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## THE PATHOLOGY OF LARYNGEAL HEMIPLEGIA

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IN THE HORSE

VOLUME 11

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Figure 1: A line drawing of the paranodal region of a myelinated nerve fibre, illustrating constriction of the nodal axon (after Waxman, 1978)

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- Figure 2: Drawings of the sequence of events in
  - A. axonal degeneration and regeneration, and
  - B. segmental demyelination and remyelination.
  - A. Degeneration of the distal portion of nerve fibre, followed by axonal sprouting and the formation of short uniform regenerated internodes.
  - B. Demyelination of an internode of a nerve fibre, followed by the formation of several short remyelinated internodes.

(after Weller and Cervos-Navarro, 1977).





Figure 3:

Line drawings of the formation of axon-Schwann cell networks, during the phagocytosis of axonal debris.

A ridge forms in the adaxonal Schwann cell cytoplasm, which enlarges to form a thin sheet. The invaginating sheet of cytoplasm surrounds abnormal axoplasmic organelles, to form an interdigitated network. Axoplasmic material is taken up by Schwann cell cytoplasm, which retracts to its original position. (after Spencer & Thomas, 1974)





Figure 4: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing a cluster of regenerating fibres. Note the surrounding Schwann cell processes (1) and the redundant loop of myelin on one of the myelinated fibres (2). x 3700

Figure 5: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing a mast cell. x 13600

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Figure 6: Line drawing of a longitudinal view of a large myelinated nerve fibre, illustrating the anatomy of nodes of Ranvier and internodal segments (after Sunderland, 1968).



Figure 7: Line drawing of a transverse section of a small myelinated nerve fibre, illustrating the relationship of the Schwann cell and myelin sheath with the axon (after Waxman, 1978).



Figure 8: Electron micrograph of equine recurrent laryngeal nerve showing a protagon granule within the Schwann cell cytoplasm of a myelinated nerve fibre. x 48600

Figure 9: Electron micrograph of equine recurrent laryngeal nerve showing a band of Bungner. The collapsed Schwann cell band is composed of many interdigitated Schwann cell processes, with surrounding basement membrane. x 11200



Figure 10: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing a lamellated myelin body (→) in Schwann cell cytoplasm of a myelinated nerve fibre. x 13600

Figure 11: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing lipid drops (1) in the Schwann cell cytoplasm of a myelinated fibre. Note the lamellated protagon granules (2). x 10500



Figure 12: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing excess convoluted basement membrane associated with a Schwann cell process. x 17500



Figure 13: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing an "onion bulb" formation. Note the layers of Schwann cell processes ( + ) surrounding a central myelinated fibre. x 6300

Figure 14: Electron micrograph of a transverse section of equine recurrent laryngeal nerve showing a collagen pocket. x 26200



Figure 15: Sites of recurrent laryngeal and vagal nerve samples.

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Figure 16: Course of the recurrent laryngeal nerve immediately caudal to the larynx. Lateral lamina of the thyroid cartilage has been removed (after Bradley, 1946)



Figure 17: Anatomical relationships of the recurrent laryngeal and vagal nerves in the midcervical region.



Figure 18: Technique of weighted nerve fixation. A suture is placed at the proximal pole of the nerve biopsy, and an aluminium weight at the distal end, after dissection of surrounding tissue. The nerve is sectioned at both ends, and immediately suspended in fixative (after Dyck & Lofgren, 1966).

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Figure 19: Technique for obtaining single teased nerve fibres (after Dyck, 1975a)

- A. Nerve is placed on a microscope slide in a drop of glycerine, under a dissecting microscope.
- B. The nerve trunk is divided into fascicles
- C. Epineurial collagen and the perineurium are removed
- D. The fascicle is divided in half repeatedly to obtain single fibres
- E. Single fibres are transferred on to a clean microscope slide
- F. Coverslips are placed on groups of 10 fibres on each slide.



Figure 20: Teased fibre classification (after Dyck, 1975a)

- A. fibre of normal appearance
- B. fibre with excessive irregularity of myelin
- C. fibre with segmental demyelination
- D. fibre with segmental demyelination and remyelination
- E. fibre that has undergone degeneration into linear row of ovoids and balls
- F. fibre with remyelinated internodes
- G. fibre with excessive variability of myelin sheath to form thickenings
- H. fibre with myelin ovoids or balls adjacent to it
- I. fibre with linear row of ovoids and balls distally, with or without paranodal demyelination on proximal internodes.



Figure 21:

A photomicrograph of a transverse section of the right recurrent laryngeal nerve in the area of the thoracic inlet, from a control animal. This illustrates the normal density and diameters of myelinated nerve fibres in equine recurrent laryngeal nerve. Phenylenediamine staining. x 200


## Figure 22: Photomicrographs of transverse sections of the left recurrent laryngeal nerve from a clinical laryngeal hemiplegic horse.

- A. Immediately caudal to the larynx. Note the severe loss of myelinated fibres.
  "Onion bulb" formations can be seen (→) one around a myelinated fibre and another without a central fibre.
- B. In the midcervical area. A decrease in density of myelinated fibres is apparent, although less severe than that in A. "Onion bulb" formations (1) and regenerating clusters (2) are common.
- C. At the level of the aortic arch. Myelinated fibre density appears only slightly decreased. "Onion bulb" formations (1) and regenerating clusters (2) with thinly myelinated fibres are common findings. Myelin debris is apparent in the Schwann cell cytoplasm of some fibres (3).

Phenylenediamine staining. x 200



- Figure 23: Photomicrographs of transverse sections of the right recurrent laryngeal nerve from a clinical laryngeal hemiplegic horse.
  - A. Immediately caudal to the larynx. Loss of myelinated fibres is apparent. "Onion bulbs" (1) and regenerating clusters (2), with thinly myelinated fibres are present.
  - B. In the midcervical area. A decrease in density of myelinated fibres and all of the pathological features observed in A can be seen.
  - C. At the level of the thoracic inlet. While no loss of myelinated fibres is readily observable, regenerating clusters (1) and thinly myelinated fibres (2) are present.

Phenylenediamine staining. x 200



Figure 24: A photomicrograph of a transverse section of the left recurrent laryngeal nerve in the midcervical area from a clinical laryngeal hemiplegic horse. Note the large clusters of regenerating fibres (1) and "onion bulb" formations (2). Phenylenediamine staining. x 400

Figure 25: A photomicrograph of a transverse section of the right recurrent laryngeal nerve immediately caudal to the larynx from a clinical laryngeal hemiplegic horse. Note the regenerating clusters (1) and "onion bulb" formations(2). Phenylenediamine staining. x 400





Figure 26: Α photomicrograph of a transverse section of the left recurrent laryngeal nerve in the midcervical area from a clinical laryngical hemiplegic horse. Note the large rounded granular cell  $(\rightarrow)$ , possibly а swollen demyelinated fibre. Thinly myelinated fibres and regenerating clusters are also present. Phenylenediamine staining. x 400

Figure 27:

A photomicrograph of a transverse section of the left recurrent laryngeal nerve in the midcervical area, from a subclinical laryngeal hemiplegic horse. Note the cluster of regenerating fibres, some with long redundant loops of myelin (1). A degenerating myelinated fibre is also present (2), and another with dense axoplasm (3). Phenylenediamine staining. x 400





Figure 28: Photomicrographs of transverse sections of fascicles from the branches of the right recurrent laryngeal nerve innervating the abductor and adductor muscles from a clinical laryngeal hemiplegic horse.

A. Abductor branch

B. Adductor branch

Note the increased severity of pathological changes in the adductor branch, including loss of myelinated fibres, regenerating clusters and "onion bulb" formations. Phenylenediamine staining. x 200



Figure 29: A photomicrograph of a transverse section of the left superficial peroneal nerve from a clinical laryngeal hemiplegic horse. Note the fibres with disproportionately thin myelin sheaths (1), and the "onion bulb" formation around a thinly myelinated fibre (2). Phenylenediamine staining. x 200

Figure 30: A photomicrograph of a transverse section of the left tibial nerve from a clinical laryngeal hemiplegic horse. Several thinly myelinated fibres are present (  $\rightarrow$  ). Phenylenediamine staining. x 200

Figure 31: A photomicrograph of a transverse section of the branch of peroneal nerve innervating the extensor digitorum longus muscle from a clinical laryngeal hemiplegic horse. Note the cluster of regenerating fibres  $( \rightarrow )$ . Phenylenediamine staining. x 400



Figure 32: A photomicrograph of a transverse section of the right common peroneal nerve from a clinical laryngeal hemiplegic horse. Note the regenerating clusters (1) and thinly myelinated fibre (2). Phenylenediamine staining. x 200

Figure 33: A photomicrograph of a transverse section of the branch of the peroneal nerve innervating the *extenson digitonum longus* muscle from a clinical laryngeal hemiplegic horse. Note the thinly myelinated fibres, one of which has surrounding Schwann cell processes (+). Phenylenediamine staining. x 200





Figure 34: A photomicrograph of a transverse section of the right recurrent laryngeal nerve in the midcervical area of a subclinical laryngeal hemiplegic horse. Note the large myelinated fibre with split myelin sheath and granular contents ( → ). Phenylenediamine staining. x 400



Figure 35: A photomicrograph of a transverse section of the left recurrent laryngeal nerve immediately caudal to the larynx of a control horse. The swollen fibre distended with dense granular axoplasm has an attenuated myelin sheath and many surrounding layers of Schwann cell processes. Phenylendiamine staining. x 400

Figure 36: A photomicrograph of a transverse section of the right recurrent laryngeal nerve immediately caudal to the larynx from a clinical laryngeal hemiplegic horse. A large "onion bulb" surrounds two fibres with thin myelin sheaths and dense axoplasm. Phenylenediamine staining. x 400





Figure 37: A photomicrograph of a transverse section of the right recurrent laryngeal nerve immediately caudal to the larynx from a 1 day old foal showing Renaut: bodies ( → ). Phenylenediamine staining. x 400

Figure 38: A photomicrograph of a transverse section of the right recurrent laryngeal nerve at the level of the thoracic inlet from a clinical laryngeal hemiplegic horse. Note the extensive Renaut body formations, one of which is not subperineurial in position (+). Phenylendiamine staining. x 100

Figure 39: A photomicrograph of a transverse section of the right recurrent laryngeal nerve in the midcervical area from a control horse. The nerve has a flattened ribbon-like appearance, being only one fascicle in width. (Haslam's anomaly). Phenylendiamine staining. x 40



Figure 40: Histograms of the myelinated nerve fibre diameter distributions of the recurrent laryngeal nerve immediately caudal to the larynx.

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DISTAL RECURRENT LARYNGEAL NERVE

Figure 41: Histograms of the myelinated nerve fibre diameter distributions of the recurrent laryngeal nerve in the midcervical area.



MIDCERVICAL RECURRENT LARYNGEAL NERVE

Figure 42: Histograms of the myelinated nerve fibre diameter distributions of the recurrent laryngeal nerve in the area of the aortic arch.

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PROXIMAL RECURRENT LARYNGEAL NERVE

Figure 43: Histograms of the myelinated nerve fibre

Histograms of the myelinated nerve fibre diameter distributions of intralaryngeal branches of the recurrent laryngeal nerves.



## INTRALARYNGEAL NERVES

Fibre diameter (µm)

Figure 44: Photomicrographs of consecutive portions of a teased myelinated fibre from the tibial nerve of a clinical laryngeal hemiplegic horse, showing type B change. Note the roughening and irregularity of the myelin sheath. (x 40)



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Figure 45: Photomicrographs of consecutive portions of a teased myelinated fibre from the left recurrent laryngeal nerve of a clinical laryngeal hemiplegic horse showing type C change. The regions of paranodal demyelination are located between the arrows. x 40



Figure 46:

Photomicrographs of consecutive portions of a teased myelinated fibre from the left recurrent laryngeal nerve immediately caudal to the larynx of a subclinical laryngeal hemiplegic horse showing type D change. There is evidence of demyelination (1) and remyelination (2). x 40



Figure 47: Photomicrographs of consecutive portions of a teased myelinated fibre from the left recurrent laryngeal nerve in the midcervical area of a clinical laryngeal hemiplegic horse showing type F change. Note the intercalated internodes located between the arrows. x 40


Figure 48:

Photomicrographs of consecutive portions of a teased myelinated fibres from the right recurrent laryngeal nerve immediately caudal to the larynx, of a clinical laryngeal hemiplegic horse, showing type G change. In addition to the thickenings of the myelin sheath, intercalated segments are present  $( \rightarrow )$  x 40



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Figure 49:

Photomicrographs of consecutive portions of a teased myelinated fibre from the left recurrent laryngeal nerve immediately caudal to the larynx of a control horse showing type I change. Note the axonal degeneration distal to a node of Ranvier (+), with preservation of the proximal portion of the fibre. x 40



Figure 50: A photomicrograph of a teased fibre from the right recurrent laryngeal nerve at the level of the thoracic inlet of a subclinical laryngealhemiplegic horse, showing chronic degeneration. Myelin debris is scattered along remnants of a nerve fibre, indicating degeneration some weeks or months previously x 40

Figure 51: Photomicrographs of consecutive portions of a teased myelinated fibre from the left recurrent laryngeal nerve in the midcervical area of a subclinical laryngeal hemiplegic horse showing recent axonal degeneration. Note the linear row of large myelin ovoids. x 40



Figure 52: A photomicrograph of a teased myelinated fibre from the left recurrent laryngeal nerve in the midcervical area of a clinical laryngeal hemiplegic horse, showing paranodal demyelination x 100

Figure 53:

Photomicrographs of teased myelinated fibres of the left recurrent laryngeal nerve immediately caudal to the larynx of a subclinical laryngeal hemiplegic horse, showing thickenings of the myelin sheath. x 40



Figure 54: Electron micrographs of transverse sections of the recurrent laryngeal nerves from laryngeal hemiplegic horses.

- A. an "onion bulb" formation of Schwann cell processes surrounding a myelinated fibre, in the midcervical area of the left recurrent laryngeal nerve. x 7800.
- B. a denervated "onion bulb" formation, in the left recurrent laryngeal nerve immediately caudal to the larynx. x 6300.
- C. a regenerating cluster, of four thinly myelinated fibres and Schwann cell the right processes, in recurrent immediately laryngeal nerve caudal to the larynx. x 5000.
- D. a band of Bungner, composed of numerous Schwann cell processes containing two lipid droplets (+), in the left recurrent laryngeal nerve immediately caudal to the larynx. x 7800.
- E. a redundant loop of myelin associated with a fibre from a regenerating cluster in the left recurrent laryngeal nerve in the midcervical area. x 7800.



