog 13 recorded at 80 HPI features a second degree heart block and a wandering pacemaker. At 96 HPI ventricular tachycardia is prominent, while atrial fibrillation is noted at 102 HPI.

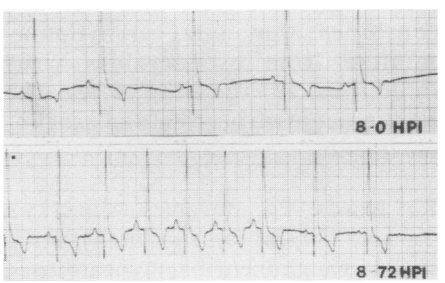

Fig. 3. The electrocardiogram (Lead II) of dog 8 recorded at 72 HPI features an atrial tachycardia.

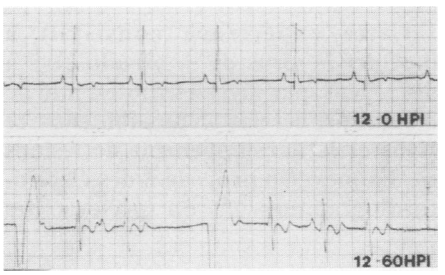

Fig. 4. The electrocardiogram (Lead II) of dog 12 recorded at 60 HPI features premature ventricular complexes and an atrioventricular dissociation.

embedded in EM-bed-812 (Electron Microscopy Sciences, Fort Washington, Pennsylvania) and cut on an LKB ultratome (LKB Instruments, Inc., Rockville, Maryland). Thick sections were stained with 1% toluidine blue. Specific areas were selected, and thin sections were cut at 50 nm, stained with uranyl acetate and lead citrate and viewed with a Hitachi HS-9 electron microscope (Hitachi, Ltd., Tokyo, Japan).

NECROPSY

All dogs were necropsied promptly after death except dogs 3, 8 and 15 which died unexpectedly overnight. Dogs 2, 11, 12, 18 and 19 died during the observation period, and dogs 1, 4, 5, 6, 7, 9, 10, 13, 14, 16, 17 and 20 were euthanized. Gross lesions were recorded, and tissues were taken for histopathology and electron microscopy as listed previously.

RESULTS

ELECTROCARDIOGRAPHY

All inoculated and control dogs had normal heart rhythm patterns in Lead II at the beginning of the experiment. Electrocardiographic abnormalities documented after inoculation are listed in Table II.

Every inoculated dog except dog 10 exhibited an electrocardiographic change. The majority of these alterations occurred between 60 and 96 hours postinoculation (HPI).

LACTATE DEHYDROGENASE ANALYSES

The results of the statistical evaluation of total LDH and LDH isoenzyme values are shown in Table III. This evaluation of the variables was modified by recalculating and analyzing the log of the response rather than the response itself. This variance stabilizing transformation was needed due to the lack of homogeneity and large variances within the groups. Statistical significance at $P < 0.05$ was used in contrasting control and inoculated groups' means using a one-way analysis of variance.

TABLE II. Electrocardiographic Abnormalities in Dogs Inoculated with PRV

Electrocardiographic Abnormality	Group	Dog Identification No. II	III	IV	V
Ventricular premature complexes		11	5,18	6[a], 13,17	4,12,15
Ventricular tachycardia			5,18	13[b]	4
Idioventricular rhythm				2	
Sinus arrest			3		
Atrial tachycardia		8[c]			
Atrial fibrillation				13[b]	
Second degree heart block		1,11	3,5	13[b]	
Fusion beats		1,14	5	17	4,19
Wandering pacemaker		1,14	3,5	17	19
AV dissociation			5		4,12[d],19
T-wave abnormalities[e]		1,8,11,14	3,5,18	13,17	4,12
P-wave abnormalities		1,8,11,14	3,5	6,17	4

[a]Fig. 1
[b]Fig. 2
[c]Fig. 3
[d]Fig. 4
[e]T- and P-wave abnormalities indicate a change in duration and/or amplitude of contraction

