A STUDY OF THE NERVE SUPPLY AND INTRINSIC MUSCULATURE OF THE EQUINE LARYNX

A thesis presented in partial fulfilment of the
requirements for the degree of Master of Veterinary
Science in Veterinary Surgery at Massey University

by

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ABSTRACT

The purpose of the study was to elucidate the structure and function of the normal equine laryngeal nerve supply and musculature. This was undertaken as a preliminary step for further examination of equine laryngeal hemiplegia or recurrent nerve paralysis.

A literature review of anatomy and function of the nerve supply and intrinsic musculature was prepared.

The experimental study was divided into two main sections, the first being an anatomical study, the second being confined to laryngeal function.

ANATOMICAL STUDY:

Six horses were used to supply the gross anatomy and nerve supply of the intrinsic laryngeal muscles. Transverse sections were taken of the vagus, and recurrent laryngeal nerves at various points, these being submitted for histological examination. It was apparent that the gross anatomy of the larynx and its nerve supply was similar to that described in the literature. However, the intralaryngeal nerve distribution had not been described so this was subsequently studied in detail. The nerve supply appeared to be unilateral and both cranial and recurrent laryngeal nerves supplied the intrinsic musculature.

Histological examination of the nerves showed that in the vagus, nerve fibres were of a mixed population composed of fine, medium and heavily myelinated fibres. These appeared to decussate and coalesce in a complex manner along the nerve. In the recurrent nerve, however, the majority of nerve fibres were of a medium myelinated type. In the

intralaryngeal nerve only medium fibres were found.

FUNCTIONAL STUDY:

In this study fourteen horses were investigated using electromyographic techniques, to establish the activity of the intrinsic laryngeal muscles during respiration. The dorsal cricoarytenoid muscle was confirmed in its "abductor" activity while the cricothyroid muscle in some horses also showed increased activity during inspiration. All other intrinsic laryngeal muscles showed "adductor" or expiratory activity. Once the normal resting respiratory activity of the laryngeal muscles was established, selective denervation of individual muscles was undertaken to confirm the anatomical findings. The dorsal cricoarytenoid muscle was supplied by a branch of the recurrent laryngeal nerve. The cricothyroid muscle was supplied by the external branch of the cranial laryngeal nerve. All other intrinsic laryngeal muscles were supplied from two branches of the recurrent nerve. Motor nerve supply was unilateral.

A further study was performed using a random sample of twenty four horses. In this investigation the intrinsic laryngeal muscles were dissected off their respective cartilages and weighed, the muscles from the left side being compared with those from the right. For those muscles supplied by the recurrent nerve, the muscles of the left side were significantly lighter than those of the right. Comparison of the cricothyroid muscles, however, showed no significant difference between left and right sides. Comparison of the denervated right muscles with the contralateral muscle after six weeks did not seem to alter this finding. Conventional histological examination of denervated muscle at six weeks post nerve section failed to show any significant signs of denervation atrophy.

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INTRODUCTION

The function of the larynx has puzzled man since the day Galen found that vocalisation originated in the larynx and not in the heart as previously thought. Possibly because of the importance of vocalisation in man's social intercourse, this function in particular and all functions in general of the human larynx have come under scrutiny by medical researchers.

During the last century considerable information on laryngeal function in man and experimental animals has been obtained. Numerous texts exist which contain descriptions of the gross anatomy of the equine larynx but there remains a paucity of information on its function.

Pressman and Kelemen (1955) in their review of laryngeal physiology make few references to the horse. However, they emphasised that marked differences in laryngeal function exist between the species.

In the horse functional laryngeal paralysis has been recognised for over 120 years (Mason, 1973). It is thought that up to 5% of thoroughbred horses are affected (Duncan et al., 1974).

The present study was undertaken to establish a reliable and accurate knowledge of the anatomy and function of the motor nerve supply to the normal equine larynx, as a prerequisite to investigations into the condition known as laryngeal hemiplegia. To describe the function of the motor supply to the equine larynx, a certain familiarity with the anatomy of the laryngeal cartilages and muscles is necessary. For this reason a brief review of the gross anatomy and function of the larynx is included in the following literature review.

LITERATURE REVIEW

ANATOMY OF THE LARYNX

The basic structure of the larynx is common to all domestic animals; however species differences do exist in laryngeal nerve supply and function (Pressman and Kelemen, 1955; Rex, 1969; Nickel *et al*., 1973).

Laryngeal cartilages:

In the horse the skeleton of the larynx consists of five cartilages which provide a tubular connection between the pharynx and the trachea (Fig. 1).

The epiglottic cartilage lies in the midline directly cranial to the glottis. The base of the epiglottis is attached to the thyrohyoid ligament and the base of the thyroid cartilage. Arising dorsally from the base of the epiglottis are the lateral cuneiform processes, which project into the aryepiglottic folds. The pointed apex of the epiglottis curves cranially over the soft palate into the nasopharynx.

The thyroid cartilage is the largest laryngeal cartilage in the horse and supplies the lateral walls and ventral floor of the larynx. The thyroid cartilage consists of two lateral walls or laminae forming quadrilateral plates, connected ventrally by the body of the thyroid cartilage. The dorsal borders of the laminae are extended cranially and caudally to form cornua which serve as synovial intercartilaginous articulations. The cranial cornua are attached to the thyrohyoid bones. The fissure between the cranial cornu and the cranial border of the lamina is bridged incompletely by connective tissue cranially, thereby forming

Figure 1. The equine laryngeal cartilages and their relationship to one another

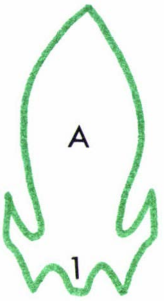
a. The epiglottic cartilage, a dorsal view showing the apex and lateral cuneiform projections from the base (1).

b. The arytenoid cartilage, lateral and medial aspects. On the medial aspect the cricoid articulation can be seen (2).

c. The thyroid cartilage lateral aspect. The caudal cornu (3) acts as the articulation for the cricothyroid joint.

d. The cricoid cartilage. The articulations with the arytenoid cartilage (4) and thyroid cartilage (5) are shown.

e. A median section through a composite of all laryngeal cartilages. Left lateral aspect.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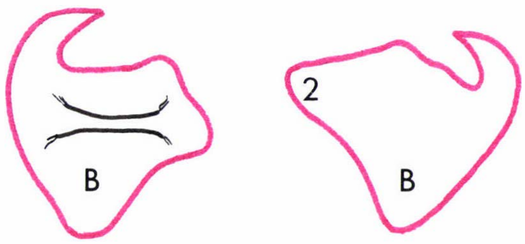
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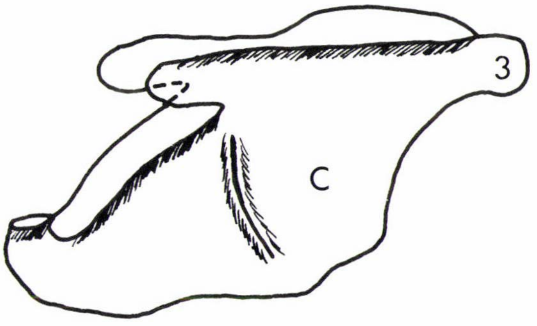
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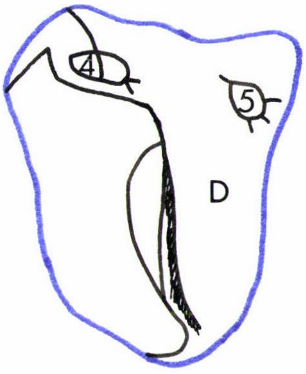
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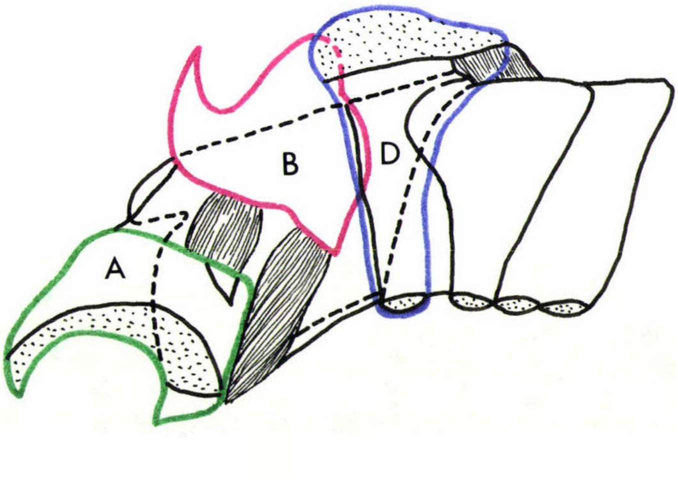
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the thyroid foramen, which serves as entry into the larynx of the internal branch of the cranial laryngeal nerve. The caudal cornu articulates bilaterally with the caudal prominence of the cricoid cartilage.

