The pathogenesis of pseudorabies (Aujeszky's disease) virus infection in sheep following intratracheal exposure

Stephen Peter Schmidt
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THE PATHOGENESIS OF PSEUDORABIES (AUJESZKY'S DISEASE) VIRUS INFECTION IN SHEEP FOLLOWING INTRATRACHEAL EXPOSURE

Iowa State University

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The pathogenesis of pseudorabies (Aujeszky's disease) virus infection in sheep following intratracheal exposure

by

Stephen Peter Schmidt

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Veterinary Pathology

Approved:

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For the Graduate College

Iowa State University
Ames, Iowa
1985
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GENERAL INTRODUCTION

Pseudorabies (Aujeszky's disease), described originally in a dog, cat, and ox by Aujeszky in 1902 (2), is a disease in which swine are the natural host and a source of the infection for nonporcine species (10). Recognized since 1910 as capable of affecting sheep, the ovine disease is characterized by severe focal pruritus, self-mutilation, and death (115). Reports of pseudorabies (Pr) outbreaks in sheep, predominantly from continental Europe (11, 15, 17, 71, 81, 113, 117, 121), have also come from South America (20), the British Isles (77), and the United States (61, 122). The disease is economically important in the midwestern United States due to its increasing incidence and virulence in swine and also because of subsequent outbreaks in nonporcine species (42). From 1969 to 1973, an average of 5 submissions per year were diagnosed by the Iowa State University Veterinary Diagnostic Laboratory (VDL) in Ames, Iowa, followed by a steady increase from 28 submissions in 1974 to 352 in 1977 (50). From 1980 to 1984, the number of submissions ranged from 321 to 581; the nonporcine submissions, of which sheep comprised from 2 to 16%, varied from 122 to 197.

Initially, rodents (81) or dogs (71, 121) were presumed to carry the virus. After Kojnok's epidemiological studies in sheep and cattle, porcine secretions containing pseudorabies virus (PrV) were recognized as a source of PrV spread from swine to sheep (69). Dow and McFerran's experimental work on the pathogenesis of Pr in sheep substantiated the susceptibility of the species to subcutaneous, oral, intranasal, and intraconjunctival inoculation (35, 36, 87). They described the clinical
signs, characterized the nature and distribution of histological lesions (35,36), and demonstrated the viral distribution in neural and nonneural tissues of infected sheep (87).

Aerosol transmission is responsible for the spread of many pathogenic and nonpathogenic viruses among animals (64). The spread of aerosolized PrV among swine on the same and different premises has been documented by Donaldson et al. (32) and Gloster et al. (43). In one particular Iowa outbreak of Pr in sheep, there was evidence for the aerogenous spread of PrV from sows to ewes and lambs (114, Part I). Necrotizing bronchopneumonia with intranuclear herpetiform inclusion bodies and nucleocapsids in the parabronchial ganglionic neurons were evidence of primary viral inoculation via inhalation.

The virus isolated from this outbreak was propagated by the personnel of the VDL, Ames, Iowa. Restriction endonuclease analysis of the isolate was performed by the staff of the National Animal Disease Center, Ames, Iowa. From this propagated virus, an inoculum was prepared to inoculate intratracheally 24 experimental animals for the pathogenesis study.

The purpose of this research was to investigate the pathogenesis of PrV infection in sheep using the ovine isolate under conditions similar to those which were observed and could potentially recur in the field. The intratracheal route was selected to simulate respiratory exposure to PrV contaminated air. The objectives of the investigation were 1) to trace the route and time sequence of PrV spread from the lungs and respiratory tract to the brain by virus localization and lesion
development, 2) to correlate neural lesions with clinical signs, 3) to identify the location of virus within the affected tissues, and 4) to evaluate nasal mucus and urine as sources of virus for potential horizontal spread of PrV among sheep.

The first manuscript in this dissertation reports the field outbreak of ovine pseudorabies from which the PrV used in these experiments originated. Included in the paper are the clinical history of the sheep, their histological lesions, and the restriction endonuclease analysis of the virus. The article further provides a vivid example of how a disease outbreak impacting an Iowa food and fiber producer raised specific questions which were investigated through research.

The second manuscript describes the author's attempt to use the virus isolated from the outbreak to duplicate in experimental sheep the natural disease process. The intratracheal route of inoculation was chosen to bypass the mucosal surfaces innervated by the dendrites of neurons in the trigeminal ganglion and to ensure the distribution of aerosolized PrV into the lower respiratory system. It was hoped that this procedure would mimic the inhalation of virus-contaminated air under field conditions. The clinical signs, histological lesions, viral distribution, and epidemiological implications of possible viral shedding in nasal mucus are discussed.

The third manuscript is a short anatomical study of the sympathetic ganglionic and general visceral afferent neural tissues affected by PrV.
in intratracheally inoculated sheep. This article describes the location and distribution of these neurological structures.

The three manuscripts constitute this dissertation which is presented in the alternate format. Each paper complies with the format required by the Canadian Journal of Comparative Medicine. This General Introduction and an extensive Literature Review precede the manuscripts. After the third manuscript is a General Discussion and Summary followed by a Bibliography containing references cited throughout this dissertation. These references are in alphabetical order and are consistent with the format of the Canadian Journal of Comparative Medicine. The dissertation concludes with an appendix which contains detailed tables summarizing the histopathological results.

The Ph.D. candidate, Stephen Peter Schmidt, was the principal investigator in each of the studies and senior author of each of the manuscripts. The contributions of the co-workers and technical assistance from other sources for all the manuscripts are listed in the Acknowledgments following the Bibliography.

The Iowa State University Committee on the Use of Animal Subjects in Research reviewed and approved funding for this project and concluded that the rights and welfare of the animal subjects were adequately protected, that risks were outweighed by the potential benefits and expected value of the knowledge sought, that confidentiality of data was assured and that informed consent was obtained by appropriate procedures.
LITERATURE REVIEW

Historical Aspects

In 1902, Aladar Aujeszky, a Hungarian physician, described a disease similar to rabies in an ox, dog, and cat (2). In each case, he reproduced the disease in rabbits after subcutaneously injecting emulsified brain tissues. Furthermore, he described the clinical signs, proposed that natural viral entry resulted from injury to skin or oral mucosa, and recognized that the central nervous system (CNS) contained the virus. He also observed that the disease was not contagious among laboratory animals and could be differentiated from rabies by clinical signs in experimental animals. Today the disease is called Aujeszky's disease in Europe and pseudorabies (Pr) in North America. Other synonyms are mad itch and infectious bulbar paralysis.

In 1910, Schmiedhoffer cited outbreaks of Pr in Hungary and Croatia (115). Working under Aujeszky, he recovered pseudorabies virus (PrV) from the various organs and excretions of experimental rabbits. He also studied the resistance of this virus to storage, temperature, and disinfectants, as well as investigated the susceptibility of dogs, cats, foxes, sheep, cattle, horses, donkeys, pigeons, and chickens to pseudorabies virus. He concluded that the virus entered the body through a break in the skin or mucosa, replicated at the point of entry, and spread by lymphatics or blood to the brain.

In 1911, Zwick and Zeller cited several new Hungarian cases of Pr in cats, dogs, cattle, oxen, and rats (129). They described the clinical signs; tested laboratory animals, including one sheep, for
sensitivity to PrV; and evaluated the contagiousness of the agent and its resistance to drying, heat, storage, and disinfectants.

In 1914, Von Ratz cited Pr outbreaks in Siberia and Brazil (105). Most significant, however, was his observation of Pr in both a wild and a domestic pig.

The earliest known cases of Pr in America were cited by Hanson, who wrote a history of Pr in the United States (52). He noted several articles reporting enzootics of mad itch in the 1800s, the earliest published in 1813.

In 1930, Shope reported an outbreak of Pr among cattle in contact with swine; it was the first North American case of Pr confirmed in a laboratory (118). He proved that a filterable virus neutralizable by immune serum caused the disease. By using cross neutralization, he also demonstrated that the virus was closely related to that isolated by Aujeszky. Shope concluded that Pr was highly contagious and that swine were a source of infection to cattle.

By 1935, enzootics of Pr were reported in North America, France, Switzerland, Holland, and Germany (40). During the 1930s, researchers began PrV inoculation studies in swine (40, 57, 73). For example, Köves and Hirt sought to demonstrate that porcine PrV could occur without nervous signs (73). They evaluated the incubation time, duration of illness, clinical signs, pathological changes, and distribution of the virus in fattening swine.

In 1935, Hirt reported that shortly after Pr appeared in sows, suckling pigs developed nervous disorders and died (57). In some
instances, sows whose litters had perished were free of clinical signs. He concluded that piglets were more susceptible than adults to the virus.

The last significant contribution in the 1930s was Gerlach and Schweinburg's sequel (41) to their work published in 1935 (40). In this 1937 article, they evaluated different strains of PrV by using several routes of inoculation in various hosts (41).

Identification of Pr as a major disease has been increasing worldwide since 1961, particularly in the United States, where swine producers nationwide recognize the disease (47). Because infected swine are the natural reservoir of the virus and a source of infection to other animal species (10), a discussion of the clinical signs, pathogenesis, lesions, latency, and viral shedding is in order.

**Pseudorabies in Swine**

The variability of Pr in swine depends upon the age and immune status of the host, strain of virus, mode of infection, and viral dose (5,7,9,10). The management practices and weather significantly influence the disease (58).

Mortality due to pseudorabies is highest in suckling pigs. The clinical signs are dyspnea, gastrointestinal upsets, trembling, and CNS disorders terminating in coma and death (47,73,82). In weaned pigs, mortality is usually less, but clinical signs in the fatal syndrome are similar to those observed in neonates (47). In adult swine, mortality is very low but morbidity is high. Many have respiratory disturbances, fever, gastrointestinal upsets, and possibly CNS disorders (47,73,82).
In pregnant swine, the virus crosses the placenta to enter the fetus and causes embryonic death, fetal maceration, abortions, and stillbirths (47,68,127).

The pathogenesis of Pr begins when virulent virus contacts the pigs' oronasal mucosa by inhalation or ingestion (47,111). The primary viral attachment site during the incubation period is the epithelium of the nasopharynx and tonsil (111). The virus replicates in these cells, which consequently undergo necrosis and release more virus to the adjacent tissues including the lymphatics and nerves (112). In the lymphatics the virus is transported to the regional lymph nodes, then replicates in the sinusoidal endothelial cells and lymphoid cells (103). The virus enters the blood (10) and causes a transient leukocyte associated viremia one to 8 days post infection (10,126).

The virus enters the CNS by ascending the cranial nerves by centripetal axoplasmic transport (86). The virus can travel up the olfactory nerves into the olfactory bulbs or via the glossopharyngeal nerve from the pharynx to the solitary nucleus in the medulla (88,111). It can also enter the trigeminal nerve, ascend to the trigeminal ganglion, and move into the trigeminal nuclei of the pons and medulla (88).

The macroscopic lesions in the CNS are minimal; however, meningeal congestion occurs (68) along with ependymal petechiae of the olfactory bulb (24). Small white foci of necrosis appear in the spleen, liver, kidneys, lungs, regional lymph nodes, and adrenal glands (24,60). Lesions are also observed within the upper and lower respiratory tract.
and include fibrinopurulent exudates and mucosal necrosis of nasal turbinates, epiglottis, and pharynx (1,68). The fibrinopurulent exudates extend distally into the trachea and bronchi. A fibrinous pericarditis and pleuritis have also been observed (68).

The microscopic lesions in the CNS are characterized by a diffuse nonsuppurative meningoencephalitis and ganglioneuritis (1,34,99). The perivascular cuffing of neural vessels by lymphocytes, macrophages, eosinophils, and neutrophils from one to 8 cell layers thick is common. The neuroparenchyma frequently contains focal necrosis, malacia, demyelination, edema, microglial nodule formation, oligodendroglial proliferation, and neuronophagia (1,34,99). Cell degeneration and intranuclear inclusion bodies have been observed in the neurons (24). Chromatolysis is also evident in neuronal and microglial nuclei (34).

The distribution of lesions is widespread in the cerebrum, with the gray and white matter equally affected (34). In the medulla, the changes are more numerous in the rostral portion than in the spinal cord. In the cerebellum, the lesions are common in the folia.

Within the spinal dorsal root (16,124), cardiac (13), and mesenteric ganglia (95), focal areas of necrosis and mononuclear cell infiltration have been observed. In cardiac ganglia, distinct intranuclear inclusion bodies were found in some of the neurons (13).

The turbinate and pharyngeal mucosa contain necrotic foci infiltrated with mixed leukocytes (1,9,26). Necrosis of the epithelium and lymphoid follicles of the tonsils may extend deep into the tissues (93). A necrotizing vasculitis of tonsillar vessels has also been
observed (92). The lower respiratory tract lesions include necrotizing tracheitis, bronchitis, bronchiolitis, and alveolitis (4,6,8). Eosinophilic intranuclear inclusion bodies are frequently observed in the proliferating and sloughed epithelial cells. In the later stages of infection, a lymphoreticular reaction develops peripheral to the necrotic foci. The other visceral organs including spleen, liver, heart, intestine, adrenal gland, testicle, eye, lymph nodes, placenta, and kidney may have areas of coagulative necrosis and vasculitis (1,24,58,60,68,92,93,94,99).

Pseudorabies virus, which has an affinity for ganglionic tissues, can produce latent and recurrent infections in swine (25,49). In latently infected swine, the PrV can be demonstrated by co-culture, tissue fragment, and RNA-DNA hybridization techniques (14,48,110).

Swine are the natural host of pseudorabies virus (10). The shedding of this virus by acutely or latently infected swine is the source of infection not only to other pigs (10,70) but also to other nonporcine hosts including cats (2,50), dogs (37), cattle (70,77,118,122), and sheep (11,69,117,122).

**Pseudorabies in Sheep**

The earliest record of Pr in sheep is from Schmiedhoffer in 1910 (115). He subcutaneously injected sheep with brain emulsions and blood from animals that had died of pseudorabies. Zwick and Zeller in 1911 (129) and Gerlach and Schweinburg in 1935 (40) also tested sheep's susceptibility to pseudorabies virus.

