

Marek's disease virus-induced transient paralysis in chickens: electron microscopic lesions*

J. N. Kornegay¹ and E. J. Gorgacz². **

¹ Department of Companion Animal and Special Species Medicine, School of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA

² Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

Summary. A study was made to determine the causative lesion of Marek's disease virus (MDV)-induced transient paralysis (TP) in chickens by comparing the ultrastructure of brain tissue from MDV-infected genetically susceptible and resistant birds. There were numerous intramyelinic vacuoles in the brains of TP-affected birds. Many of these vacuoles contained particulate material compatible with precipitated protein from edema. Astrocyte processes often were distended with similar particulate material. Most intramyelinic vacuoles were either adjacent to the axolemma or within inner myelin lamellae. Myelin sheaths of affected axons, while being displaced, were relatively normal with no vesiculation. Most affected axons were also otherwise normal. Cell processes adjacent to occasional affected axons were distended by degenerating mitochondria, vacuoles, and amorphous material. Some of these processes appeared to be inner loops of oligodendrocyte cytoplasm. The cell bodies of most oligodendrocytes were normal, but a few contained vacuoles similar to those seen in processes adjacent to axons. There were scattered necrotic cells. While most of these could not be specifically identified, some appeared to be oligodendrocytes. Mononuclear inflammatory cells were present both perivascularly and within the parenchyma. Although these cells occasionally contacted myelinated axons and there was myelin phagocytosis, there was no indication that they initiated demyelination. Brain tissue from virus-inoculated resistant birds had perivascular aggregates of mononuclear cells, but there were no intramyelinic vacuoles and few necrotic cells. These findings suggest that intramyelinic vacuolation contributes to the patho-

genesis of transient paralysis. Potential pathophysiological mechanisms contributing to the vacuoles, including brain edema and oligodendrocyte injury, are discussed.

Key words: Intramyelinic edema — Oligodendrocyte — Marek's disease virus — Transient paralysis

Marek's disease in chickens is caused by an oncogenic herpesvirus [11, 30] and is associated with visceral lymphomas and neural lymphoid lesions [4, 8, 28, 31]. Most Marek's disease virus (MDV)-infected chickens have definitive brain lesions [45] that do not cause neurological clinical signs. However, MDV infection can cause a reversible encephalopathy, termed transient paralysis (TP), in certain lines of chickens [10, 18, 34, 39, 42, 46–48]. Affected birds become depressed and recumbent 8 to 12 days after either inoculation or natural exposure to the virus. Although some severely affected birds die at this time, the clinical signs usually resolve in 24 to 48 h. Most birds that have recovered from TP develop the typical visceral and neural lesions of Marek's disease 2 to 6 weeks later. The incidence of TP in farm flocks is generally quite low.

Susceptibility to TP is a recessive trait controlled by major histocompatibility complex (MHC) genes [39]. The occurrence of clinical signs probably has an immunological basis [32]. In previous descriptions of the histological lesions in brains from chickens having TP [10, 18, 34, 39, 42, 46–48], lymphocytic perivascular cuffing was the only change consistently identified. Since lymphocytic perivascular cuffing has been identified in the brains of genetically resistant [39] and clinically recovered birds [34], it seems likely that this is not the causative lesion of TP. In a recent light microscopic study of brain lesions in TP-susceptible and resistant lines of chickens, we made compari-

* Supported in part by the National Institute of Neurological and Communicative Disorders and Stroke, NIH, Grant No. NS16770 and by the State of North Carolina

** Present address: Experimental Pathology Laboratories Inc., P.O. Box 487, Ross, OH 45061, USA

Offprint requests to: J. N. Kornegay (address see above)

sons at 11 days after MDV inoculation, when clinical signs were evident in susceptible birds, and at 17 days when these signs had remitted [19]. Brains obtained from susceptible birds at the height of clinical involvement consistently had vacuolation of both gray and white matter throughout the brain, while this lesion was not present in brains of genetically resistant or clinically recovered birds. There was apparent vasculitis in some of the clinically affected susceptible birds, suggesting that the vacuoles might have resulted, at least in part, from vasogenic brain edema. In this report, ultrastructural brain lesions occurring after MDV inoculation of TP-susceptible and TP-resistant chickens are described.

Materials and methods

Experimental animals

Chickens from two closely related inbred White Leghorn lines (G-B1 and G-B2) were used. These two lines differ genetically at the B complex; i.e., the chicken MHC. While G-B1 birds have the B13 MHC haplotype and are resistant to TP, G-B2 birds have the B6 haplotype and are susceptible [39]. All birds were wingbanded at hatching and reared in Horsfall-Bauer isolation chambers with filtered air under positive pressure.

Virus

The clone-purified GA isolate of MDV was used. To induce TP, intraperitoneal inoculations were made with cell-associated virus in spleen cells from MDV-infected G-B1 or G-B2 birds. Each inoculated bird received 1.0 ml of a cell suspension containing 1×10^7 to 2×10^7 cells/ml. This was equivalent to approximately 0.8×10^3 to 1.6×10^3 focus forming units of MDV/bird. The birds were 2–3 weeks old at the time of inoculation.

Neurological examination

Thirteen G-B1 birds (10 inoculated, 3 control) and 25 G-B2 birds (22 inoculated, 3 control) were evaluated. Neurological examinations were done on all inoculated birds on the day prior to inoculation and 8 to 11 days after inoculation. Control birds were examined on the day tissues were obtained. This examination was modified from one used in mammalian domestic animals [35] and included evaluation of mental attitude, postural reactions, and reflexes [20].