## Figure 55: Electron micrographs of transverse sections of recurrent laryngeal nerve from laryngeal hemiplegic horses.

- A, a Schwann cell containing a large lamellated myelin body. Note the peripheral vesicles and loose convoluted basement membrane, (from the right nerve in the midcervical area) x 8200
- B, a small myelinated fibre with accumulated mitochondria and vesicles. Note the loosely compacted inner myelin lamellae. (from the left nerve in the area of the aortic arch). x 15300
- C, a complex axon-Schwann cell network (from the left nerve in the midcervical area) x 21200
- D, axoplasm with densely packed disoriented neurofilaments, from a fibre with prominent axon-Schwann cell networks (from the left nerve in the area of the aortic arch) x 35700



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Figure 56: Electron micrograph of transverse section of right recurrent laryngeal nerve in the midcervical area from a subclinical laryngeal hemiplegic animal. Note the thick myelin sheath relative to the small axonal compartment (1). The adaxonal Schwann cell cytoplasm is distended with clumped floccular material (2) x 15300

Figure 57: Electron micrograph of a transverse section of right recurrent laryngeal nerve in the midcervical area from a clinical laryngeal hemiplegic animal. This atrophic fibre has a disproportionately thick myelin sheath and small axon. x 19200

Figure 58: Electron micrograph of a transverse section right recurrent laryngeal nerve of in themidcervical area from a control horse. This thinly myelinated fibre appears to have two separate axonal compartments (>), and an adaxonal dilation filled with tubular and vesicular structures and a large dense body. x 15300

Figure 56: Electron micrograph of transverse section of right recurrent laryngeal nerve in the midcervical area from a subclinical laryngeal Note the thick hemiplegic animal. myelin sheath relative to the small axonal compartment (1). The adaxonal Schwann cell cytoplasm is distended with clumped floccular material (2) x 15300

Figure 57: Electron micrograph of a transverse section of right recurrent laryngeal nerve in the midcervical area from a clinical laryngeal hemiplegic animal. This atrophic fibre has a disproportionately thick myelin sheath and small axon. x 19200

Figure 58: Electron micrograph of a transverse section of right recurrent laryngeal nerve in the midcervical area from a control horse. This thinly myelinated fibre appears to have two separate axonal compartments (>), and an filled adaxonal dilation with tubular and vesicular structures and a large dense body. x 15300



Figure 59:

Electron micrographs of transverse sections of equine recurrent laryngeal nerve showing nerve fibres distended with accumulated organelles.

A-C, a swollen fibre from the left recurrent laryngeal nerve immediately caudal to the larynx of a control animal. A, the thinly myelinated fibre with dense axoplasm is surrounded by layers of Schwann cell processes. x 2600. B, the axoplasm contains mainly dense membranous bodies. x 8200.

C, the neurofilaments and microtubules were concentrated into a small area of the axon. x 35700.

D-F, details of the axoplasm of swollen axons, from other nerves. D, axoplasm of a fibre devoid of myelin, also from the left recurrent immediately caudal laryngeal nerve to the larynx of a control animal. Many tubular vesicular structures are present, and in addition to membranous bodies. 10500. х E, axoplasm from a fibre in the left recurrent laryngeal nerve immediately caudal to the larynx of a clinical laryngeal hemiplegic animal, with many tubular and vesicular structures and mitochondria. x 15300. F, axoplasm from a fibre in the left recurrent laryngeal nerve immediately caudal to the larynx of a subclinical laryngeal hemiplegic animal, with mainly vesicular structures. x 7800

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Figure 60: Electron micrograph of a transverse section of the right tibial nerve from a clinical laryngeal hemiplegic animal. Schwann cell processes are surrounding a thinly myelinated fibre. Two areas of excess adaxonal Schwann cell cytoplasm can be seen (+ ). x 6300.

Figure 61: Electron micrograph of a transverse section of the right common peroneal nerve from a clinical laryngeal hemiplegic animal. A multiloculated structure, thought to result from the breakdown of myelin, is present within a Schwann cell process. Note the excess convoluted basement membrane associated with the process. x 19200.



Figure 62: Mean regression lines for axis cylinder perimeter (log to base e) and number of myelin lamellae of the left recurrent laryngeal nerve immediately caudal to the larynx in control, subclinical and clinical laryngeal hemiplegic animals.



Figure 63: Mean regression lines for axis cylinder perimeter (log to base e) and number of myelin lamellæ of the left recurrent laryngeal nerve in the midcervical area in control, subclinical and clinical laryngeal hemiplegic animals.



Figure 64: Mean regression lines for axis cylinder perimeter (log to base e) and number of myelin lamellae of the left recurrent laryngeal nerve in the region of the aortic arch in control, subclinical and clinical animals.



Figure 65:

Mean regression lines for axis cylinder perimeter (log to base e) and number of myelin lamellae of the right recurrent laryngeal nerve immediately caudal to the larynx in control, subclinical and clinical laryngeal hemiplegic animals.



Figure 66: Mean regression lines for axis cylinder perimeter (log to base e) and number of myelin lamellae of the right recurrent laryngeal nerve in the midcervical area of control, subclinical and clinical laryngeal hemiplegic animals.



Figure 67: Mean regression lines for axis cylinder perimeter (log to base e) and number of myelin lamellae of the right recurrent laryngeal nerve at the level of the thoracic inlet in control, subclinical and clinical laryngeal hemiplegic animals.



Figure 68: Longitudinal and transverse views, illustrating the plexus formation which results in alteration in the number and size of fascicles over a short length of nerve (after Sunderland, 1968).







Figure 69: A photomicrograph of a transverse section of the left *cnicoanytenoidous latenalis* muscle of a control horse. Groups of myosin ATPase low fibres can be seen. Myosin ATPase staining x 40

Figure 70: A photomicrograph of a transverse section of the left *cnicoanytenoidous latenalis* muscle of a subclinical laryngeal hemiplegic horse. Note the extensive atrophy and hypertrophy of fibres, with increased perimysial and endomysial connective tissue. Myosin ATPase staining. x 100

Figure 71: A photomicrograph of transverse section of the left *cnicoanytenoideus latenalis* muscle of a clinical laryngeal hemiplegic horse. Fascicular atrophy involves the majority of fascicles, and there is increased perimysial and endomysial connective tissue. Myosin ATPase staining. x 100



Figure 72: A photomicrograph of a transverse section of the left cricoarytenoideus lateralis muscle from a clinical laryngeal hemiplegic horse. Note the extensive fibre atrophy involving all fascicles, with increased perimysial and endomyial connective tissue. Myosin ATPase staining. x 40

Figure 73:

A photomicrograph of a transverse section of the right cricoarytenoideus lateralis muscle from the same clinical laryngeal hemiplegic horse as in Fig. 72. Note the variation in fibre size, with atrophic and hypertrophic fibres. Myosin ATPase staining. x 40

Figure 74: A photomicrograph of a transverse section of the left cricoarytenoideus dorsalis muscle from the same clinical laryngeal hemiplegic horse as in Fig. 72. Note the extensive fibre type grouping, and fascicular atrophy. Myosin ATPase staining. x 40


Figure 75: A photomicrograph of a transverse section of the normal extenson digitonum longus muscle from a control horse. Myosin ATPase staining x 40

Figure 76:

A photomicrograph of a transverse section of the *extensor digitorum longus* muscle from a subclinical laryngeal hemiplegic horse. Note the large groups of myosin ATPase low fibres. Myosin ATPase staining. x 40.





Figure 77: Photomicrographs of transverse section of the *extensor digitorum longus* muscles from clinical laryngeal hemiplegic horses.

- A. Fibre type grouping involves whole fascicles. x 40
- B. Another area from the same muscle as A, shows less extensive fibre type grouping. x 40
- C. Note the atrophic fibres and increased endomysial connective tissue of one fascicle, and the presence of hypertrophic fibres in the adjacent fascicle. x 100.
  Myosin ATPase staining.

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Figure 78: Sites of tissue sampling in the medulla oblongata.



Figure 79: Anatomical relationships of the nucleus ambiguus in the medulla oblongata.



Figure 80: A photomicrograph of the right nucleus amkiguus from the horse with a parasitic lesion of the right vagal nerve at its outlet from the brainstem. Note the chromatolytic neuronal cell bodies (+), with loss of Nissl substance and eccentrically placed nucleolus. Luxol fast blue/cresyl violet. x 100

Figure 81: A photomicrograph of the normal left *nucleus amkiguus* from a clinical laryngeal hemiplegic animal. Luxol fast blue/cresyl violet. x 40

Figure 82: A photomicrograph of the normal right *nucleus amkiguus* from the above clinical laryngeal hemiplegic animal. Luxol fast blue/cresyl violet. x 40



Figure 83: A photomicrograph of the normal left nucleus um& lynns from a control horse. Luxol fast blue/cresyl violet. x 40

Figure 84: A photomicrograph of the normal left nucleus ambiguus from a subclinical laryngeal hemiplegic horse. Luxol fast blue/cresyl violet. x 40



Figure 85: A photomicrograph of the right lateral cuneate nucleus from a subclinical laryngeal horse, showing numerous axonal spheroids  $(\rightarrow)$  H + E. x 100

Figure 86: A photomicrograph of the left lateral cuneate nucleus from a subclinical laryngeal horse, showing numerous axonal spheroids.  $(\Rightarrow)$  H + E. x 100



Figure 87: Sampling levels of the peroneal nerve.



A photomicrograph of a transverse section of the left cricoarytenoideus lateralis muscle from a horse affected with stringhalt. Fascicular atrophy of fibres, and increased perimysial and endomysial connective tissue is evident. Some fascicles of hypertrophic fibres are seen, with noticeable grouping of fibre types. Myosin ATPase. x 40

Figure 89: A transverse section of the right cricoarytenoideus lateralis muscle from a horse affected with stringhalt. Extensive atrophy and hypertrophy of fascicles is present. The atrophy is seen involve entire fascicles or is to limited to the edges of others. Myosin ATPase. x 40

Figure 88:



Figure 90: A photomicrograph of a transverse section of left *tikialis cranialis* muscle from a horse affected with stringhalt. Note the atrophy and hypertrophy of muscle fibres. Myosin ATPase. x 40.

Figure 91: A transverse section of the right *flexon carpi nodialis* muscle from a horse affected with stringhalt. The grouping of muscles fibre types and peripheral fascicular atrophy (+ ) are apparent Myosin ATPase. x 40



Figure 92: A photomicrograph of a transverse section of the normal left recurrent laryngeal nerve in the area of the aortic arch, showing the typical myelinated fibre density and diameters. Phenylenediamine staining. x 200

Figure 93: A photomicrograph of a transverse section of the left recurrent laryngeal nerve immediately caudal to the larynx, from a horse stringhalt. affected with Note the marked decrease in fibre density. Myelin debris is present (1). A large fibre with a split myelin sheath can be observed (2). A Renaut body is also present. Phenylenediamine staining. x 200

Figure 94: A photomicrograph of a transverse section of the left recurrent laryngeal nerve in the area of the aortic arch, from a horse affected with stringhalt. A loss of myelinated fibres, with considerable regenerative activity is evident. Many regenerating clusters and "onion bulbs" (2) are present. Some myelin debris can also be seen (3). Phenylenediamine staining. x 200



Figure 95:

A photomicrograph of a transverse section of the right tibial nerve from a horse affected by stringhalt. There is a noticeable loss of myelinated fibres, and myelin debris is prominent (1). Several regenerating clusters, (2) and other thinly myelinated fibres (3) are present. Phenylenediamine staining. x 200

Figure 96: A photomicrograph of a transverse section of the right median nerve from a horse affected by stringhalt. A slight decrease in fibre density is apparent, and some myelin debris can be seen (1). Many thinly myelinated fibres are present (2). Phenylenediamine staining. x 200

Figure 97:

A photomicrograph of a transverse section of the left recurrent laryngeal nerve in the area of the aortic arch, from a horse affected by stringhalt. At this higher power more detailed pathological structures can be seen. "Onion bulb" formations (1) and regenerating clusters (2) are surrounded by several layers of cell processes. Myelin debris is present in the Schwann cells of some myelinated fibres (3). Many of the fibres have disproportionately thin myelin sheaths. Phenylenediamine staining x 400



A photomicrograph of a teased fibre from the Figure 98: left recurrent laryngeal nerve in the midcervical area, from a horse with stringhalt. Note the myelin debris associated with the remnants of myelinated fibres indicating axonal degeneration of some weeks duration. x 100

Figure 99:

A photomicrograph of a teased fibre from the right deep peroneal nerve from a horse with stringhalt. Recent axonal degeneration is evidenced by the linear row of large myelin ovoids. x 100





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Figure 100: Photomicrographs of consecutive portions of a teased fibre myelinated from the right recurrent laryngeal nerve at the level of the thoracic inlet from a horse with stringhalt Areas of demyelination (type C change) are indicated by the arrows. x 40

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Figure 101: Photomicrographs of consecutive portions of a teased myelinated fibre from the right median nerve of a horse with stringhalt. There has been axonal degeneration distal to a node of Ranvier (1) and in the proximal preserved part of the fibre paranodal demyelination is evident (2). This represents type I change. x 40



- Figure 102: Electron micrographs of transverse sections from the hindlimb nerves of a horse affected with stringhalt.
  - A. left superficial peroneal nerve showing a Bungner band of numerous Schwann cell processes, and a multiloculated structure, (+), thought to be a result of myelin degradation. x 5200.
  - B. a high power view of the structure in A. x 21200.
  - C. left superficial peroneal nerve showing a Bungner band containing myelin debris in several stages of degradation (1), and a large axonal sprout (2). x 3400.
  - D. the axonal sprout shown in C containing disoriented neurofilaments. x 7800.
  - E. a myelinated fibre from the left superficial peroneal nerve showing margination of the microtubules. x 21200.
  - F. a myelinated fibre from the right tibial nerve showing a distension of the adaxonal Schwann cell cytoplasm. Note the lipid bodies and protagon granules in the outer Schwann cell cytoplasm. x 15300.



Figure 103: Electron micrographs of transverse sections from the recurrent laryngeal and limb nerves of a horse affected with stringhalt.

- A. the right tibial nerve showing a cluster of thinly myelinated regenerating fibres. Note the apparently atrophic fibre with small axonal calibre relative to its myelin sheath thickness (+). x 5200.
- B. the left recurrent laryngeal nerve in the area of the aortic arch showing a fibre from a regenerating cluster with accumulation of membranous dense bodies in its axoplasm. x 15300.
- C. a fibre from a regenerating cluster in the left recurrent laryngeal nerve in the area of the aortic arch with a lamellated myelin body in the outer Schwann cell cytoplasm. x 15300.
- D. the right recurrent laryngeal nerve level of the the thoracic at inlet showing a thinly myelinated fibre with surrounding Schwann cell process. Note the excess convoluted basement membrane, and the protagon granule (+) in the outer Schwann cell cytoplasm. x 7800.
- E. the right median nerve showing a thinly myelinated fibre with associated empty profiles of basement membrane. x 11200.
- F. the left recurrent laryngeal nerve in the area of the aortic arch showing a thinly myelinated fibre with surrounding Schwann cell processes and loose folded basement membrane. 11200.