The early field cases of ovine Pr were reported by Kolonits in 1912.
followed by the deaths of lambs, ewes, and cows (122). The sheep had been separated from the swine by a board fence. Further deaths were prevented by separating the cattle and sheep from the swine by a double row of fencing. The surviving sheep were found sero-negative to PrV by serum neutralization tests.

Recently, the extensive use of live PrV vaccines in swine has resulted in unusual iatrogenic lamb exposures to vaccine strain pseudorabies virus. Syringes previously used to vaccinate pigs for Pr and then improperly sterilized have been used to vaccinate lambs, usually with disastrous results (72,123).

The major experimental work on ovine Pr was done by Dow and McFerran (35,36,87). After administering the virus by scarification, subcutaneous injection, and intranasal, oral, and conjunctival inoculation, they described the resulting clinical signs and the gross and microscopic lesions (35). Later, they discussed the viral isolations from throat, nasal, and fecal swabs, blood, and urine of the experimental sheep (87). Samples from the thoracic and abdominal organs along with serial sections of the CNS from the cauda equina to the anterior cerebrum were examined for PrV lesions and distribution (87).

Dow and McFerran also evaluated the virus and lesion distribution of PrV as it related to high and low dose subcutaneous inoculation in sheep (36). The animals receiving low doses of virus had a generalized nonsuppurative encephalomyelitis while those receiving high doses had lesions suggestive of centripetal virus dissemination via cutaneous peripheral nerves to the brainstem. Nervous system lesions and viral
isolates were correlated.

Pondhuis et al. (102) and Lee et al. (78) conducted challenge experiments with PrV in vaccinated and nonvaccinated sheep. Pondhuis et al. determined that sheep were suitable for vaccine study because they were susceptible to PrV and would respond well to protective vaccines. Lee et al. used a formalin inactivated vaccine containing an experimental adjuvant to protect sheep from an intranasal challenge of pseudorabies virus. This vaccine protected 83.3% of the vaccinated sheep while all of the controls died. One to 2 days prior to the animals' deaths, Lee et al. isolated virus from nasal secretions of 60% of the control sheep.

The clinical signs observed in field cases of ovine Pr included indigestion, apathy, tympany, grinding of teeth, weakness, trembling, rubbing, pyrexia, recumbency, labored breathing, and increased mucus and salivary flow (11,81,113,117,121).

The clinical signs reported in sheep inoculated with field strain PrV were pruritus at the inoculation site, pyrexia, uneasiness, muscular spasms, abdominal distress, increased respiratory rates, ataxia, and coma followed by death (35,36,40,115,129). Those animals inoculated with vaccine strain PrV had clinical signs of depression, trembling, staggering, increased salivation, facial muscle fasciculations and chorea, recumbency, paddling, and death (21,123). Pruritus at the injection sites was not reported.

There were few macroscopic lesions in sheep naturally and experimentally infected with pseudorabies virus (35,36,81,117).
Postmortem findings were excoriation and hemorrhage at the inoculation sites, pulmonary edema, congestion, increased cerebrospinal fluid, meningeal congestion, pericardial and pleural hemorrhages, subepicardial petechiae, and mild impactions of the rumen or abomasum (21,35,36,81,117,123).

The microscopic lesions in sheep from natural Pr outbreaks were traumatic skin lesions, focal interstitial pneumonia, glial nodules in the cerebral cortex and mesencephalon, mild nonsuppurative encephalitis, intense glial proliferation in outer layers of the brainstem, and multifocal meningitis (117). Neuronal degeneration and necrosis along with multifocal lymphocytic infiltration of the mesenteric ganglion and plexus have also been reported (12).

In sheep experimentally exposed to PrV, microscopic lesions were traumatic skin changes as well as inflammation and hemorrhage in the lymph nodes draining the inoculation sites (35,36). The most significant changes were inflammation of the dorsal root or cranial nerve ganglia corresponding to the inoculation sites. The ganglionic lesions included edema, hemorrhage, infiltrates of macrophages, lymphocytes, neutrophils and eosinophils, and occasionally fibrinoid changes in the ganglionic vessels. The ganglionic neurons were either degenerate or necrotic. Two types of intranuclear inclusion bodies were in the ganglia. One type was round, multiple, and brightly eosinophilic; the other was round and basophilic, filling the entire nucleus.

The location of lesions in the brainstem depended upon the
inoculation site. After subcutaneous injection of PrV, the brainstem lesions were in the nucleus cuneatus and internal arcuate fibers. After oral, intranasal, or conjunctival inoculation, lesions were in the solitary tract and solitary nucleus, spinal tract nucleus and main sensory nucleus of the trigeminal nerve, and adjacent nuclei in the medulla.

The brainstem had neurons in various stages of degeneration and necrosis. Many neurons contained either a basophilic or an eosinophilic intranuclear inclusion body. There were also intranuclear inclusion bodies in glial cells. Microglial infiltration tended to be mild and diffuse; however, some areas had multifocal dense microglial nodules. Perivascular cuffing by lymphocytes and macrophages was prominent in the affected areas.

The lesions induced by vaccine PrV were in the spinal cord, brainstem, cerebellum, midbrain, and cerebrum (21,123). The histological changes were characterized by severe neuronal degeneration, neuronophagia, glial nodule formation, mononuclear perivascular cuffs, and large basophilic intranuclear bodies within neurons and glial cells.

Virus has been isolated from the brain (11,15,17,20,69,81,113, 117,122) and blood (81) of sheep from natural Pr outbreaks. McFerran and Dow described the PrV distribution in tissues from experimental animals (87). Virus isolations were negative from throat, nasal, and fecal swabs, blood, and urine of infected animals. Following scarification of PrV on the thigh or side, virus was located in the corresponding spinal cord segments, dorsal root ganglia, medulla, and
mesencephalon. Following intranasal inoculation, virus was occasionally isolated from the nasal mucosa, pharynx, and submaxillary lymph nodes in addition to CNS tissues. Using vaccine virus, Clark et al. made isolations from the prescapular lymph nodes and brain (21). Lee et al., evaluating the efficacy of inactivated Pr vaccine in swine and sheep, located field strain challenge virus in the CNS and nasal secretions from 3 of 5 unvaccinated controls (78).

The pathogenesis of PrV infection in sheep has been discussed by Dow and McFerran (35,36,87). Histological observations and virus isolations suggested that the virus acts on nervous tissues associated with inoculated areas. After scarification, the histopathologic changes indicated a centripetal viral spread along peripheral nerves to the dorsal root ganglia and into the dorsal horn of the respective spinal cord segments. Although the ascending routes in the spinal cord are unknown, lesions extended further cranially than caudally.

When PrV was inoculated intraconjunctivally, the major lesions were in the trigeminal ganglia and the main sensory and spinal tract nuclei of the trigeminal nerve. Following oral and intranasal virus inoculation, the lesions in the solitary and adjacent nuclei in the medulla oblongata were compatible with virus ascending the glossopharyngeal nerve. The infrequency of viral isolations from the drainage lymph nodes, nasal mucosa, pharynx, and conjunctiva suggested that extraneural viral replication did not occur at the inoculation sites (87).
Axoplasm Transport

The neural transport mechanism of PrV and other herpesviruses to the CNS has been studied by several workers (23,38,39,56,86). Field and Hill observed that if low doses of PrV were injected into the pinnae of mice, local viral replication was necessary prior to neural spread (39). McCracken et al. discovered in calves that PrV replicated in the fascia at the injection site for 48 to 60 hours (86). Subsequently, the virus appeared in the local nerve and spinal cord segment innervating the infected area, then ascended and descended throughout the central nervous system. He concluded that centripetal viral spread occurred along the peripheral nerve to the central nervous system. Field and Hill studied the centripetal spread of PrV in mice from rear foot pad and ear pinnae inoculation to the central nervous system (38,39). Cook and Stevens discovered that herpes simplex virus inoculated into the rear foot pads of mice progressed via the peripheral nerves to the central nervous system (23).

There have been proposed two main methods for virus progression up nerves: progressive replication in Schwann cells, endoneurial connective tissue cells, or perineural epithelial cells; and transport in perineural fluid, neural lymphatic fluid, or axoplasm (23,38,39,56,62,86).

Cook and Stevens determined that neurons, satellite cells, and nonmyelinating Schwann cells of the peripheral nervous system produced viral specific products; however, numerous virions were located in the neuronal perikaryon but only a few in the axons (23). They also
observed a difference in cell susceptibility between Schwann cells and fibroblasts associated with myelinated and nonmyelinated axons. Those Schwann cells adjacent to myelinated axons rarely became infected or contained complete virions while Schwann cells associated with nonmyelinated nerves contained capsids, nucleocapsids, and excessively proliferated nuclear membranes. The cells, however, were never observed to produce morphologically complete virions.

The most significant observation was the low number of incomplete virions in the supporting cells in contrast to the large number of complete virions in the neurons of the ganglia (23). Virions were also observed first in the ganglionic neurons and then in the nerves. Cook and Stevens reasoned that fibroblasts located in the extracellular spaces should become infected if the virus were free in the extracellular space. Since these fibroblasts were uninfected, Cook and Stevens eliminated the importance of cell to cell replication and perineural fluid transport as means of viral progression. They further noted that since infection progressed in the presence of neutralizing antibodies, the endoneurial blood and lymph vessels were not involved in the transport of virus to the ganglia. The direction of lymphatic flow was also found to be centrifugal in the endoneurial lymphatics and would therefore sweep virions away from the ganglia.

McCracken et al. reached similar conclusions with pseudorabies virus (86). After inoculation, there was local viral replication in connective tissue followed by centripetal spread to the central nervous system. The virus was rarely in the interstitial and Schwann cells and
never in the perineural epithelial cells. The simultaneous appearance of virus throughout the nerve-related ganglion and spinal cord segment from 60 to 84 hours post inoculation was found incompatible with centripetal stepwise progression by cellular pathways. Furthermore, because the replication of PrV requires 5 hours, virus probably could not have spread cell to cell along a 75 cm nerve in the observed time.

Virus has not been observed in the perineural fluid (86). Although viral antigen has occurred in the endoneurium, the virus is unlikely to enter the CNS by this route. The large number of virions within the ganglionic neurons but not within satellite cells makes viral entry to the neurons from the extracellular space improbable. This conclusion is strengthened by the absence of virus in the extracellular space of the endoneurium, which suggests that the satellite cells may even prevent virus movement. Additional evidence against endoneurial fluid transport of virus is the random lesion distribution and the absence of infected neurons in the hilar area of the ganglia (86).

Because virus has been observed to travel rapidly from the peripheral to the CNS, the most likely means of viral movement is axoplasmal transport (23,38,39,56,62,86). The rates, 5.2 mm per hour in mice and 20 mm per hour in calves (23,38,86), were compatible with viral movement within an axonal fluid medium.

Recent work has proved that substances, mostly proteins, are transported anterograde and retrograde within the axons of mammalian nerves (97). The anterograde system moves macromolecules rapidly or slowly within the axons. These substances maintain synaptic
excitability, effect neurotransmission, and when released at nerve terminals control the postsynaptic cells. Proteins synthesized by neurons are compartmentalized into small particulate and soluble fractions (97). Soluble protein fractions are hypothesized to move by slow transport and particulate fractions by fast transport (97).

Fast anterograde transport moves proteins a constant 410 mm per day regardless of nerve size, species of animal, and type of nerve (97). This rate is independent of fiber diameter and has been found to occur in the nonmyelinated fibers of the vagus. The proposed mechanism is like that described in the sliding filament theory of muscle (97). Crossbridges move a transport filament, functioning as a carrier, along microtubules or neurofilaments.

A slow transport mechanism was proposed from observations of swelling and beading of nerve axons proximal to constricted areas (97). It was hypothesized that the swelling represented the accumulation of proteins transported anterograde within the fiber. This rate was calculated at one to 3 mm per day. On the basis of exponentially declining outflow characteristics, typical of slow transport, a mitochondrial shuttle mechanism was suggested (97).

The retrograde transport system, in contrast, may be a means to move feedback substances to alter and control the level of protein synthesis in the perikaryons (97). Kristensson et al. reviewed extensively the retrograde transport system of macromolecules in the neuronal axons (74,75). By using protein tracers, he noted marked proteins in the neuronal perikaryon after their uptake at the axon
terminals. This observation confirms that axonal transport occurs also in the retrograde direction. The uptake and retrograde transport of proteins has been seen in the peripheral, central, and autonomic nervous systems and may provide the means for neurotrophic viruses and toxins to ascend in the nervous system (74).

The perineurium and endoneurial vessels prevent diffusion in the peripheral nervous system (74). At the distal ends of the axons, including those of the autonomic nervous system, the terminals are exposed to a variety of macromolecules. The synaptic uptake of macromolecules is a selective process with size and charge as important factors (74). Those molecules with increasing molecular size and positive charge are more easily absorbed into the axon. The selective uptake of materials is mediated by membrane receptors at the axon terminals. The existence of receptors may also explain the neurotropism of selected viruses for the central nervous system (62).

Two major mechanisms incorporate macromolecules into the axon (74). The first, micropinocytic uptake, is directly related to synaptic activity and neurotransmitter release. Micropinocytosis is considered the most likely means by which macromolecules and viruses are absorbed after they contact the axon terminal membrane. These macromolecules, when taken up at the synapse, become membrane bound in vesicles within the axoplasm. The other mechanism is the diffusion of substances through an injured axon's membrane. After cell injury, the membrane permeability increases and materials diffuse into the axon.
Neuroanatomy of the Cranial Nerves

The trigeminal nerve arises as a large sensory root and a smaller motor root from the dorsorostral aspect of the pons (85). The nerve has exteroceptive and proprioceptive general somatic afferent (GSA) fibers and special visceral efferent (SVE) fibers (85).

The exteroceptive GSA fibers arise from neurons in the trigeminal ganglion. The neurons have dendrites in the mucous membranes and skin of the head. Axons from the neurons course via the sensory root to the main sensory nucleus and spinal tract nucleus of the trigeminal nerve in the brainstem. The proprioceptive GSA fibers arise in the muscles of mastication and pass as sensory fibers in the mandibular nerve to terminate in the mesencephalic nucleus. The SVE fibers originate in the trigeminal motor nucleus of the dorsal pons and pass in the motor root of the mandibular nerve to the masticatory muscles.