Electron microscopic examination

Brain tissue was obtained at 8 to 11 days after the birds were inoculated with virus. The chickens were anesthetized using a combination of xylazine (60 mg/kg body weight, IM) and diazepam (1.5 mg/kg of body weight, IM). The sternum and ventral rib cage were removed, the pericardial sac was incised, and the right auricle was torn. A 16-gauge needle then was inserted into the right brachiocephalic artery at the base of the heart and 250 ml of a solution containing 1% paraformaldehyde and 1% glutaraldehyde in 0.2 M sodium cacodylate buffer was drip perfused from a height of 1.5 m over a 30-min span. Then 50 ml of a more concentrated solution containing 4% paraformaldehyde and 5% glutaraldehyde in 0.2 M sodium cacodylate buffer was infused manually with a syringe. The brain

of each bird was removed immediately following perfusion and serially sectioned at approximately 2-mm intervals. Portions of the neostriatum and cerebellar white matter were diced into approximately 1 mm³ sections and immersed in the more concentrated fixative for 24 h. These tissues were rinsed in 0.1 M phosphate buffer, then postfixed in 1% OsO₄ in 0.1 M phosphate buffer. After rinsing with deionized water, tissues were dehydrated through an ethanol and acetone series and embedded in Spurr resin [40]. Semi-thin (0.25–0.5 µm) sections were cut with a glass knife and stained with 1% toluidine blue in 1% sodium borate. All slides were examined with a light microscope. Thin sections approximately 80 nm thick were cut with a diamond knife, stained with methanolic uranyl acetate and Reynold's lead citrate [36], and examined with a transmission electron microscope.

Results

Virus-inoculated, TP-susceptible chickens

Neurological clinical signs were evident in 18 of the 22 inoculated birds examined 8 to 11 days after inoculation. These signs varied from mild depression and ataxia to stupor and marked paresis. There were light and electron microscopic lesions in both the cerebellar and striatal sections in all birds. Lesions were most pronounced in the cerebellum. The severity of these lesions varied directly with the degree of clinical involvement, being least pronounced in the four clinically-normal birds. Viral particles were not identified in any of the birds.

There were scattered vacuoles, particularly in the cerebellar white matter, on light microscopic examination (Fig. 1A). At the electron microscopic level, many of these vacuoles were within myelin sheaths either directly adjacent to the axolemma (Fig. 1B) or within inner myelin lamellae (Fig. 2A, B). Although the location of the intramyelinic spaces relative to myelin periodicity could not usually be defined, some appeared to be at the intraperiod line (Fig. 2B). These intramyelinic spaces were usually relatively electron lucent, except for widely dispersed membranous debris and particulate material presumed to be precipitated protein from edema. Myelin displaced by these vacuoles often remained attached to the axon and was relatively normal morphologically, with no vesiculation. Lymphocytes, plasma cells, and macrophages were present in affected areas. However, while there was phagocytosis of myelin (Fig. 3A, B), there was no indication that these cells initiated demyelination. Few axons were completely demyelinated. Most affected axons were morphologically normal, but some were more electron lucent than normal, contained swollen mitochondria, and had discontinuous axolemmas. There were scattered axonal spheroids (Fig. 4A) and axoplasm was occasionally sequestered by inner loops of oligodendrocyte cytoplasm (Fig. 4B).

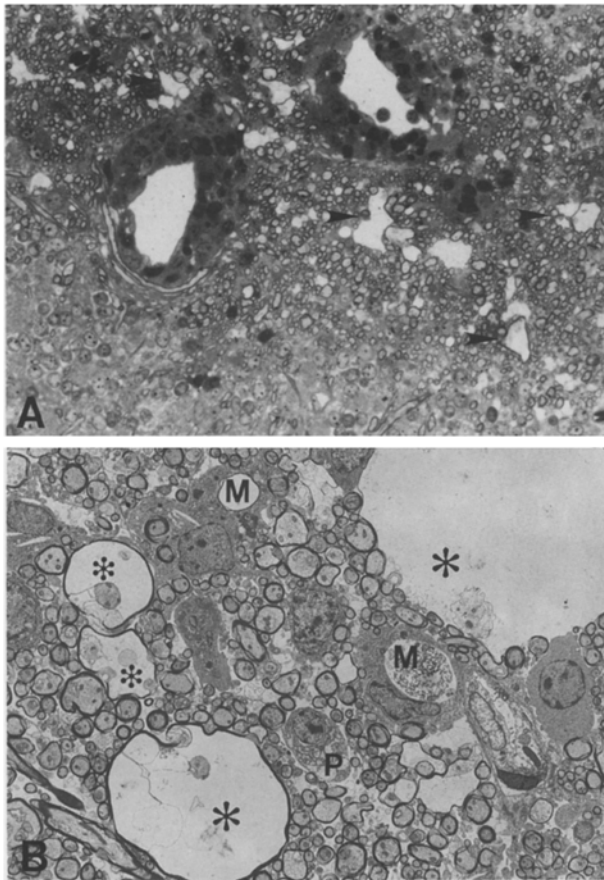


Fig. 1 A, B. Inoculated G-B2 chicken, cerebellum. **A** There is a perivascular mononuclear inflammatory cell infiltrate. Numerous vacuoles are seen within the adjacent white matter (*arrowheads*). **B** Several periaxonal spaces are markedly expanded (*asterisks*). Two macrophages (*M*) containing necrotic cellular debris of myelin and a plasma cell (*P*) can be identified. **A** Toluidine blue, $\times 231$; **B** $\times 1,654$