The cricoid cartilage lies between the laminae of the thyroid cartilage. It is shaped like a signet ring. The dorsal lamina forms the roof of the larynx and cranially the bilateral cricoarytenoid articulations. Caudally it supplies the formerly described cricothyroid articulation. Laterally and ventrally the cartilage is thin and circular, forming the cricoid arch. This arch serves as an attachment caudally for the first tracheal ring by means of the cricotracheal ligament, while cranially it is connected to the thyroid cartilage by the cricothyroid ligament.

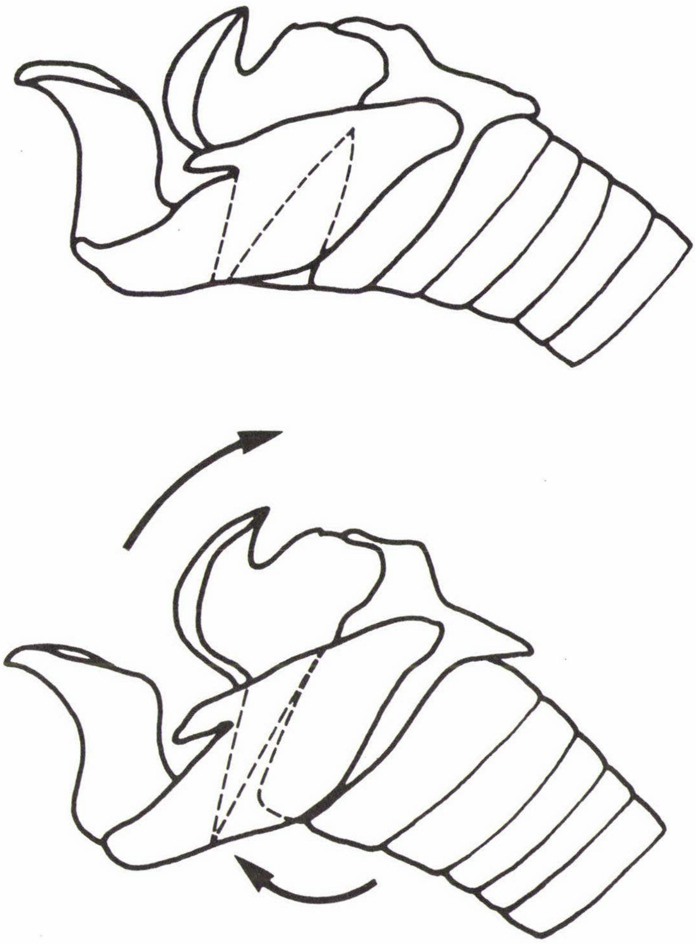
The arytenoid cartilages are paired structures which lie on either side of the rima glottidis. The irregularly shaped cartilages are articulated at their bases to the cricoid cartilage at the cricoarytenoid articulation. The ventrally located vocal process is attached rostrally to the epiglottis and caudally to the thyroid cartilage by the intrinsic laryngeal musculature in two planes. The cricoarytenoid articulations allow movement of the arytenoid cartilages medially and laterally, affecting the size of the glottis. The cricothyroid articulations allow ventrodorsal movement of the cartilages through an axis passing between the cricothyroid articulations (Fig. 2). (Sisson and Grossman, 1953; Nickel _et al_., 1973).

Figure 2. Laryngeal movement:

a. Lateral aspect of larynx showing the movement of the laryngeal cartilages during contraction of the cricothyroid muscles which act to tense the vocal cords and increase the dorsoventral glottic distance.

b. Craniocaudal aspect of the larynx showing the movement of arytenoid cartilages and vocal folds during contraction of the dorsal cricoarytenoid muscles which act to rotate laterally the arytenoid cartilages and increase the horizontal glottic diameter.

(a)



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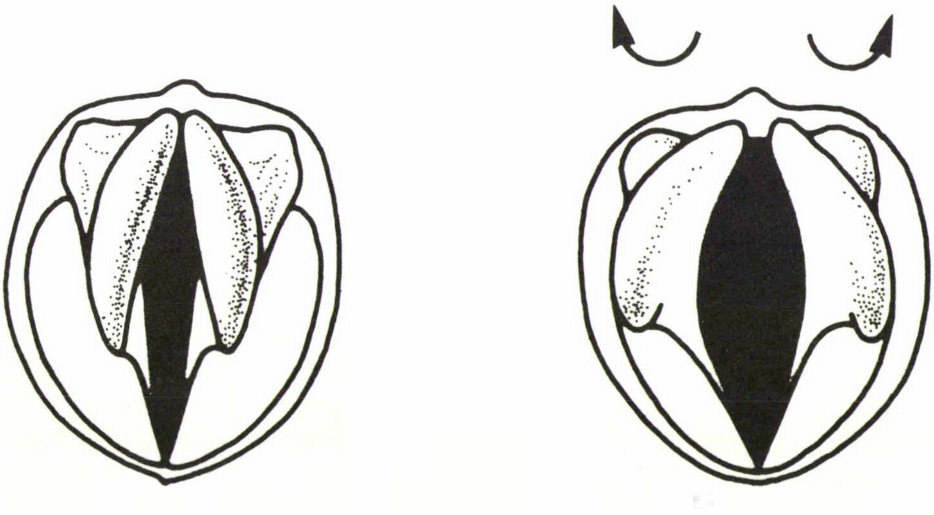


Intrinsic laryngeal musculature

Each cricothyroid muscle (Fig. 3b) arises from the lateral surface of the cricoid arch and passes craniodorsally to the thyroid lamina inserting on to the lateral surface, caudal edge and caudal cornu of the lamina. The action of the muscles is to rotate the cricoid cartilage rostrally towards the thyroid cartilage, thus increasing the distance between the laryngeal floor and roof. This results in an increase in the length of the vocal cords. (Fig. 2a), (Sisson and Grossman, 1953; Nickel et al., 1973).

Each dorsal cricoarytenoid muscle (Fig. 3c) arises from the caudodorsal surface of the cricoid lamina. The two muscles are separated by the median crest of the cricoid cartilage and each passes cranially to insert on to the muscular process of the arytenoid cartilage. The action of each muscle causes a dorsolateral rotation of the muscular process thereby carrying the vocal process of the arytenoid cartilage away from the median plane and therefore increasing the size of the glottis (Fig. 2b), (Sisson and Grossman, 1953; Nickel et al., 1973). This muscle has been described as the abductor muscle in the horse (Sisson and Grossman, 1953), the respiratory muscle in the dog (Hast, 1967) and the inspiratory muscle of the cat (Suzuki and Kirchner, 1969). Semon and Horsley (1890) and Cook (1966) relate the action of this muscle to that of the inspiratory phase of respiration.

The transverse arytenoid muscle (Fig. 3b) in the horse is a paired muscle. It arises from the muscular process and cranially from the dorsolateral border of the arytenoid cartilage. The fibres of the muscle pass dorsomedially to fuse with each other at the midline. The action of these muscles is to adduct the dorsal portions of the arytenoid cartilages, thus closing the dorsal part of the glottis (Sisson and Grossman, 1953; Nickel et al., 1973).

The lateral cricoarytenoid muscles (Fig. 3c) lie medial to the thyroid laminae. Each muscle originates from the craniolateral edge of the cricoid arch, the fibres passing craniodorsally to attach on to the caudal area of the muscular process of the arytenoid cartilage. The action of these muscles causes the muscular process of the arytenoid cartilages to move in a dorsoventral manner. This in turn causes adduction of the vocal processes and closes the glottis when the vocal folds are relaxed (Sisson and Grossman, 1953; Nickel et al., 1973).

The thyroarytenoid muscles (Fig. 3a) in the horse are divided into ventricular and vocal muscles. The more cranial ventricular muscle arises from the junction of the thyroid laminae and cricothyroid ligament and passes dorsally, cranial to the lateral ventricle, to insert mainly on to the cranial part of the muscular process of the arytenoid cartilages. Some fibres pass dorsally over the transverse arytenoid muscle to join the contralateral muscle medially. The vocal muscle lies in the vocal fold lateral and caudal to the vocal ligament. The muscle arises from the ventral thyroid laminae and the cricothyroid membrane. The fibres pass dorsally to insert on to the ventral side of the muscular process and the vocal process of the arytenoid cartilage. Sisson and Grossman (1953) claim that both muscles act as adductors closing the glottis by slackening the vocal folds. However, Nickel et al. (1973) state that the ventricular muscle closes the "vestibular cleft" while the vocal muscle in conjunction with the cricothyroid muscle acts as a tensor of the vocal folds.