The sensory root joins the trigeminal ganglion, which overlies the oval foramen and occupies the caudal portion of the ophthalmic and maxillary nerve sulcus in the basisphenoid bone. This ganglion is embedded in the fibrous dura covering the foramen and is medial to the wall of the cavernous sinus (85). From the rostral end of the ganglion arise three sensory nerves: the ophthalmic, maxillary, and sensory branch of the mandibular (44,85).

The ophthalmic nerve gives rise to the nasociliary nerve, which passes rostrally and divides into the ethmoidal and infratrochlear nerves (44). The ethmoidal branch accompanies the ethmoidal artery to distribute in mucosa of the nasal cavity, dorsal aspect of the nasal
The maxillary nerve arises with the ophthalmic nerve as a common trunk from the trigeminal ganglion (44). The pterygopalatine nerve, which originates medially from the maxillary nerve, divides into the caudal nasal and greater palatine nerves. The caudal nasal nerve passes rostrally on the floor of the nasal cavity to innervate the ventral nasal concha, nasal septum, and areas of the rostral nasal cavity.

The greater palatine nerve emerges from the greater palatine foramen and associates with the greater palatine artery (44). The nerve innervates the gums and hard palate mucosa prior to terminating in the dental pad. The lesser palatine nerve, which innervates the soft palate, branches from the greater palatine nerve at the posterior palatine foramen. Accessory palatine branches from the greater palatine nerve also distribute to the transition area between the soft and hard palate.

After progressing rostrally as the infraorbital nerve, the maxillary nerve divides into dorsal and ventral fibers at the infraorbital foramen (44). The dorsal group of fibers separate into external and internal branches. The internal nasal branch terminates in the mucosa and skin of the nasal vestibule.

From the rostral border of the mandibular nerve originates the buccal nerve, which sends fibers into the ventral portion of the parotid gland and to the mucous membrane and buccal glands of the oral cavity (44). The lingual nerve is a rostral branch from the terminal division of the mandibular nerve. After giving off the sublingual nerve, the
lingual nerve distributes throughout the rostral two-thirds of the tongue. The sublingual nerve emerges, follows the lingual nerve, and innervates the ventral and rostral areas of the buccal mucosa.

The glossopharyngeal nerve carries four fiber types (85): general visceral afferent (GVA), special visceral afferent (SVA), general visceral efferent (GVE), and special visceral efferent. General visceral afferent fibers have their neurons in the distal ganglion and their peripheral fibers in the pharynx and caudal one-third of the tongue. Their central axons synapse in the solitary nucleus of the medulla oblongata. The SVA fibers also have their neurons in the distal ganglion and central axons terminating in the solitary nucleus; however, their dendritic zones are in the taste buds. General visceral afferent fibers arise from neurons in the salivatory nucleus and pass to the otic ganglion and salivary gland. Special visceral efferent fibers arise from neurons in the nucleus ambiguus and terminate in the stylopharyngeus muscle.

The glossopharyngeal nerve emerges from the dorsolateral aspect of the medulla oblongata as a rostrocaudal linear series of rootlets continuous with those of the vagus and accessory nerves (44). The glossopharyngeal rootlets form a single trunk which penetrates the jugular foramen along with the vagus and accessory nerves. The glossopharyngeal nerve contains two ganglia: a small proximal ganglion at the exit of the jugular foramen, and a distal ganglion near the caudomedial wall of the tympanic bulla. The glossopharyngeal nerve also supplies a branch to the carotid sinus and exchanges fibers with the
vagus nerve proximal to the carotid sinus branch. A pharyngeal nerve arises from the glossopharyngeal nerve to join a pharyngeal branch of the vagus and sympathetic fibers from the cranial cervical ganglion to form the pharyngeal plexus (44,45). Fibers from this plexus supply the muscles and mucosa of the pharynx and soft palate. The glossopharyngeal nerve widens into the lateropharyngeal ganglion and continues along the lateral aspect of the pharynx to innervate the pharyngeal mucosa and tonsils (44). The nerve eventually terminates as a lingual branch to innervate the caudal one-third of the tongue.

The vagus nerve contains GSA, GVA, SVA, GVE, and SVE fibers (85). The GSA fibers to the skin of the external ear arise from neurons in the proximal ganglion. Axons from these neurons synapse in the spinal tract nucleus of the trigeminal nerve. Both the GVA fibers to the pharynx, larynx, esophagus, and thoracic and abdominal viscera and the SVA fibers to the taste buds of the epiglottis arise from neurons in the distal ganglion. Axons from these neurons synapse in the solitary nucleus. The GVE fibers to the parasympathetic plexuses of the cranial, thoracic, and abdominal regions arise from neurons in the dorsal motor nucleus. The SVE fibers to the larynx and pharynx arise from neurons in the nucleus ambiguus.

The vagus nerve emerges from the dorsolateral aspect of the medulla oblongata caudal to the glossopharyngeal nerve and leaves the cranium by the jugular foramen in intimate association with the accessory nerve (44,45). Two ganglia occur along the vagus. A proximal ganglion is within the nerve at the point of emergence from the jugular foramen.
A poorly delineated distal ganglion is located either proximal or distal to the origin of the cranial laryngeal nerve (85). The vagus exits the cranium and courses distally in close association with the accessory nerve (44,45).

In the retropharyngeal area, the vagus gives rise to the pharyngeal branch (44,45). This branch courses toward the pharynx, where it separates into a rostral and a caudal division. The rostral division joins the pharyngeal plexus. The caudal division, or pharyngoesophageal nerve, innervates several pharyngeal muscles and terminates in the rostral end of the esophagus.

Distal to the pharyngeal branch, the cranial laryngeal nerve leaves the vagus, descends lateral to the pharynx, and separates into an external and internal branch (44). The external branch innervates pharyngeal musculature and supplies branches to the thyroid gland. The internal branch penetrates the larynx and divides into two groups of fibers: a large rostral group that innervates the laryngeal mucosa and a caudal group that innervates pharyngeal mucosa and joins the pharyngoesophageal nerve (44).

The recurrent laryngeal nerves originate from the intrathoracic portion of the vagus (85). The right nerve leaves the vagus at the level of the first intercostal space to course caudally around the right subclavian artery to the right ventrolateral tracheal surface. The left nerve leaves the vagus at the aortic arch, passes around the caudal surface of the aorta, then proceeds cranially along the ventral border of the trachea. These nerves supply fibers to the cardiac, tracheal,
and esophageal plexuses.

The cardiac plexuses are intimately connected with the pulmonary plexuses (85). Both structures are formed by intercommunicating branches from the sympathetic ganglia and vagus and caudal laryngeal nerves (99). From these plexuses, numerous branches course along the bronchi and vessels to the pulmonary parenchyma.

**Neuroanatomy of the Lungs**

The lungs are innervated by the parasympathetic fibers from the vagus nerve and by the sympathetic nerves from the second through fourth thoracic ganglia (107). This dual innervation features an excitatory and inhibitory supply to the smooth muscles of the airways, blood vessels, and glands (107). Stimulation of the vagus nerve constricts the airway, increases glandular or goblet cell secretion, and dilates the pulmonary vessels. In contrast, stimulation of adrenergic nerves relaxes the smooth muscle of airways, constricts the pulmonary and bronchial blood vessels, and inhibits the glandular secretions. There are also partially characterized afferent nerves stimulated by pressure, stretch, or irritation.

The sympathetic and parasympathetic nerves form plexuses around the hilus and send branches along the bronchial tree and vessels (107). Both sets of nerves contain afferent and efferent fibers. The preganglionic fibers from the vagal nuclei descend in the vagus nerve to ganglia around blood vessels and airways. The postganglionic fibers which leave these ganglia innervate the airway, vascular smooth muscle, gland epithelium, and goblet cells. The postganglionic fibers from the
sympathetic ganglia enter the lung and innervate the same structures. Afferent nerve endings and their neurons in the mucosa are distributed throughout the lung. These afferent endings have been described between epithelial cells of airways, in the lamina propria, and subjacent to the basement membrane. The sensory endings in smooth muscle respond to changes in tension while epithelial endings detect irritants. Axons from these epithelial endings are myelinated and terminate in the distal ganglia of the vagus or brainstem.

Neuroanatomy of the Sympathetic Nervous System

The autonomic nervous system is divided into two mutually antagonistic systems, the parasympathetic and sympathetic (3). In these systems, two neurons intervene between the CNS and the innervated viscus. The first neuron, the preganglionic, is between the CNS and autonomic ganglia, while the second neuron, the postganglionic, is in the ganglia.

The preganglionic neurons for the sympathetic system are in the lateral column of the gray matter in the spinal cord segments between the first thoracic and first lumbar vertebrae (3). Myelinated preganglionic fibers pass in the white rami communicantes from the ventral root to the sympathetic ganglia. Fibers either terminate in the nearest ganglion or pass rostrally or caudally in the sympathetic chain to other ganglia.

The thoracic sympathetic trunk is a bilateral structure located ventral to the articulations of the ribs (89). The cervicothoracic ganglion, the most cranial ganglion of the trunk, lies on the
ventrolateral surface of the longus colli muscle and ventral to the costovertebral joint of the first rib and intercostal space (85,89). On the left the ganglion is located directly ventral to the head of the first rib, but on the right it rests slightly caudal between the heads of the first and second ribs.

The ovine cervicothoracic ganglion is approximately 18 mm long, 5 mm wide, and 3 mm thick (89). The caudal cervical and first two thoracic ganglia combine to form the cervicothoracic ganglion. The ansa subclavia connects the cervicothoracic ganglion to the cervical sympathetic trunk and cranial ganglia (89).

The left cervicothoracic cardiac nerves arise from the caudal region of the cervicothoracic ganglion and pass to the left intervascular triangle (89). Filaments from this nerve go to the thoracic aorta and pretracheal portion of the cardiac plexus (85). The right cervicothoracic cardiac nerves arise from the cervicothoracic ganglion (89), then combine with the thoracic cardiac nerves to enter the pretracheal portion of the cardiac and pulmonary plexuses (89).

The cranial cervical ganglion is a pale brown fusiform structure approximately 8 mm long, 6 mm wide, and 3 mm thick (44,85,89). The ganglion is embedded in adipose tissue on the lateral aspect of the rectus capitis ventralis muscle and is located ventromedial to the tympanic bulla, ventral to the posterior foramen lacerum, and medial to the glossopharyngeal and vagus nerves, condyloid artery, and jugular process (44,85). The sympathetic trunk enters the ventral pole of the ganglion after coursing medial to the common carotid and occipital
arteries (85,89).

The internal carotid nerve emerges from the dorsal pole of the cranial cervical ganglion, courses dorsorostrally, and enters the rostral portion of the jugular foramen (44,116). After traversing the foramen to enter the cranial vault, the nerve continues rostrally within the cavernous plexus located in the floor of the middle cranial fossa (44). The nerve then supplies a branch, the deep petrosal nerve, which joins the greater petrosal nerve to become the nerve of the pterygoid canal, and terminates in the pterygopalatine ganglia. The postganglionic fibers from the cranial cervical ganglion, however, pass through these ganglia without synapsing, accompany the pterygopalatine nerve, and branch to the mucous membrane of the nasal cavity and palate. The internal carotid nerve also gives off smaller branch nerves to the trigeminal ganglion, abducent nerve, and hypophysis (44).

The jugular nerve emerges from the caudal surface of the internal carotid nerve to give the majority of its fibers to the hypoglossal, accessory, vagus, and glossopharyngeal nerves (44). The connection to the glossopharyngeal nerve is at the distal ganglion while the connection to the vagus nerve is at the proximal ganglion (96,116).

The external carotid nerve originates from the ventral pole of the cranial cervical ganglion. The nerve innervates and travels caudally along the lingual, external, and common carotid arteries to innervate the large salivary glands (44,96).

The pharyngeal plexus is formed by the glossopharyngeal and vagal nerve branches and rami from the ventral pole of the cranial cervical
ganglion (116). The fibers from this plexus innervate the muscles and mucous membranes of the pharynx and the cranial one-third of the trachea (116). A few fibers from the cranial cervical ganglion, sympathetic trunk, and pharyngeal plexus reach the cranial esophagus.

**Neuroanatomy of the Brainstem**

The GVA system is composed of neurons with dendrites in the viscera and with perikaryons in the spinal and cranial nerve ganglia. These neurons regulate body temperature, blood pressure, blood gas concentration and pressure, and visceral movement (28). The receptors for this system are both encapsulated and free in the innervated organs, and the axons follow those peripheral nerves most available to the organs (28). These nerves include the facial nerve for the cephalic blood vessels and middle ear; the glossopharyngeal nerve to the caudal tongue, pharynx, carotid body, and sinus; and the vagus nerve to the larynx, pharynx, and thoracic and abdominal viscera (28). Their axons course from the cranial nerve ganglia to the ventrolateral medulla (28), then enter the neuroparenchyma to project rostrolaterally and caudally in the solitary tract.

The solitary tract is composed of fibers from the trigeminal, facial, glossopharyngeal, and vagus nerves (22,125). The rostral end of the tract is formed by fibers from the facial nerve, the middle section by fibers from all four nerves, and the caudal end by fibers from the vagus. Caudal to the vagus nerve, the tract rapidly becomes smaller and shifts laterally to merge with the ventral portion of the dorsal funiculus (125).
The solitary nucleus, composed of neurons encircling the solitary tract, receives input from axons of those nerve fibers comprising the tract. This nucleus develops in the alar plate of the medulla oblongata adjacent and parallel to the column of the general visceral efferent motor nuclei of the vagus and glossopharyngeal nerves (28,125). From the rostral end of the nucleus ambiguus and inferior olive, the nucleus extends rostrocaudally to the decussation of the pyramids (46,125). The nucleus participates in reflex activity responsible for respiration, cardiovascular output, and swallowing by sending fibers either directly or indirectly to the general visceral efferent column (28,53,120).

The GSA system, a part of the trigeminal nerve, contains neurons that perceive pain, touch, and temperature (28). The receptor organs are a mixture of encapsulated and nonencapsulated exteroceptors stimulated by mechanical and physical contacts with the environment.