Figure 104: Histograms of the myelinated nerve fibre diameter distributions of the recurrent laryngeal nerves from a horse with stringhalt.


#### COMPOSITION OF SOLUTIONS FOR PROCESSING NERVE SAMPLES

#### 0.1M cacodylate buffer

21.4g sodium cacodylate trihydrate<sup>1</sup>
986ml distilled water
14ml 0.2M hydrochloric acid

Adjust pH to 7.35-7.4 with hydrochloric acid or sodium hydroxide.

#### 2% glutaraldehyde

80ml 25% glutaraldehyde<sup>2</sup> 920ml 0.1M cacodylate buffer

Adjust pH to 7.35-7.4 with 0.1M hydrochloric acid or 0.1M sodium hydroxide.

#### 1% osmium tetroxide

1g osmium tetroxide<sup>3</sup> 100ml 0.1M cacodylate buffer

1. Sodium cacodylate, BDH Chemicals Ltd, Poole, England.

2. Glutaraldehyde, 25% solution in water, Koch-Light Laboratories Ltd, Buckinghamshire, England.

3. Osmium tetroxide, Sigma Chemical Co., Missouri, U.S.A.

## 1% phenylenediamine

0.1g p-phenylenediamine<sup>1</sup> 10ml 70% alcohol

## 66% glycerol

165ml 99% glycerol<sup>2</sup> 85ml distilled water

1. P-phenylenediamine grade 2, Sigma Chemical Co., Missouri, U.S.A.

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2. Glycerol, Ajax Chemicals, Sydney, Australia.

## EMBEDDING RESIN

#### Solution A

polarbed 812 resin<sup>1</sup> 62ml dodecenyl succinic anhydride (DDSA)<sup>2</sup> 100ml

## Solution B

polarbe	ed 812	resin		100ml
methyl	nadic	anhydride	(MNA) <sup>3</sup>	89ml

## Resin for embedding

solution A	25ml
solution B	75ml
2,4,6-tri(dimethylaminomethyl)phenol <sup>4</sup>	2ml
(DMP-30)	

Solutions A and B, and the final resin were all prepared in advance and stored frozen. When required for processing, the polarbed was removed from the freezer 2-3 hours before it was required.

1.	Epon 812 resin or Polarbed 812 resin, Polaron Equipment Ltd, Watford, England.
2.	DDSA, Polaron EquipmentLtd, Watford, England.
3.	MNA, Polaron Equipment Ltd, Watford, England.
4.	DMP-30, Polaron Equipment Ltd, Watford, England.

## STAINING METHOD FOR ELECTRON MICROSCOPY

#### Uranyl acetate stain

A saturated solution of uranyl acetate<sup>1</sup> was prepared in 50% ethanol.

#### Lead citrate stain

The following substances are combined and shaken vigorously until dissolved.

0.25g lead citrate<sup>2</sup> 0.1ml 10N sodium hydroxide<sup>3</sup> 10ml distilled water

#### METHOD

- Float the grid, sections down, on a drop of uranyl acetate for 3 minutes.
- Wash the grid in 70% ethanol, and then in distilled water. Dry with filter paper.
- Float the grid on a drop of lead citrate stain for 5 minutes.
- Wash the grid in distilled water, dry and place in a grid box.

1. Uranyl acetate, BDH Chemicals Ltd, Poole, England.

- 2. Lead citrate, BDH Chemicals Ltd, Poole, England.
- 3. Sodium hydroxide, Ajax Chemicals, Sydney, Australia.

## STAINING METHODS FOR MUSCLE TISSUE

<u>Myosin ATPase</u> (according to the technique of Padykula and Herman, 1955, as modified by Davies and Gunn, 1972).

SOLUTION 1

Substrate	1.0M tris(hydroxymethyl)aminomethane						12ml			
	0.18	BM cal	lcium	chlo	oride	(hexa	hydrat	$(e)^2$		6ml
	ATP	disod	lium s	salt	3					90mg
	dist	illed	a wate	er					to	45ml
Ad	just	the p	pH to	9.5	with	0.1N	hydrod	chlori	c ac	id.
	dist	cilled	a wate	er					to	60ml

SOLUTION 2

Cacodylate buffered formaldehyde pH 7

2.14g sodium cacodylate(trihydrate)	in	50ml
distilled water.		
0.2M hydrochloric acid		6.3ml
40% formaldehyde		20ml
distilled water		to 200ml

SOLUTION 3

2% cobalt chloride<sup>4</sup>

SOLUTION 4

1% ammonium sulphide<sup>5</sup>

1.	Tris (hydroxymethyl) aminomethane, Sigma Chemical Co., St Louis, USA.
2.	Calcium chloride, hexahydrate, BDH Chemicals Ltd, Poole, England.
3.	Adenosine-5'-triphosphone acid, BDH Chemicals Ltd, Poole, England.
4.	Cobalt chloride, Koch-Light Laboratories Ltd, Buckinghamshire,
5.	Ammonium sulphide 10%, Ajax Chemicals, Sydney, Australia.

## Method

1.	Place sections in solution 2 for two minutes.
2.	Wash in two changes of distilled water.
3.	Incubate in solution 1 for 20 minutes at 37 <sup>0</sup> C.
4.	Wash in two changes of distilled water.
5.	Place the sections in solution 3 for three minutes.
6.	Wash in two changes of distilled water.
7.	Place the sections in solution 4 for 30 seconds.
8.	Wash and mount in glycerine jelly.
Haen	notoxylin and eosin (Culling, 1974)
1.	Fix in 70% alcohol.
2.	Wash.
3.	Stain in Ehrlich's haematoxylin <sup>1</sup> for 10 minutes.
4.	Rinse in tapwater to remove excess stain.
5.	Differentiate in acid alcohol for 10 seconds.
6.	Regain blue colour and stop decolourisation by washing
	in Scott's tapwater for 2 minutes.
7.	Rinse in tapwater.
8.	Stain in eosin <sup>2</sup> for 40 seconds.
9.	Rinse rapidly in water.
10.	Dehydrate in alcohols.
11.	Clear in xylol.
12.	Mount. <sup>3</sup>

- 1. Haematoxylin, May & Baker Ltd, Dagenham, England.
- 2. Eosin, Harleco, Philadelphia, U.S.A.
- 3. DPX mountant, BDH Chemicals Ltd, Poole, England.

# PROCESSING AND STAINING METHODS FOR CENTRAL NERVOUS SYSTEM TISSUE

#### Processing method

Tissue blocks were embedded in paraffin wax using an automatic processor.<sup>1</sup> The schedule followed was:

50% alcohol for 1 hour 70% alcohol for 1 hour 95% alcohol for 1 hour absolute alcohol 1 for 1 hour absolute alcohol 2 for 1 hour absolute alcohol 3 for 2 hours absolute alcohol 4 for 2 hours chloroform for 4 hours xylene 1 for 1 hour xylene 2 for 1 hour xylene 3 for 1.5 hours wax for 2 hours wax for 2 hours

Staining method (Culling, 1974)

#### SOLUTIONS

#### Luxol fast blue

luxol fast blue	1g
95% alcohol	1000ml
10% acetic acid	5ml

- 1. Shandon Elliot Automatic Tissue Processor, SE 400, Shandon Scientific Co. Ltd, London, England.
- Luxol fast blue, Gurr's, Searle Scientific Services, High Wycombe, Bucks, England.

Cresyl violet

cresyl violet <sup>1</sup>	0.1g
distilled water	100ml

Lithium carbonate

0.005% aqueous solution lithium carbonate<sup>2</sup>

Cresyl violet differentiator

95% alcohol	90ml
chloroform	10ml
glacial acetic acid	3 drops

#### METHOD

- To remove wax, take the sections through xylene and 95% alcohol.
- 2. Stain in luxol fast blue overnight at 37<sup>0</sup>C.
- 3. Wash in 95% alcohol, then in distilled water.
- Commence differentiation by immersing the sections in lithium carbonate solution for not more than 20 seconds
- Differentiate in 70% alcohol until grey and white matters are clearly distinguished (30-60 seconds).
- Rinse in distilled water and examine under the microscope. If differentiation is not complete, repeat steps 4, 5 and 6 with reduced times.
- 7. Wash well in distilled water.
- Stain in cresyl violet for 10 minutes at room temperature.
- 9. Wash in distilled water.
- Cresyl fast violet, Gurr's, Searle Scientific Services, High Wycombe, Bucks, England.
- 2. Lithium carbonate, May & Baker Ltd, Dagenham, England.

- 10. Wash in 70% alcohol.
- Differentiate in cresyl violet differentiator for 1-2 seconds.
- 12. Rinse in 95% alcohol to remove differentiator.
- 13. Rinse in absolute alcohol and clear in xylol.
- 14. Check differentiation under a microscope to ensure that only the nuclei and Nissl substances are stained. Repeat steps 11, 12, 13 and 14 if necessary.
- 15. Mount in DPX.

#### LIGHT MICROSCOPIC MORPHOLOGY OF EQUINE PERIPHERAL NERVE

Control horses

HORSE 1 Left distal recurrent laryngeal nerve (RLN) FASCICLES No. 12 RENAUT BODIES - present, but small and not well developed. FIBRE DENSITY - normal OTHER FEATURES - myelinated fibres were smaller in diameter than in older horses, and the myelin sheaths thinner. No abnormalities. Left midcervical RLN 7 of medium myelinated fibres, motor to the intrinsic laryngeal muscles, and 2 of small myelinated fibres, sensory to the trachea and No. FASCICLES oesophagus. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES IN MEDIUM - fibres smaller than adult proportions. MYELINATED FIBRES No abnormalities. Left proximal RLN No. FASCICLES - 3 3 of medium myelinated fibres, 11 of small fibres and 3 mixed. **RENAUT BODIES - absent** FIBRE DENSITY - normal OTHER FEATURES IN MEDIUM - fibres smaller than adult proportions. MYELINATED FIBRES No abnormalities. Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve, were 3 fascicles composed entirely of medium myelinated fibres, assumed to be the motor fibres of the RLN. No abnormalities. Left vagus midcervical At one edge of an elliptical transverse section of the whole nerve, there appeared to be a collection of medium myelinated fibres into several fascicles. No abnormalities. Right distal RLN No. FASCICLES - 8 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - fibres smaller than adult proportions. No abnormalities. Right midcervical RLN No. FASCICLES - 7 of medium myelinated fibres, 1 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - flattened appearance of the nerve on MYELINATED FIBRES transverse sectioning, only 1 fascicle wide. Fibres smaller than adult proportions. No. abnormalities. No abnormalities. Right proximal RLN No. FASCICLES - 8 of medium myelinated fibres, 6 of small fibres. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - fibres smaller than adult proportions. MYELINATED FIBRES No abnormalities. Right vagus proximal to separation of RLN At one edge of an elliptical transverse section of the nerve was one large fascicle of medium myelinated fibres, and some unmyelinated fibres. Right vagus midcervical Along one edge of an elliptical transverse section of the whole nerve there was a greater concentration of medium myelinated fibres, but not collected into separate fascicles. HORSE 2 Left distal RLN No. FASCICLES - 9 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - slight increase in endoneurial nuclei. A regenerating cluster, redundant myelin loop associated with one fibre. A split myelin sheath. A myelin ovoid in the Schwann cell cytoplasm of a myelinated fibre. 7 of medium myelinated fibres, 6 of small fibres and 2 mixed. No. FASCICLES -

RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a regenerating cluster. MYELINATED FIBRES Several early "onion bulbs". A myelin ovoid in the Schwann cell cytplasm of a myelinated fibre. Right distal RLN No. FASCICLES -15 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - an early "onion bulb". Several myelin ovoids in the Schwann cell cytoplasm of myelinated fibres. Right midcervical RLN No. FASCICLES - 7 of medium myelinated fibres, 3 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a myelin ovoid in the Schwann cell MYELINATED FIBRES cytoplasm of a myelinated fibre. Right proximal RLN No. FASCICLES - 9 of medium myelinated fibres, 9 of small fibres. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a small fibre with an inappropriately MYELINATED FIBRES thick myelin sheath. HORSE 3 Left distal RLN No. FASCICLES - 9 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - slight increase in nuclei. Several thinly myelinated fibres. A regenerating cluster. Several "onion bulbs", some early and some large. Several myelin ovoids in Schwann cell cytoplasm of myelinated fibres. No. FASCICLES - 4 of medium myelinated fibres, 9 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres in MYELINATED FIBRES one fascicle.Several myelin ovoids and Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 5 fascicles composed entirely of medium myelinated fibres. No abnormalities. Left vagus midcervical Along one edge of an elliptical transverse section of the whole nerve, medium myelinated fibres were scattered through the fascicles. Right distal RLN No. FASCICLES -11 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - several thinly myelinated fibres. Several regenerating clusters. Several early "onion bulbs". Rare myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Right midcervical RLN No. FASCICLES - no transverse section of whole nerve. RENAUT BODIES - present FIBRE DENSITY - normal 

 FIBRE DENSITY - normal

 OTHER FEATURES OF MEDIUM - several thinly myelinated fibres.

 MYELINATED FIBRES
 Several early "onion bulbs".

 An "onion bulb" with a core of a large fibre with either a very thin myelin sheath or devoid of myelin. Several myelin ovoids in Schwann cell cytoplasm of myelinated fibres.

 Right proximal RLN

 No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned.

 fascicles sectioned. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several myelin ovoids in Schwann cell MYELINATED FIBRES cytoplasm of myelinated fibres.