TABLE III. Postinoculation Means[a] of LDH in Controls and Dogs Inoculated with PRV

Group	Total LDH	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
			Untransformed Means			
I	22.00	3.30	2.27	5.02	3.50	6.02
II	59.66	20.86	11.33	14.20	5.93	7.66
III	216.75	49.47	50.00	51.75	22.65	41.72
IV	222.50	51.30	49.97	54.90	21.67	44.45
V	85.25	23.45	15.55	23.72	8.70	13.67
II-V	146.04	36.27	31.71	35.89	14.73	26.87
			Transformed Means			
I	1.31	0.61	0.45	0.73	0.54	0.68
II	1.78	1.33	1.06	1.17	0.83	0.88
III	2.00	1.37	1.28	1.35	1.09	1.26
IV	2.32	1.65	1.64	1.72	1.28	1.58
V	1.81	1.22	1.03	1.30	0.91	1.08
II-V	1.97[b]	1.39[b]	1.25[b]	1.38[b]	1.02	1.20

[a]Means recorded in IU/L
[b]Statistically significant ($P < 0.05$)

Statistical significance was documented between the inoculated groups (II-V) and the control group in the following categories: total LDH, LDH-1, LDH-2 and LDH-3.

GROSS LESIONS

Various degrees of endocardial, myocardial and epicardial hemorrhages were evident in the right and/or left ventricles in the following dogs: 1, 2, 3, 5, 11, 12, 13, 15 and 18. The right atrium had hemorrhagic foci in dogs 3 and 5. Most of these dogs had diffuse pulmonary congestion, and dogs 8, 11, 13 and 18 had a marked amount of pulmonary edema.

MICROSCOPIC LESIONS

All members of the intravenously inoculated group (dogs 1, 8, 11 and 14) had necrosis along with inflammatory cell infiltrates of neutrophils and lymphocytes associated with autonomic ganglia and nerve tracts of the right atrium. Pale basophilic intranuclear inclusions were pronounced within neurons (Fig. 5). Vasculitis and perivasculitis with extension into the surrounding myocardium was marked. This inflammatory response consisted of lymphocytic and neutrophilic infiltrates along with hemorrhagic foci. The sarcoplasm of some individual myofibers was swollen, granular and deeply eosinophilic. Hemorrhage was accompanied by myocardial degeneration in both the right and left ventricles of dogs 1 and 11 and to a lesser degree in dog 8. Hemorrhage was also obvious in the subendocardial regions of the ventricles in these three dogs. The inflammatory response was similar to that described in the atria but with more hemorrhage and less cellular infiltration. The stellate ganglia in dogs 1, 8 and 11 had prominent lymphocytic infiltrates, multifocal areas of necrosis and intranuclear inclusion bodies within neurons (Fig. 6).

Atrial lesions as previously described were present in dogs 3 and 5 of the intramuscularly inoculated group. The lesions were more extensive on the right side. Ventricular lesions were well marked in dogs 3, 5 and 18, with most of these changes in the left ventricle. Dogs 5 and 18 had a very striking fibrinoid vasculitis within the myocardium. The stellate ganglia of dogs 3 and 5 displayed changes as noted earlier in other groups.