The sensory nuclei of the trigeminal nerve receive axons from neurons in the trigeminal ganglia (104). These axons enter the neuroparenchyma of the pons and distribute ascending fibers to the main sensory nucleus and descending fibers to the spinal tract nucleus of the trigeminal nerve. The main sensory nucleus, which mediates tactile sensibility, is located in the middle of the pontine level in the lateral portion of the reticular formation and dorsolateral to the trigeminal motor nucleus.

The spinal tract nucleus of the trigeminal nerve continues rostrally with the main sensory nucleus and receives descending fibers from neurons originating in the trigeminal ganglion (18). This large
elongate nucleus lies deep to the restiform body but caudally approaches the surface where the nucleus and tract form the tuber cinereum. The nucleus continues further caudally to join the gelatinous substance over the dorsal horns of the spinal cord.

The spinal tract nucleus of the trigeminal nerve receives some cutaneous afferent fibers of pain, temperature, and tactile input from the facial, glossopharyngeal, and vagus nerves (18,76,104). The fibers from these nerves join the nucleus and occupy the dorsal-most position and can be traced as far caudally as the second cervical vertebra (104). The main sensory nucleus and the spinal tract nucleus receive fibers from the primary sensory cortex and many afferent fibers from the reticular formation (104).
PART I. A NECROTIZING PNEUMONIA IN LAMBS CAUSED BY PSEUDORABIES VIRUS (AUJESZKY'S DISEASE VIRUS)
A NECROTIZING PNEUMONIA IN LAMBS CAUSED BY PSEUDORABIES VIRUS (AUJESZKY'S DISEASE VIRUS)

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ABSTRACT

An outbreak of pseudorabies occurred in sheep housed in the same building with swine. Although the sheep and swine were not in direct contact, the lambs and ewes were exposed to air from the sows' section. Three dead lambs were submitted to the Iowa State University Veterinary Diagnostic Laboratory for necropsy.

Grossly there were pulmonary congestion and multifocal pulmonary hemorrhages. Microscopic lesions were severe acute multifocal necrotizing bronchopneumonia with necrotizing vasculitis and intranuclear inclusion bodies within the neurons of the parabronchial ganglia.

Bacterial cultures were negative for pathogenic agents; pseudorabies virus was isolated from ovine brain. Viral antigen was demonstrated in the neurons of the parabronchial ganglia by immunoperoxidase staining. Electron microscopy revealed nucleocapsids in the parabronchial ganglionic neurons which contained basophilic intranuclear inclusion bodies. Viral DNA prepared from the ovine pseudorabies virus isolate was found by restriction endonuclease analysis to be closely related to the Indiana Funkhauser strain of pseudorabies virus.
INTRODUCTION

Pseudorabies (Aujeszky's disease), a disease of worldwide distribution affecting swine and other domestic animals, has been increasing in incidence and virulence in the midwestern United States (42). From 1969 to 1973, an average of 5 cases a year were diagnosed at the Iowa State University Veterinary Diagnostic Laboratory (VDL) in Ames, Iowa. Pseudorabies (Pr) cases increased from 28 in 1974 to 352 in 1977 (50). From 1980 to 1984, the number of positive cases based on pseudorabies virus (PrV) isolation ranged from 321 to 581; the nonporcine cases varied from 120 to 197 of the total, with ovine isolates comprising 2 to 16% of the nonporcine cases.

Since the discovery of Pr by Aujeszky in 1902 (2), relatively few papers have described spontaneous outbreaks in sheep. The earliest reports were from Eastern Europe in 1912 (71), 1927 (121), and 1933 (81), followed by England in 1938 (119) and Brazil in 1939 (20). Kojnok observed that swine were the source of PrV for ruminant infection in 1962 (69). This finding was substantiated by other authors' reports of natural Pr outbreaks in sheep during the 1960s until the present (11,15,17,61,113,117,122).

Incubation periods varying from 70 to 100 hours (113) and clinical signs lasting from 12 to 38 hours (15,17,113) were observed in sheep. The typical early clinical signs were uneasiness (20,113,117), lethargy (121), depression (15,81) and anorexia (15,20,121). These signs were followed by generalized tremors (17,81,113,117,121), respiratory difficulties (15,17,113), rumen atony (113), cardiac arrhythmias (113),
excessive salivation (15,17,81,113,117), ocular and nasal discharge (81), intense pruritus (11,15,20,81,113,117,121), and recumbency and death (11,15,17,81,113,117,121). The rectal temperatures were 40.8-41.6°C (15,81,113) and sometimes returned to normal prior to death (113).

At the pruritic sites, the macroscopic lesions were characterized by excoriation, edema, and hemorrhage (20,81,117). The other observations were hepatic, meningeal, and pulmonary congestion (117,121); pulmonary and tracheal edema (15,117); abomasal impaction and catarrhal enteritis (81); and subepicardial petechiae (117). Histological lesions in nonneural tissues, excluding the pruritic sites, were pulmonary edema and focal intralobular granuloma-like interstitial pneumonia in 2 animals (117). Neural tissues in spontaneous and experimental infections contained neuronal necrosis in the cervicothoracic (stellate) ganglia, mesenteric plexus, and parabronchial ganglia (12). Nonsuppurative ganglionitis was also reported from dorsal root and trigeminal ganglia (35). Within the central nervous system in naturally and experimentally infected sheep, nonsuppurative encephalitis (35,36,117) or necrotizing encephalitis (61) was reported in the cerebral cortex (117), brainstem (35,36,61,87,117), and cerebellum (61,117).

A review of the literature disclosed no descriptions of necrotizing pneumonia or necrotizing pulmonary vasculitis in sheep and only one report of necrosis in the parabronchial ganglia (12). The objective of this study was to characterize the pneumonic lesions in an outbreak of
Pr in young lambs which were presumed to have been infected by aerosolized PrV from farrowing sows.
MATERIALS AND METHODS

Origin of Ovine Tissues

Three dead 2- to 3-week-old lambs were submitted for necropsy to the Iowa State University Veterinary Diagnostic Laboratory. The lambs were from a flock of 35 ewes and 48 lambs.

A remodeled barn housed the animals (Fig. 1). The larger end of the building was subdivided into 2 units, one for farrowing sows (vaccinated against Pr with a modified live vaccine (Pr-Vac, Norden Laboratories, Inc., Lincoln, Nebraska)) and the other for lambing ewes. A solid plank wall, built from the floor to the bottom edge of the ceiling joists, separated the two areas. This construction permitted a 20 cm open space between the top of the wall and the haymow floor boards. A ventilation fan, constructed high in the wall, blew air from the farrowing to the lambing side. In the room adjacent to the lambing unit were penned ewes with older lambs. The next room contained market weight swine. The walls between these areas were as previously described, and the rooms were open at one end.

Seven ewes, 32 lambs (2-3 weeks old), and one cat died during the outbreak. The first Pr cases occurred among the ewes and older lambs while the remaining cases were in the neonatal lambs and lambing ewes. The initial losses were lambs followed by deaths of ewes later in the outbreak. The clinical signs, beginning with muscle spasms and ending with pruritus, had a sudden onset. The lambs died within 12 hours after clinical signs began. Pruritus was common in the lambs but was evident in only one ewe.
Histotechnique

Representative tissues were harvested at necropsy for histological examination. Portions of cerebrum, cerebellum, brainstem (diencephalon, mesencephalon, metencephalon, and medulla oblongata), lung, heart, liver, and kidney were fixed in 10% neutral buffered formalin. Tissues were processed by routine paraffin techniques, sectioned at 6μ and stained with hematoxylin and eosin (H & E) by the Harris method (79).

Virus Isolation

Ovine brain tissue was processed for the isolation and identification of pseudorabies virus (55).

Restriction Endonuclease Analysis

Virus isolated from the outbreak (VDL 82P2294), along with Norden vaccine virus (PR-Vac, Norden Laboratories, Inc., Lincoln, Nebraska) and Indiana Funkhauser (Ind(FH)) strain of PrV were subjected to restriction endonuclease (RE) analysis (101). Viral DNA was prepared, purified, and cleaved with 3 RE enzymes (Bam HI, Sal I, and Hinf I, Bethesda Research Laboratories, Gaithersburg, Maryland) as previously described (100,101).

Immunoperoxidase Staining

In an attempt to localize and prove that viral antigen was present in the lungs and brainstem of the submitted lambs, an immunoperoxidase staining procedure (Vectastain™ ABC Kit, Vector Laboratories, Inc., Burlingame, California) was performed on formalin fixed paraffin embedded tissues. Several slides were prepared from the same blocks from which the H & E slides were processed. Tissue sections, cut at 6μ,
were mounted on glass slides and incubated for 12 hours at 60°C in dry heat. The sections were deparaffinized and immunoperoxidase stained as outlined by the Vectastain™ protocol. Rabbit antipseudorabies serum was the primary antiserum, with 10 mM phosphate-buffered saline used as buffer and diaminobenzidine used as oxidizable peroxidase substrate. Harris hematoxylin was the counterstain.

Electron Microscopy

Selected sections from the H & E slides were processed for electron microscopy to demonstrate viral particles in the neurons of the parabronchial ganglia (51).
RESULTS

Gross and Microscopic Findings

Necropsy disclosed diffuse pulmonary congestion and multifocal pulmonary hemorrhages.

Histological examination of the pulmonary parenchyma revealed severe generalized edema, hemorrhage, and congestion. The alveoli contained serofibrinous exudate mixed with erythrocytes while the thickened alveolar septae contained a moderate increase in macrophages. A few of the pulmonary vessels, bronchioles, and adjacent parenchyma contained multifocal areas of necrosis (Fig. 2). These necrotic foci were characterized by the loss of tissue cytoarchitecture and contained mixed leukocyte infiltrates of neutrophils, a few macrophages and lymphocytes (Fig. 3). In some of the foci, a few degenerate and unidentifiable cells contained eosinophilic intranuclear inclusion bodies. Within the parabronchial ganglia were degenerate neurons with nuclei characterized by marginated chromatin and clumped eosinophilic inclusion bodies (Fig. 4). In other ganglionic neurons, the nuclei were filled with a basophilic pale granular nucleoplasm encircled by fragmented and particulate chromatin at the nuclear membrane. The brainstem, slightly caudal to the cerebellar peduncles, contained degenerate neurons with basophilic intranuclear inclusion bodies. These neurons were medial and dorsal to the facial nucleus. A mild lymphocytic perivascular infiltrate involved some of the neuroparenchymal vessels.
Virus Isolation

Pseudorabies was confirmed by PrV isolation from emulsified ovine brain tissue. The virus (82P2294) was plaque purified and propagated on porcine kidney cells. A pool of virus from the third passage with a titer of $2.2 \times 10^8$ plaque forming units per mL was frozen at $-70^\circ$C.

Restriction Endonuclease Banding Patterns

The VDL 82P2294 ovine isolate and Ind(Fh) strain had relatively similar DNA banding patterns with all 3 restriction enzymes (Fig. 5). The banding patterns of the Norden vaccine strain were different from both VDL 82P2294 and Ind(Fh) strains. In the Bam HI pattern (Lane B), there was an area of heterogeneity between 5.0 and 6.0 megadaltons. In the Sal I pattern (Lane B), there were numerous differences in numbers and migration of bands. In the Hinf I pattern, the Norden PrV DNA had no 4.2 megadalton band, differentiating Norden from the VDL 82P2294 and Ind(Fh) strains.

Immunoperoxidase Staining

Viral antigen positive neurons were in the transverse tissue sections from the medulla oblongata slightly caudal to the cerebellar peduncles. The affected neurons tended to be located medial and dorsal to the facial nucleus in the nucleus reticularis gigantocellularis.
Electron Microscopy

Nonenveloped nucleocapsids were demonstrated within the nuclei of the neurons in the parabronchial ganglia (Fig. 6). The virus particles were in the nuclei which contained granular pale basophilic intranuclear inclusion bodies in the H & E stained sections.
DISCUSSION

The history, clinical signs, macroscopic and histologic lesions, virus distribution, and RE analysis of viral DNA have been described for an outbreak of Pr in ewes and 2- to 3-week-old lambs. Although PrV isolations or serology were not attempted from the swine, the close association between the sheep and the pigs suggested the possible shedding of virulent aerosolized PrV (32) from vaccinated sows with latent or subclinical PrV infections (27,49,91).

The results of RE analysis clearly indicate that the ovine PrV isolate was not related to a vaccine strain of PrV, but was closely related to the virulent Ind(Fh) strain. The necrotizing pneumonia and the presence of viral antigen and nucleocapsids in the parabronchial ganglionic neurons is evidence for the aerogenous spread of PrV in this outbreak. With this type of viral transmission, ventilation and airflow patterns should be considered when Pr is suspected in swine or other farm animals. The specific involvement of the lungs warrants submission of this tissue for histological and virological examination when aerogenous spread of Pr is suspected.
Fig. 1. Floor plan of the barn. The solid black lines represent outer walls while inner walls, marked with oblique lines, partitioned the barn. A ventilation fan (A) was located between the areas for farrowing sows and lambing ewes. The arrows indicate the air flow from the farrowing to the lambing unit.
Fig. 2. Lung from lamb. Note the area of pneumonia peripheral to a bronchiole (arrow). H & E.
Bar=125\mu

Fig. 3. Vessel wall in pulmonary parenchyma. Note the focal vasculitis obliterating the cytoarchitecture of the vessel wall (arrow).
H & E. Bar=86\mu
Fig. 4. Parabronchial ganglion in lamb lung. Note the degenerate neurons and eosinophilic intranuclear inclusion bodies (arrows). H & E. Bar=50 μ
Fig. 5. Electrophoretic banding patterns of Pr viral DNA, VDL virus (Lane A). Norden vaccine virus (Lane B), and Ind(FH) (Lane C). The molecular weights on the left apply to the Bam HI restriction enzyme banding patterns, and the molecular weights on the right apply to Sal I and Hinf I banding patterns.
Fig. 6. Parabronchial ganglion cell from lamb. Electron micrograph from an H & E stained section.