Laryngeal innervation:

The laryngeal nerves all originate from the vagal trunk. The cranial laryngeal nerve arises from the vagal trunk immediately caudal to the cranial cervical ganglion. The nerve passes medial to the internal carotid artery in the wall of the pharynx to enter the larynx by the thyroid foramen. Within the larynx the nerve divides into fine branches which end in the mucosa of the larynx, pharynx and esophageal entrance. A small anastomotic branch joins with the recurrent laryngeal nerve lateral to the thyroarytenoid muscle.

Arising from the same area of the vagal trunk is a small nerve, the external branch of the cranial laryngeal nerve. This nerve supplies the cricothyroid and cricopharyngeus muscles. The origin of this nerve is variable. It may arise directly from the vagus at the point of branching of the cranial laryngeal nerve, or caudal to this point. It may also be found originating from the pharyngeal branch of the vagus (Sisson and Grossman, 1953).

The recurrent laryngeal nerve arises from the vagus in the thorax and is reflected medially around the subclavian artery on the right side and medially around the ductus arteriosus and aorta on the left. From these points the nerves follow a common pattern on the dorsolateral surfaces of the trachea, passing cranially up the trachea to enter the caudal part of the larynx. Each recurrent laryngeal

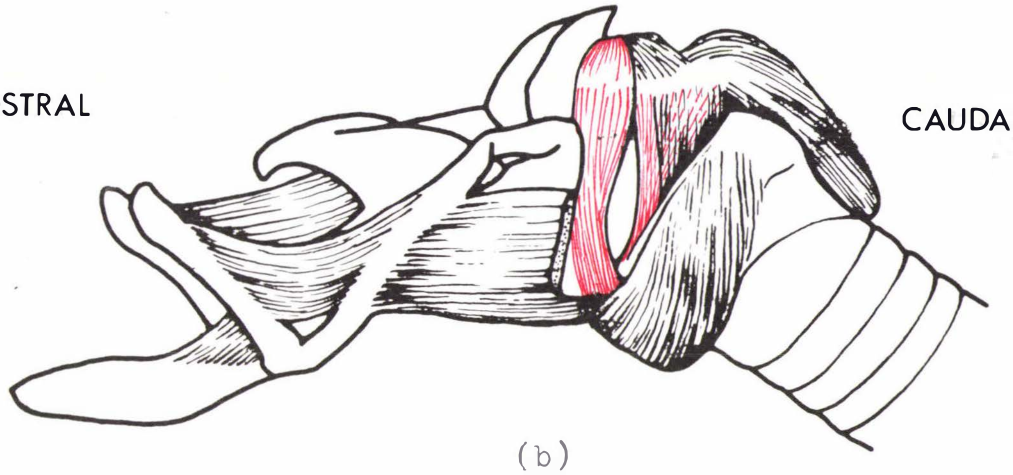
Figure 3. Intrinsic laryngeal musculature

a. Left lateral aspect of larynx with caudal part of the thyroid cartilage removed. The muscles in red are the ventricularis (rostral) and vocalis (caudal).

b. Left lateral aspect of larynx with caudal part of the thyroid cartilage removed. The muscles in red are the transverse arytenoid muscle (dorsal) and the cricothyroid muscle (ventral).

c. Left lateral aspect of the larynx with caudal part of the thyroid cartilage removed. The muscles in red are the dorsal cricoarytenoid muscle (caudal) and the lateral cricoarytenoid muscle (rostral).

(a)



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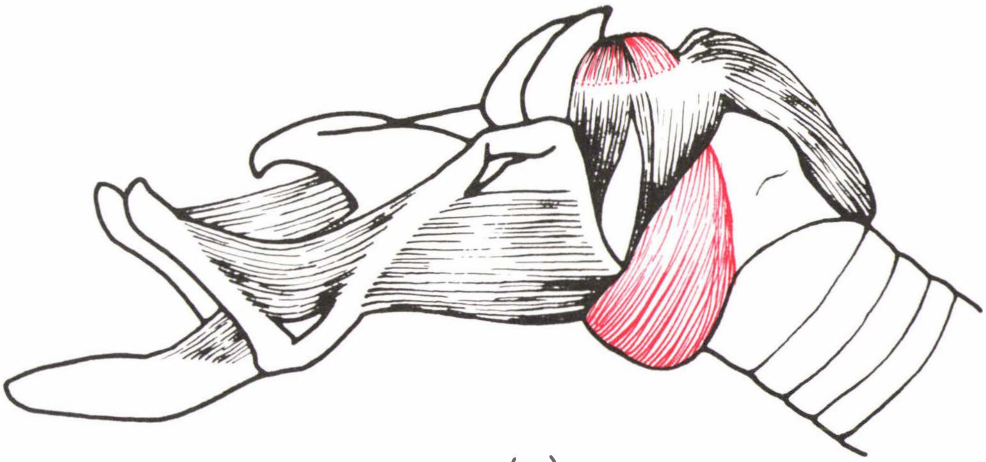

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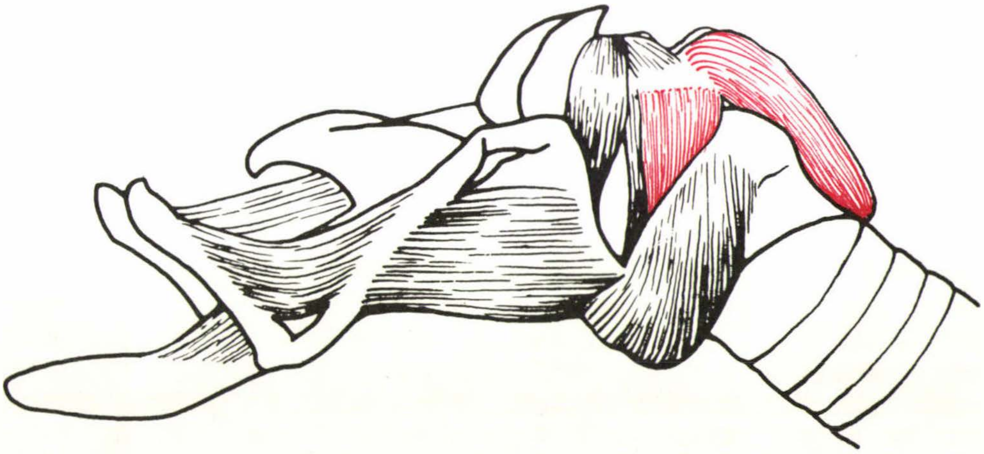


nerve unilaterally supplies all intrinsic laryngeal muscles except the cricothyroid muscle and also joins with the cranial laryngeal nerve by the anastomotic branch described earlier (Sisson and Grossman, 1953).

Sisson and Grossman (1953) state that the cranial laryngeal nerve in the horse is sensory, while the recurrent laryngeal nerve supplies motor function to all intrinsic laryngeal muscles except the cricothyroid which is supplied by the external branch of the cranial laryngeal nerve.

Intracranially, laryngeal motor activity has been demonstrated in the medulla oblongata in dogs (Semon and Horsley, 1890), horses (Argyle, 1934) and cat (Reisen-Russell, 1895). Safanie (1967) claimed that fibres from both cranial nerves X and XI fuse at the distal vagal ganglion to form the vagal trunk. Mettam (1899) stated that in the horse, the cranial nerve XI or accessory nerve contributed fibres to the recurrent laryngeal nerve, while Lemere (1932a, b) claimed that in the dog, laryngeal motor function originated from the "bulbar roots" of the accessory nerve. Semon (1890) postulated that abductor and adductor nerve fibres were spatially separate within the recurrent laryngeal nerve. Russell (1892) claimed he was able to separate nerve bundles within the recurrent laryngeal nerve and could trace the distinct bundles to individual adductor and abductor muscles. However Sunderland and Swaney (1952) were unable to show discrete abductor and adductor trunks in the recurrent nerve of man. These authors suggested that in man there was constant coalescence and division of fascicles along the recurrent laryngeal nerve.

Vermeulen (1914) found an average of eight fascicles in the recurrent nerve in the horse. Cole (1946) described two types of fibre trunks in the recurrent nerve of the horse. He described an average of six fascicles of heavily myelinated fibres and five fascicles of finely myelinated fibres.