Numerous necrotic cells that were too distorted to allow definite identification of cell type were present. Some were relatively electron dense, contained large mitochondria and maloriented tubules, and had no visible filaments, suggesting that they were oligodendrocytes (Fig. 5A). A few more normal-appearing oligodendrocytes had vacuoles and swollen mitochondria within their cell bodies (Fig. 5B). Cytoplasmic processes containing swollen mitochondria, vacuoles, and amorphous material were occasionally present within dilated periaxonal spaces (Fig. 6A, B). Many of these processes were located adjacent to the axon and appeared to be inner tongues of oligodendrocyte cytoplasm contributing to the myelin sheath (Fig. 6A). Similar processes seen more rarely external to affected myelin sheaths may have been outer loops of oligodendrocyte cytoplasm. Some of these processes were also probably from macrophages.

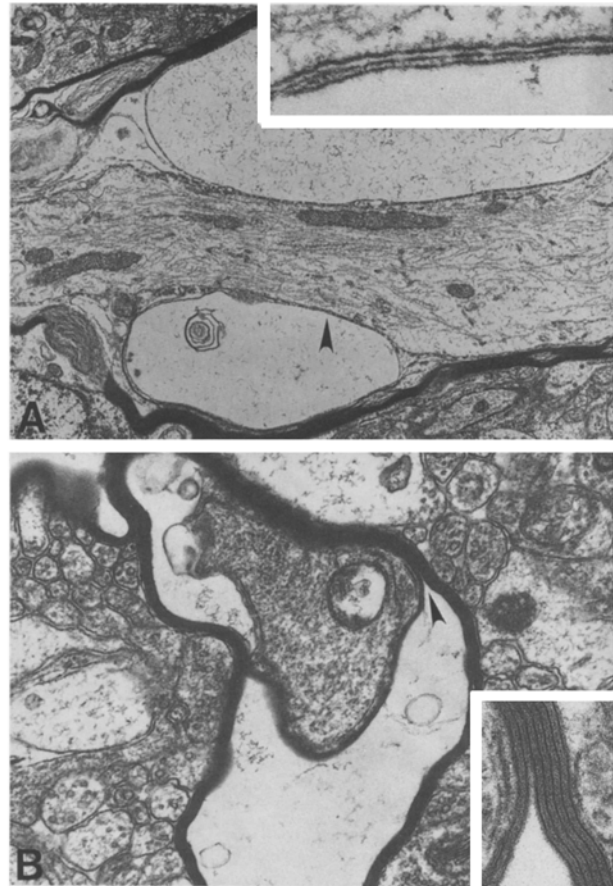


Fig. 2A, B. Inoculated G-B2 chicken. **A** Cerebellum. Spaces on either side of the axon are dilated and contain particulate material. Area at *arrowhead* is enlarged in inset. Inset shows interface between vacuole and axon with remaining thin layer of myelin. **B** Striatum. Dilated intramyelinic space. Area at *arrowhead* is enlarged in inset. Inset shows separation of myelin sheath at the intraperiod line. **A** $\times 7,150$, *inset* $\times 55,184$; **B** $\times 21,703$, *inset* $\times 71,312$

Astrocyte nuclei and cell bodies seen with light microscopy were often more vesicular than normal. On electron microscopic examination, there were numerous cell processes that contained particulate material similar to that seen in intramyelinic vacuoles. While many of these cells had few organelles and no identifiable filaments, they were believed to be astrocytes based on their nuclear morphology and overall electron density. Processes from some of these cells abutted apparently collapsed intramyelinic vacuoles (Fig. 7), suggesting they may have been involved in removal of intramyelinic edema. Most neurons were normal, but some had swollen mitochondria and rough endoplasmic reticulum.

Small lymphocytes and macrophages often were seen within the perivascular space between the endothelial and astrocytic basal laminae (Fig. 8A). Cellular debris and relatively electron-lucent spaces, presumed

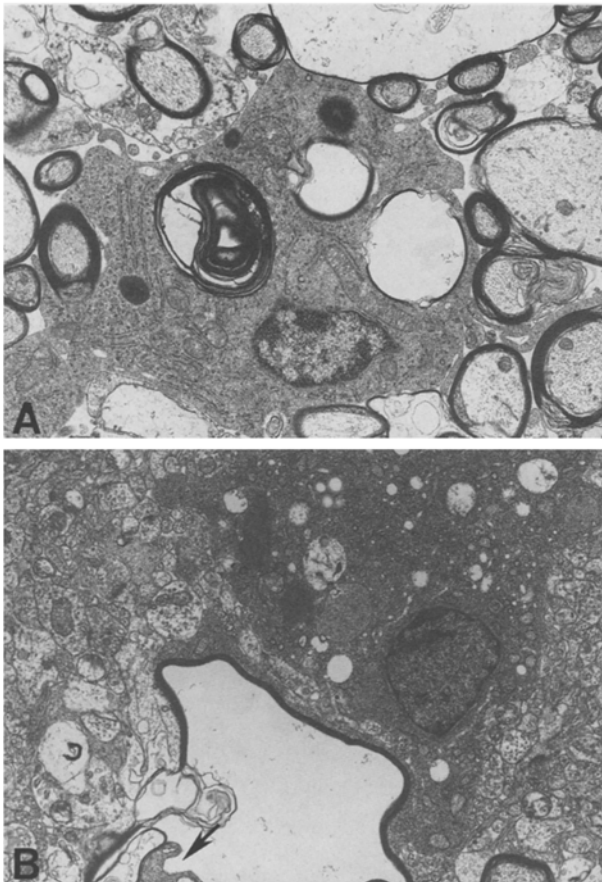


Fig. 3A, B. Inoculated G-B2 chicken, cerebellum. **A** A macrophage contains myelin debris. **B** A dilated periaxonal space (axon at the *arrow*) and adjacent macrophage are seen. **A, B** $\times 7,337$