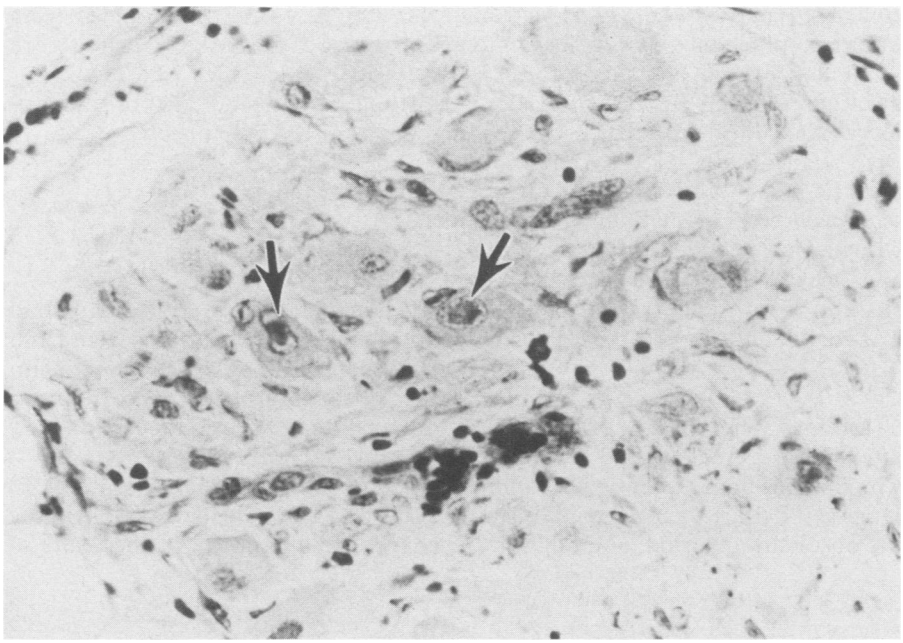

Fig. 5. An autonomic ganglion in the right atrium of dog 1 features distinct intranuclear inclusion bodies (arrows) within neurons. X450.

Dogs 2, 6 and 13 of the subcutaneously inoculated group had atrial lesions as previously described. Endocardial and myocardial ventricular lesions were prominent in dogs 2 and 13. Dog 6 showed only minor changes in the ventricles. Ganglioneuritis of the stellate ganglia and sympathetic nerve trunks was evident in dogs 2, 13 and 17.

The oral-nasally inoculated group (dogs 4, 12, 15 and 19) all had

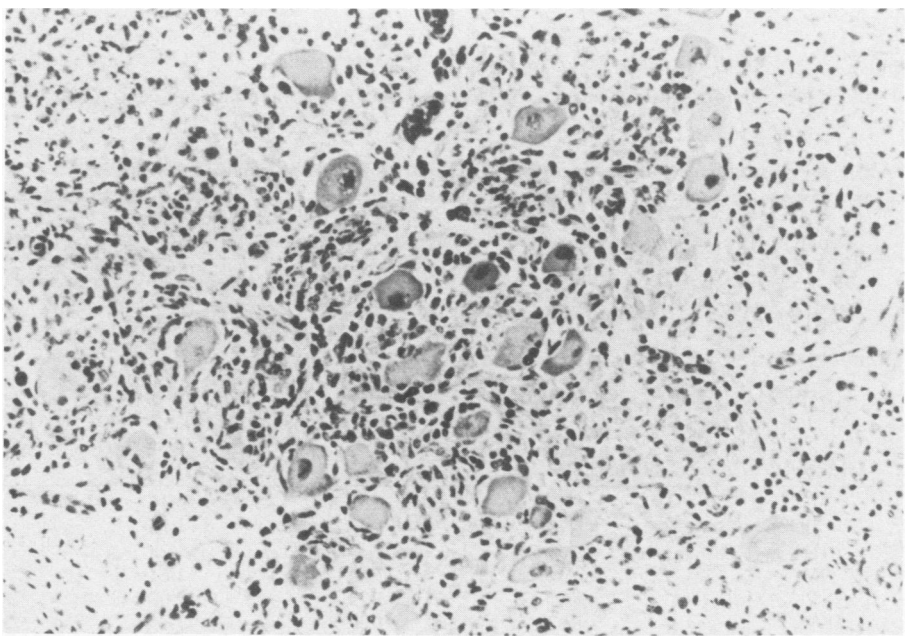

Fig. 6. The left stellate ganglion of dog 1 features a pronounced lymphoplasmacytic infiltrate, neuronal degeneration and intranuclear inclusion bodies. X180.