Stimulation experiments of the recurrent laryngeal nerve of the horse have shown that weak stimulation produced abduction (Cole, 1946). In cats and goats, Murtagh and Campbell (1948) showed that electrical stimulation invariably caused adduction and that abduction was infrequent and only occurred after repeated shocks. Mechanical stimulation of the nerve, however, resulted in adduction. From these findings Murtagh and Campbell (1948) concluded that the greater number of fibres in the nerve were large and supplied the adductor muscles and presumed that abductor fibres would be smaller and less numerous. Later studies showed that in cases of laryngeal paralysis and in traumatised recurrent laryngeal nerves abductor function loss corresponded with loss of finely myelinated fibres (Murtagh and Campbell, 1951, 1952). From these findings the authors claimed that in man, cat and goat, finer heavily myelinated fibres of 2.8 μ diameter controlled abductor function. These fibres made up 40% of the nerve fibre population. The remaining larger fibres controlled adduction. In 1956 these authors emphasised that the recurrent nerve was not solely motor but had mixed sensory and motor components.

The cranial laryngeal nerve in the dog was shown to contain both large and small fibres (Chase and Ranson, 1914).

FUNCTION OF THE LARYNX

Intrinsic laryngeal musculature

The activity of individual intrinsic laryngeal muscles has been a point of conjecture for many years. Semon and Horsley (1890), after observations on live and postmortem larynges in man, stated that the dorsal cricoarytenoid muscle was in a constant state of contraction in respiration. They claimed that this muscle was the primary respiratory muscle of the larynx. The adductor group of muscles was claimed to have no respiratory function and was primarily involved with vocalisation and deglutition (Semon and Horsley, 1890; Nickel et al., 1973).

With the advent of electromyography, the activity of laryngeal muscles both normal and abnormal, in man and a range of experimental animals has been investigated (Siribodhi et al., 1963; Hiroto et al., 1964; Tomita, 1967; Asigian et al., 1970; Dedo, 1970; Kotby-Nasser and Haugen, 1970; Drasnoveau and Popoviciu, 1971; Murakami and Kirchner, 1971; Hirose and Gay, 1972). Experimental studies on specific muscles has shown in dogs (Nakamura et al., 1958; Hast, 1967) and cats (Green and Neil, 1955; Suzuki and Kirchner, 1969) that the dorsal cricoarytenoid muscle was most active just prior to and during the inspiratory phase of respiration, while the adductor group of muscles show electrical activity during expiration. Anatomically the cricothyroid muscle has been included with the adductor group of muscles, causing adduction and tensing of the vocal folds in horses (Sisson and Grossman, 1953), dogs (Freedman, 1955), (Murtagh, 1945), goats and cats (Murtagh, 1945). However, later studies have advanced the possibility of multiple function of this muscle. It has been shown that electrical activity in the muscle varies between inspiratory and expiratory phases depending on the type of respiration (Suzuki et al., 1970).

Laryngeal innervation

Sensory stimuli from the laryngeal mucosa in cats is carried to the brain through the cranial laryngeal nerve (Rex, 1969). This concept agrees with that of Pressman and Kelemen (1955) who considered the cranial laryngeal nerve contained mainly sensory fibres.

In the horse Sisson and Grossman (1953) state that the internal branch of the cranial laryngeal nerve contains only sensory fibres and that some motor fibres, which supply the cricothyroid and cricopharyngeal muscles, are present in the external branch of the cranial laryngeal nerve. These authors also state that the remaining intrinsic laryngeal muscles receive their motor nerve supply unilaterally through the recurrent laryngeal nerves. Pressman and Kelemen (1955) however consider that in man the transverse arytenoid muscle receives a bilateral motor nerve supply from the recurrent laryngeal nerves.

MATERIALS AND METHODS

ANATOMICAL STUDY OF THE LARYNGEAL NERVES

Gross anatomy of the laryngeal nerves

Six horses were used to trace the gross nerve supply to the larynx. The age, sex and breed of these animals is shown in Table 1. Immediately after slaughter a dissection of the vagus and all its branches from the point of emergence from the jugular foramen to a point caudal to the heart was performed on both left and right sides. All laryngeal branches were traced.

Histology of the laryngeal nerves

Following exposure of the nerves in the 6 horses used in the study of the gross anatomy of the laryngeal nerve supply. Transverse sections were taken at the points shown in Table 2.

All nerve sections were fixed in Bouin's solution. After wax embedding and cutting, the transverse sections were stained using trichome blue by the modified Masson's Method (Birtles, 1974).

FUNCTIONAL STUDY OF THE LARYNGEAL NERVES

Utilising the findings of the anatomical study of the laryngeal nerves, a series of experiments was devised to establish the motor control of the individual laryngeal muscles. For this purpose four experiments were undertaken, each being repeated on three horses. On these twelve horses, and on two further animals, electromyographic observations were made of the right intrinsic laryngeal muscles to establish the pattern of electrical activity.

Clinical and routine haematological examination prior

TABLE 1

DESCRIPTION OF HORSES USED FOR GROSS LARYNGEAL NERVE STUDY

NUMBER	TYPE	SEX	AGE IN YEARS
1	Arab	Gelding	6
2	Thoroughbred	Colt	2
3	Thoroughbred/ Pony cross	Gelding	10
4	Thoroughbred	Mare	14
5	Pony	Mare	10
6	Pony	Mare	10