to be caused by collections of proteinaceous fluid, often were seen among the inflammatory cells. Many microvilli extended from endothelial cells into vessel lumina. There were also increased numbers of small coated vesicles that appeared to originate subsequent to invagination of the luminal surface of the endothelial cell (Fig. 8B). The numbers of endothelial microvilli and vesicles were increased when compared to uninoculated controls, but were similar in degree to that found in MDV-inoculated G-B1 birds. Perivascular astrocyte foot processes occasionally were swollen and electron lucent (Fig. 8B). Widening of endothelial cell tight junctions was not demonstrated, and perivascular extracellular spaces were not dilated.

Virus-inoculated, TP-resistant chickens

Although none of these birds had clinical signs, all had lesions in both the cerebellum and striatum that are characteristic for MDV-infected chickens. Brain

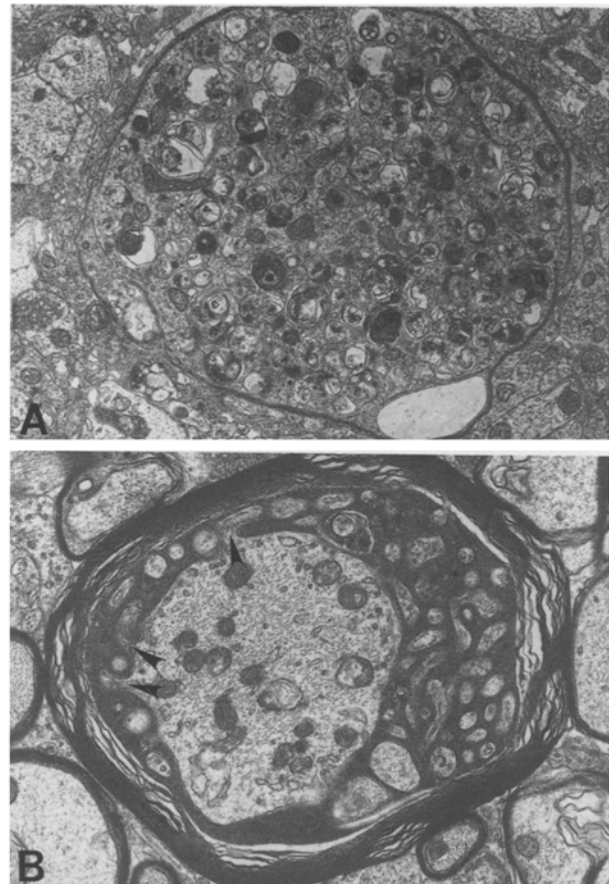


Fig. 4A, B. Inoculated G-B2 chicken. **A** Striatum. A large axonal spheroid containing mitochondria and membranous material is seen. **B** Cerebellum. Portions of axoplasm are sequestered by oligodendrocyte cytoplasm. Origination of these axoplasmic islands can be seen at the *arrowheads*. **A** $\times 7,337$; **B** $\times 10,748$

lesions in G-B1 birds were considerably less pronounced than those identified in the G-B2 birds. There were no intramyelinic vacuoles and few necrotic cells. Small lymphocytes often had accumulated perivascularly. Pinocytotic activity appeared to be greater than that of uninoculated controls and was generally similar to that of inoculated G-B2 chickens. Viral particles were not identified in any of the birds.

Uninoculated controls

Neurological clinical signs were not evident in any control chickens, and brain lesions found in MDV-inoculated birds were not present.

Discussion

We have previously compared the light microscopic lesions induced by MDV inoculation in G-B2 chickens known to be susceptible to TP to those in resistant