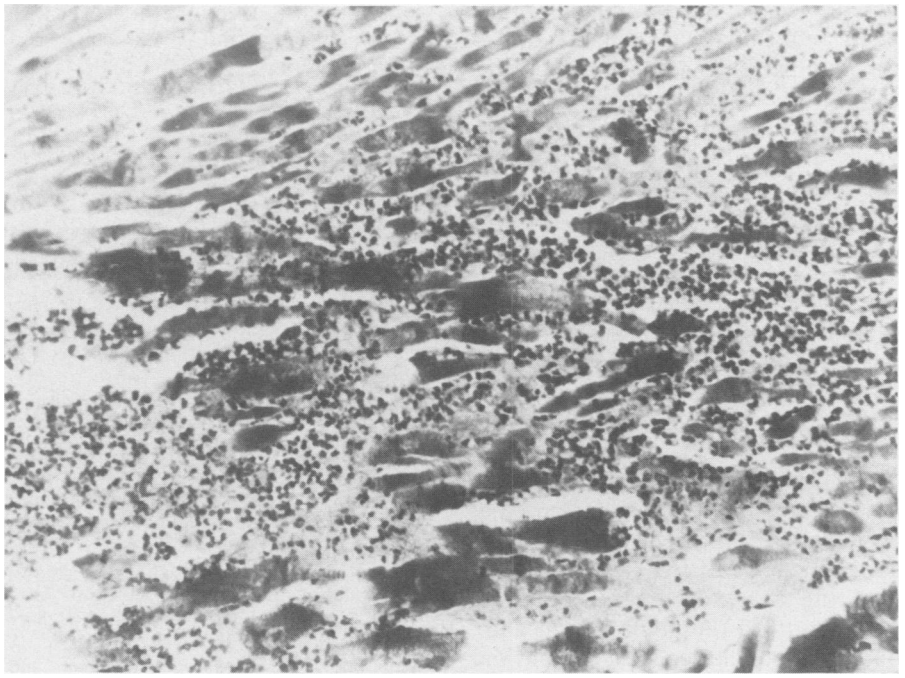

Fig. 7. Extensive focus of hemorrhage and coagulative necrosis in the left ventricle of dog 12. X180.

myocardial lesions in both the right and left ventricles (Fig. 7). Atrial changes were noted in dogs 4, 12 and 19. A stellate ganglioneuritis in dogs 4, 12 and 15 was very apparent.

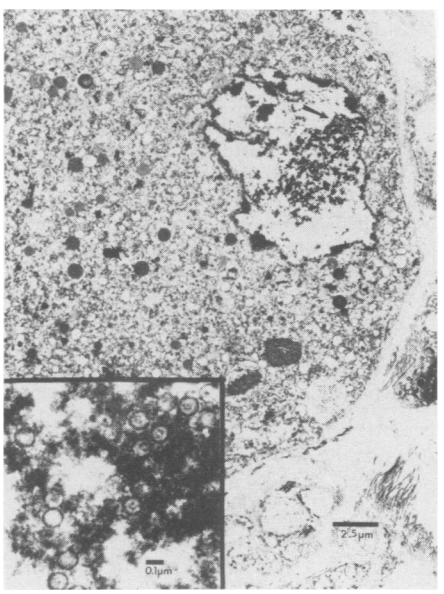

Fig. 8. Electron micrograph of the left stellate ganglion of dog 2. Note margination of nuclear chromatin and the presence of nucleocapsids in the nucleus. X6,000. Arrow indicates area of the insert.
Insert: Higher magnification of herpes-like nucleocapsids in the karyoplasm. X77,000.

No microscopic lesions were noted in the control group.

ELECTRON MICROSCOPY

In the selected tissues examined from each of the groups, herpes-like viral particles were demonstrated in neurons and satellite cells in the sympathetic nerve trunks, stellate ganglia and autonomic ganglia of the right atrium (Fig. 8). Virus was also found within endothelial cells in the myocardium. Viral particles were approximately 120 nm in size and were noted singly and in small clusters. The position of these particles was mainly intranuclear, but cytoplasmic particles were also seen. The cells which contained the icosahedral nucleocapsids had marked margination of nuclear chromatin. After an extensive search of over 150 grids of selected heart samples, no viral particles were found in myocardial cells.