Fight vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 2 fascicles of medium myelinated fibres. No abnormalities. Fight vagus midcervical At one edge of an elliptical transverse section of the whole nerve were 2 fascicles of medium myelinated fibres. Two fibres contained myelin ovoids in the Schwann cell cytoplasm. HORSE 4 Left distal RLN No. FASCICLES - 9 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - an early "onion bulb" with a denervated Schwann cell or a fibre devoid of myelin at its centre. A large "onion bulb" containing a large swollen fibre with dense axoplasm, and a thin attenuated myelin sheath. Left midcervical RLN No. FASCICLES - 9 of medium myelinated fibres, 8 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a regenerating cluster of 2 small fibres S with concentric Schwann cell processes. Several myelin ovoids and lipid granules in the MYELINATED FIBRES Schwann cell cytoplasm of myelinated fibres. Left proximal RLN 5 of medium myelinated fibres, 10 of small fibres, and 1 mixed. No. FASCICLES -RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a myelin ovoid in Schwann cell cytoplasm MYELINATED FIBRES of a myelinated fibre. Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 5 fascicles of medium myelinated fibres. No abnormalities. Left vagus midcervical At one edge of an elliptical transverse section of the whole nerve was 1 fascicle with a collection of medium myelinated fibres. Right distal RLN No. FASCICLES - 14 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - several thinly myelinated fibres. A myelin ovoid in Schwann cell cytoplasm of a myelinated fibre. Right midcervical RLN No. FASCICLES - 9 of medium myelinated fibres, 4 of small fibres. **RENAUT BODIES - present** FIBRE DENSITY - normalOTHER FEATURES OF MEDIUM - several thinly myelinated fibres.MYELINATED FIBRESA regenerating cluster with severa OTHER FEATURES OF MEDIUM - several thinly myelinated fibres.MYELINATED FIBRESA regenerating cluster with several layers<br/>of Schwann cell processes. Several early<br/>"onion bulbs". A myelin ovoid in Schwann cell<br/>cytoplasm of a myelinated fibre.Right proximal RLNNo. FASCICLES - 12 of medium myelinated fibres, 10 of small fibres.<br/>RENAUT BODIES - extensive<br/>FIBRE DENSITY - normal<br/>OTHER FEATURES OF MEDIUM - a myelin ovoid in Schwann cell cytoplasm<br/>MYELINATED FIBRES<br/>of a myelinated fibre.<br/>Right vagus proximal to separation of RLN Right vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 6 fascicles of mainly medium myelinated fibres. No abnormalities. Fight vagus midcervical At one edge of an elliptical transverse section of the whole nerve were 4 fascicles of medium myelinated fibres. HORSE 5 Left distal RLN No. FASCICLES - 14 of medium myelinated fibres. RENAUT BODIES - extensive FIBRE DENSITY - slight decrease 

 FIBRE DENSITI - Slight Gecrease

 OTHER FEATURES - several thinly myelinated fibres. Several regenerating clusters. Several early and one large "onion bulb". Split myelin sheaths with dense contents in 2 fibres. Several myelin ovoids in Schwann cell cytoplasm of myelinated fibres.

Left midcervical RLN Left midcervical RLN No. FASCICLES - 9 of medium myelinated fibres, 5 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres. MYELINATED FIBRES Several regenerating clusters. Several early "onion bulbs". Several myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Right distal RLN Right distal RLN No. FASCICLES - 9 of medium myelinated fibres. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES - An early "onion bulb". Rare thinly myelinated fibres. Rare myelin ovoids and lipid granules in Schwann cell cytoplasm of myelinated fibres. Right midcervical RLN No. FASCICLES - no transverse section of whole nerve. No. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - rare thinly myelinated fibres. MYELINATED FIBRES Rare myelinated fibres with u granules in Schwann cell cytoplasm. Right proximal RLN No. FASCICLES - 12 of medium myelinated fibres, 9 of small fibres. RENAUT BODIES - extensive FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres. S Several regenerating clusters. Early "onion bulbs" around some thinly myelinated MYELINATED FIBRES fibres. HORSE 6 Left distal RLN No. FASCICLES - 10 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - slight decrease OTHER FEATURES - several thinly myelinated fibres. Several regenerating clusters. Several early "onion bulbs". HORSE 6 Left midcervical RLN No. FASCICLES - 5 of medium myelinated fibres, 2 mixed with small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres. MYELINATED FIBRES Several regenerating clusters. Several early "onion bulbs". Several myelinated fibres with lipid granules in Schwann cell cytoplasm. Left proximal RLN No. FASCICLES - 5 of medium myelinated fibres, 3 of small fibres. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several myelin ovoids in Schwann cell MYELINATED FIBRES cytoplasm of myelinated fibres. Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 5 fascicles of medium myelinated fibres. No abnormalities. Right distal RLN No. FASCICLES - 10 of medium myelinated fibres. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES - a thinly myelinated fibre. A regenerating cluster. Several early "onion bulbs". Several myelin ovoids in Schwann cell cytoplasm Right midcervical RLN No. FASCICLES - 6 of medium myelinated fibres, 3 of small fibres. No. FASCICLES - 6 of medium myelinated fibres, 3 of small fibres. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a regenerating cluster. An early MYELINATED FIBRES "onion bulb". A myelin ovoid in Schwann cell cytoplasm of a myelinated fibre. Right proximal RLN No. FASCICLES - 8 of medium myelinated fibres, 8 of small fibres. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a large "onion bulb". MYELINATED FIBRES Several myelinated fibres with myelin ovoids and lipid granules in Schwann cell cytoplasm ovoids and lipid granules in Schwann cell cytoplasm. Fight vagus proximal to separation of RLN At one edge of an elliptical transverse section of the whole nerve were 4 fascicles of medium myelinated fibres. No abnormalities. Right vagus midcervical apparent collection of medium myelinated fibres.

#### Subclinical Horses

No

HORSE 7 Left distal RLN No. FASCICLES - 11 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - mild decrease, especially in some fascicles. OTHER FEATURES - increased endoneurial nuclei. Thinly myelinated fibres not uncommon. Early "onion bulbs" not uncommon. Several regenerating clusters. Myelin ovoids and lipid granules in Schwann cell cytoplasm not uncommon. A fibre with split myelin sheath. A swollen fibre with donue granular axoplasm, and a very thin attenuated myelin sheath. Left midcervical RLN No. FASCICLES - no transverse section of whole nerve. Left distal RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - slight increase in endoneurial nuclei. MYELINATED FIBRES Thinly myelinated fibres, regenerating clusters and early "onion bulbs" not uncommon. Several large denervated "onion bulbs". Myelin ovoids and u granules in Schwann cell cytoplasm of myelinated fibres common. Left proximal RLN No. FASCICLES - 9 of m RENAUT BODIES - absent 9 of medium myelinated fibres, 9 of small fibres. FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres and OTHER FEATORES OF MEDION - Several thinly myelinated fibres and<br/>early "onion bulbs".MYELINATED FIBRESearly "onion bulbs".Several myelinated fibres with myelin ovoids and<br/>lipid granules in Schwann cell cytoplasm.Left vagus proximal to separation of RLN<br/>At one end of an elliptical transverse section of the whole nerve<br/>were 6 fascicles of medium myelinated fibres. Several fibres with u<br/>granules in Schwann cell cytoplasm granules in Schwann cell cytoplasm. Left vagus midcervical At one end of an elliptical transverse section of the whole nerve medium myelinated fibres were collected into several fascicles. No abnormalities. abnormalities. Right distal RLN No. FASCICLES - 12 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - appeared slightly decreased. OTHER FEATURES - slight increase in endoneurial nuclei. Several thinly myelinated fibres, regenerating clusters and early "onion bulbs". Several myelinated fibres with myelin ovoids and lipid granules in Schwann cell cytoplasm. A fibre with split myelin sheath. Right midcervical RLN No. FASCICLES - no transverse section of whole nerve. No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, MYELINATED FIBRES regenerating clusters and early "onion bulbs". Several myelinated fibres with lipid granules in Schwann cell cytoplasm. Several swollen fibres with vacuolar axoplasm and attenuated myelin sheaths. Right proximal RLN No. FASCICLES - 11 of medium myelinated fibres, 3 of small fibres and 2 mixed. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - rare thinly myelinated fibres and MYELINATED FIBRES Several myelinated fibres with myelin ovoids and lipid granules in Schwann cell cytoplasm.

290 Hight vagus proximal to separation of RLN At one edge of an elliptical transverse section of the whole nerve were 4 fascicles of medium myelinated fibres. A fibre with a thin myelin sheath, a regenerating cluster and several myelin ovoids in Schwann cell cytoplasm of myelinated fibres were present. Right vagus midcervical At one edge of an elliptical transverse section of the whole nerve were 4 fascicles of medium myelinated fibres. One fibre contained lipid granules in the Schwann cell cytoplasm. HORSE 8 left distal RLN no transverse section of whole nerve. Individual fascicles sectioned. No. FASCICLES -RENAUT BODIES - present FIBRE DENSITY - mild decrease OTHER FEATURES - increased endoneurial nuclei. Thinly myelinated fibres, regenerating clusters and early "onion bulbs" not uncommon. A swollen fibre with dense axoplasm and attenuated myelin sheath. Several large fibres devoid of myelin with vacuolar axoplasm. Left midcervical RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres. MYELINATED FIBRES Rare early "onion bulbs". A myelin ovoid completely occupying a Schwann cell. Right distal RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - several thinly myelinated fibres, regenerating clusters and early "onion bulbs". Right midcervical RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - extensive FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - thinly myelinated fibres and early MYELINATED FIBRES "onion bulbs" not uncommon. Right proximal RLN No. FASCICLES - no transverse section of whole nerve No. Individual fascicles sectioned. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - a regenerating cluster. MYELINATED FIBRES HORSE 9 Left distal RLN No. FASCICLES - 12 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - mild decrease, less obvious in 2 fascicles. OTHER FEATURES - increased endoneurial nuclei. Thinly myelinated fibres and "onion bulbs" common. Sourceal regenerating clusters. Several HORSE 9 Several regenerating clusters. Several myelinated fibres with lipid granules in Schwann cell cytoplasm. A large round cell with myelin debris. Several myelin ovoids occupying entire Schwann cell. Left midcervical RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - present RENAUT BODIES - process FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - slight increase in endoneurial nuclei. MYELINATED FIBRES MYELINATED FIBRES Dulbs" not uncommon. Dulbs" not uncommon. cell cytoplasm not uncommon. Left proximal RLN No. FASCICLES -9 of medium myelinated fibres, 16 of small fibres and 2 mixed. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - thinly myelinated fibres, regenerating clusters and early "onion bulbs" not uncommon. MYELINATED FIBRES

Myelinated fibres with lipid granules in Schwann cell cytoplasm and myelin ovoids common. Left vagus proximal to separation of RLN

At one end of an elliptical transverse section of the whole nerve were 5 fascicles of medium fibres. Several thinly myelinated myelinated fibres, regenerating clusters and "onion bulbs". Several myelinated fibres with lipid granules in Schwann cell cytoplasm. Left vagus midcervical No apparent collection of medium myelinated fibres. Right distal RLN No. FASCICLES - 14 of medium myelinated fibres. Right distal RLN No. FASCICLES - 14 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - slight decrease OTHER FEATURES - slight increase in endoneurial nuclei. Several thinly myelinated fibres, regenerating clusters and early "onion bulbs". Several large "onion bulbs". Several fibres with split myelin sheaths. Several myelinated fibres with myelin ovoids and lipid granules in Schwann cell cytoplasm. Right midcervical RLN No. FASCICLES - no transverse section of whole nerve No. FASCICLES - no transverse section of whole nerve RENAUT BODIES - present FIBRE DENSITY - normal FIBRE DENSITI - HOLMALOTHER FEATURES OF MEDIUM - several thinly myelinated fibres,MYELINATED FIBRESregenerating clusters and myelinated fibreswith lipid granules in Schwann cell cytoplasm. Right proximal RLN No. FASCICLES - 10 of medium myelinated fibres, 16 of small fibres. RENAUT BODIES - extensive FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - flattened, ribbon-like arrangement of fascicles. Several thinly myelinated fibres, regenerating clusters and myelinated fibres with myelin ovoids and lipid granules in Schwann cell cytoplasm. MYELINATED FIBRES Right vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve medium myelinated fibres were collected into several fascicles. No abnormalities. Right vagus midcervical At one edge of an elliptical transverse section of the whole nerve medium myelinated fibres were collected into several fascicles. HORSE 10 Left distal RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - absent FIBRE DENSITY - mild decrease OTHER FEATURES - increased endoneurial nuclei. Thinly myelinated fibres common. Several early "onion bulbs, some denervated. A regenerating cluster. Left midcervical RLN No. FASCICLES - 6 of medium myelinated fibres, 3 of small fibres. RENAUT BODIES - present FIBRE DENSITY - slight decrease OTHER FEATURES OF MEDIUM - regenerating clusters common. MYELINATED FIBRES Several thinly myelinated fibres, early "onion bulbs" and myelinated fibres with myelin ovoids in Schwarn cell ovtoplasm myelin ovoids in Schwann cell cytoplasm. Left proximal RLN No. FASCICLES - 5 of medium myelinated fibres, 5 of small fibres. RENAUT BODIES - absent RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - regenerating clusters a little less MYELINATED FIBRES common than at the midcervical level. Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 4 fascicles of medium myelinated fibres. Regenerating clusters not uncommon, some of their fibres with redundant myelin loops. Several thinly myelinated fibres. Left vagus midcervical At one edge of an elliptical transverse section of the whole nerve At one edge of an elliptical transverse section of the whole nerve medium myelinated fibres were collected into several fascicles. One degenerating fibre and one myelinated fibre with a myelin ovoid

in Schwann cell cytoplasm were seen.

292 Right distal RLN No. FASCICLES - no transverse section whole nerve. Individual fascicles sectioned. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES - several thinly myelinated fibres, early "onion bulbs" and myelinated fibres with myelin ovoids in Schwann cell cytoplasm. A small myelinated fibre with a thick myelin sheath at the centre of an "onion bulb". Right midcervical RLN No. FASCICLES - 7 of medium myelinated fibres, 2 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, MYELINATED FIBRES regenerating clusters and myelinated fibres with myelin ovoids in Schwann cell cytoplasm. A 1 layer "onion bulb" encircling a myelinated fibre. A fibre with a encircling a myelinated fibre. A fibre with a split myelin sheath containing granular material. Right proximal RLN No. FASCICLES - 12 of medium myelinated fibres, 4 of small fibres. RENAUT BODIES - extensive FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several regenerating clusters and MYELINATED FIBRES myelinated fibres with myelin ovoids in Schwann cell cytoplasm. Right vagus midcervical At one edge of an elliptical transverse section of the whole nerve were 7 fascicles with medium myelinated fibres collected. No abnormalities. HORSE 11 Left distal RLN FASCICLES - no transverse section of whole nerve. No. Individual fascicles sectioned. RENAUT BODIES - present FIBRE DENSITY - mild decrease OTHER FEATURES - increased endoneurial nuclei. Thinly myelinated fibres, regenerating clusters and early "onion bulbs" not uncommon. A swollen fibre with dense granular axoplasm and or attenuated myelin sheath. an attenuated myelin sheath. A myelin ovoid and myelin debris in a round cell. Left midcervical RLN no transverse section of whole nerve. No. FASCICLES -Individual fascicles sectioned. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, MYELINATED FIBRES regenerating clusters and "onion bulbs". Myelin debris and lipid granules in a Schwann cell or a macrophage. Left proximal RLN NO. FASCICLES no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - thinly myelinated fibres, regenerating MYELINATED FIBRES clusters and early "onion bulbs" not Right distal RLN NO. FASCICLES - no transverse section of whole nerve. RENAUT BODIES - uncommon FIBRE DENSITY - normal OTHER FEATURES - several thinly myelinated fibres and early "onion bulbs". Right midcervical RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres and regenerating clusters. Right proximal RLN No. FASCICLES - no transverse section of whole nerve. Individual fascicles sectioned. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several regenerating clusters and early

MYELINATED FIBRES

"onion bulbs".