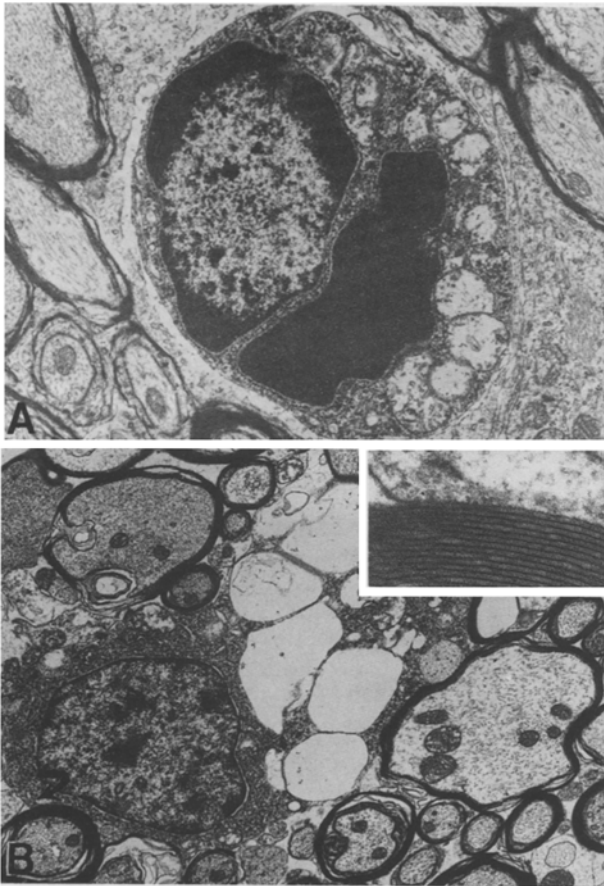


Fig. 5A, B. Inoculated G-B2 chicken, cerebellum. **A** Necrotic cell, believed to be an oligodendrocyte, has a pycnotic nucleus and numerous degenerating mitochondria. **B** Cell, believed to be an oligodendrocyte, contains several large vacuoles that are subdivided by strands of cytoplasm. The relationship of this cell and an adjacent myelin sheath in the lower left corner (*arrow*) is seen in the *inset*. *Inset* shows close association of cell and myelin sheath. **A** $\times 14,469$; **B** $\times 7,844$, *inset* $\times 71,285$

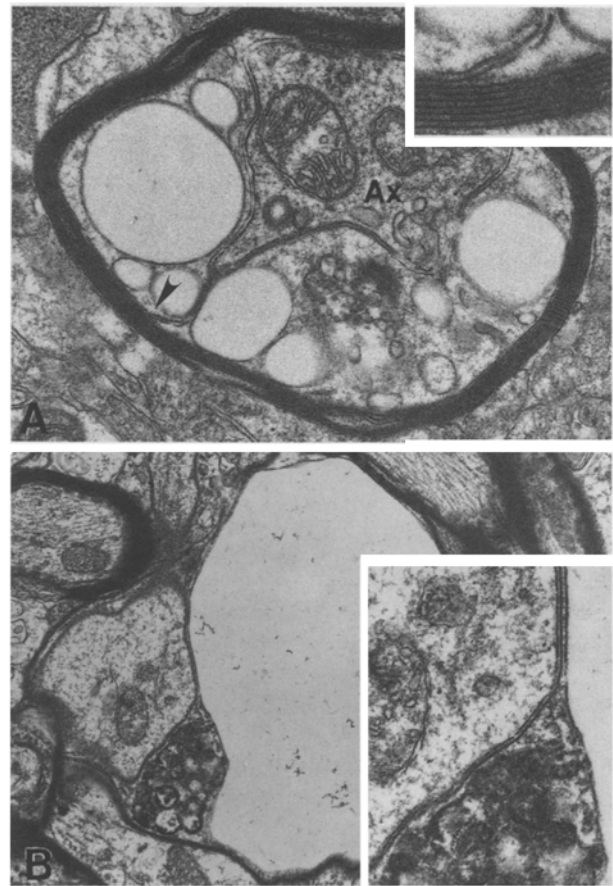


Fig. 6A, B. Inoculated G-B2 chicken. **A** Striatum. Inner loop of oligodendrocyte cytoplasm adjacent to axon (*Ax*) contains several vacuoles and necrotic debris. Connection of this process to the surrounding myelin sheath (*arrowhead*) is enlarged in the *inset*. *Inset* shows the contribution of the membrane of this cell to the myelin sheath. **B** Cerebellum. Cell process, believed to be inner loop of oligodendrocyte cytoplasm, adjacent to axon within a dilated space contains degenerating mitochondria. *Inset* shows interface of this process and the adjacent axon. **A** $\times 25,042$, *inset* $\times 71,311$; **B** $\times 12,402$, *inset* $\times 32,038$

G-B1 birds [19]. The most significant difference was the presence of vacuoles in both the striatum and cerebellar white matter of the G-B2 chickens with TP. These vacuoles were felt most likely to represent edema, perhaps occurring as a result of apparent vasculitis in some birds. Similar vacuoles were seen on light microscopic examination of TP-affected birds described here. Ultrastructural findings indicated that many of them were dilated intramyelinic spaces. These spaces and numerous astrocyte processes contained fine particulate material that presumably was precipitated protein from edema. The myelin sheaths of affected axons, although generally widely displaced from the axolemma, were often otherwise relatively normal morphologically. Active phagocytosis of myelin seen in TP-affected birds suggested that at least

some affected axons eventually became demyelinated. However, few completely demyelinated axons were seen.

The origin of the dilated spaces seen within myelin sheaths of TP-affected birds is not clear. Similar intramyelinic vacuoles have been described in a variety of naturally occurring and experimental diseases. No single pathogenetic mechanism appears to be responsible for this lesion. Intramyelinic vacuoles and demyelination seen in van Bogaert-Bertrand spongy degeneration in human infants are thought to occur subsequent to accumulation of edema in astrocytes [1, 2, 15]. The edema, which may occur because of lowered astrocytic ATPase activity, is also believed ultimately to accumulate within myelin sheaths because of the hydrophilic nature of the intraperiod line.