DISCUSSION

Electrocardiographic abnormalities are common in various viral cardiomyopathies and were observed in 15 of 16 inoculated dogs in this study. With coxsackievirus infections in humans, electrocardiographic tracings are virtually always abnormal. Arrhythmias with ST segment and T wave changes, often ventricular in origin with AV conduction disturbances, are common (8). Similar electrocardiographic changes have been noted in patients with influenza and poliomyelitis infections (9,10). Cytomegalovirus and herpes simplex virus have also produced similar variations (11). Electrocardiographic abnormalities associated with parvovirus in the canine are well documented (12).

Many of these same electrocardiographic changes were common in this research. Most of these alterations were transient and nonspecific, but some were persistent and refractory. Refractory arrhythmias can cause sudden death and are the most likely reason for some unexplained deaths with PRV.

The correlation of electrocardiographic changes with myocardial damage in this study was striking. All the animals which presented electrocardiographic abnormalities had heart lesions of varying degrees.

The intravenous and intramuscular routes of infection were associated with the most severe lesions, while the oral-nasal and subcutaneous groups were affected but to a lesser degree. Ventricular endocardial and myocardial hemorrhage, myocytolysis, and necrosis with inflammation were the most frequent findings. The left ventricle was more consistently affected than the right. Atrial lesions were similar but not as extensive in most cases. Many of the lesions were associated with inflammatory reactions involving blood vessels, nerves or ganglia. Myocardial damage appeared to radiate from these areas.

The examination of lactate dehydrogenase levels revealed significant ($P < 0.05$) increases in total LDH and in the isoenzymes LDH-1, LDH-2 and LDH-3, in inoculated dogs. Since these three fractions contain mostly polypeptide chains of myocardial origin, with LDH-1 being heart-specific, the levels observed would suggest myocardial necrosis (13).

It has been suggested by Dow and McFerran that cardiac lesions might be dependent on a hematogenous dissemination of the virus (4). In this project, cardiac lesions were docu-

mented by all routes of inoculation, including the more natural oral-nasal route.

Since PRV was demonstrated in injured endothelial cells in the myocardium by electron microscopy and not in myocardial cells, the cause for myocardial hemorrhage and necrosis may be endothelial damage after hematogenous dissemination. However, we favor the hypothesis that excessive sympathetic cardiac stimulation, associated with neuritis and ganglioneuritis, can cause the same types of lesions.

Nearly 80% of the dogs with myocardial lesions also featured marked changes in the stellate ganglia with various types of infiltrating inflammatory cells, necrosis and herpes-like intranuclear inclusion bodies within neurons and satellite cells. The remaining dogs had milder lesions in these same structures.

Investigations on electrical stimulation of stellate ganglia leading to myocardial hemorrhage and necrosis suggests that the reduced end-systolic volume due to an increased force of contraction results in mechanical damage within the heart (14). Also, the oxygen demand of the overstimulated heart is markedly increased and may lead to relative ischemia which contributes to lesion formation (14).

Cardiac sympathetic sensory endings with myelinated and unmyelinated fibers are excited by ischemia and are capable of eliciting a cardiocardiac sympathetic reflex. This sympathetic reflex, which takes place within a few seconds after the onset of ischemia, plays an important role in the genesis of early ventricular arrhythmias. The excitation of cardiac sympathetic afferents can also reflexly inhibit the activity of efferent vagal cardiac fibers, which slow the heart rate. Inhibition of vagal impulses impairs the maintenance of an optimal heart rate and facilitates the occurrence of dangerous tachycardia (15,16). Also, pathological axons have an extensive repetoire of inappropriate ectopic excitability, including spontaneous activation, repetitive responses to single stimuli and mechanosensitivity (17). In rats infected intraocularly with pseudorabies virus, the neurons of the superior sympathetic ganglia show a spontaneous activity characterized by periodic bursts of action potentials recorded on both the post and presynaptic nerves (18).