Note the nucleocapsids near the nuclear membrane (arrows). Bar=0.2 μm
PART II. PATHOGENESIS OF OVINE PSEUDORABIES
(AUJESZKY'S DISEASE) FOLLOWING
INTRATRACHEAL INOCULATION
PATHOGENESIS OF OVINE PSEUDORABIES
(AUJESZKY'S DISEASE) FOLLOWING
INTRATRACHEAL INOCULATION

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ABSTRACT

Thirty-two sheep, 24 inoculated intratracheally with an aerosolized Iowa field strain of pseudorabies virus and 8 control animals, were used in the study. Eight animals (2 control and 6 principals) were killed at 24, 48, 72, and 86–96 hours post inoculation.

Clinical signs in inoculated sheep living more than 72 hours were pyrexia, depression, frequent swallowing, facial fasciculations, chorea, excessive salivation, mild tympanites, labored breathing, and focal pruritus followed by death. Macroscopic lesions were severe focal facial trauma, petechiae in several sympathetic ganglia, and dilated esophaguses. The trigeminal, cranial cervical, cervicothoracic, and parabronchial ganglia and the medulla oblongata contained nonsuppurative inflammatory changes which increased in frequency and severity with time. Within the medulla oblongata, the lesions were limited to the spinal tract nucleus of the trigeminal nerve, solitary tract, and solitary nucleus.

The neural tissues with virus and viral antigen were the trigeminal, cranial cervical, and cervicothoracic ganglia and the medulla oblongata. The virus distribution within the central and sympathetic nervous systems was localized and dependent upon time after inoculation. Direct fluorescent antibody staining demonstrated the presence of viral antigen in sympathetic ganglionic neurons. The nasal mucus contained virus from 33% of the animals examined between 86–96 hours post inoculation, but no virus was recovered from urine or cerebrospinal fluid.
Although there were lung and bronchial lymph node viral isolates, the extensive neural distribution of lesions and virus suggested that the virus traveled from the respiratory mucosa to the nervous system by two routes: (1) in the vagus and glossopharyngeal nerves to the medulla oblongata and (2) in the postganglionic fibers to the sympathetic ganglia.
INTRODUCTION

In 1902, Aujeszky described pseudorabies (Pr) in a cat, dog, and ox (2). The first reports of Pr in sheep were from experimental work in 1910 (115) and 1911 (129). There were no reports of natural Pr in sheep prior to 1912, but 3 outbreaks occurred in sheep flocks from 1912-1933 (71,81,121). Kojnok's observations of outbreaks in cattle and sheep led to recognition that swine could be a source of pseudorabies virus (PrV) for sheep (69). Through the 1960s, several published accounts from Eastern Europe recorded large losses of sheep from pseudorabies (11,15,17,113,117). The articles described clinical signs and macroscopic and microscopic lesions. In 1964, Dow and McFerran reported the characteristics and distribution of lesions and the distribution of virus in PrV infected sheep (35,87). They also proposed the pathogenesis of ovine Pr from viral exposure by several different routes.

Researchers have suggested that PrV carrier rats (81) and virus contaminated fomites (77), urine (15,17), swine pen effluents (113), swine secretions (69), and direct contact with pigs (122) could infect sheep. Sheep have contracted Pr after experimental PrV inoculation by scarification, subcutaneous injection, intranasal and oral administration, and intraconjunctival instillation (35,87). Air can be a vector for the transmission of pathogenic and nonpathogenic viruses (64), and the airborne spread of aerosolized PrV among swine on the same and different premises has been documented by Donaldson et al. (32) and Gloster et al. (43). Air could be a suitable vector for transmitting
PrV to nonporcine hosts.

The purpose of this research was to determine the pathogenesis of PrV in sheep exposed intratracheally to aerosolized virus. The virus was an Iowa field strain originally isolated from lambs which presumably had been exposed to virus aerosols from adjacently housed sows. This paper describes the incubation period, clinical signs, macroscopic and histological lesions, and the tissue distribution of virus and viral antigen. Nasal secretions and urine were examined for virus to ascertain the potential for horizontal virus transmission within infected sheep flocks once an outbreak occurred.
MATERIALS AND METHODS

Animals and Experimental Design

Thirty-two male or female Suffolk and Suffolk-cross domestic sheep approximately 5 to 6 months old were used in the project. The sheep were maintained for 1 1/2 months on a mixed grass pasture with water provided ad libitum prior to their use in the experiment. During the experiment, the animals were housed in concrete floored isolation units. The 32 sheep were randomly assigned to 8 groups of 4 animals each. There were 6 PrV inoculated groups and 2 control groups. For inoculation, a group of 4 animals was removed from the pasture, transferred to an isolation unit, and inoculated at the same time intratracheally with a virulent field strain of pseudorabies virus. Animals were killed at 24, 48, 72, and, if they survived that long, 86-96 hours post inoculation (HPI). Both groups of control animals received sterile cell culture media intratracheally and were killed at 24, 48, 72, or 96 hours post inoculation. This permitted 2 control and 6 principal replicates at each postinoculation time period. Before inoculation with PrV, the animals were tested for Pr antibodies using the microtitration serum neutralization test (55) and found to be negative.

Virus Inoculum

A field strain of PrV was isolated from lambs submitted to the Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa, from an Iowa outbreak of Pr and provided the seed virus for this study. The isolate was plaque purified and propagated in a monolayer of 48-hour-old
porcine kidney (PK 15) cells. The virus was dispensed in 1 mL aliquots containing $1 \times 10^7$ plaque forming units (PFU) of PrV and frozen at -70°C.

**Virus Isolation from Tissue**

Tissue samples for virus isolation (VI) were harvested at necropsy and stored at -70°C until processed. The non-neural tissues submitted for VI were nasal turbinates, tonsils, retropharyngeal lymph nodes, lung, bronchial lymph nodes, and adrenal glands. Nervous system tissues were trigeminal ganglia, cranial cervical ganglia, cervicothoracic ganglia, olfactory bulbs, cerebral cortex, basal ganglia, mesencephalon, metencephalon, medulla oblongata, cervical spinal cord, thoracic spinal cord, and lumbar spinal cord.

The tissues were emulsified, then processed for PrV isolation and identification on Madin-Darby bovine kidney cell monolayers (55). One blind passage was made prior to the isolates being called negative; for those samples causing herpesvirus-like cytopathic effects in the cells, direct fluorescent antibody (DFA) tests were used to confirm the presence of pseudorabies virus.

**Virus Isolation from Nasal Secretions, Cerebrospinal Fluid, and Urine**

Nasal mucus was collected one day prior to PrV inoculation and at 24-hour intervals beginning at 24 HPI and continuing until the animals were euthanatized. Cerebrospinal fluid (CSF) and urine were harvested only from animals sacrificed between 86-96 hours post inoculation.
Samples of nasal mucus were obtained by inserting sterile cotton-tipped applicators 7 to 10 cm through the nostril to the ventral nasal meatus. The swabs were rotated gently and allowed to absorb mucus for approximately 10 seconds. After removal, the swabs were placed in sterile tubes containing 1 mL of Earle's balanced salt solution and antibiotics. The urine and CSF were obtained at necropsy in sterile syringes and transferred to serum tubes. The CSF was removed from the ventral aspect of the foramen magnum and the urine aspirated from the urinary bladder. The nasal swabs, urine, and CSF were stored at -70°C until processed.

**Virus Inoculation**

Sheep were injected with xylazine (Bayvet, Cutter Laboratories, Inc., Shawnee, Kansas) (intravenous dose of 0.05 mg per kg body weight) followed 1-2 minutes later by intravenous ketamine hydrochloride (Bristol Laboratories, Syracuse, New York) (2-5 mg per kg body weight). When recumbent, the animals were intubated with a 9 mm inside diameter Lo-Pro cuffed tracheal tube (National Center Company, Argyle, New York). A Stephen Slater Valve (Anesthesia Associates, Inc., San Marcos, California) was attached to the tracheal tube; affixed to the input port of the valve was a Pelco all-glass nebulizer (Ted Pella, Inc., Tustin, California). Two mL of either a control solution of Earle's balanced salt solution or tissue culture fluid containing PrV at 1.0 x 10⁷ PFU per mL were nebulized 5-7 minutes by an 8 L per minute airflow at 1 atmosphere pressure generated by a Gast portable pressure/vacuum pump (Fisher Scientific, Itasca, Illinois).
Clinical Signs

Following inoculation, animals were observed for clinical signs twice daily. Rectal temperatures were recorded from all animals at 12 hour intervals and analyzed using a repeated measures split-plot analysis of variance (ANOVA). The ANOVA was run using the Statistical Analysis System (106). When significant effects were observed, individual differences between means were tested for statistical significance using Tukey's Honestly Significant Difference Test (67). The harmonic mean was used for all mean comparisons involving an unequal number of observations. In all analyses, statistical significance at the \( q < 0.05 \) level was accepted.

Necropsy

The animals were killed at 24, 48, and 72 HPI; however, 3 animals were killed when death was imminent between 86-96 hours post inoculation. Representative tissues were harvested, and macroscopic lesions were recorded. The brain was bisected along the longitudinal fissure. One half was fixed in 10% neutral buffered formalin. The other half was subdivided into cerebral cortex, basal ganglia, mesencephalon, metencephalon, and myelencephalon for VI or DFA examination. The spinal cord was divided into the cervical, thoracic, and lumbar regions. Each segment was further subdivided into 4 equal pieces of tissue. The first and third pieces were formalin fixed for histological study while the second and fourth were saved for VI and DFA examination. Other tissues for histopathology were fixed in 10% neutral-buffered formalin except for the eyes, which were fixed in
Bouin's solution for 24 hours. Tissues saved for VI and DFA examination were frozen in liquid nitrogen and stored at -70°C until processed at the Iowa State University Veterinary Diagnostic Laboratory.

**Histotechnique**

Tissues harvested for histological examination were central nervous system—olfactory bulbs, cerebral cortex, basal ganglia, hypothalamus, pituitary, mesencephalon, metencephalon, medulla oblongata, cervical spinal cord at the first and fourth through fifth cervical vertebrae, thoracic spinal cord at the first through fourth and eighth through eleventh thoracic vertebrae, and lumbar spinal cord at the first through second and fourth through sixth lumbar vertebrae; ganglia—trigeminal, cranial cervical, and cervicothoracic; and respiratory system—nasal turbinates, trachea, and all lung lobes. The other tissues harvested were eyes, tonsils, retropharyngeal and bronchial lymph nodes, spleen, thymus, salivary glands, heart, adrenal glands, kidneys, liver, pancreas, duodenum, jejunum, ileum, and spiral colon. The nasal turbinates, following fixation, were decalcified in a saturated aqueous solution of ethylenediamine tetraacetate for 48-72 hours. The tissues were processed by routine paraffin techniques, sectioned at 6 μ, and stained with hematoxylin and eosin (H & E) by the Harris method (79).

**Immunoperoxidase Staining**

All 6 PrV inoculated animals killed between 86-96 HPI and 2/6 animals killed at 72 HPI with moderate to severe lesions in the medulla oblongata were tested for PrV antigen. The tissue sections came from
the medulla at the level of the obex. Several slides were prepared from
the identical blocks from which H & E sections were processed. Tissue
sections, cut at 6 μ, were mounted on glass slides and incubated for 12
hours at 60°C in dry heat. The sections were then deparaffinized and
immunoperoxidase stained as outlined by the Vectastain™ protocol
(Vectastain™ ABC Kit, Vector Laboratories, Inc., Burlingame, California). Rabbit antipseudorabies serum was the primary antiserum,
with 10mM phosphate buffered saline (PBS) used as the buffer and
diaminobenzidine as the oxidizable peroxidase substrate. Harris
hematoxylin was the counter stain.

Direct Fluorescent Antibody Test

The following nonneural tissues were saved for DFA examination:
ventral nasal turbinates, tonsils, lung, bronchial lymph nodes, and
adrenal glands. The neural tissues were trigeminal ganglia, cranial
cervical ganglia, cervicothoracic ganglia, olfactory bulbs, basal
ganglia at the level of the cruciate sulcus, mesencephalon immediately
caudal to the posterior colliculi, medulla oblongata at the level of the
obex, cervical spinal cord at the level of the 2nd cervical vertebra,
thoracic spinal cord at the level of the 5th thoracic vertebra, and
lumbar spinal cord at the level of the 3rd lumbar vertebra. These
tissues were placed in 5 mL sample vials (Nalge Co., Rochester, New
York), quick frozen in liquid nitrogen, and maintained at -70°C until
processed.

The procedure for DFA staining and blocking test has been described
(55). The tissue samples were sectioned to 6μ, mounted on glass
slides, and fixed in cold acetone at 3°C for 5 minutes. Pseudorabies virus conjugate was applied evenly to one of the tissue sections while PrV conjugate mixed with unlabeled PrV antiserum, with a titer of 1:128, was applied to the other tissue section. The slide was incubated for 30 minutes at 37°C, rinsed in PBS for 5 minutes, and then rinsed for 5 minutes in distilled water. The slides were air dried and coverslipped using a buffered glycerol mounting medium. The preparations were examined with a fluorescence microscope and classified positive or negative depending upon the presence or absence of bright yellow-green fluorescence.

**Electron Microscopy**

Tissues used for electron microscopy were from the spinal tract nucleus of the trigeminal nerve in the medulla oblongata at the level of the obex, cranial cervical ganglia, and cervicothoracic ganglia. These formalin fixed neural tissues, harvested at necropsy for histological study, were cut into 1 mm³ pieces, placed into 3% glutaraldehyde in 0.1 M cacodylate buffer, and post-fixed in 1% osmium tetroxide. The osmium fixed tissues were dehydrated, infiltrated, embedded, and cut into 3 μ thick sections. Sections were stained with 1% toluidine blue in 1% aqueous sodium borate and examined with light microscopy to identify areas containing cellular lesions for trimming and thin sectioning. Thin sections approximately 50 nm were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined in a Hitachi HS-9 electron microscope (Hitachi, Ltd., Tokyo, Japan).
RESULTS

Clinical Signs

The average rectal temperature range for the control animals was 39.1-39.4°C during the experiment and for the principals approximately 39.4-39.6°C until 48 hours post inoculation (Fig. 1). From 48 to 84 HPI, the temperature rose steadily to approximately 41.5°C and declined to 41.2°C at 96 hours post inoculation. The mean temperature differences between the control and inoculated sheep approached statistical significance at 60 HPI (q<0.05) and was significant at 72, 84, and 96 hours post inoculation.