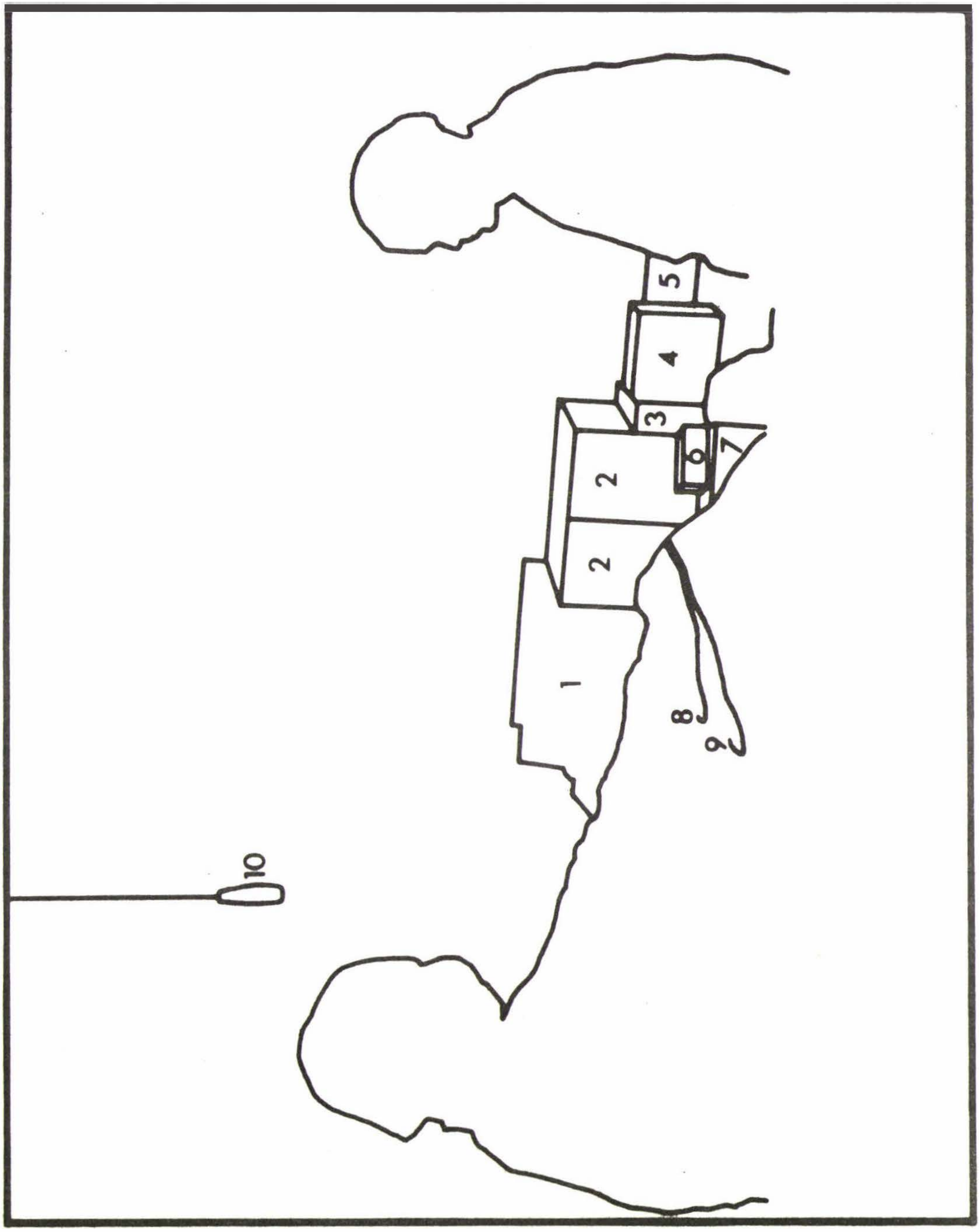
TABLE 2

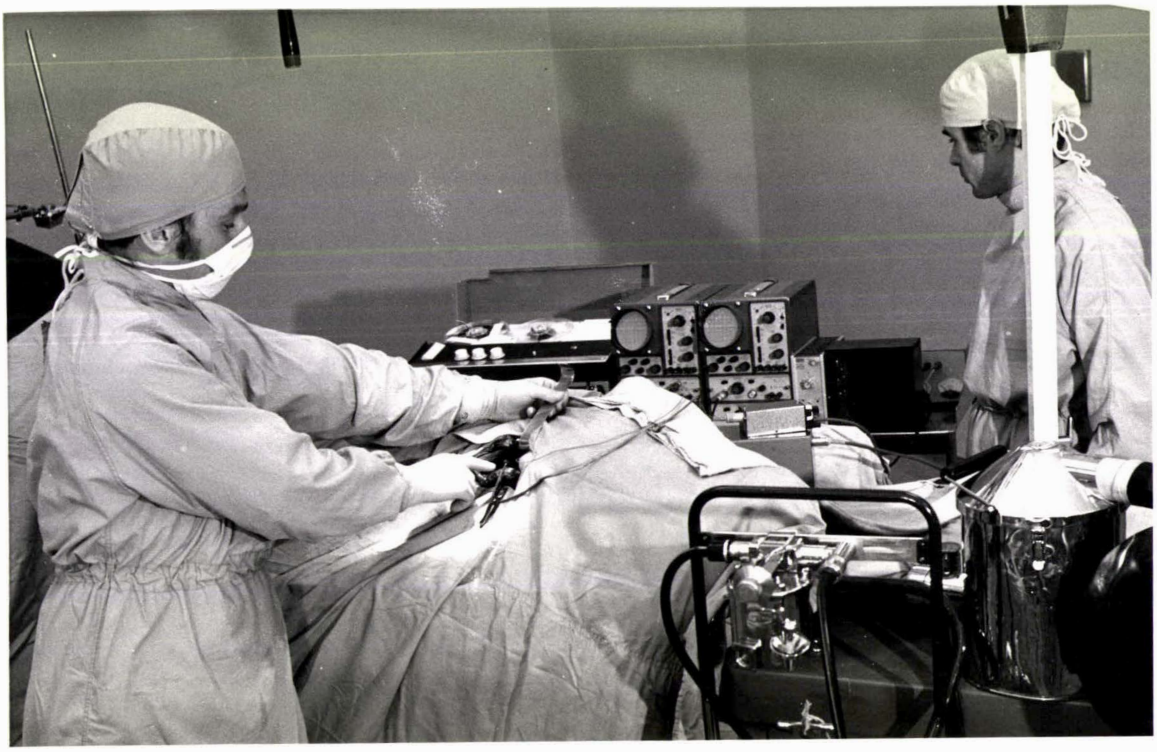
POSITIONS OF THE VAGUS AND LARYNGEAL BRANCHES AT WHICH SAMPLES FOR HISTOLOGICAL EXAMINATION WERE TAKEN

NERVE	POSITION
Vagus	At the jugular foramen
Vagus	Before branching of the recurrent laryngeal nerve
Vagus	After branching of the recurrent laryngeal nerve
Recurrent Laryngeal Nerve	Near origin from vagus nerve
Recurrent Laryngeal Nerve	Near the larynx
Recurrent Laryngeal Nerve	Branch to abductor muscles
Recurrent Laryngeal Nerve	Branch to adductor muscles
Cranial Laryngeal Nerve	At thyroid foramen
External Cranial Laryngeal Nerve	At the cricothyroid muscle

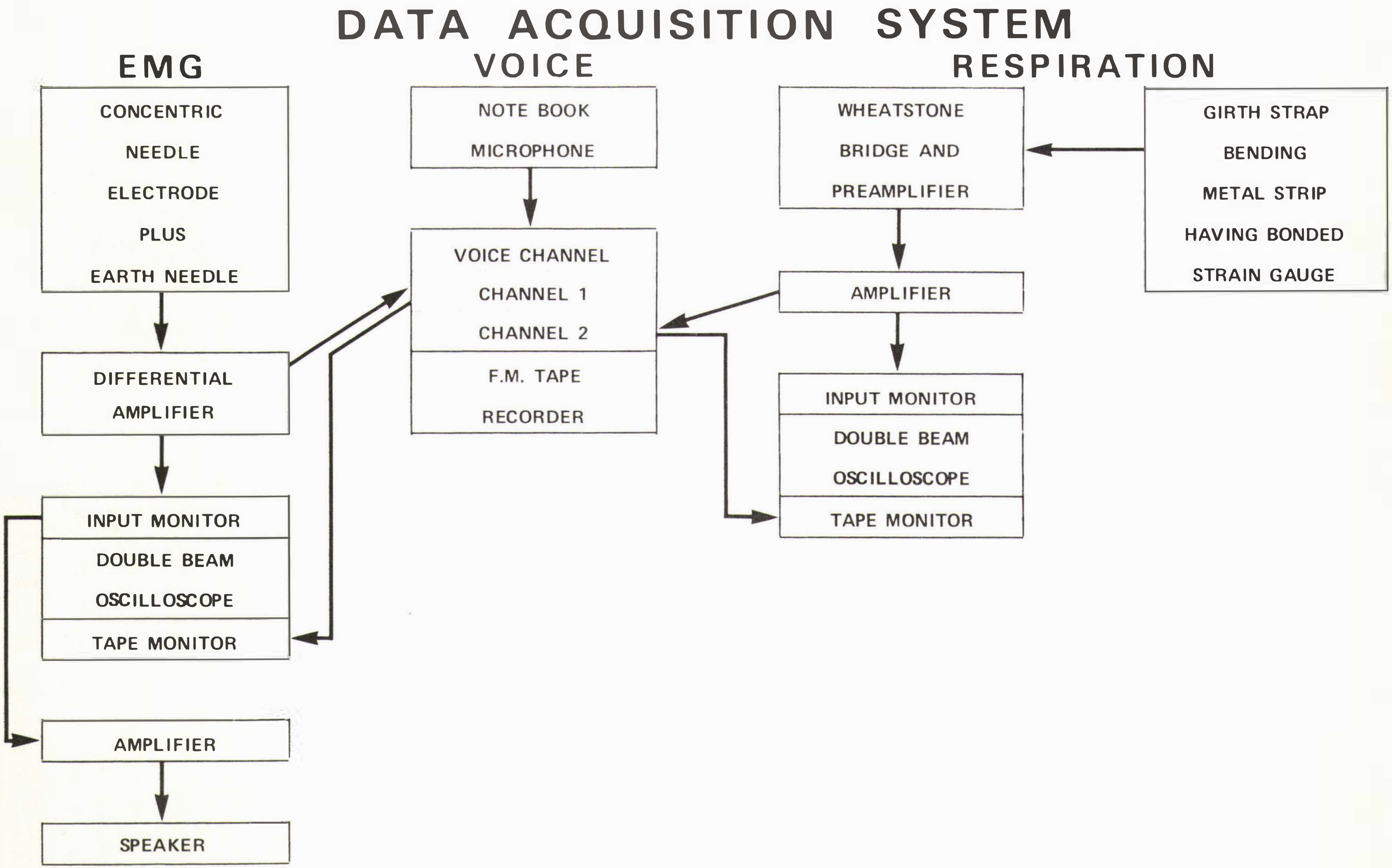
Figure 4.