We concluded that PRV infections in the dog likely cause myocardial damage by producing a marked ganglioneuritis of the stellate ganglia resulting in excessive sympathetic stimulation to the myocardium and/or by endothelial cell disruption and resultant myocardial ischemic damage. The lesions resulting from either or both of these mechanisms can initiate arrhythmias which may lead to sudden death.

ACKNOWLEDGMENTS

The authors wish to thank Kay Pierce, Jane Fagerland, Debora Hoyt, Michael Meetz, James DeLano and Jacqueline Piepkorn for their technical assistance in this study.

REFERENCES

1. **PENSAERT MB, COMMEYNE S, ANDRIES K.** Vaccination of dogs against pseudorabies, using an inactivated virus vaccine. Am J Vet Res 1980; 41: 2016-2019.
2. **FLIR K.** Zur Pathologie der Morbus Aujeszky beim Hund. Arch Exp Veterinarmed 1955; 9: 949-956.
3. **EIDSON ME, KISSLING RE, TIERKEL ES.** Pseudorabies virus infection in dogs. J Am Vet Med Assoc 1953; 123: 34-35.
4. **DOW C, McFERRAN JB.** Aujeszky's disease in the dog and cat. Vet Rec 1963; 75: 1099-1102.
5. **GORE R, OSBORNE AD, DARKE PG, TODD JN.** Aujeszky's disease in a pack of hounds. Vet Rec 1977; 101: 93-95.
6. **GUSTAFSON DP.** Pseudorabies in dogs and cats. Current therapy VIII. 8th ed. Philadelphia: W.B. Saunders Co., 1983.
7. **BECKER CH, BERGMAN V.** Primary lesions in cardiac ganglia of the pig after *Herpesvirus suis* infection. Am J Vet Res 1968; 29: 745-748.
8. **SMITH WG.** Coxsackie B myopericarditis in adults. Am Heart J 1970; 80: 34.
9. **VEREL D, WARRACK AJ, POTTER CW, WARD C, RICKARDS DF.** Observations on the A2 England influenza epidemic: A clinicopathological study. Am Heart J 1976; 92: 290.
10. **WERNSTEIN L, SHELOKOV A.** Cardiovascular manifestations of acute poliomyelitis. New Engl J Med 1951; 244: 281-284.
11. **BRAUNWALD E.** Heart disease. A textbook of cardiovascular medicine. 2nd ed. Philadelphia: W.B. Saunders Co., 1980.
12. **LENGHAUS C, STUDDERT MJ.** Acute and chronic viral myocarditis. Am J Pathol 1984; 115: 316-319.
13. **LOTT JA, STANG JM.** Serum enzymes and isoenzymes in the diagnosis and differential of myocardial ischemia and necrosis. Clin Chem 1980; 26: 1241-1250.
14. **KLOUDA A, BRYNJOLFSSON G.** Cardiotoxic effects of electrical stimulation of the stellate ganglia. Ann NY Acad Sci 1969; 156: 271-280.
15. **SCHWARTZ PF, STONE HL.** Left stellectomy in the prevention of ventricular fibrillation caused by acute myocardial ischemia in conscious dogs with anterior myocardial infarction. Circulation 1980; 62: 1256-1265.
16. **SCHWARTZ PF, STONE HL, BROWN AA.** Effects of unilateral stellate ganglion blockage on the arrhythmias associated with coronary occlusion. Am Heart J 1976; 92: 589-599.
17. **RASMINSKY M.** Ectopic impulse generation in pathological nerve fibers. Trends Neurosci 1983; 6: 388-390.
18. **DEMPHER J, LARRABEE MG, BANG FB, BODIAN D.** Physiological changes in sympathetic ganglia infected with pseudorabies virus. Am J Physiol 1955; 182: 203-216.