The first visible clinical signs occurred in 2 animals at 72 HPI and were mild depression, abnormal facial movements, salivation, and frequent swallowing. By 84-96 HPI, the remaining 5 animals were either having clinical signs or were dead. The severity of the signs increased with time to severe depression, facial fasciculations, chorea, excessive salivation, labored breathing, moderate tympany, self-mutilation, and finally death. The control animals had no clinical signs of pseudorabies.

Macroscopic Lesions

In general, there were no significant lesions in the control sheep; however, in the right lung of a 24 HPI control, there was a 6 cm well-encapsulated abscess from which Corynebacterium pseudotuberculosis was isolated.

The lesions in the principals were epicardial hemorrhage, enlarged bronchial lymph nodes, dilated esophaguses, petechiae in the
cervicothoracic ganglia, and facial trauma. The epicardial ecchymoses were distributed over the left ventricle in 1/6 animals at 24 hours post inoculation. Enlarged bronchial lymph nodes also occurred in 1/6 animals at 24 and 1/6 animals from 86-96 hours post inoculation. Two animals, 1/6 at 72 HPI and 1/6 from 86-96 HPI, had dilated esophaguses. Cervicothoracic ganglionic petechiae were distributed in 1/6 animals killed at 48 and 72 HPI and in 2/6 animals from 86-96 hours post inoculation. In 3/6 animals from 86-96 HPI, there were severe facial excoriations rostral and ventral to the ears. The underlying subcutaneous tissues and musculature were edematous and hemorrhagic.

**Microscopic Lesions**

There were no microscopic lesions compatible with Pr from the principal sheep in the eyes, nasal turbinates, tonsils, thymus, salivary glands, adrenal glands, kidneys, liver, pancreas, duodenum, jejunum, ileum, and spiral colon. In 1/6 animals from 86-96 HPI, a linear microfocus of necrosis was adjacent to the smooth muscle layer in the trachea. Atrial lesions occurred in 2/6 animals from 86-96 hours post inoculation. One animal had a very mild myocardial infiltrate of neutrophils while the other had a focus of histiocytes adjacent to a nerve. The septal myocardium in 1/6 animals at 48 HPI had a microfocus of histiocytes infiltrating several Purkinje fibers.

In the retropharyngeal and bronchial lymph nodes, several microfoci of necrosis were in the subcapsular and paracortical sinuses. The spleen contained necrotic foci in the germinal centers in 2/6 principal sheep from 86-96 hours post inoculation.
All but one control animal at 72 HPI had no lesions in neural tissues. The exception had wide lymphocytic perivascular cuffs approximately 5-7 cell layers deep in the cerebrum, basal ganglia, and mesencephalon. The following neural tissues contained microscopic lesions typical for Pr: parabronchial ganglia, trigeminal ganglia, cranial cervical ganglia, cervicothoracic ganglia, mesencephalon, metencephalon, and medulla oblongata (Table 1). In 2 parabronchial ganglia in 1/6 sheep from 86-96 HPI, there were eosinophilic and basophilic intranuclear inclusion bodies (Fig. 2). The lesions in the trigeminal ganglia ranged in severity from foci of mononuclear cells encompassing degenerate neurons (Fig. 3) to randomly scattered neuronal intranuclear inclusion bodies. The inclusion bodies were of 2 types: (1) a common, large, pale basophilic intranuclear body composed of fine granular material and encircled by particulate chromatin at the nuclear membrane and (2) an infrequent, fragmented eosinophilic intranuclear body occupying the central portion of the nucleus and surrounded by a halo.

Cranial cervical ganglia (Figs. 4 and 5) and cervicothoracic ganglia (Figs. 6 and 7) contained mild to severe lesions. Mild lesions were characterized by one or 2 microfoci of mononuclear cells, usually 10 or less, associated with degenerate neurons and located within the parenchyma. Occasional intranuclear inclusion bodies were also observed in neurons. Moderate lesions contained mononuclear infiltrates of approximately 25 cells and had numerous degenerate to necrotic neurons within affected foci. Neuronal intranuclear inclusion bodies were
frequent, predominantly basophilic and either associated with the mononuclear infiltrates or randomly dispersed within the parenchyma. Severe lesions were characterized by hemorrhage, multifocal mononuclear infiltrates, degenerate to necrotic neurons, and numerous intranuclear inclusion bodies within neurons (Figs. 4-7).

The mesencephalon and metencephalon contained a few widely scattered neurons with basophilic intranuclear inclusion bodies. The medulla oblongata contained severe and consistent lesions limited to the solitary tract, dorsomedial solitary nucleus, and spinal tract nucleus of the trigeminal nerve (Fig. 8). The lesions were most common one mm caudal to the obex.

In the neuroparenchyma of the brainstem, the lesions were less dramatic and more localized than in the ganglia. The mild lesions were limited to neurons and were reflected by alterations in the nucleoplasm. In general, the nucleus was distended by fine granular basophilic material which obliterated the nucleolus and was segmentally encircled by particulate chromatin material at the nuclear membrane. Moderate lesions contained edema, diffuse gliosis, microglial nodules, lymphocytic perivascular cuffing, basophilic intranuclear inclusion bodies in neurons, and eosinophilic intranuclear inclusion bodies in neurons and glial cells. In addition, severe lesions had wider lymphocytic perivascular cuffs (Fig. 9), larger and more frequent microglial nodules (Fig. 10), and more extensive gliosis and edema. Intranuclear inclusion bodies of both types were focally numerous in the neurons and neuroglia.
Immunoperoxidase Staining

No PrV antigen was seen in the tissues from the control animals. Sections of medulla oblongata from 2/6 sheep killed at 72 HPI and 6/6 at 86–96 HPI found to have lesions by light microscopy were examined by the immunoperoxidase method. Traces of Pr viral antigen were demonstrated in the solitary tract, solitary nucleus, and spinal tract nucleus of the trigeminal nerve. At 72 HPI, both animals had PrV positive neurons and glial cells in the solitary tract and solitary nucleus while only one animal had positive cells in the spinal tract nucleus of the trigeminal nerve. From 86–96 HPI, all 6 animals had Pr viral antigen in the solitary tract and solitary nucleus, and 4/6 animals had viral antigen in the spinal tract nucleus of the trigeminal nerve. In both groups, the preponderant distribution of virus infected cells was in the neuroparenchyma dorsomedial to the solitary tract. Of the 2 sympathetic ganglia tested from 2/6 animals from 86–96 HPI, a positive reaction for viral antigen was observed in the cranial cervical ganglion and was limited exclusively to the neurons.

Direct Fluorescent Antibody Test

Numerous positive fluorescent tissues were found in the animals from the last 2 time periods (Table 1). The cranial cervical and cervicothoracic sympathetic ganglia were consistently positive (Fig. 11). The brainstem contained many fluorescent foci. The most intense positive fluorescence was from the tissues of the medulla oblongata at the level of the obex.
Virus Isolation from Tissue

In the nonneural tissues, the positive PrV isolations were dispersed over all of the time periods (Table 1). The pulmonary tissues were positive for virus early in the experiment, and bronchial lymph node isolations increased with time.

Pseudorabies virus isolations occurred most frequently from the trigeminal ganglia, cranial cervical ganglia, cervicothoracic ganglia, brainstem, and spinal cord (Table I). The number of isolations increased with time. More PrV isolations were made from the medulla oblongata than from the combined tissues of mesencephalon and metencephalon.

Virus Isolation from Nasal Secretions, Cerebrospinal Fluid, and Urine

Pseudorabies virus was isolated from the nasal mucus of 2 animals killed in agonal stages between 86-96 hours post inoculation. No virus was isolated from urine of 2/2 animals or from the CSF of 5/5 animals from 86-96 hour post inoculation.

Electron Microscopy

In one sheep killed from 86-96 HPI, there were numerous herpes nucleocapsids located in neurons of the cranial cervical ganglia, cervicothoracic ganglia, and spinal tract nucleus of the trigeminal nerve (Fig. 12). No nucleocapsids were observed in the ganglionic satellite cells or in the glial cells of the medulla oblongata.
DISCUSSION

The experimental sheep were susceptible to PrV by intratracheal aerosol exposure. The incubation period was 72-84 hours and the duration of clinical signs approximately 12 hours. The susceptibility of sheep to PrV by the intratracheal route correlated closely with their infectibility to oral, nasal, and intraconjunctival viral exposure (35).

A temperature range of 40°-41.4°C occurred from 60-96 HPI in the principals. Elevated temperatures from 40.5-42.2°C were reported from natural outbreaks (15,81,113) and other experimental work (35,36,87). Three authors noted a temperature decline to normal or subnormal during the terminal stage of disease (35,36,113). A similar pattern occurred in one animal whose temperature declined from 40.5°C at 84 HPI to 38.8°C at 96 hours post inoculation. The pathogenesis of fever in sheep with Pr is unknown; however, the absence of lesions in the hypothalamus is suggestive of temperature elevation caused by endogenous pyrogen release from necrotic neurons or inflammatory cells (108).

The clinical signs which appeared 72-96 HPI were depression, abnormal facial movements and twitches, occasional chorea, and frequent swallowing. The signs increased in frequency and severity with time and included tympanites, excessive salivation, labored breathing, and rubbing of the head. These and similar signs have been previously reported in natural outbreaks (11,15,17,81,113,117,121) and in experimental infections (35,36). Clinical pneumonia did not occur in the intratracheally inoculated sheep.

The general depression may have been caused by the multifocal
lesions interrupting reflexes and impulses from the solitary tract, solitary nucleus, and spinal tract nucleus of the trigeminal nerve to the rostral midbrain and diencephalon. For example, the caudal spinal tract nucleus of the trigeminal nerve projects axons to the thalamus or the reticular system (109). Furthermore, cell bodies of the solitary nucleus send axons rostrally in the solitariothalamic pathway (for conscious projection) to the thalamic nuclei which send axons to the somesthetic cortex (28).

The caudal portion of the spinal tract nucleus of the trigeminal nerve is important for transmission of pain and thermal sensation from the head (109). The specific level of lesions (0 to one mm caudal to the obex) corresponds to the level of the spinal tract nucleus of the trigeminal nerve which receives input from the head and face (128). Lesions in this nucleus may have accounted for the perception of focal pruritus which caused these animals to traumatize the facial skin by scratching and rubbing. The frequent swallowing and tympanites may have been due to the interruption of the eructation mechanism (33), whose central and reflex mechanisms operate through viscerotopically organized interneurons located in the dorsal vagal nucleus, adjacent reticular formation, and solitary nucleus between the transverse planes 3 mm rostral and one mm caudal to the obex (53,66). Lesions in the solitary tract and solitary nucleus near the obex could have interrupted the cyclical activity and caused tympanites in the principals.

The solitary subnuclei regulate the visceral reflex activity for respiration (28), swallowing (28), cardiovascular output, and vascular
tone (120). The dorsomedial solitary nuclear lesions corresponded to the distribution of the vagal afferent fibers from the respiratory tract (22,65). Furthermore, the respiratory and cardiovascular centers converge in this area (80,120); therefore, death could have resulted from cardiac dysrhythmias, vascular collapse, or possibly respiratory failure. The extensive neuronal necrosis in the major sympathetic ganglia would be compatible with loss of sympathetic output (30) and be more evidence for a fatal cardiac dysrhythmia or hypotension (3).

There were no gross pulmonary lesions consistent with PrV from principals. The macroscopic findings attributable to PrV were focal self-inflicted trauma, dilated esophaguses, and multifocal hemorrhages in the cervicothoracic ganglia. Previously reported findings of pulmonary congestion (117,121), pulmonary edema (15,117), meningeal congestion (121), hepatic congestion (121), abomasal impaction (81), subepicardial petechiae (35,36,117), and increased cerebrospinal fluid (36) were not observed in these experimental animals. The dilated esophaguses probably resulted from lesions interrupting the solitary nucleus reflex arcs to the esophagus (33,53). The multifocal hemorrhages in the ganglia resulted from severe inflammatory lesions of the ganglionic parenchyma.

Previous reports of natural PrV infections characterized neural lesions as either a nonsuppurative encephalitis involving all major anatomical divisions of the brain (117) or a multifocal necrotizing encephalitis in the brainstem and cerebellum (61). In other reported Pr cases, the presence of lesions in the parabronchial (12,114) and
cervicothoracic ganglia (12) were evidence that PrV can be effectively distributed in the ovine autonomic nervous system. Dow and McFerran reported a nonsuppurative encephalomyelitis in the PrV infected experimental animals (36). In animals inoculated intranasally and orally, lesions occurred in the solitary tract, solitary nucleus, spinal tract nucleus of the trigeminal nerve, and other nuclei of the medulla (35).

The most significant findings were the neuroparenchymal lesions in the medulla oblongata and sympathetic ganglia of the principals killed at 72 and 86-96 hours post inoculation. The major difference between animals killed at 72 HPI and those killed in the agonal stages (86-96 HPI) was the severity of lesions and increase in the number of affected animals. In the mesencephalon and metencephalon, the randomly scattered histological changes were characterized as basophilic intranuclear changes limited exclusively to neurons. The lesions in the medulla oblongata were frequent and limited to the solitary tract, dorsomedial solitary nucleus, and spinal tract nucleus of the trigeminal nerve. The lesions were classified as a nonsuppurative encephalitis.

The precise mechanism and the exact location whereby the virus entered the nervous system could not be determined in this study. There were no sequential lesions attributable to or compatible with PrV entry in the nonneural epithelial tissues of the oropharynx or respiratory tract. The only nonneural tissues containing lesions were the retropharyngeal and bronchial lymph nodes and spleens from animals killed from 86-96 hours post inoculation. The micronecroses in the
Kolonits reported the death of one ewe during a bovine outbreak of pseudorabies. Szilárd discussed the deaths of a ram and a pregnant ewe but did not speculate on the source of the virus. Marcis described an outbreak of Pr in a flock of pregnant ewes. The affected sheep had been housed in a shelter previously occupied by swine. Ewes in late stages of pregnancy were the first sheep affected by the disease, followed by recently bred ewes and lastly by those animals that had recently lambed. A laboratory confirmed the diagnosis of Pr by inoculating rabbits with suspect brain emulsions and blood from the affected sheep. Marcis hypothesized that infected rodents were the source of the virus.