DATA AQUISITION SYSTEM





1. F.M. Tape Recorder (Epsylon Lacorder)
2. Double Beam Oscilloscopes (Telequipment)
3. D.C. Amplifier (Phillips)
4. Speaker
5. Power supply
6. Differential Amplifier - E.M.G. and High gain
7. Amplifier power supply
8. Earth electrode
9. Concentric Needle Electrode (Shielded cable)
10. Microphone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EMG
VOICE
RESPIRATION
CONCENTRIC NEEDLE ELECTRODE PLUS EARTH NEEDLE
DIFFERENTIAL AMPLIFIER
INPUT MONITOR
DOUBLE BEAM OSCILLOSCOPE
TAPE MONITOR
AMPLIFIER
SPEAKER
NOTE BOOK MICROPHONE
VOICE CHANNEL
CHANNEL 1
CHANNEL 2
F.M. TAPE RECORDER
WHEATSTONE BRIDGE AND PREAMPLIFIER
GIRTH STRAP BENDING METAL STRIP HAVING BONDED STRAIN GAUGE
AMPLIFIER
INPUT MONITOR
DOUBLE BEAM OSCILLOSCOPE
TAPE MONITOR

Figure 5.

DATA RETRIEVAL SYSTEM

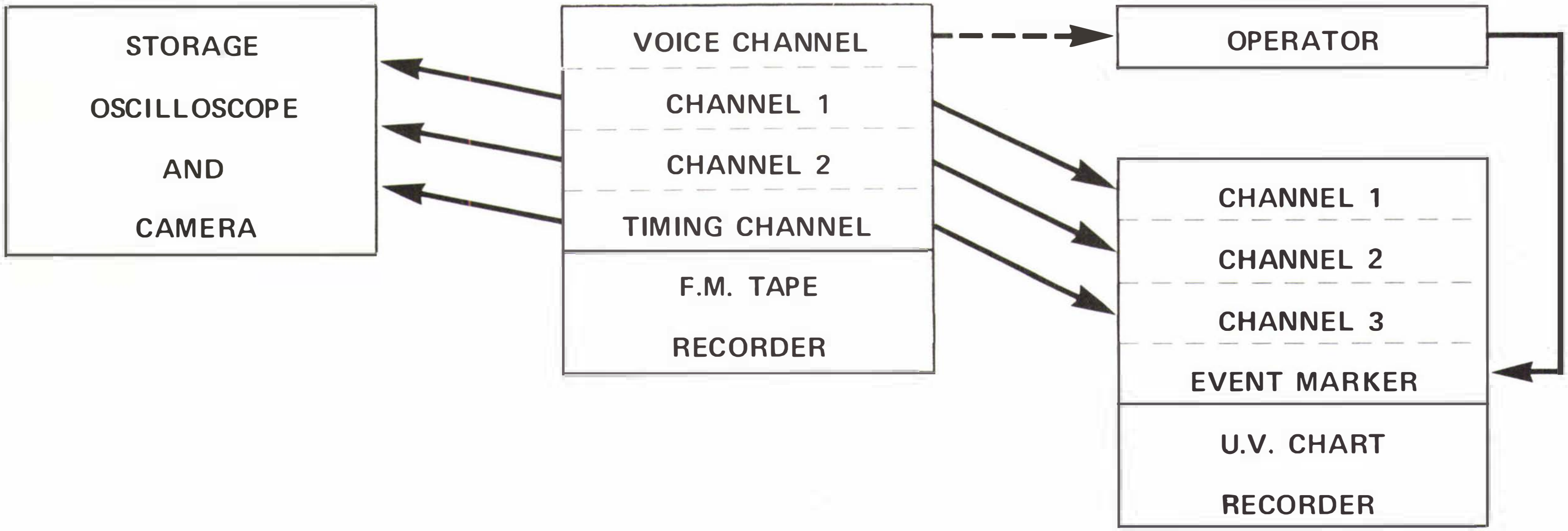

Figure 6.

to the experiments on these animals failed to reveal any abnormality.

Each animal was prepared for anaesthesia by an intramuscular injection of 20 mg acetyl promazine. Anaesthesia was induced by intravenous administration of 10% thiopentone sodium (1g/100g live weight). An endotracheal tube was placed in position and attached to a large animal Water's canister and rebreathing bag. Anaesthesia was maintained by use of a halothane-oxygen mixture delivered through a Fluotec vaporiser. The animals were placed in left lateral recumbency and the area of skin over the right lateral surface of the larynx was prepared for surgery. The intrinsic laryngeal muscles on the right side were selected for recording because only rarely are they affected by the pathological processes so commonly found in the left intrinsic muscles (Duncan et al., 1974). After suitable draping, a skin incision was made immediately ventral and parallel to the external maxillary vein extending forward for 10 cm from the level of the sterno-mandibular muscle. After cutting through the cutaneous muscle, the external maxillary vein and the omohyoid muscle were identified. Blunt dissection was carried out between these two structures thus exposing the cricopharyngeal and thyropharyngeal muscles and the postero-dorsal aspect of the larynx. For earthing purposes a monopolar needle electrode was implanted in the skin of the horse over the posterior extremity of the horizontal ramus of the mandible. An active concentric needle electrode was then placed in the cricothyroid and dorsal cricoarytenoid muscles in turn and the electromyograms from these muscles were recorded on frequency modulated magnetic tape.

The sulcus between the crico- and thyropharyngeal muscles was then carefully opened taking care to ensure that the external branches of the superior laryngeal nerves were not injured. Following this, the concentric needle electrode could be placed in the lateral cricoarytenoid, the transverse arytenoid and the vocalis and ventricularis muscles without the exterior of the needle being in contact with any other muscles. Recordings were obtained from up to three separate regions of each muscle.

In each recording the electrodes were connected to an amplifier which was in turn connected to both a loudspeaker and a two channel oscilloscope thus allowing both audio and visual appraisal of the electrical activity (Fig. 4).

In the first few animals, respiration was simultaneously monitored by use of a thermistor placed in the endotracheal tube. In later experiments this arrangement was replaced by a tension transducer attached to a belt around the horse's girth. This monitored the excursions of the thoraco-abdominal wall during inspiration and expiration. The tension transducer was also connected to the two channel oscilloscope allowing simultaneous viewing of both electromyographic and respiratory action. A permanent record of these was obtained by connecting each system to a frequency modulated tape recorder[*]. A voice channel was available on this machine and allowed comments to be recorded on tape during the procedure (Fig. 5). The tapes were later replayed into an ultra-violet oscillograph thus producing a static record

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[*] Epsylon MR 800 A Labcorder.

that could be visually examined (Fig. 6).

Denervation experiments

After the normal electrical activity pattern on the right intrinsic laryngeal muscles was recorded, various nerves were transected. Utilising the findings of the gross anatomical study, electromyographic recordings were taken during nerve transection from the muscle supplied by the transected nerve. Two or three recordings were again taken from each muscle immediately after nerve section.

During the actual nerve section the arytenoid movement was observed through a rhinolaryngoscope.

On completion of the recordings the surgical incision was closed in two layers, dead space was occluded with 2/0 chromic catgut and the skin was closed with heavy Vetafil. Sutures were removed in ten days. After six weeks the horses were returned and subjected to further surgery. Using the same technique as earlier described, both left and right intrinsic laryngeal muscles were electromyographically monitored and recorded, thus allowing a comparison of operated and unoperated sides.

Experiment I: Abductor Activity

The right recurrent laryngeal nerve was identified as it passed dorsal to the right thyroid gland. Care was taken to avoid the laryngeal branches of the thyroid artery which lay dorsolateral to the nerve at this point. After the nerve was identified the trunk was carefully dissected cranially to the point of first division. This branch was then traced to its insertion into the right dorsal cricoarytenoid muscle. During the experiment the dorsal cricoarytenoid nerve was sectioned and two centimetres of nerve was removed.

Experiment II: Adductor Activity

The right recurrent laryngeal nerve was identified as described in Experiment I. The recurrent nerve was traced to the point of division of the "Abductor" branch. The nerve was traced cranial to this branch, thus exposing that part of the nerve supplying the adductor group of muscles. Electromyographic recordings of adductor activity were taken from the lateral cricoarytenoid muscle. This muscle was used for ease of recording during nerve section as it is the most accessible of the adductor group of muscles. A two centimetre section of nerve was removed in all cases.

Experiment III: Cricothyroid Activity

The external branch of the right cranial laryngeal nerve was identified as it passed laterally over the extrinsic laryngeal muscles. Transection of the nerve, examination, and sampling were made as in Experiments I and II.

Experiment IV: Cranial Laryngeal Nerve Function

The internal branch of the right cranial laryngeal nerve was identified as it passed ventrocaudally from the vagus to the thyroid foramen of the larynx. In this experiment, all intrinsic laryngeal muscles were monitored before and immediately after nerve section. Transection and sampling of the nerve was made as described in Experiment I and II.