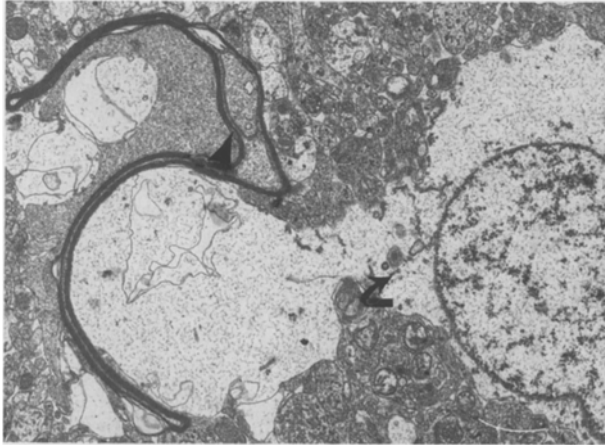


Fig. 7. Inoculated G-B2 chicken, striatum. A process extending from the cell body at the right (*arrow*) abuts an apparently collapsed intramyelinic vacuole. An axon is seen at the *arrowhead*. The process and cell body contain fine particulate material compatible with precipitated protein from edema. There are no identifiable cellular organelles but the nucleus resembles that of an astrocyte. $\times 6,996$

Similar pathogenetic mechanisms could be involved in analogous spongiform encephalopathies in cattle and cats [16, 17]. The changes reported here in TP-affected birds are similar to those of van Bogaert-Bertrand spongy degeneration, but we were unable to define whether the intramyelinic vacuoles preceded or occurred subsequent to the astrocyte distension. It is possible that an MDV-induced effect on astrocytes could lead to intracellular edema and subsequent intramyelinic vacuolation. It seems at least equally plausible that astrocyte distension, seen in TP-affected birds, occurred because these cells were involved in the removal of edema. Presumably, accumulation of extracellular edema could also lead to intramyelinic vacuolation, especially in young birds in which myelination is still active. However, there was often minimal, if any, expansion of the extracellular space in areas of intramyelinic vacuolation in birds of this study.

Studies of toxins that induce intramyelinic vacuolation have been useful in defining the role of myelin or oligodendrocyte injury in the evolution of this lesion. Intoxication with tin [3, 26, 38] and hexachlorophene [9, 43] is thought to alter the biochemical composition of myelin. This evidently increases permeability of myelin lamellae to extracellular fluid, resulting in large vacuoles at the intraperiod line. Most of these vacuoles occur at the periphery of the myelin sheath, which seems consistent with their origin from extracellular fluid. Myelin structural changes induced by the MDV or an associated immune-mediated mechanism could conceivably allow similar intramyelinic fluid accumulation, especially considering that demyelination in the

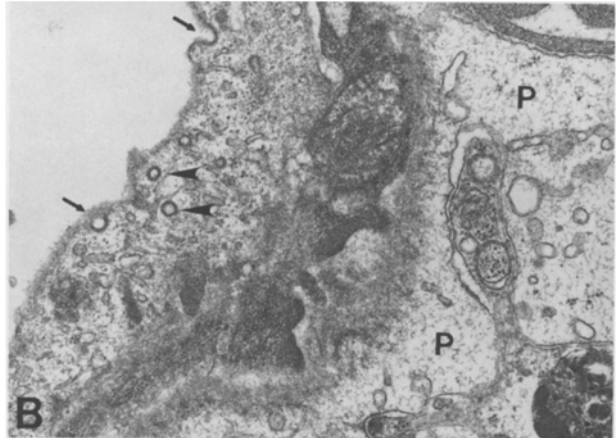
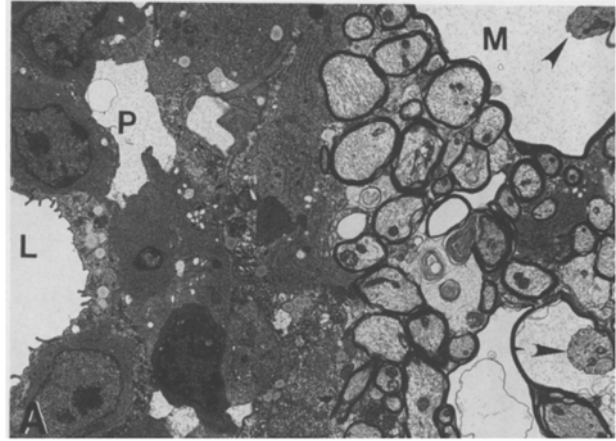


Fig. 8A, B. Inoculated G-B2 chicken. **A** Cerebellum. Vessel (lumen at *L*) and adjacent white matter. Several lymphocytes and collections of particulate material (*P*) are present within the perivascular space. Portions of several myelin-lined spaces (*M*) are seen adjacent to the vessel. Axons within these spaces are identified by *arrowheads*. **B** Striatum. Vessel and adjacent astrocyte process. Several coated vesicles are present in the endothelial cytoplasm (*arrowheads*). The cytoplasm appears to be invaginating at two sites (*arrows*). The adjacent astrocyte foot process (*P*) is dilated. **A** $\times 2,926$; **B** $\times 17,466$

peripheral nervous system is well documented in MD [23, 33]. However, demyelination was not a prominent feature in TP-affected birds, and the intramyelinic vacuoles were usually periaxonal or dissected inner myelin lamellae, with the peripheral myelin being spared.

Changes similar to those seen in TP-affected birds have been reported subsequent to intoxication with agents that have a cytopathic effect against oligodendrocytes, such as cuprizone [5, 6, 27, 41] and isonicotinic acid hydrazide [7, 21]. Numerous dilated spaces form adjacent to axons and within inner myelin lamellae subsequent to these toxins. Inner and outer tongues of oligodendrocyte cytoplasm adjacent to affected axons are often distended with degenerating organelles, and axoplasm of some axons is sequestered

by oligodendrocyte cytoplasm. Many affected axons are eventually demyelinated. The intramyelinic vacuolation apparently results from the coupled effects of disruption of normal myelination and myelin hydrophilia. Certain lesions described here suggest that oligodendrocyte injury may also lead to intramyelinic vacuolation in TP-affected birds. Some of the many necrotic cells appeared to be oligodendrocytes, inner loops of oligodendrocytes were distended with necrotic debris, and there were large vacuoles in a few oligodendrocyte cell bodies. However, despite the similarities between these lesions and those caused by oligodendrocyte toxins, we were unable to show definite morphological evidence that oligodendrocyte lesions led to intramyelinic vacuolation.