Only two field cases of ovine Pr were reported from 1930 to 1960 (20,77). In 1961, Becker (11) along with Bogdan (17) and Berecz (15) reported outbreaks in sheep. Becker and Berecz observed that those outbreaks were associated with outbreaks in swine on the same premises. Bogdan and Berecz concluded that urine was significant in the horizontal spread of the virus.

In 1962, Kojnok observed that outbreaks of Pr in cattle and sheep coincided with outbreaks in pigs housed in the same buildings or yards as the other animals (69). He concluded that infected swine were a source of PrV to cattle and sheep. Senf and Seffner (117) in addition to Schäfer and Gudat (113) stated that in numerous Pr outbreaks on separate state farms, the sheep were in close contact with swine or had previously occupied their quarters.

In 1979, Thawley et al. reported a Pr outbreak in swine which was
trigeminal nerve. The neural distribution of lesions, virus, and viral antigen in the solitary tract, solitary nucleus, and spinal tract nucleus of the trigeminal nerve was consistent with viral dissemination along afferent fibers of the glossopharyngeal nerve from the pharynx (45) and the afferent fibers in the vagus nerve from the lung and airways (65,107). This distribution is compatible with the ability of the virus to be transported axoplasmally in the neural tissues (83,86).

In the cranial cervical and cervicothoracic ganglia, the random distribution of PrV antigen positive neurons and the absence of viral antigen in satellite cells are compatible with the axoplasmic transport of virus (83,86) in the sympathetic postganglionic fibers distributed in the pharyngeal (45) and respiratory mucosa (65,107). Virus in the cervical spinal cord segments could correspond to the caudal projections of the spinal tract nucleus of the trigeminal nerve (84). Virus in the cranial thoracic spinal cord would correspond to the distribution of the preganglionic neurons which synapse with the neurons in the cranial cervical ganglia and cervicothoracic ganglia (83).

The presence of PrV cytopathic effects, viral antigen, and nucleocapsids in the neurons corresponded to viral replication. These necrotic neurons along with ganglionic inflammation reduce the functional capacity of the ganglia. The spontaneous intermittent discharges from PrV infected neurons, which extend beyond normal bounds (3,31), may be the physiological cause of the clinical signs and death.

The lesions in the sympathetic ganglia were strikingly different from those in the medulla oblongata. In the medulla oblongata
there were edema, diffuse gliosis, microglial nodules, lymphocytic perivascular cuffing, mild spongiosis, eosinophilic intranuclear inclusion bodies in neurons and glial cells, and basophilic intranuclear inclusion bodies in neurons. The sympathetic ganglia were characterized by large multifocal accumulations of mononuclear cells and numerous degenerated and necrotic neurons. Many of the neurons contained intranuclear inclusion bodies.

The lesion differences probably resulted from the length of time the different structures were infected with virus. There were lesions in the cranial cervical and cervicothoracic ganglia prior to changes in the medulla oblongata at 48 hours post inoculation. The length of the postganglionic fibers from the cranial cervical and cervicothoracic ganglia to the respiratory mucosa is shorter than the length of fibers from the pulmonary and tracheal mucosal irritant receptors to the respective nuclei in the medulla oblongata. In addition, the virus before entering the medullary nuclei and tracts would have to replicate first in the neurons of the distal ganglia of the vagus and glossopharyngeal nerves. The effect is that the lesions in the sympathetic ganglia would be older and consequently the inflammatory changes more developed. At the same time, the cytopathic effects in the neurons of the medulla oblongata would be fatal before inflammatory foci as extensive as those in the sympathetic ganglia could occur.

This study indicates the potential for horizontal transmission of PrV in a flock of sheep. In contrast to McFerran and Dow (87) and in agreement with Lee et al. (78), 2/6 animals or 33% of the sheep shed
virus in the nasal mucus during the agonal phase of the disease. No other positive isolations were made from earlier mucus samples. Contrary to the proposals of Bogdan (17) and Berecz (15) and in agreement with the data of McFerran and Dow (87), no virus was isolated from urine samples from 86–96 hours post inoculation. Furthermore, the absence of PrV in the CSF tends to agree with McFerran and Dow's findings of no viremia (87).

Under the conditions of this study, sheep were found to be very susceptible to an Iowa field isolate of pseudorabies virus. Both the specific focal nonsuppurative lesions in the medulla oblongata and cranial cervical and cervicothoracic ganglia and the numerous sequential viral isolations indicate the ability of PrV to enter both the general visceral afferent and sympathetic nervous systems from presumably intact mucosal surfaces. Based upon the lesion distribution, the ease of location, and the striking nature of the sympathetic ganglionic inflammatory lesions, the harvesting of these tissues for histological and virological examination is warranted.

The results of this study suggest that prior to virus isolation and identification Pr can often be differentiated from other ovine encephalitides by history, clinical signs, and histological lesions in the central and sympathetic nervous systems.
Fig. 1. Average rectal temperatures at 12 hour intervals from the control and PrV inoculated sheep. Arrows indicate the times at which various clinical signs occurred.
Bloating, salivation, labored breathing, rubbing

Depression, facial fasciculations, frequent swallowing

Significant temperature differences
Fig. 2. Parabronchial ganglion from a PrV inoculated sheep killed at 88 HPI. Note the basophilic intranuclear inclusion body encircled by particulate chromatin at the nuclear membrane (large arrow), and the eosinophilic inclusion body occupying the central portion of the nucleus (small arrow). H & E. Bar=47μ

Fig. 3. Trigeminal ganglion from a PrV inoculated sheep killed at 48 HPI. Note focus of gliosis encircling a degenerate neuron. H & E. Bar=40μ
Fig. 4. Cranial cervical ganglion from a PrV inoculated sheep killed at 96 HPI. Note multifocal glial infiltrates (arrow). H & E. Bar=222 μ

Fig. 5. Higher magnification of Fig. 4. Note the neurons containing a basophilic intranuclear inclusion body (large arrow), and the eosinophilic intranuclear inclusion body (small arrow). H & E. Bar=38μ
Fig. 6. Cervicothoracic ganglion from a PrV inoculated sheep killed at 96 HPI. Note severe multifocal glial infiltration adjacent to capsular hemorrhage (large arrow), and intranuclear inclusion bodies (small arrows). H & E. Bar=106 μ

Fig. 7. Higher magnification of Fig. 6. Note glial focus and a basophilic intranuclear inclusion body encircled by a particulate margin of chromatin (arrow). H & E. Bar=49 μ
Fig. 8. Medulla oblongata one mm caudal to the obex from a PrV inoculated sheep killed at 90 HPI. Note location of solitary tract and solitary nucleus (small arrow) and spinal tract nucleus of the trigeminal nerve (large arrow). H & E. Bar 1.6 mm

Fig. 9. Higher magnification of spinal tract nucleus of the trigeminal nerve in Fig. 8. Note severe lymphocytic perivascular cuffing, diffuse gliosis, and edema. H & E. Bar=107μ
Fig. 10. Spinal tract nucleus of the trigeminal nerve (transection one mm caudal to the obex) from a PrV inoculated sheep killed at 86 HPI. Note microglial nodule within the neuropil. H & E. Bar=20 μ

Fig. 11. Cervicothoracic ganglion from a PrV inoculated sheep killed at 96 HPI. Note the neurons with the prominent intranuclear, nuclear membrane, and perinuclear cytoplasmic fluorescence (arrow). DFA. Bar=40 μ
Fig. 12. Cranial cervical ganglion cell from a PrV inoculated sheep killed at 88 HPI. Note the nucleocapsids near the nucleolus of a neuron. Electron microscopy. Bar=0.5 μm
Table 1. Summary of tissue viral isolations, fluorescent antibody staining, and lesions from PrV inoculated sheep

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^aNasal turbinate (NT); Tonsil (TON); Retropharyngeal lymph node (RLN) Lung (LU); Bronchial lymph node (BLN); Adrenal gland (AD); Trigeminal ganglia (TG); Cranial cervical ganglia (CCG); Cervicothoracic ganglia (CTG); Mesencephalon (MES); Metencephalon (MET); Medulla oblongata (MO); Cervical spinal cord (CSC); Thoracic spinal cord (TSC); Lumbar spinal cord (LSC).

^bVirus isolation.

^cDirect fluorescent antibody.

^dHistological lesions.

^eNumber positive/Number examined.

^fNot done.
PART III. THE ANATOMICAL LOCATION OF NEURAL STRUCTURES
MOST OPTIMALLY SAMPLED FOR PSEUDORABIES
(AUJESZKY'S DISEASE) IN SHEEP
THE ANATOMICAL LOCATION OF NEURAL STRUCTURES
MOST OPTIMALLY SAMPLED FOR PSEUDORABIES
(AUJESZKY'S DISEASE) IN SHEEP

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ABSTRACT

This paper describes the location and dissection of important neurologic structures for histological and virological diagnosis of pseudorabies in sheep. Six sheep were dissected to expose and photograph cranial cervical ganglia, cervicothoracic ganglia, the medulla oblongata, and trigeminal ganglia.
Since its description by Aujeszky in 1902 (2), pseudorabies (Aujeszky's disease) has become recognized as a worldwide disease in which pigs, the natural host (10), are a source of infection for ruminants (69). Virus and lesions in sheep have been localized to specific neurologic structures. Senf and Seffner reported a nonsuppurative encephalitis (117) and Janovitz a necrotizing encephalitis (61) from spontaneous pseudorabies (Pr) outbreaks in sheep flocks. The lesions were in the cerebral cortex (117), cerebellum (61,117) and brainstem (61,117). Dow and McFerran localized histological lesions and virus in neural structures associated with the route of inoculation (35,36,87). Nonsuppurative ganglionitis and encephalitis were in the trigeminal ganglia and medulla oblongata of experimental sheep inoculated by the intraconjunctival, oral, and intranasal routes (35,87). Becker found viral induced neuronal necrosis in the cervicothoracic ganglia of sheep in natural and experimental pseudorabies virus (PrV) infections (12). Schmidt reported that sheep inoculated intratracheally with aerosolized PrV had histological lesions, viral antigen, and virus in the medulla oblongata and trigeminal, cranial cervical, and cervicothoracic ganglia (114, Part II).

The purpose of this article is to illustrate the location of neural structures that contain lesions and virus in experimental and natural infections.
MATERIALS AND METHODS

Six adult ewes were euthanatized and dissected to expose the cranial cervical ganglia, cervicothoracic ganglia, brainstem, and trigeminal ganglia. These tissues were easily obtained with standard necropsy instruments and without significantly altering necropsy protocols (98).
The cranial cervical ganglia (Fig. 1-4) are bilaterally symmetrical sympathetic ganglia at the anterior end of the sympathetic trunk (96). Approximately 8 mm long and 6 mm wide, these grayish to pale brown, fusiform neural structures are embedded in the adipose tissue of the retropharyngeal area (44). Each ganglion lies ventral to the posterior foramen lacerum (85), ventromedial to the tympanic bulla (89), medial to the jugular process of the occipital bone (89), and on the lateral aspect of the rectus capitis ventralis muscle (44).

The cranial cervical ganglia are excised prior to removing the head. After removing or reflecting the tongue, pharynx, larynx, trachea, and esophagus, the rectus capitis ventralis muscles are visualized and then transected at the level of the atlantooccipital articulation. The cranial cervical ganglia are found by first locating the exposed ventral ovoid space at the atlantooccipital articulation. The space is formed rostrally by the caudal aspect of the occipital bone at the ventral edge of the foramen magnum and caudally by the ventral edge of the cranial atlas. After this space is identified, the rectus capitis ventralis muscles are removed from their insertion on the basilar portion of the occipital bone. This permits observation of the ventral rostral edge of the occipital condyles. From this area, the ganglia lie approximately one to 2 cm rostrolateral to areas slightly medial to the jugular processes.

The cervicothoracic sympathetic ganglia (Fig. 5) are formed by the fusion of the caudal cervical and the first one or 2 thoracic
sympathetic ganglia (90). The irregularly star-shaped, grey to pale brown ganglia are approximately 10–15 mm wide and 3–4 mm thick. The ganglia extend between the heads of the first and second ribs and rest directly ventral to the head of the first rib on the left and slightly more caudal on the right (90). The ganglia, loosely embedded in the subpleural adipose tissue of the thoracic inlet, rest on the lateral surface of the longus colli muscle to reach the dorsal surface of the esophagus on the left and the dorsal surface of the trachea on the right (90).

The cervicothoracic ganglia are found after the thoracic viscera are removed and the head of the first rib is located. The firm, tough, star-shaped ganglia are found by using blunt dissection in the subpleural loose connective tissue in the area formed by the angle of the first intercostal space and the longus colli muscle. The ganglia rest at the rostral ends of the sympathetic trunks which are visible through the pleura ventral to the costovertebral articulations.

The medulla oblongata (Fig. 6) is the region of the brainstem between the pons and the rostral cervical spinal cord (29).

The brain and trigeminal ganglia are removed after the head is disarticulated and separated from the carcass. The brain is removed from the cranium as a single unit (98). Then the cerebral hemispheres and cerebellum are removed to expose the medulla oblongata.

The trigeminal ganglia (Fig. 7) are bilateral, symmetric elongate structures composed of white and gray neural tissue (44). Approximately 15 mm long and 4 mm wide, each overlies and is attached to the fibrous
dura mater covering the oval foramen (85). The ganglia occupy the caudal maxillary sulcus of the basisphenoid bone and are lateral to both the wall of the cavernous sinus and the rostral epidural rete mirabile (44).

The trigeminal ganglia are easily extracted by locating the large transected sensory roots (largest cut nerves) caudal and lateral to the pituitary gland on the floor of the cranium. The dura overlying the ganglion is removed. A small scissors is subsequently slid rostrolaterally under the sensory root to excise the ganglionic tissue.