WEIGHTS OF INTRINSIC LARYNGEAL MUSCLES

A. Twenty-four larynges obtained from dead horses of various ages, breeds and sexes were placed in 10% formalin for 7 days. An additional larynx was obtained from a diagnosed roarer for purposes of comparison. The intrinsic muscles from these larynges were removed from their cartilaginous attachments and weighed. Since it is possible that the use of wet, fixed tissue introduces an error in muscle weight measurement, the muscles were subsequently freeze-dried and weighed again. The significance of the weight difference between paired right and left muscles in each horse was tested using Student's t test within the following groups:

(1) Abductor muscles (dorsal cricoarytenoid)

(2) Adductor muscles supplied by the recurrent laryngeal nerve (lateral cricoarytenoid, transverse arytenoid, vocalis and ventricularis)

(3) Cricothyroid

B. The larynges were removed postmortem from the 12 horses involved in the denervation experiments and treated in the same manner as previously mentioned in A.

HISTOLOGY OF INTRINSIC LARYNGEAL MUSCLES

Sections were taken from the horses involved in the denervation experiments. Sections of the denervated muscles and contralateral equivalent muscle were stained with Haematoxylin and eosin and examined.

RESULTS

ANATOMICAL STUDY OF THE LARYNGEAL NERVES

Gross anatomy of the laryngeal nerves

1. Vagus

The gross anatomy of the vagus and its laryngeal branches was outstanding in its uniformity, and was similar to the descriptions in the literature (Bradley, 1923; Sisson and Grossman, 1953; Taylor, 1955).

The vagus nerve was first identified at the jugular foramen. It emerged in conjunction with the glossopharyngeal (IX) nerve and the external brand of the accessory (XI) nerve. The glossopharyngeal and vagus nerves immediately passed together through a fold of the guttural pouch mucosa. The external branch of the accessory nerve curved caudally towards the lateral cervical region. The vagus nerve became associated with the internal carotid artery at this point and passed parallel to this vessel before becoming associated with the cranial cervical ganglion. The vagosympathetic trunk then travelled caudally down the neck to the level of the thoracic inlet on the dorso lateral surfaces of the trachea.

2. Cranial laryngeal nerves

The first laryngeal branch of the vagus arose from the oesophageal division of the pharyngeal branch of the vagus. The oesophageal branch of the pharyngeal nerve passed caudally from its point of origin from the vagus slightly caudal to the cranial cervical ganglion, to the cranial oesophagus. In all cases this nerve supplied the external branch of the cranial laryngeal nerve. The external branch of the cranial laryngeal nerve was traced laterally in the pharyngeal wall to the point where it lay lateral, but in close proximity to, the cricopharyngeal and thyropharyngeal muscles. In this region the nerve branched extensively (Fig. 7). One division of the nerve progressed ventrally to insert into the cricothyroid and thyropharyngeal muscles. Other smaller branches ramified into the musculature of the cricopharyngal muscle.

Caudal to the pharyngeal branch, a large nerve arose from the vagus. This nerve passed in a ventral direction medial to the external carotid artery in the lateral wall of the pharynx and then continued ventrocaudally to enter the larynx at the thyroid foramen. This nerve was identified as the internal branch of the cranial laryngeal nerve.

The laryngeal nerve distribution in the cranial and cervical region was unilateral but similar on both left and right sides.

3. Recurrent laryngeal nerves

In contrast, the branching of the recurrent laryngeal nerves differed on either side. The right nerve branched from the vagus at the level of the first intercostal space, passing medially around either the right sub-clavian or the costocervical artery to lie on the right lateral wall of the trachea. At the thoracic inlet a series of fine branches of the recurrent nerve entered the trachea. There was also an interconnection at this point with the cervicothoracic ganglion. From this position the nerve passed cranially on the trachea crossing deep to the common carotid artery before coming to lie on the dorsolateral aspect of the trachea in the region of the thyroid gland (Fig. 8). The nerve passed dorsal to the gland but ventral to the thyroid and laryngeal blood vessels. It then entered the larynx medial to the extrinsic laryngeal muscles (Fig. 7).

The left recurrent nerve branched from the left vagus at the level of the base of the heart, i.e. about 25 to 30 cm caudal to the equivalent point of branching of the right recurrent laryngeal nerve. Fine branches of both the vagus and recurrent laryngeal nerves were found at this point. These were traced into the walls of the aorta and sinus area of the heart. The recurrent laryngeal nerve divided into two branches which curved medially around the aorta and ligamentum arteriosum to lie on the trachea. The smaller of these two branches appeared to be associated with the sympathetic nerve at this point before rejoining the muscle or nerve fibres. This branch was identified as the transverse arytenoid or adductor branch of the recurrent laryngeal nerve.

The remaining branch of the recurrent nerve passed rostro-ventrally medial to the thyroid lamina. The nerve passed obliquely along the lateral surface of the lateral cricoarytenoid and gave off two branches into the substance of this muscle. The recurrent nerve continued rostro-ventrally to lie lateral to the lateral ventricle where it finally divided into three branches. The most caudal branch was reflected caudally, medial to the lateral cricoarytenoid where it entered the substance of the vocalis muscle. The cranial division passed cranially over the lateral ventricle to pass into the substance of the ventricularis muscle. The remaining branch i.e. the dorsal branch interconnected with a large branch of the internal cranial laryngeal nerve (Fig. 10).

Figure 7. Extrinsic innervation of the larynx

Right lateral aspect of the larynx. Showing the external distribution of all nerves associated with the larynx in the laryngeal region.

Key:
🟥 Vagus nerve
🟩 Sympathetic nerve
🟪 External branch of the cranial laryngeal nerve
🟧 Internal branch of the cranial laryngeal nerve
🟦 Recurrent laryngeal nerve
🟪 Oesophageal branch
🟫 Pharyngeal nerve

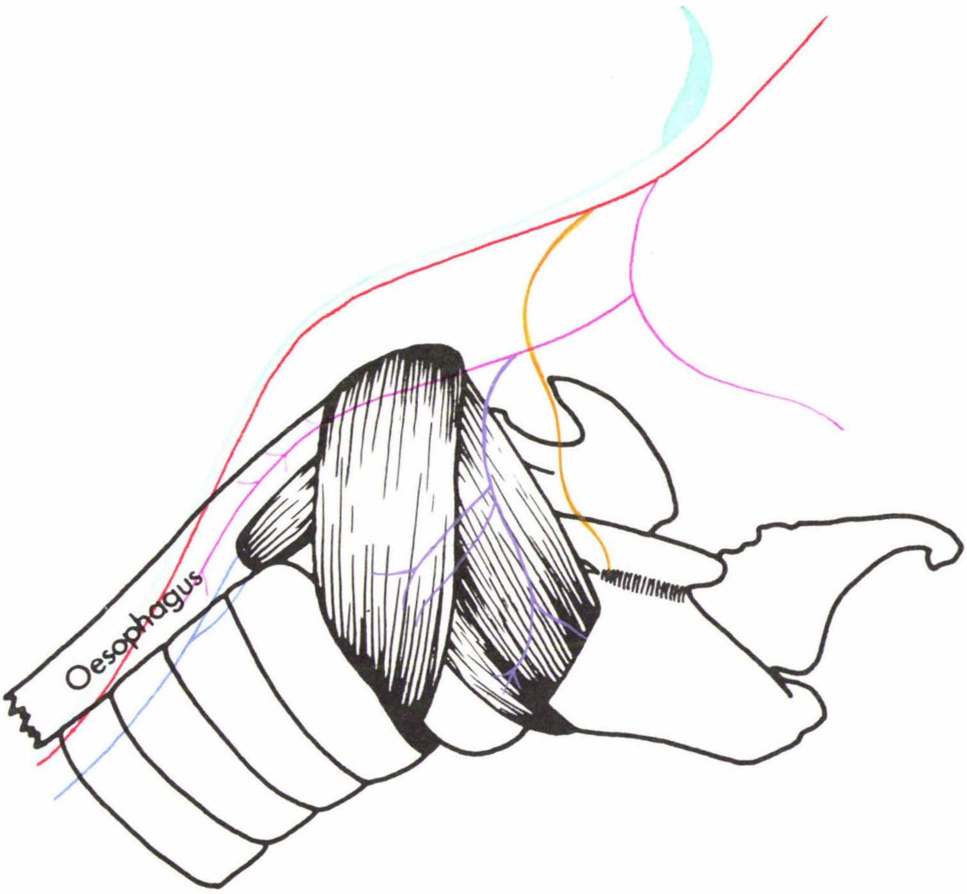

Oesophagus

Figure 8. Distribution of the vago-sympathetic trunk and recurrent laryngeal nerve in the thoracic region