Intramyelinic vacuolation is evidently not a prominent feature of other viral and immune-mediated encephalitides. Apparent intramyelinic vacuoles similar to those seen in TP-affected birds were illustrated but not specifically discussed in papers describing demyelination associated with infection with the JHM corona virus [29], vesicular stomatitis virus [13], and Chandipura virus [14]. That intramyelinic vacuoles have been seen subsequent to JHM corona virus infection is particularly noteworthy, since oligodendrocyte injury may be involved in the pathogenesis of this condition [22, 44]. However, intramyelinic vacuolation has not been described subsequent to Theiler's virus infection [12], even though virus-induced lysis of oligodendrocytes may be responsible for demyelination [37]. While intramyelinic vacuolation is not a common feature of experimental allergic encephalitis, large intramyelinic vacuoles similar to those seen in TP-affected birds have been reported in association with neuronal and glial cell death [24].

We are unsure of the significance of the vascular changes seen in TP-affected birds. Most of the lymphocytes within perivascular spaces were probably simply migrating extravascularly, as has been reported previously in MDV-inoculated, TP-resistant chickens [25]. There was no separation of endothelial cell tight junctions or marked vessel wall distortion to suggest vasculitis. However, there was evidence of increased pinocytotic activity in TP-affected birds compared to uninoculated controls. While studies using electron-dense markers would be needed to provide definite proof, this increase in pinocytosis suggests that the blood-brain barrier may also be altered in TP-affected birds. Such an increase in vascular permeability could be the source of the intramyelinic and astrocytic edema seen in these birds.

Acknowledgements. We gratefully acknowledge the assistance of the staffs of the electron microscopy laboratories of the University of Georgia College of Veterinary Medicine and the School of Veterinary Medicine at North Carolina State University.

References

1. Adachi M, Wallace BJ, Schneck L, Volk BW (1966) Fine structure of spongy degeneration of the central nervous system (van Bogaert and Bertrand type). *J Neuropathol Exp Neurol* 25:598–616
2. Adornato BT, O'Brien JS, Lampert PW, Roe TF, Neustein HB (1972) Cerebral spongy degeneration of infancy. A biochemical and ultrastructural study of affected twins. *Neurology* 22:202–210
3. Aleu FP, Katzman R, Terry RD (1963) Fine structure and electrolyte analysis of cerebral edema induced by alkyl tin intoxication. *J Neuropathol Exp Neurol* 22:403–413
4. Biggs PM (1973) Marek's disease. In: Kaplan AS (ed) *The herpesviruses*. Academic Press, New York, pp 557–594
5. Blakemore WF (1972) Observations on oligodendrocyte degeneration, the resolution of status spongiosus and remyelination in cuprizone intoxication in mice. *J Neurocytol* 1:413–426
6. Blakemore WF (1973) Demyelination of the superior cerebellar peduncle in the mouse induced by cuprizone. *J Neurol Sci* 20:63–72
7. Blakemore WF, Palmer AC, Noel PRB (1972) Ultrastructural changes in isoniazid-induced brain oedema in the dog. *J Neurocytol* 1:263–278
8. Calnek BW (1980) Marek's disease virus and lymphoma. In: Rapp F (ed) *Oncogenic herpesviruses*, vol 1. CRC Press, Boca Raton, pp 103–143
9. Cammer W, Rose AL, Norton WT (1975) Biochemical and pathological studies of myelin in hexachlorophene intoxication. *Brain Res* 98:547–559
10. Cho BR, Kenzy SG, Mathey WJ (1970) Histologic and microbiologic studies of chickens with transient paralysis. *Avian Dis* 14:587–598
11. Churchill AE, Biggs PM (1967) Agent of Marek's disease in tissue culture. *Nature* 215:528–530
12. Dal Canto MC, Lipton HL (1975) Primary demyelination in Theiler's virus infection. *Lab Invest* 33:626–637
13. Dal Canto MC, Rabinowitz SG (1981) Murine central nervous system infection by a viral temperature-sensitive mutant. A subacute disease leading to demyelination. *Am J Pathol* 102:412–426
14. Dal Canto MC, Rabinowitz SG, Johnson TC (1979) Virus-induced demyelination. Production by a viral temperature-sensitive mutant. *J Neurol Sci* 42:155–168
15. Gambetti P, Mellman WJ, Gonatas NK (1969) Familial spongy degeneration of the central nervous system (Van Bogaert-Bertrand disease). *Acta Neuropathol (Berl)* 12:103–115
16. Jolly RD (1974) Congenital brain oedema of Hereford calves. *J Pathol* 114:199–204
17. Kelly DF, Gaskell CJ (1976) Spongy degeneration of the central nervous system of kittens. *Acta Neuropathol (Berl)* 35:151–158
18. Kenzy SG, Cho BR, Kim Y (1973) Oncogenic Marek's disease herpesvirus in avian encephalitis (temporary paralysis). *J Natl Cancer Inst* 51:977–982
19. Kornegay JN, Gorgacz EJ, Parker MA, Duncan JR, Schierman LW (1983) Marek's disease virus-induced transient paralysis: a comparison of lesions in susceptible and resistant lines of chickens. *Acta Neuropathol (Berl)* 61:263–269
20. Kornegay JN, Gorgacz EJ, Parker MA, Brown J, Schierman LW (1983) Marek's disease virus-induced transient paralysis: Clinical and electrophysiological findings in susceptible and resistant lines of chickens. *Am J Vet Res* 44:1541–1544