A large swollen fibre with attenuated myelin sheath.

Clinical Horses

HORSE 12 Left distal RLN No. FASCICLES - 11 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - severely decreased OTHER FEATURES - marked increase in endoneurial nuclei. Few myelinated fibres remain. Remaining fibres are thinly myelinated, part of early "onion bulbs" or regenerating clusters. HORSE 12 of early "onion bulbs" or regenerating clusters. Left midcervical RLN No. FASCICLES - 10 of medium myelinated fibres, 3 of small fibres and 1 mixed. RENAUT BODIES - absent FIBRE DENSITY - slight decreaseOTHER FEATURES OF MEDIUM - slight increase in endoneurial nuclei.MYELINATED FIBRESThinly myelinated fibres, regeneration Thinly myelinated fibres, regenerating clusters and "onion bulbs" common, more so than distal level. A large fibre so than distal level. A large fibre devoid of myelin. Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 6 fascicles composed of medium myelinated fibres. Fibre density was normal, but there were several thinly myelinated fibres, early "onion bulbs" and myelinated fibres with myelin ovoids and lipid granules in Schwann cell cytoplasm. In one fascicle thinly myelinated fibres and regenerating clusters were common. Right distal RLN No. FASCICLES - 13 of medium received No. FASCICLES - 13 of medium myelinated fibres. RENAUT BODIES - extensive FIBRE DENSITY - normal FIBRE DENSITY - normal OTHER FEATURES - several thinly myelinated fibres, regenerating clusters and early "onion bulbs". A very thinly myelniated fibre with dense axoplasm. Myelin debris in a Schwann cell or macrophage. Right midcervical RLN NO. FASCICLES - 5 of medium myelinated fibres, 4 of small fibres and 1 mixed. DENAUT PODIES - present ANG I MIAGE. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, early MYELINATED FIBRES "onion bulbs" and myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Right proximal RLN No. FASCICLES - 11 of medium myelinated fibres, 5 of small fibres and 1 mixed. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres. MYELINATED FIBRES Right vagus midcervical At one edge of an elliptical transverse section of the whole nerve medium myelinated fibres were collected into several fascicles. No abnormalities. HORSE 13 Left distal RLN No. FASCICLES - 9 of medium myelinated fibres RENAUT BODIES - present FIBRE DENSITY - severely decreased OTHER FEATURES - marked increase in endoneurial nuclei. Very few myelinated fibres remain. Several thinly myelinated fibres. "Onion bulbs" a prominent feature, many not containing a central myelinated fibre. An "onion bulb" around a small fibre with a thick myelin sheath. Left proximal RLN NO. FASCICLES no transverse section of whole nerve. Individual fascicles sectioned. **RENAUT BODIES - absent** FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several "onion bulbs" and myelin ovoids MYELINATED FIBRES in Schwann cell cytoplasm of myelinated fibres.

Left vagus proximal to separation of RLN At one end of an elliptical transverse section of the whole nerve were 6 fascicles of medium myelinated fibres. Fibre density normal, but several early "onion bulbs" and myelinated fibres with myelin ovoids in Schwann cell cytoplasm. A Schwann cell completely occupied by a myelin ovoid. Left vagus midcervical 294 Left vagus midcervical No discernible collection of medium myelinated fibres. Right distal RLN No. FASCICLES - 14 of medium myelinated fibres. RENAUT BODIES - uncommon FIBRE DENSITY - slight decrease apparent in some fascicles. OTHER FEATURES - slight increase in endoneurial nuclei. Thinly myelinated fibres, regenerating clusters and "onion bulbs" not uncommon. Fibres with redundant myelin loops in clusters. Fibres with redundant myelin loops in clusters. Myelinated fibres with myelin ovoids in Schwann cell cytoplasm not uncommon. A swollen fibre with attenuated myelin sheath. A large "onion bulb" with 2 central swollen fibres with dense axoplasm and thin myelin sheaths. A fibre with a split myelin sheath with dense granular contents. Right midcervical RLN No. FASCICLES - 8 of medium myelineter of the No. FASCICLES - 8 of medium myelinated fibres, 3 of small fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - Thinly myelinated fibres, "onion bulbs" S and regenerating clusters not uncommon. An "onion bulb" with 2 central fibres with no or MYELINATED FIBRES very thin myelin sheaths. An "onion bulb" with a central fibre with dense axoplasm and no myelin sheath. Right proximal RLN No. FASCICLES - 7 of medium myelinated fibres, 2 of small fibres and 1 mixed. RENAUT BODIES - present RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - regenerating clusters not uncommon. MYELINATED FIBRES Several "onion bulbs" and myelinated fibres with myelin ovoids in Schwann cell cytoplasm. Fight vagus proximal to separation of RLN At one edge of an elliptical transverse section of the whole nerve were 3 fascicles of medium myelinated fibres. Several thinly myelinated fibres. A myelinated fibre with a myelin ovoid in Schwann cell cytoplasm. A myelinated fibre with a myelin ovoid in Schwann cell cytoplasm. HORSE 14 Left distal RLN FASCICLES - 10 of medium myelinated fibres NO. RENAUT BODIES - present FIBRE DENSITY - severely decreased, especially in some fascicles. OTHER FEATURES - marked increase in endoneurial nuclei. Few myelinated fibres remain. Thinly myelinated fibres and early "onion bulbs" common amongst remaining fibres. 2 "onion bulbs" with central swollen fibres with Left midcervical RLN No. FASCICLES - 8 of medium myelinated fibres, 3 of small fibres. RENAUT BODIES - present FIBRE DENSITY - mild decrease OTHER FEATURES OF MEDIUM - thinly myelinated fibres, regenerating MYELINATED FIBRES clusters and "onion bulb" formations dense axoplasm and very thin or no myelin. Left proximal RLN of medium myelinated fibres, 8 of small fibres and 1 mixed. NO. FASCICLES -RENAUT BODIES - uncommon RENAUT BODIES - GROOMMENT FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, MYELINATED FIBRES MYELINATED FIBRES and myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Left vagus midcervical No discernible collection of RLN fibres.

Right distal RLN No. FASCICLES - 13 of mediu myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - normal OTHER FEATURES - slight increase in endoneurial nuclei. Thinly myelinated fibres, regenerating clusters and "onion bulbs" not uncommon. Some fascicles more affected than others. Redundant loops of myelin in fibres in clusters. A myelinated fibre with a split myelin sheath with dense contents. Right midcervical RLN No. FASCICLES - 10 of medium myelinated fibres, 6 of small fibres and 1 mixed. RENAUT BODIES - present 295 FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, MYELINATED FIBRES regenerating clusters and "onion bulbs". Right proximal RLN No. FASCICLES - 9 of medium myelinated fibres, 5 of small fibres. RENAUT BODIES - extensive RENAUT BODIES - extensive FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - several thinly myelinated fibres, MYELINATED FIBRES regenerating clusters, "onion bulbs", myelin ovoids and myelin debris in Schwann cell cytoplasm of myelinated fibres. Right vagus proximal to separation of RLN At one end of an elliptical transverse section of whole nerve were 3 whole and 2 part fascicles of medium myelinated fibres. Fibre density was normal. A thinly myelinated fibre, a myelinated fibre with a myelin ovoid in Schwann cell cytoplasm, and a swollen fibre with dense axoplasm and surrounding Schwann cell processes. Right vagus midcervical Right vagus midcervical No discernible collection of medium myelinated fibres. Peroneal nerve, to extensor digitorum longus muscle Fibre density normal. No Renaut bodies. A regenerating cluster. Several thinly myelinated fibres and "onion bulbs". HORSE 15 Left distal RLN No. FASCICLES - 9 of medium myelinated fibres. RENAUT BODIES - absent FIBRE DENSITY - severely decreased OTHER FEATURES - marked increase in endoneurial nuclei. Only 10-20 fibres remaining in each fascicle. Several thinly myelinated fibres and "onion bulbs", often denervated. Interted fibres. 2 of small fibres. HORSE 15 Left midcervical RLN No. FASCICLES - 9 of medium myelinated fibres, 2 of small fibres. TIBRE DENSITY - mild decrease OTHER FEATURES OF MEDIUM - increase in endoneurial nuclei MYELINATED FIBRES Thinly myelinated fibres, regenerat: Thinly myelinated fibres, regenerating clusters and "onion bulbs" common. "Onion bulbs" with normal and thinly myelinated fibres at centre, and denervated. Redundant myelin loops present in fibres from clusters. clusters. Left proximal RLN No. FASCICLES - 7 of me RENAUT BODIES - absent 7 of medium myelinated fibres, 9 of small fibres. FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - thinly myelinated fibres and "onion bulbs" MYELINATED FIBRES of all types common. Regenerating clusters not uncommon. Several myelinated fibres with myelin ovoids in Schwann cell cytoplasm. Left vagus proximal to separation of RLN At one end of an elliptical transverse Section of the whole nerve were 7 fascicles of medium myelinated fibres. Fibre density normal, but thinly myelinated fibres and large "onion bulbs" were common. Several regenerating clusters and myelinated fibres with myelin ovoids in Schwann cell cytoplasm. Redundant myelin loops in some fibres in clusters. Left vagus midcervical FIBRE DENSITY - normal Left vagus midcervical At one edge of an elliptical transverse section of the whole nerve medium myelinated fibres were collected into several fascicles. Thinly myelinated fibres not uncommon.

Right distal RLN Right distal RLN No. FASCICLES - 14 of medium myelinated fibres. RENAUT BODIES - present FIBRE DENSITY - slight decrease, especially in some fascicles. OTHER FEATURES - thinly myelinated fibres and "onion bulbs" common. Regenerating clusters not uncommon. Regenerating clusters not uncommon. Right midcervical RLN No. FASCICLES - 9 of medium myelinated fibres, 2 of small fibres. RENAUT BODIES - uncommon FIBRE DENSITY - slight decrease OTHER FEATURES OF MEDIUM - large "onion bulbs" common. MYELINATED FIBRES Regenerating clusters not unco S Regenerating clusters not uncommon. Thinly myelinated fibres not uncommon. Right proximal RLN No. FASCICLES - 7 of medium myelinated fibres, 8 of small fibres. RENAUT BODIES - absent FIBRE DENSITY - normal OTHER FEATURES OF MEDIUM - thinly myelinated fibres, regenerating MYELINATED FIBRES clusters and early "onion bulbs" not MYELINATED FIBRES uncommon. At one edge of an elliptical transverse section of the whole nerve were 3 fascicles of medium myelinated fibres. Fibre density normal, but several thinly myelinated fibres, regenerating clusters and early "onion bulbs". Left median nerve Several thinly myelinated fibres, early "onion bulbs", regenerating clusters and myelinated fibres with myelin ovoid in Schwann cell cytoplasm per fascicle. Left tibial nerve Several thinly myelinated fibres, "onion bulbs" and myelinated fibres with myelin ovoids in Schwann cell cytoplasm per fascicle. A fibre with split myelin sheath. Right tibial nerve Several thinly myelinated fibres and early "onion bulbs". A regenerating cluster. Left superficial peroneal nerve Several thinly myelinated fibres and "onion bulbs" per fascicle. A myelin ovoid in Schwann cell cytoplasm of a myelinated fibre. A regenerating cluster. Right superficial peroneal nerve Several thinly myelinated fibres and early "onion bulbs" per fascicle. Several large "onion bulbs". Left common peroneal nerve Several thinly myelinated fibres and "onion bulbs". Right common peroneal nerve Several thinly myelinated Tibres and early "onion bulbs", less frequent than more distal samples. Several regenerating clusters. Peroneal to extensor digitorum longus muscle Left and right contained several thinly myelinated fibres, early as well as large "onion bulbs", and regenerating clusters per fascicle.

## LIGHT MICROSCOPIC MORPHOMETRY OF EQUINE RECURRENT LARYNGEAL NERVE

## Kolmogorov-Smirnov test

The Kolmogorov-Smirnov test determines whether the distribution of myelinated nerve fibre diameters is different between groups. The probability that the two groups are the same is determined.

				the some	
	Probability that	t dis	tributions of two	groups are the same	
COMPARISON OVERALL			SMALL	LARGE	
	a province and the second second				
ITD THE 2LD	p<.01		p<.01	p<.01	
ILMC VS 2LMC	n ( 01		p<.01	p>.2	
ITD WE SID	$\mathbf{p}$ ( 01		p<.01	p<.01	
ILP VS ZLP	p < 01	- 1	p>.2	p<.01	
IRD VS ZRD	p(.01		p<.01	p<.01	
RMC VS ZRMC	pt.01		01 ( ) ( 1	p<.01	
TRP VS ZRP	pt.01		n< 01	p>.2	
ILD VS 3LD	p<.01		p(0)	p<.01	
1LMC vs 3LMC	pk.UI		p( 01	p<.01	
1LP vs 3LP	p<.01			D<.01	
1RD vs 3RD	p<.01		.02(p(.05	DC.01	
1RMC vs 3RMC	p<.01		pt.01		
1RP vs 3RP	p<.01		.02 <p<.05< td=""><td></td></p<.05<>		
1LD vs 1RD	p<.01		p<.01		
1LMC vs 1RMC	p<.01	1	p<.01	05(p) 1	
1LP vs 1RP	p<.01	- 1	p<.01	.05(p(.1	
1LD vs 1LP	p<.01	- 1	p<.01		
1LMC vs 1LD&F	p<.01		.1 <p<.2< td=""><td>.02(p(.05</td></p<.2<>	.02(p(.05	
1RD VS 1RP	p<.01		p<.01	.1 <p<.2< td=""></p<.2<>	
1RMC VS 1RD&E	p<.01	1	• p<.01	.02 <p<.05< td=""></p<.05<>	
2T.D VS 2RD	p<.01	- 1	p<.01	p<.01	
2LMC VS 2RMC	p<.01		p<.01	p<.01	
2LP VS 2RP	p<.01		.1 <p<.2< td=""><td>p&lt;.01</td></p<.2<>	p<.01	
2LD VS 2LP	p<.01	1	p<.01	p<.01	
DIMC VS 2LDL	p(_01		p<.01	p<.01	
	p(.01	3	p<.01	p<.01	
DDMC VS 2RP	$p \in 01$		p<.01	p<.01	
DID WE 200	p( 01		p<.01	p<.01	
DIN VS SKD	p( 01		p<.01	p<.01	
DIMC VS JKMC	p( 01		.05 <p<.1< td=""><td>p&lt;.01</td></p<.1<>	p<.01	
JLP VS JRP	p(.01		p<.01	.01 <p<.02< td=""></p<.02<>	
BLD VS 3LP			nc.01	p<.01	
BLMC VS 3LD&			n< 01	p>.2	
3RD VS 3RP	.U2(p(.U5		01(p( 02	p<.01	
3RMC vs 3RD&			.012	F	
L	D distal P proximal				
L left	D	a	ideonuice l	Prontmat	
R right	M	C m	lacervical		

TABLE 19:	Differences in the distributions of myelinated	(2)
INDER 19	fibre diameters between control (1), subclinical	(2)
	and clinical (3) laryngeal hemiplegic animals.	