These ganglionic and central nervous system tissues can be subdivided and saved for histological examination, fluorescent antibody examination, and virus isolation.
DISCUSSION

The experimental work of Dow and McFerran (35,36,87) and Schmidt (114, Part II), and the field report of Senf and Seffner (117) suggest that the medulla oblongata and the trigeminal ganglia are important tissues to harvest for histological and virus isolation procedures. Data from Becker (12) and the experimental work of Schmidt (114, Part II) indicate that the cranial cervical and cervicothoracic ganglia also have merit as tissues for the diagnosis of pseudorabies in sheep. These neurologic structures, which are easy to locate and remove using standard necropsy materials and procedure, should be included in sampling.
Fig. 1. Longitudinal section of skull along midline. Note the cranial cervical ganglion (arrow). Bar=50 cm

Fig. 2. Higher magnification of Fig. 1. Note the cranial cervical ganglion (arrow) rostral to the vagus (V) and hypoglossal (H) nerves. A, atlantooccipital articulation. Bar=2 cm
Fig. 3. Ventral view of atlantooccipital articulation. Note the cranial cervical ganglia (arrows) lateral to the rectus capitis ventralis muscle (M) and caudal to the medial retropharyngeal lymph node (N).
Bar=2.6 cm

Fig. 4. Same as Fig. 3 with the rectus capitis ventralis muscle excised. Note the cranial cervical ganglia (arrows) rostrolateral to the rostral border of the occipital condyle (O). Bar=2.6 cm
Fig. 5. Sagittal view of thorax. Note the cervicothoracic ganglion (arrow) ventral to the first rib (R) and at the cranial end of the thoracic sympathetic trunk. The ganglion rests on the longus colli muscle (M). Bar=3 cm

Fig. 6. Dorsal view of the brainstem. Note the obex (arrow). Bar=3 cm
Fig. 7. Dorsal view into the base of the cranium. Note the transected end of the trigeminal nerve sensory root (small arrow), and the exposed trigeminal ganglion (large arrow) lateral to the pituitary gland (P). O, optic chiasma. Bar=2 cm.
GENERAL DISCUSSION AND SUMMARY

The potential problem of ovine Pr contracted from inapparently infected sows shedding virus to sheep is exemplified by an Iowa outbreak of pseudorabies. A remodeled barn housed farrowing sows which were separated from lambing ewes by a high solid wall. A ventilation fan in the wall blew PrV contaminated air from the farrowing to the lambing area.

The sows had been vaccinated with modified live PrV vaccine. Because this virus is pathogenic for sheep (21,72,123), it was necessary to preclude the shedding of vaccine virus from the sows to the lambs and ewes. Restriction endonuclease analysis proved that the virus isolated from the ovine outbreak was related to the Ind(Fh) strain of pseudorabies but not to the vaccine.

Severe necrotizing pneumonia, pulmonary vasculitis, and herpetiform intranuclear inclusion bodies in the parabronchial ganglionic neurons were observed in the lambs submitted for necropsy. These pulmonary lesions were evidence that the lungs served as the main portal of virus entry in this particular outbreak. Viral antigen was localized in the parabronchial neurons by immunoperoxidase staining, and nucleocapsids were demonstrated at the nuclear membrane by electron microscopy.

To investigate the pathogenesis of PrV infection, the virus isolated from the ovine outbreak was propagated, then inoculated intratracheally into experimental sheep. These animals were susceptible to PrV by the intratracheal route. The incubation period was 72-84 hours, and the duration of illness before death was approximately 12
hours. Early clinical signs were fever, depression, abnormal facial movements, mild infrequent facial twitches, chorea, and frequent swallowing; later signs were bloat, excessive salivation, dyspnea, and self-mutilation.

The lesions in the medulla oblongata and sympathetic ganglia were probably responsible for the clinical signs. The general depression could have resulted from lesions interrupting impulses from the solitary nucleus and spinal tract nucleus of the trigeminal nerve to the rostral midbrain and diencephalon (28). The spinal tract nucleus of the trigeminal nerve at the level of the obex receives input from cutaneous afferent fibers of the facial, glossopharyngeal, and vagus nerves (18,22,46,65,76,125). The fibers from these nerves join the nucleus at the dorsal-most position and carry cutaneous afferent fibers of pain, temperature, and tactile sensation from the head. The specific level of lesions at the obex corresponds to the portion of the nucleus which receives input from the head and face (109,128). Lesions in this nucleus accounted for the perception of focal pruritus which caused these animals to traumatize the facial skin by scratching and rubbing.

Frequent swallowing and tympanites could have arisen from the interruption of neural reflexes involving neurons in the solitary nucleus, dorsal vagal nucleus, and adjacent reticular formation in the area of the obex (53). The solitary nucleus also regulates visceral reflex activity for respiration (80), cardiovascular output, and vascular tone (120). Therefore, death could have occurred from cardiac dysrhythmias, vascular collapse, or respiratory failure. In addition,
the extensive lesions in the major sympathetic ganglia of the head and thorax could cause a fatal cardiac dysrhythmia or hypotension from the loss of sympathetic output (3).

Inflammation, degenerate and necrotic neurons, and PrV in neurons interfere with the function of neural tissues. Pseudorabies virus replication causes the affected neurons to emit spontaneous intermittent discharges (30,31) that may be the physiological cause of the clinical signs and death.

The gross lesions attributable to PrV were focal self-inflicted excoriations, dilated esophaguses, and multifocal hemorrhages in the cervicothoracic ganglia. The dilated esophaguses probably resulted from interruption of the solitary nucleus reflex arcs (53), while multifocal hemorrhages in the ganglia resulted from severe inflammatory changes of the ganglionic parenchyma.

The severity of nervous system lesions increased with time. The findings in the CNS were in the medulla oblongata and sympathetic ganglia of the principals killed between 72-96 hours post inoculation. The medullary neuroparenchyma was characterized by edema, diffuse gliosis, microglial nodules, lymphocytic perivascular cuffing, mild spongiosis, eosinophilic intranuclear inclusion bodies in neurons and glial cells, and basophilic intranuclear inclusion bodies in neurons. The ganglia contained large multifocal accumulations of mononuclear cells and numerous degenerated and necrotic neurons. Many of the neurons had either an eosinophilic or a basophilic intranuclear inclusion body.
The lesion differences between the ganglia and the medulla oblongata probably resulted from the length of time the structures were infected with virus. The distance of the postganglionic fibers from the cranial cervical and cervicothoracic ganglia to the respiratory mucosa is shorter than the distance from the irritant receptors in the pulmonary and tracheal mucosa to the solitary nucleus. Furthermore, the virus entering the medulla oblongata would replicate first in the neurons of the distal ganglia of the vagus and glossopharyngeal nerves. Consequently, the lesions in the sympathetic ganglia would be more severe than lesions in the medulla oblongata. The cytopathic effects in the medullary neurons would be fatal to the host prior to observing inflammatory changes like those in the ganglia.

The site and means of virus entry to the nervous system could not be determined from this study. In the nonneural tissues of the oropharynx or respiratory tract, sequential lesions were not found compatible with PrV entry. The early viral isolations from the lung were probably residual inoculum and not viral replication since the number of isolations declined with time. Virus isolations from the bronchial lymph nodes increased as time progressed. The absence of lesions in the tracheal, bronchial, bronchiolar, and alveolar epithelium along with the presence of virus in the bronchial lymph nodes suggests that virus entered the submucosal lymphatics probably without prior epithelial replication. Entry into the submucosa is possible by four routes: 1) the replication of virus in epithelial cells, causing focal necrosis and virus release which were not in the examined sections, 2)
transcellular and paracellular transport of virus through epithelial cells into the interstitial tissues (63), 3) phagocytosis of virus by alveolar macrophages which then migrate to the interstitial space (63), and 4) the replication and release of virus from the parabronchial ganglia to the interstitial space. The evidence in this work favors the last possibility because virus was isolated from the lung containing intranuclear inclusion bodies in the parabronchial ganglionic neurons. However, entry routes 2 and particularly 3 (alveolar macrophage) have not been disproven and therefore need further study.

The site of virus distribution within the central and sympathetic nervous systems depended upon the hours post inoculation and is compatible with the axoplasmal transport of the virus in the neural tissues (83). The few mild lesions and small quantity of PrV in the trigeminal ganglia suggest that PrV bypassed the dendritic fields of the trigeminal fibers in the nasal and buccal mucosa. The distribution of lesions, virus, and viral antigen in the solitary tract, solitary nucleus, and spinal tract nucleus of the trigeminal nerve was consistent with viral dissemination via the afferent fibers in the glossopharyngeal nerve from the pharynx (65) and via the afferent fibers in the vagus nerve from the lung and airways (65). In the cranial cervical and cervicothoracic ganglia, the random location of PrV antigen positive neurons and the absence of viral antigen in satellite cells were compatible with virus distribution (83) in the postganglionic fibers supplied to the pharyngeal (65) and respiratory mucosa (65). Virus in the cervical spinal cord segments would correspond with the caudal
projections from the spinal tract nucleus of the trigeminal nerve (84). Virus could have traveled to the cranial thoracic spinal cord by following the axonal distribution of the preganglionic neurons that synapse with the neurons in the cranial cervical and cervicothoracic ganglia.

The parabronchial ganglionic lesions were reproducible but not the fulminating pneumonia observed in the field. Three major factors—virus, age, and temperature—may account for the absence of pneumonia in the principals. The virus isolated from the outbreak could have represented a mixed population from which plaque purification may have selected a nonpneumotrophic virus strain later used for the inoculum.

Age is probably important because pneumonia occurred in neonatal lambs, and the principals were 5 to 6 months old. In older animals, the mucociliary clearance mechanism and protective mucus layers may have been better developed than in younger animals, thereby preventing virus from contacting epithelial cell receptors. In addition, cell membrane receptors for the virus may exist on epithelial cell membranes in neonates but not in adult animals. Neonatal alveolar macrophages could also be important in the pathogenesis of pneumonia. The alveolar macrophages from the neonatal sheep may be more permissive for viral replication than the alveolar macrophages from the older experimental animals. This condition would allow for the replication of virus in the alveoli and subepithelial tissues, and would contribute to the fulminating pneumonia observed in the young animals. Temperature, particularly hypothermia, has been demonstrated as a factor in the
pathogenesis of canine herpesvirus infection in dogs (19). By enhancing
PrV replication (19) or by slowing cilia (63), hypothermia in neonatal
sheep may also be significant in the pathogenesis of pneumonia. Because
the ovine Pr outbreak occurred during the winter, hypothermia in
neonates (54) was probably intensified and may account for their severe
pneumonia.

Horizontal transmission of PrV in a flock of sheep may potentially
occur during an outbreak. In contrast to McFerran and Dow's work (87)
and in agreement with Lee et al. (78), virus was isolated from nasal
mucus during the agonal phase of the disease. Contrary to Berecz's (15)
and Bogdan's (17) hypothesis and in concurrence with McFerran and Dow's
findings (87), virus was not isolated from urine samples.

The extensive viral and histopathological involvement of the
trigeminal, cranial cervical, and cervicothoracic ganglia from the
experimental sheep led to the inclusion of a short anatomy paper in this
dissertation. By describing the morphology and illustrating the
anatomical location of the ganglia, the article serves as a simplified
and concise reference for harvesting the specimens for examination.


44. GODINHO HP. A comparative anatomical study of the cranial nerves in goat, sheep and bovine (Capra hircus, Ovis aries and Bos taurus); their distribution and related autonomic components. Ph.D. Thesis. Iowa State University. 1968.


71. KOLONITS B. Paralysis bulbaris infectiosa acuta kutyan, juhon es szarvasmarhakon. Allatorvosi Lapok 1912; 35; 615-617.


129. ZWICK, ZELLER. Untersuchungen über die sogenannte Pseudowut. Arb Kaiserl Gesundht 1911; 36: 382-408.
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A special thank-you goes to my wife, Bonnie, for her love, support, patience, and sacrifice during this endeavor. To her I dedicate this dissertation.
APPENDIX: TABLES
Table A-1. Summary of experimental design

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\(^a\)Infected (i).
\(^b\)Control (c).
\(^c\)Animal number.
\(^d\)Hours post inoculation.
Table A-2. Histopathological observations from selected tissues of PrV inoculated sheep

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aHours post inoculation.

bLungs (LU); Mesencephalon (MES); Metencephalon (MET); Cerebellum (CB); Medulla oblongata (MO); Solitary Tract Nucleus (STN); Spinal Tract Nucleus of the Trigeminal Nerve (STT); Trigeminal ganglia (TG); Cranial cervical ganglia (CCG); Cervicothoracic ganglia (CTG).

cNegative.

dNot done.

ePositive: (+) intranuclear inclusion bodies in neurons; (++) or (+++) intranuclear inclusion bodies in neurons and glial cells, gliosis, microglial nodules, and lymphohistiocytic perivascular cuffing.
Table A-3. Virus isolation from selected tissues of PrV inoculated sheep

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\[^a\text{Hours post inoculation.}\]

\[^b\text{Nasal turbinate (NT); Tonsil (TON); Retropharyngeal lymph node (RLN), Lung (LU); Bronchial lymph node (BLN); Adrenal gland (AD); Trigeminal ganglia (TG); Cranial cervical ganglia (CCG); Cervicothoracic ganglia (CTG); Olfactory bulbs (OB); Cerebral cortex and basal ganglia (CC); Mesencephalon and Metencephalon (MM); Medula oblongata (MO); Cervical Spinal Cord (CSC); Thoracic spinal cord (TSC); Lumbar spinal cord (LSC).}\]

\[^c\text{Negative.}\]

\[^d\text{Positive.}\]
Table A-4. Fluorescent antibody staining from selected tissues of PrV inoculated sheep

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^aHours post inoculation.

^bLung (LU); Bronchial lymph node (BLN); Adrenal gland (AD); Trigeminal ganglia (TG); Cranial cervical ganglia (CCG); Cervicothoracic ganglia (CTG); Olfactory bulbs (OB); Basal Ganglia (BG); Mesencephalon (MES); Medulla oblongata (MO); Cervical spinal cord (CSC); Thoracic spinal cord (TSC); Lumbar spinal cord (LSC).

^cNot done.

^dNegative.

^ePositive.