21. Lampert PW, Schochet SS (1968) Electron microscopic observations on experimental spongy degeneration of the cerebellar white matter. *J Neuropathol Exp Neurol* 27:210–220
22. Lampert PW, Sims JK, Kniazeff AJ (1973) Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. *Acta Neuropathol (Berl)* 24:76–85
23. Lampert P, Garrett R, Powell H (1977) Demyelination in allergic and Marek's disease virus induced neuritis. Comparative electron microscopic studies. *Acta Neuropathol (Berl)* 40:103–110
24. Lassmann H, Kitz K, Wisniewski HM (1980) Structural variability of demyelinating lesions in different models of subacute and chronic experimental allergic encephalomyelitis. *Acta Neuropathol (Berl)* 51:191–201
25. Lawn AM, Watson JS (1982) Ultrastructure of the central nervous system in Marek's disease and the effect of route of infection on lesion incidence in the central nervous system. *Avian Pathol* 11:213–225
26. Leow ACT, Anderson RMcD, Little RA, Leaver DD (1979) A sequential study of changes in the brain and cerebrospinal fluid of the rat following triethyltin poisoning. *Acta Neuropathol (Berl)* 47:117–121
27. Ludwin SK (1978) Central nervous system demyelination and remyelination in the mouse. An ultrastructural study of cuprizone toxicity. *Lab Invest* 39:597–612
28. Marek J (1907) Mehrfache Nervenentzündung (Polyneuritis) bei Hühnern. *Dtsch Tierärztl Wochenschr* 15:417–421
29. Nagashima K, Wege H, Meyermann R, ter Meulen V (1978) Corona virus-induced subacute demyelinating encephalomyelitis in rats: a morphological analysis. *Acta Neuropathol (Berl)* 44:63–70
30. Nazerian K, Solomon JJ, Witter RL, Burmester BR (1968) Studies on etiology of Marek's disease. II. Finding of a herpesvirus in cell culture. *Proc Soc Exp Biol Med* 127:177–182
31. Pappenheimer AM, Dunn LC, Cone V (1929) Studies on fowl paralysis (neurolymphomatosis gallinarum). I. Clinical features and pathology. *J Exp Med* 49:63–86
32. Parker MA, Schierman LW (1983) Suppression of humoral immunity in chickens prevents transient paralysis caused by a herpesvirus. *J Immunol* 130:2000–2001
33. Prineas JW, Wright RG (1972) The fine structure of peripheral nerve lesions in a virus-induced demyelinating disease in fowl (Marek's disease). *Lab Invest* 26:548–557
34. Purcell DA, Taylor SM (1971) The histopathology of transient paralysis of the fowl. *Br Vet J* 127:313–317
35. Redding RW, Braund KG (1978) Neurological examination. In: Hoerlein BF (ed) *Canine neurology*. Saunders, Philadelphia, pp 53–70
36. Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* 17:208–212
37. Rosenthal A, Fujinami RS, Lampert PW (1986) Mechanism of Theiler's virus-induced demyelination in nude mice. *Lab Invest* 54:515–522
38. Scheinberg LC, Taylor JM, Herzog I, Mandell S (1966) Optic and peripheral nerve response to triethyltin intoxication in the rabbit: biochemical and ultrastructural studies. *J Neuropathol Exp Neurol* 25:202–213
39. Schierman LW, Fletcher OJ (1980) Genetic control of Marek's disease virus-induced transient paralysis: association with the major histocompatibility complex. In: Biggs PM (ed) *Resistance and immunity to Marek's disease*. Commission of the European Communities, Luxembourg, pp 429–442
40. Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res* 26:31–43
41. Suzuki K, Kikkawa Y (1969) Status spongiosus of CNS and hepatic changes induced by cuprizone (biscyclohexanone oxalyldihydrazone). *Am J Pathol* 54:307–325
42. Walker D, Grattan DAP (1968) A temporary paralytic syndrome of growing pullets. *Vet Rec* 82:43–47
43. Webster HdeF, Ulsamer AG, O'Connell MF (1974) Hexachlorophene-induced myelin lesions in the developing nervous system of *Xenopus* tadpoles: morphological and biochemical observations. *J Neuropathol Exp Neurol* 33:144–163
44. Weiner LP (1973) Pathogenesis of demyelination induced by a mouse hepatitis virus (JHM virus). *Arch Neurol* 28:298–303
45. Wight PAL (1962) The histopathology of the central nervous system in fowl paralysis. *J Comp Pathol* 72:348–359
46. Wight PAL (1968) The histopathology of transient paralysis of the domestic fowl. *Vet Rec* 82:749–755
47. Willemart JP, Montlaur D, Verger M, Labrousse F, Antony deC (1967) La paralysie transitoire des poules domestiques. *Rec Med Vet* 143:253–261
48. Zander DV (1959) Experiences with epidemic tremor control. Proceedings of the 8th Annual Western Poultry Disease Conference, Davis, Calif, pp 18–23

Received August 11, 1987/Revised October 27, 1987/
Accepted November 10, 1987