# GRADING OF TEASED MYELINATED FIBRES FROM EQUINE PERIPHERAL NERVE

## TABLE 20: <u>Grading (after Dyck, 1975a) of teased myelinated</u> <u>fibres in the recurrent laryngeal nerves of</u> <u>control horses</u>

NEDVE			FI	BRE	TYP	ES (%	)		
NERVE	A	В	c	D	E	F	G	I	ABNORMAL
HORSE 2 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	98 98 100 100 100 100	2			1	1 2			2 2 0 0 0 0
HORSE 3 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	99 - 99 96 99 99		1		1	1 3			1 1 4 1 1
HORSE 4 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	95 97 100 97 100 100	1		1	3 1 1	2 1 1			5 3 0 3 0 0
HORSE 5 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	92 99 100 100 98 96		2	2		3 1 2 3		1	8 1 0 2 4
HORSE 6 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	94 98 97 95 98 97		3 1	1	1 2	3 1 4 2 3			6 2 3 5 2 3

#### TABLE 21:

## 21: Grading (after Dyck, 1975a) of teased myelinated fibres in recurrent laryngeal nerves of subclinical laryngeal hemiplegic animals

NEDVE				FIBF	E TYP	PES (8	5)		
NERVE	A	В	С	D	E	F	G	I	ABNORMAL
HORSE 7 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	46 96 100 94 99 90		6 1 2 1	15	1 1 2	33 3 3 5			54 4 0 6 1 10
HORSE 8 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	76 89 - 100 98 99		3		20 10	1 1 2 1			24 11 0 2 1
HORSE 9 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	28 62 91 90 94 94	1	2 4 1	13 6	5 6 2 2	51 21 8 7 4 6	1		72 38 9 10 6
HORSE 10 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	31 74 93 99 94 97		53	16 3 1	5 2 1	43 17 6 1 5 3	1		69 26 7 1 6 3
HORSE 11 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	60 74 70 84 91 81	1 1 1	3 1 2	2 1 2 2	7 8 12 3 2	23 13 15 10 6 15	4 2 2	2	40 26 30 16 9 19

## TABLE 22: Grading (after Dyck, 1975a) of teased myelinated fibres in recurrent laryngeal nerves of clinical laryngeal hemiplegic horses

NEDVE			FI	BRE TY	PES (%	)	
NERVE	A	с	D	E	F	G	ABNORMAL
HORSE 12 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	34 44 97 93 97 100	1	2	4	64 55 2 2 2	1	66 56 3 7 3 0
HORSE 13 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	17 - 94 87 89 96	8 1 3 2	14	1	60 5 9 7 4	1	83 6 13 11 4
HORSE 14 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	44 58 98 90 92 70	3 1 1	4 7	1	51 30 2 8 5 30	1 1 1 2	56 42 2 10 8 30
HORSE 15 L distal L midcervical L proximal Rt distal Rt midcervical Rt proximal	57 49 76 73 86 88	1	2 1	4 2 2 2 4 4	37 43 20 19 10 8	2 4 6	43 51 24 27 14 12

## TABLE 23: Grading (after Dyck, 1975a) of teased myelinated fibres in limb nerves from clinical laryngeal hemiplegic horses

NEDVE		FIBRE TYPES (%)									
NERVE	A	В	С	D	E	F	G	ABNORMAL			
HORSE 14 deep peroneal	84	3	1			4	8	16			
HORSE 15 median common peroneal	90 88					4	6 2	10 12			
superficial peroneal deep peroneal tibial	85 82 92		3 1	1	3 2	9 7 5	7 3	15 18 8			
					-						

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## INTERNODE LENGTHS IN EQUINE RECURRENT LARYNGEAL NERVE

TABLE 24: Internode length statistics of normal (A) fibres in equine recurrent laryngeal nerve

	SM	ALL FIBRE	S	LA	RGE FIBRE	S	OVERALL FIBRES			
GROUP	meanIL (µm)	SD (µm)	meanCV (%)	meanIL (µm)	SD (µm)	meanCV (%)	meanIL (μm)	SD (µm)	meanCV (%)	
Left distal RLN control subclinical clinical	710 640 550	77 124 188	9.3 11.6 15.0	944 937 909	113 107 84	7.7 8.7 9.6	865 770 658	151 188 233	8.3 10.3 13.4	
<u>Left proximal RLN</u> control subclinical clinical	867 847 804	94 128 165	7.9 8.3 10.1	1084 1088 1149	84 90 115	6.3 6.3 7.1	975 993 1046	141 159 206	7.1 7.1 8.0	
<u>Right distal RLN</u> control subclinical clinical	677 675 673	102 102 112	8.6 10.4 10.5	947 991 991	117 125 146	7.3 7.9 8.8	828 890 862	174 189 205	7.8 8.7 9.5	
<u>Right proximal RLN</u> control subclinical clinical	852 814 798	105 144 162	7.4 11.0 9.7	1094 1153 1105	106 118 94	6.7 6.8 6.6	975 1023 968	161 209 200	7.1 8.4 8.0	

IL

internode length
standard deviation SD

CV coefficient of variation

RLN recurrent laryngeal nerve

	SMALL FIBRES			L	ARGE FIBR	ES	OVERALL FIBRES			
GROUP	meanIL (μm)	SD (µm)	meanCV (%)	meanIL (µm)	SD (µm)	meanCV (ዩ)	meanIL (μm)	SD (µm)	meanCV (%)	
Left distal RLN control subclinical clinical Left proximal RLN control subclinical	687 617 522 842 776	92 142 185 108 168	13.4 23.0 35.4 12.8 21.6	957 949 926 1091 1096	122 117 99 94 98	12.8 12.3 10.7 8.6 9.0	854 759 651 967 951	172 211 249 160 208	20.1 27.7 38.2 16.6 21.9	
clinical <u>Right distal RLN</u> control subclinical clinical <u>Right proximal RLN</u> control subclinical clinical	704 654 648 626 825 770 754	216 118 118 135 125 167 180	30.7 18.1 18.3 21.5 15.1 21.7 23.9	1154 959 998 993 1106 1155 1114	126 123 132 157 116 128 104	10.9 12.8 13.2 15.8 10.5 11.1 9.3	971 807 863 820 965 997 928	278 194 213 235 185 239 233	28.6 24.1 24.6 28.6 19.1 24.0 25.1	

## TABLE 25: Internode length statistics of normal (A) internodes in equine recurrent laryngeal nerve

CROUD	SMALL FIBRES			L	ARGE FIBR	ES	OVE	RALL FIBR	ES
GROUP	meanIL (µm)	SD (µm)	meanCV (%)	meanIL (µm)	SD (µm)	meanCV (ዩ)	meanIL (µm)	SD (µm)	meanCV (%)
Left distal RLN control subclinical clinical	700 599 526	86 127 171	11.0 34.0 33.6	944 929 896	113 104 82	8.4 14.0 9.2	849 640 577	177 258 216	9.3 28.4 30.8
Left proximal RLN control subclinical clinical	865 795 752	- 98 170 196	7.9 13.2 16.6	1084 1088 1149	84 90 115	6.3 6.4 7.1	968 928 1001	161 257 252	7.1 9.4 10.5
Right distal RLN control subclinical clinical	671 659 644	106 114 133	9.4 14.7 15.5	946 990 990	117 125 145	7.4 8.5 10.2	819 868 819	184 210 242	8.3 10.7 12.6
<u>Right proximal RLN</u> control subclinical clinical	849 797 772	108 154 169	8.3 15.0 15.4	1094 1150 1104	105 118 93	9.6 7.6 7.3	973 997 930	163 236 233	7.8 10.6 11.3

## TABLE 26: Internode length statistics of total fibres in equine recurrent laryngeal nerve

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1.1

00000	SMALL FIBRES			L	ARGE FIBRI	ES	OVERALL FIBRES			
GROUP	meanIL (μm)	SD (µm)	meanCV (%)	meanIL (µm)	SD (µm)	meanCV (ዩ)	meanIL (µm)	SD (µm)	meanCV (%)	
Left distal RLN control subclinical clinical	675 506 449	122 226 212	18.1 44.6 47.3	957 949 926	122 117 99	12.8 12.3 10.7	847 659 571	184 287 282	21.7 43.6 49.4	
Left proximal RLN control subclinical clinical	842 742 676	109 205 238	13.0 27.6 35.2	1091 1097 1154	94 98 126	8.6 9.0 10.9	967 928 951	161 237 298	16.6 25.6 31.3	
<u>Right distal RLN</u> control subclinical clinical	648 629 598	127 148 165	19.6 23.4 27.6	959 998 993	123 132 157	12.8 13.2 15.9	803 851 797	199 228 255	24.8 26.7 32.0	
Right proximal RLN control subclinical clinical	817 740 721	143 201 208	17.5 27.1 28.9	1106 1155 1114	116 128 105	10.5 11.1 9.4	960 978 904	194 262 259	20.3 26.8 28.6	

TABLE 27: Internode length statistics of total internodes in equine recurrent laryngeal nerve

#### Kolmogorov-Smirnov tests

The Kolmogorov-Smirnov test determines whether the distribution of mean fibre internode lengths (IL) or individual internode lengths, is different between groups. The probability that the two groups are the same is measured.

TABLE 28: Differences in the distributions of IL of type A fibres between groups of control (1), subclinical (2) and clinical (3) laryngeal hemiplegic horses

	Probability that IL distributions of 2 groups are the same										
GROUP	OVE	RALL	SI	MALL		LARGE					
	FIBRE	NODE	FIBRE	NODE	FIBRE	NODE					
1LD vs 1RD1LP vs 1RP1LD vs 1LP1RD vs 1RP2LD vs 2RD2LP vs 2RP2LD vs 2LP2RD vs 2RP3LD vs 3RD3LP vs 3RP3LD vs 3LP3RD vs 3RP1LD vs 2LD1LP vs 2LP1RP vs 2RD1RP vs 3RD1RP vs 3RD	.001 <p<.005 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 .001<p<.005 p&lt;.001 p&lt;.005</p<.005 </p<.005 	p<.001 p>.1 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001	.001 <p<.005 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 p&lt;.001 .005<p<.01 p&lt;.001 .01<p<.025 p&lt;.001 p&lt;.001 .025<p<.5 .05<p<.1 .001<p<.005 p&lt;.001 p&lt;.001 p&lt;.001 .01<p<.005 .05<p<.1 .001<p<.005 p&lt;.001 p&lt;.001 .001<p<.005 .001<p<.005 .001 .001<p<.005 .001 .001 .001 .001 .005</p<.005 </p<.005 </p<.005 </p<.005 </p<.1 </p<.005 </p<.005 </p<.1 </p<.5 </p<.025 </p<.01 </p<.005 	p<.001 p>.1 p<.001 p<.001 p<.001 p>.1 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001	<pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre>	p<.001 p>.1					

L left

R

MC midcervical

P proximal

D distal

right

		probability that IL distributions of 2 groups are the same											
GROUP	OVEF	RALL	SI	MALL	LARGE								
	FIBRE	NODE	FIBRE	NODE	FIBRE	NODE							
1LDvs1.RD1LPvs1.RP1LDvs1.LP1RDvs1.RP2LDvs2.RD2LPvs2.RP2LDvs2.RP2LDvs2.RP3LDvs3.RD3LPvs3.RP3LDvs3.RP3LDvs3.RP1LDvs2.LD1LPvs2.LP1RDvs2.RD1RPvs2.RP1LDvs3.LD1LPvs3.LP1RPvs3.RP1RDvs3.RD1RPvs3.RP	.005 <p<.01 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 .001<p<.005 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 .05<p<.1< td=""><td>p&lt;.001 p&gt;.1 p&lt;.001</td><td>.005<p<.01 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 p&lt;.001 .001<p<.005 p&lt;.001 .025<p<.05 p&lt;.001 p&lt;.001 p&gt;.1 .01<p<.025 p&lt;.001 p&gt;.1 .01<p<.025 p&lt;.001 p&gt;.1 .01<p<.025 p&lt;.001 p&gt;.1 .05<p<.1< td=""><td>p&lt;.001 .05<p<.1 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 .001<p<.005 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001</p<.005 </p<.1 </td><td><pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre></td><td><pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre></td></p<.1<></p<.025 </p<.025 </p<.025 </p<.05 </p<.005 </p<.01 </td></p<.1<></p<.005 </p<.01 	p<.001 p>.1 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001 p<.001	.005 <p<.01 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 p&lt;.001 .001<p<.005 p&lt;.001 .025<p<.05 p&lt;.001 p&lt;.001 p&gt;.1 .01<p<.025 p&lt;.001 p&gt;.1 .01<p<.025 p&lt;.001 p&gt;.1 .01<p<.025 p&lt;.001 p&gt;.1 .05<p<.1< td=""><td>p&lt;.001 .05<p<.1 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 .001<p<.005 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001</p<.005 </p<.1 </td><td><pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre></td><td><pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre></td></p<.1<></p<.025 </p<.025 </p<.025 </p<.05 </p<.005 </p<.01 	p<.001 .05 <p<.1 p&lt;.001 p&lt;.001 p&lt;.001 p&gt;.1 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 .001<p<.005 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001 p&lt;.001</p<.005 </p<.1 	<pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre>	<pre>p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1 p&gt;.1</pre>							

## TABLE 29: Differences in the distributions of IL of total fibres between groups of control (1), subclinical (2) and clinical (3) laryngeal hemiplegic animals

left  $\mathbf{L}$ R

MC midcervical

right distal D

Ρ

proximal

#### ELECTRON MICROSCOPIC MORPHOLOGY OF EQUINE PERIPHERAL NERVE

Left distal recurrent laryngeal nerve CONTROL Horses examined - 2, 3, 4, 6 Fibre density - normal Bands of Bungner - one seen in horse 6, with a lipid granule and swollen processes.
Excess Schwann cells and collagen pockets - occasional in horses 2
and 3, common in 6.
"Onion bulbs" - none to several single layer formations, some more extensive. Regenerating clusters - one in horses 3 and 6. Horse 3, cluster of 3 small fibres around large fibre with excess Schwann cell cytoplasm and disintegrating sheath. Thinly myelinated fibres - in regenerating clusters. Myelin debris - occasional lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a large myelinated fibre with split myelin sheath, packed with whorled membranous dense bodies, mitochondria, vesicles and floccular bodies, mitochondria, vesicles and floccular material (horse 6). - a collapsed myelin ovoid completely occupying a Schwann cell with excess cytoplasm (horse 3). - two very large swollen axons in many layered "onion bulbs". One with very thin myelin sheath and dense axoplasm, mainly vesicular and whorled membranous dense bodies. Microtubules and neurofilaments present in a central area. The second had no myelin sheath, similar axoplasm. second had no myelin sheath, similar axoplasm, although fewer dense bodies and more vesicles. SUBCLINICAL Horses examined - 7, 9 Fibre density - loss of fibres apparent. Bands of Bungner - moderately common. Excess Schwann cells and collagen pockets - common. "Onion bulbs" - common, varying from one to many layers of Schwann cell processes. Usually with a central thinly myelinated fibre. Denervated structures also seen, one around an empty basement membrane profile. Another around a fibre with excess basement membrane membrane. Regenerating clusters - several in horse 7, some fibres with redundant myelin loops. Thinly myelinated fibres - common in "onion bulbs" and clusters, others seen with very thin sheaths and loosely compacted lamellae, and excess Schwann cell cytoplasm. Myelin debris - common lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres. - lipid droplets also seen in Schwann cell processes and macrophages. Other abnormalities - a collapsed myelin ovoid completely occupying a Schwann cell (horse 9). - a degenerating unmyelinated axon, swollen with floccular axoplasm. - several fibres with adaxonal Schwann cell projections into the axonal compartment, often fibres with other abnormalities e.g. in "onion bulbs", with lipid or protagon granules, with redundant loops of myelin, excess organelles, thin myelin sheath. - several fibres with accumulated organelles, several fibres with accumulated organeties,
mitochondria and membranous dense bodies.
several fibres with inappropriately thick
sheath for axonal calibre (horse 7).
several fibres with redundant loops of myelin.
a very large swollen demyelinated axon with densely packed organelles, dilated vesicular structures with floccular contents, in "onion bulb". - a fibre with a split sheath, packed with organelles. - a regenerating axonal sprout with very thin sheath and excess mitochondria, in a 1 layer "onion bulb".

CLINICAL 309 Horses examined - 12, 13, 14, 15 Fibre density - severe decrease, few myelinated fibres left. Bands of Bungner - very common, some with axonal sprouts (horses 14 and 15), one with excess neurofilaments and branching tubular structures (15). Excess Schwann cells and collagen pockets - very common, some with excess basement membrane (horse 15). Above structures constitute majority of endoneurium. "Onion bulbs" - very common in horse 13 and 15, mainly denervated. Less common in horses 12 and 14. Small and large formations around remaining fibres, usually thinly myelinated. Regenerating clusters - occasional in horses 12 and 15. Thinly myelinated fibres - in "onion bulbs" and clusters - remaining myelinated fibres often thinly myelinated. Myelin debris - lipid and protagon granules not uncommon in bands of Bungner and Schwann cell processes. - occasional lipid granules and lamellated ovoids in Schwann cells of myelinated fibres. Other abnormalities - almost all remaining myelinated fibres. i.e. in "onion bulbs", clusters, with inappropriately thin or thick sheaths. - a swollen axon without myelin sheath, filled with dense whorled membranous bodies and tubular structures, in an "onion bulb" (horse 14). - a collapsed myelin ovoid completely occupying a Schwann cell, with split sheath containing floccular material (horse 15). - margination of microtubules in a thickly myelinated fibre. - a very distended axon with a thin layer of outer Schwann cell cytoplasm, filled with vesicles and tubules, in an "onion bulb". Left midcervical recurrent laryngeal nerve CONTROL Horses examined -2, 4, 5, 6Fibre density - normal. Bands of Bungner - absent Excess Schwann cells and collagen pockets - rare to occasional. "Onion bulbs" - absent to several 1 layer formations (horse 5). Regenerating clusters - one in horses 5 and 6. In former, large cluster with a disproportionately thickly myelinated fibre and several with thin myelin, some with excess organelles. Thinly myelinated fibres - in clusters. Myelin debris - occasional lipid granules and rare lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a collapsed myelin ovoid completely occupying a Schwann cell (horse 5). SUBCLINICAL Horses examined - 7, 9, 10 Fibre density - slight loss of fibres in horse 9. Bands of Bungner - several, one with axonal sprout with excess organelles. Excess Schwann cells and collagen pockets - common. "Onion bulbs" - occasional to common, mainly 1-2 layer formations, usually around a thinly myelinated fibre, some with excess basement membrane. One denervated around a band of Bungner (horse 9). Several large formations (horse 7). Regenerating clusters - occasional to moderately common. Fibres often with inappropriately thin or thick myelin sheaths and redundant loops of myelin. Thinly myelinated fibres - in "onion bulbs" and clusters. Myelin debris - moderately common lipid and protagon granules. and organelles. Myelin debris - moderately common lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a myelinated fibre with accumulated organelles, mitochondria and membranous dense bodies (horse 7). - fibres with disproportionately thick myelin for axonal calibre (horses 7, 9). - a well developed axon-Schwann cell network in a medium diameter myelinated fibre. - redundant myelin loop in a small myelinated fibre. - excess basement membrane lying free in the

endoneurium, and around some Schwann cell processes (horse 9). - a degenerating myelinated fibre with dense axoplasm and a disintegrating myelin sheath with excess outer Schwann cell cytoplasm (horse 10). - an adjacent Schwann cell with watery cytoplasm, completely occupied by a collapsed myelin ovoid. CLINICAL Horses examined - 12, 15 Fibre density - mild decrease. Bands of Bungner - moderately common. One containing swollen organelle dense axonal sprouts (horse 12), and several in an area of unmyelinated fibres. Excess Schwann cells and collagen pockets - common. "Onion bulbs" - 1 to 2 layer and larger formations common. Almost all myelinated fibres in "onion bulbs", some normally and some thinly myelinated. Some denervated with central bands of Bungner. One around a myelinated fibre and a band of Bungner (horse 15). myelinated fibre and a band of Bungner (horse 15). Regenerating clusters - common. In horse 12, usually a large central fibre with several small thinly myelinated fibres, while in horse 15 usually all small fibres. Often with long redundant loops of myelin. Some fibres with inappropriately thick sheaths. Thinly myelinated fibres - usually in "onion bulbs" and clusters. Myelin debris - lipid and protagon granules common, and lamellated myelin ovoids occasional in Schwann cell cytoplasm of myelinated fibres. Also in Schwann cell processes. Other abnormalities - several small fibres with redundant loops of myelin (horse 12).
 - several fibres with inappropriately thick
myelin sheaths for axonal calibre (horse 15).
 - an axon-Schwann cell network in a myelinated
fibre in an "onion bulb". Left proximal recurrent laryngeal nerve CONTROL Horses examined -3, 6Fibre density - normal. Bands of Bungner - absent. Excess Schwann cells and collagen pockets - rare to occasional. "Onion bulbs" - absent. Regenerating clusters - absent. Thinly myelinated fibres - absent. Myelin debris - occasional lipid granule in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a collapsed myelin ovoid completely occupying a Schwann cell (horse 3). SUBCLINICAL Horses examined - 7, 9, 10, 11 Fibre density - normal. Bands of Bungner - several, in all but horse 7. Excess Schwann cells and collagen pockets - occasional to moderately common. "Onion bulbs" - absent in horses 7 and 10. occasional 1-2 layer formations in horses 9 and 11, usually around a thinly myelinated fibre. Schwann cell processes very attenuated, often with excess convoluted basement membrane in horse 11. One around a band of Bungner, another around a fibre with a very small axon and a disintegrating sheath. Regenerating clusters - absent in horse 7. - occasional to common in others. Fibres with inappropriately thick or thin sheaths, redundant loops of myelin and excess organelles. Thinly myelinated fibres - in "onion bulbs" and clusters. - rarely with very thin myelin (horse 7) another with excess Schwann cell cytoplasm (11). Myelin debris - lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres occasional to common. - also present in Schwann cell processes and macrophages. Other abnormalities - adaxonal Schwann cell projection into the axoplasm seen moderately commonly, and several axon Schwann cell networks (horse 7). - large myelinated fibres with inappropriately thick myelin sheaths for axonal calibre moderately common (horses 9, 10, 11).
- a small myelinated fibre with clumped floccular material in a subaxolemmal position. - a myelinated fibre with an unusual axoplasmic organelle, circular in outline with a floccular centre and a dense outer layer of concentric membranes. - several Schwann cells completely occupied by collapsed myelin ovoids (horses 10 and 11). CLINICAL Horses examined - 13, 15 Fibre density - normal. Bands of Bungner - occasional. Bands of Bungner - occasional. Excess Schwann cells and collagen pockets - occasional to common, some with excess basement membrane. "Onion bulbs" - 1 to 2 layer formations occasional in horse 13 and common in horse 15, usually around thinly myelinated fibres. Several denervated and several extensive formations (horse 15).
Beconcepting clusters - occasional to common, some with redundant loops of myelin Regenerating clusters - several, some with redundant loops of myelin in horse 15. Thinly myelinated fibres - in "onion bulbs" and clusters usually. Myelin debris - occasional lipid granule in Schwann cell cytoplasm of myelinated fibres and in Schwann cell processes. Other abnormalities - axonal calibre inappropriately small for myelin thickness in many fibres (horse 13). - excess basement membrane around Schwann cell processes (15). - occasional small fibre with redundant loop of myelin. - axon-Schwann cell network in a fibre with densely packed disoriented neurofilaments. Right distal recurrent laryngeal nerve CONTROL Horses examined - 2, 3, 6 Fibre density - normal. Bands of Bungner - one in horse 3, containing myelin debris. Excess Schwann cells and collagen pockets - rare (horse 2), occasional (horse 6), common (horse 3). "Onion bulbs" - absent in horse 2, several 1-2 layer formations in horses 3 and 6, around myelinated fibres, sometimes with excess basement membrane. Regenerating clusters - one seen in horse 3. Thinly myelinated fibres - one seen in horse 6. Myelin debris - occasional lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm Other abnormalities - several myelinated fibres of horses 2 and 6. ell projections into the axoplasm. - a myelin ovoid completely occupying a Schwann cell. SUBCLINICAL Horses examined - 7, 9 Fibre density - normal in horse 7, slight decrease in horse 9. Bands of Bungner - occasional. Bands of Bungner - occasional. Excess schwann cells and collagen pockets - common. "Onion bulbs" - occasional 1 layer formations in horse 7. - common, and varied in type in horse 9. Regenerating clusters - occasional, fibres with inappropriately thin or thick myelin sheaths, some with myelin debris in Schwann cells, some with accumulated axonal organelles, some with redundant loops of myelin myelin. Thinly myelinated fibres - in "onion bulbs" and clusters, also others in horse 7. Myelin debris - common lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres. of myelinated fibres. - also present in macrophages. Other abnormalities - several fibres with marginated microtubules. - several degenerating myelinated fibres, one with a small axis cylinder containing dense axoplasm with many vesicles and a split sheath containing granular and floccular material. - occasional myelinated fibre with adaxonal Schwann cell projection into the axoplasm.

CLINICAL 312 Horses examined - 12, 13, 15 Fibre density - normal. Bands of Bungner - occasional. Excess Schwann cells and collagen pockets - occasional to common. "Onion bulbs" - absent in horse 12. - occasional 1-2 layer and more extensive formations in horse 13. common 1-2 layer formations in horse 15, around normally and thinly myelinated fibres, and denervated. Regenerating clusters - occasional in horses 12 and 15. Thinly myelinated fibres - only in "onion bulbs" and clusters. Myelin debris - lipid and protagon granules, and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibbres, occasional in horses 12 and 13, common in horse 15. Other abnormalities - redundant loop of myelin on a large myelinated fibre (horse 12) - a degenerating myelinated fibre (horse 15). - a thinly myelinated fibre with axon-Schwann cell network around a dense body.
 fibre with densely packed neurofilaments and
 microtubules (horse 13).
 fibre with marginated microtubules (horse 13).
 excess basement membrane around a Schwann cell process (horse 15). - fibre with a small axis cylinder and distended adaxonal space containing clumped floccular material. Right midcervical recurrent laryngeal nerve CONTROL Horses examined - 2, 4, Fibre density - normal. Bands of Bungner - one of the unmyelinated type in horse 6. Excess Schwann cells and collagen pockets - rare to occasional. "Onion bulbs" - a denervated "onion bulb" in horse 4. Regenerating clusters - absent. Thinly myelinated fibres - absent. Myelin debris - occasional lipid granule and lamellated myelin ovoid in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a small myelinated fibre with split myelin sheath containing many vesicular structures, and two apparent axonal compartments. SUBCLINICAL Horses examined - 7, 9, 10, 11 Fibre density - normal. Fibre density - normal. Bands of Bungner - rare to moderately common. One with lipid and protagon granules (horse 11), a group in an area of unmyelinated fibres (11), one with an axonal sprout with densely packed organelles. Excess Schwann cells and collagen pockets - occasional to common. "Onion bulbs" - absent in horses 10 and 11. - in others several 1-2 layer formations, usually around thinly myelinated fibres, sometimes with lipid and protagon granules. Schwann cell process Regenerating clusters - absent in 7, 9 and 11. - rare in horse 10, occasional in horse 11, fibres with thin myelin sheaths and redundant myelin loops. Thinly myelinated fibres - in "onion bulbs" and clusters. Myelin debris - lipid and protagon granules and lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres occasional to common. A vacuolated protagon granule. - also present in Schwann cell processes. s - occasional adaxonal Schwann cell projection into axoplasm of fibres with densely packed Other abnormalities . neurofilaments, loosely compacted myelin lamellae and excess Schwann cell cytoplasm (horse 10 and 11). Several fibres with axon-Schwann cell networks (horse 7). - fibres with inappropriately thick myelin for axonal calibre, one with clumped material in adaxonal Schwann cell cytoplasm, another

313 with a disintegrating myelin sheath. - an inappropriately thinly myelinated large fibre with swollen floccular axoplasm and outward slippage of the myelin sheath (11). - a thinly myelinated fibre with a disintegrating sheath, small axis cylinder packed with organelles packed with organelles, especially mitochondria. - a clumped floccular body in axoplasm of a myelinated fibre (11). - a collapsed myelin ovoid completely occupying a Schwann cell (7). - several degenerating fibres with dense axoplasm, clumped in horse 7 and with large numbers of vesicles in horse 9, and a disintegrating myelin sheath. CLINICAL Horses examined - 13, 15 Fibre density - normal. Bands of Bungner - rare in horse 15. Excess Schwann cells and collagen pockets - occasional to common. "Onion bulbs" - several large formations, usually around small myelinated fibres (13). - 1-2 layer and larger formations common in horse 15, mainly around thinly myelinated fibres, some denervated. Regenerating clusters - rare (13) to occasional (15). Some fibres with very long redundant loops of myelin. Thinly myelinated fibres - mainly in "onion bulbs" and clusters. Myelin debris - occasional lipid and protagon granules in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - excess basement membrane seen in "onion bulbs" and clusters. - a myelin ovoid completely occupying a Schwann cell with convoluted basement membrane. Right proximal recurrent laryngeal nerve CONTROL Horses examined - 3, 5, 6 Fibre density - normal. Bands of Bungner - absent. Excess Schwann cells and collagen pockets - rare to occasional. "Onion bulbs" - absent. Regenerating clusters - absent. Thinly myelinated fibres - one seen in horse 4. Myelin debris - occasional lipid granule and lamellated myelin ovoid in Schwann cell cytoplasm of myelinated fibres. SUBCLINICAL Horses examined - 7, 9, 10 Fibre density - normal. Bands of Bungner - absent. Excess Schwann cells and collagen pockets - occasional. "Onion bulbs" - several 1-2 layer formations in horse 9, one around a thinly myelinated fibre with an adaxonal Schwann cell projection into the axoplasm. Regenerating clusters - several seen in horse 9, some fibres abnormal e.g. small axis cylinder and thick sheath, adaxonal Schwann cell projection into axon. Thinly myelinated fibres - occasional in horses 7 and 9. Myelin debris - lipid and protagon granules, and lamellated myelin ovoids occasional in horses 7 and 10, and common in 9. Other abnormalities - several collapsed myelin ovoids completely occupying Schwann cells (7). - several small myelinated fibres with redundant loops of myelin (9). - adaxonal Schwann cell projections into axoplasm not uncommon (9). · a degenerating fibre with dense axoplasm containing membranous dense bodies, with a disintegrating myelin sheath. CLINICAL Horses examined - 13 Fibre density - normal. Bands of Bungner - absent. Excess Schwann cells and collagen pockets - occasional. "Onion bulbs" - occasional 1-2 layer formations. Regenerating clusters - absent. Myelin debris - occasional lipid and protagon granules, and

lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a collapsed myelin ovoid completely occupying a myelin sheath. several degenerating fibres with dense axoplasm and disintegrating sheath, and excess Schwann cell cytoplasm. - an adaxonal Schwann cell projection into the axonal compartment. Left vagus proximal to separation of recurrent laryngeal nerve SUBCLINICAL Horses examined - 9, 13 Fibre density - normal. Bands of Bungner - occasional. Excess Schwann cells and collagen pockets - not unco mon. "Onion bulbs" - common and varied in morphology. - several 1-2 layer formations, around normally, thickly and thinly myelinated fibres. - several extensive formations, sometimes with excess basement membrane. Regenerating clusters - several, some fibres with very thin sheaths some with thick sheaths and very small axis cylinders. Myelin debris - lipid and protagon granules common, and lamellated myelin ovoids occasional in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a fibre with a clump of floccular material in adaxonal Schwann cell cytoplasm. - a small myelinated fibre with excess accumulated organelles. - a small fibre with a redundant myelin loop. - occasional fibre with adaxonal projection of Schwann cell cytoplasm, one reaching across dia eter of axon (13). - several degenerating myelinated fibres, one with very dense acoplasm containing clear and dense core vesicles, and a split myelin sheath containing vesicles and disoriented neurofilaments. Another with a very thick sheath and small axis cylinder with dense axoplasm. Branch of deep peroneal to long digital extensor muscle CLINICAL Horses examined - 14, 1 Fibre density - normal. 15 Bands of Bungner - rare in horse 15. Excess Schwann cells and collagen pockets - occasional to common. "Onion bulbs" - 1-2 layer formations occasional in horse 15 and more common in horse 14, usually around thinly myelinated fibres, sometimes with excess basement membrane. One around a fibre with a very small axis cylinder and myelin debris in Schwann cell cytoplasm. Regenerating clusters - several seen in horse 15. Thinly myelinated fibres - occasional. Myelin debris - several lamellated myelin ovoids in Schwann cell cytoplasm of myelinated fibres in horse 14. Other abnormalities - a collapsed myelin ovoid completely occupying a Schwann cell in horse 15. - excess basement membrane on Schwann cell processes of an "onion bulb". - several large myelinated fibres with - several large myelinated fibres with adaxonal Schwann cell projections into axoplasm. - an axon-Schwann cell network in a thinly myelinated fibre (15). - a large myelinated fibre with several inner myelin lamellae separated by floccular material (15) - several myelinated fibres with redundant loops of myelin (14). of myelin (14). - a thinly myelinated fibre with excess Schwann cell cytoplasm. - a fibre of inappropriately small axonal calibre for myelin sheath thickness (14).

CLINICAL Horses examined - 15

Fibre density - normal.

Bands of Bungner - occasional.

Excess Schwann cells and collagen pockets - not uncommon. "Onion bulbs" - 1-2 layer formations common, around normally, thickly or thinly myelinated fibres. - some more extensive formations, some with excess basement membrane.

Regenerating clusters - several seen, some fibres abnormal, with myelin ovoids in Schwann cell cytoplasm, thin myelin sheaths, redundant loops of myelin. Thinly myelinated fibres - several apart from those in "onion bulbs" and clusters.

Myelin debris - lamellated myelin ovoids not uncommon in Schwann cell cytoplasm of myelinated fibres. Other abnormalities - a collapsed myelin ovoid occupying Schwann cell, together with partially digested myelin. - a degenerating unmyelinated fibre with

floccular axoplasm. - a small thickly myelinated fibre with floccular contents, probably a collapsing myelin ovoid.

- excess basement membrane around a denervated Schwann cell, and associated with a small myelinated fibre.

several fibres with axon-Schwann cell networks.
several small fibres with redundant myelin loops.

- a Schwann cell process with a loculated myelin breakdown product and excess convoluted basement membrane.

## APPENDIX 11

## LIGHT MICROSCOPIC MORPHOMETRY AND INTERNODE LENGTHS IN RECURRENT LARYNGEAL NERVES IN STRINGHALT

## Kolmogorov-Smirnov tests

TABLE 30: Difference in the distributions of myelinated fibre diameters between stringhalt (1) and clinical laryngeal hemiplegic (2) animals

COMPARISON	Probability that distributions of two groups are the same						
	OVERALL	SMALL	LARGE				
1LD vs 2LD	p<.01	p<.01	p>.2				
1LMC vs 2LMC	p<.01	p>.2	p<.01				
1LP vs 2LP	p<.01	p<.01	p<.01				
1RD vs 2RD	p<.01	p<.01	p>.2				
1RMC vs 2RMC	p<.01	p<.01	p<.01				
1RP vs 2RP	p<.01	p<.01	p<.01				
1LD vs 1RD	p<.01	p>.2	p>.2				
1LMC vs 1RMC	.01 <p<.02< td=""><td>p&lt;.01</td><td>p&lt;.01</td></p<.02<>	p<.01	p<.01				
1LP vs 1RP	p<.01	p<.01	p<.01				
1LD vs 1LP	p<.01	p<.01	p<.01				
1LMC vs 1LD&P	p>.2	p>.2	p>.2				
1RD vs 1RP	p<.01	.02 <p<.05< td=""><td>p&gt;.5</td></p<.05<>	p>.5				
1RMC vs 1RD&P	p<.01	p<.01	.01 <p<.02< td=""></p<.02<>				

- L left
- R right
- D distal
- MC midcervical
- P proximal

NEDVE	SMALL	INTERNOD	ES	LARG	E INTERNO	DES	OVERALL INTERNODES			
NERVE	meanIL (μm)	SD CV (µm) (%)		meanIL (μm)	meanIL SD CV (μm) (μm) (%)		meanIL (µm)	SD (µm)	CV (१)	
Normal (A) fibres										
left distal	648	71	11.2	932	92	10.2	732	153	10.9	
proximal	776	164	9.9	1128	89	6.7	1069	168	7.3	
right distal	668	93	10.8	913	67	7.8	755	145	9.8	
proximal	820	110	9.7	1058	73	6.8	935	152	8.3	
Normal (A) internodes										
left distal	634	107	16.9	960	117	12.2	719	181	25.2	
proximal	754	190	25.2	1135	106	9.4	1052	204	19.4	
right distal	641	104	16.2	907	85	9.4	743	162	21.8	
proximal	782	150	19.2	1078	78	7.2	902	192	21.3	
Total internodes										
left distal	515	243	47.1	960	117	12.2	610	287	47.1	
proximal	602	297	49.4	1135	106	9.4	982	302	30.7	
right distal	641	104	16.2	907	85	9.4	743	162	21.8	
proximal	732	221	30.2	1078	78	7.2	864	247	28.5	

TABLE 31:	Internode	length	statistics	of	recurrent	laryngeal	nerves	from	a	horse	with	stringh	nalt
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## Kolmogorov-Smirnov tests

The Kolmogorov-Smirnov test tests whether the distribution of mean fibre internode lengths (IL) or individual internode lengths, is different between groups. The probability that the two groups are the same is determined.

probability that IL distributions of 2 groups are the same									
GROUP	OVER	ALL	SI	MALL	LARGE				
	FIBRE	NODE FIBRE NODE		FIBRE	NODE				
4LD vs 4RD	p>.1	.025 <p<.05< td=""><td>p&gt;.1</td><td>.005<p<.01< td=""><td>p&gt;.1</td><td>p&gt;.1</td></p<.01<></td></p<.05<>	p>.1	.005 <p<.01< td=""><td>p&gt;.1</td><td>p&gt;.1</td></p<.01<>	p>.1	p>.1			
4LP vs 4RP	p<.001	p<.001	p<.001	p<.001	p>.1	p>.1			
4LD vs 4LP	p<.001	p<.001	p>.1	p<.001	p>.1	p>.1			
4RD vs 4RP	p<.001	p<.001	.001 <p<.005< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.005<>	p<.001	p>.1	p>.1			
1LD vs 4LD	.05 <p<.1< td=""><td>p&lt;.001</td><td>.05<p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<></td></p<.1<>	p<.001	.05 <p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<>	p<.001	p>.1	p>.1			
1LP vs 4LP	p<.001	p<.001	p<.001	p<.001	p>.1	p>.1			
1RD vs 4RD	p=.1	p<.001	p>.1	p>.1	p>.1	p>.1			
1RP vs 4RP	p>.1	p<.001	p>.1	.001 <p<.005< td=""><td>p&gt;.1</td><td>p&gt;.1</td></p<.005<>	p>.1	p>.1			
3LD vs 4LD	p>.1	p<.001	p>.1	p<.001	p>.1	p>.1			
3LP vs 4LP	p>.1	p<.001	p>.1	p>.001	p>.1	p>.1			
3RD vs 4RD	.01 <p<.025< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td><td>p&gt;.1</td><td>.05<p<.1< td=""></p<.1<></td></p<.025<>	p<.001	p>.1	p>.1	p>.1	.05 <p<.1< td=""></p<.1<>			
3RP vs 4RP	.05 <p<.1< td=""><td>.001<p<.005< td=""><td>p&gt;.1</td><td>.05<p<.1< td=""><td>P&gt;.1</td><td>p&gt;.1</td></p<.1<></td></p<.005<></td></p<.1<>	.001 <p<.005< td=""><td>p&gt;.1</td><td>.05<p<.1< td=""><td>P&gt;.1</td><td>p&gt;.1</td></p<.1<></td></p<.005<>	p>.1	.05 <p<.1< td=""><td>P&gt;.1</td><td>p&gt;.1</td></p<.1<>	P>.1	p>.1			

TABLE 32: Differences in the distributions of IL of normal (A) fibres between groups of control (1), laryngeal hemiplegic (3) and stringhalt (4) horses

L left

R right

D distal

MC midcervical

P proximal

	probability that IL distributions of 2 groups are the same								
CROUD	OVERA	LL	SMA	LARGE					
GROUP	FIBRE NODE		FIBRE	NODE	FIBRE	NODE			
4LD vs 4RD	p >.1	.01 <p<.025< td=""><td>p&gt;.1</td><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.025<>	p>.1	p<.001	p>.1	p>.1			
4LP vs 4RP	p<.001	p<.001	p<.001	p<.001	p>.1	p>.1			
4LD vs 4LP	p<.001	p<.001	.05 <p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<>	p<.001	p>.1	p>.1			
4RD vs 4RP	.001 <p<.005< td=""><td>p&lt;.001</td><td>.05<p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<></td></p<.005<>	p<.001	.05 <p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<>	p<.001	p>.1	p>.1			
1LD vs 4LD	p<.001	p<.001	p<.001	p<.001	p>.1	p>.1			
1LP vs 4LP	p<.001	p<.001	.001 <p<.005< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.005<>	p<.001	p>.1	p>.1			
1RD vs 4RD	p>.1	.001 <p<.005< td=""><td>p&gt;.1</td><td>p&gt;.1</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.005<>	p>.1	p>.1	p>.1	p>.1			
1RP vs 4RP	.05 <p<.1< td=""><td>p&lt;.001</td><td>.05<p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<></td></p<.1<>	p<.001	.05 <p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td></p<.1<>	p<.001	p>.1	p>.1			
3LD vs 4LD	p>.1	p<.001	p>.1	p<.001	p>.1	p>.1			
3LP vs 4LP	p>.1	p<.001	p>.1	p<.001	p>.1	p>.1			
3RD vs 4RD	.05 <p<.1< td=""><td>p&lt;.001</td><td>p&gt;.1</td><td>p&gt;.1</td><td>p&gt;.1</td><td>.05<p<.1< td=""></p<.1<></td></p<.1<>	p<.001	p>.1	p>.1	p>.1	.05 <p<.1< td=""></p<.1<>			
3RP vs 4RP	p=.05	p<.001	p>.1	p>.1	p>.1	p>.1			

TABLE 33: Differences in the distributions of IL of total fibres between groups of control (1), laryngeal hemiplegic (3) and stringhalt (4) horses

L left

R right

D distal

MC midcervical

P proximal