Right lateral aspect.
Showing origin of the right recurrent laryngeal nerve

Key: \ Vagus nerve
\ Sympathetic nerve
\ Recurrent laryngeal nerve

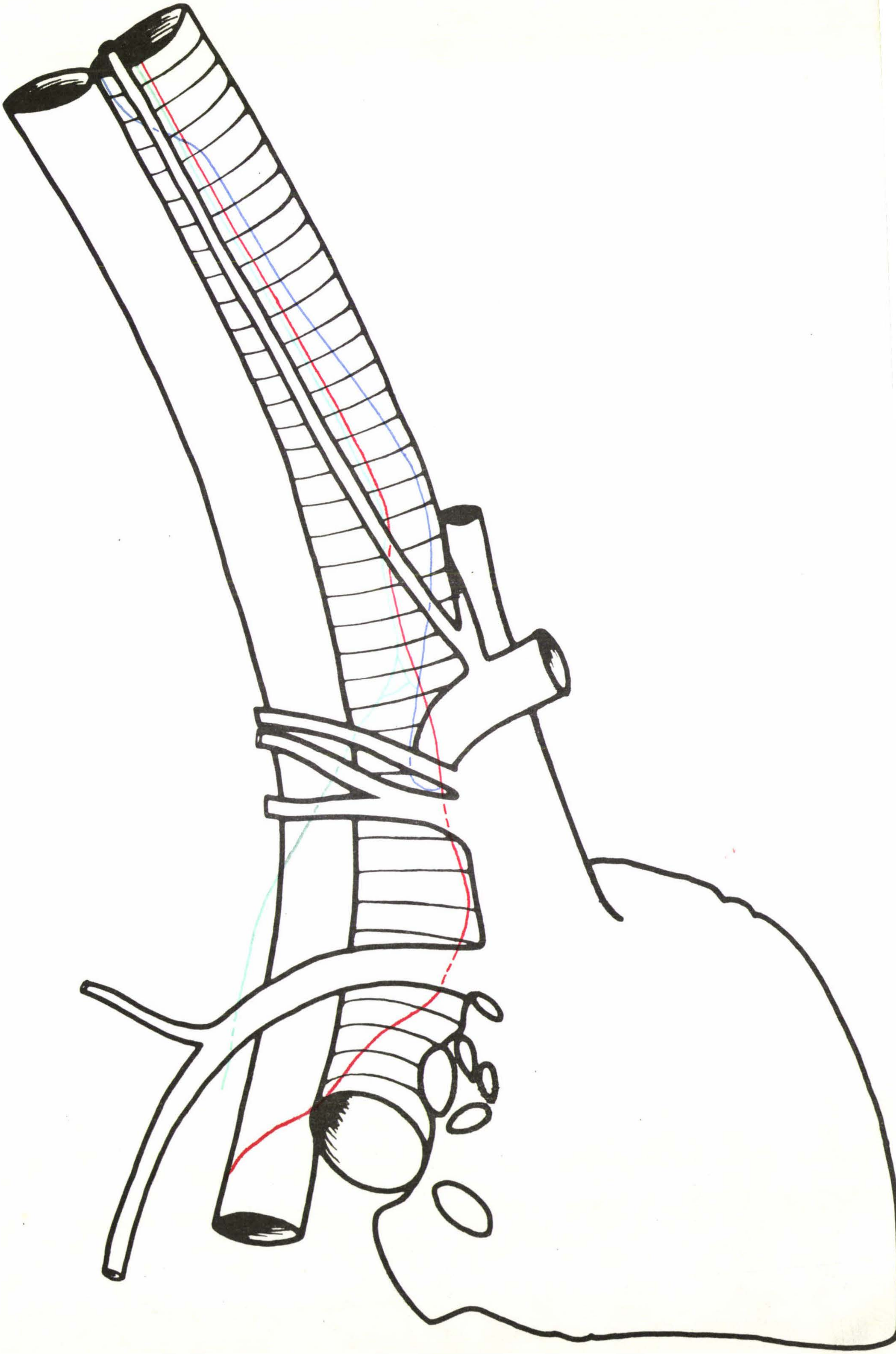


Figure 9. Distribution of the vago-sympathetic trunk and recurrent laryngeal nerve in thoracic region

Left lateral aspect.
Showing origin of the left recurrent laryngeal nerve.

Key: ╲ Vagus nerve
╲ Sympathetic nerve
╲ Recurrent laryngeal nerve

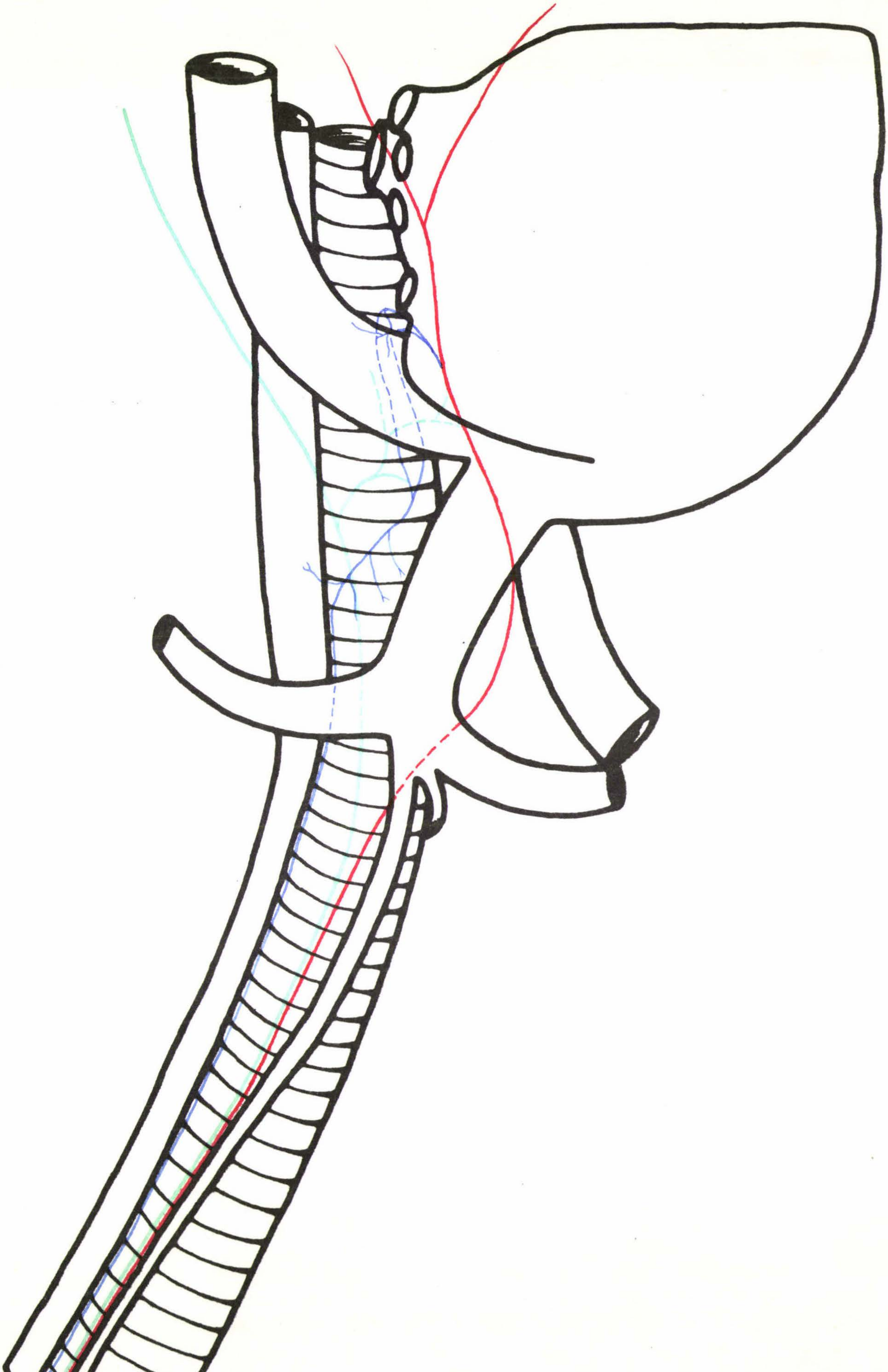
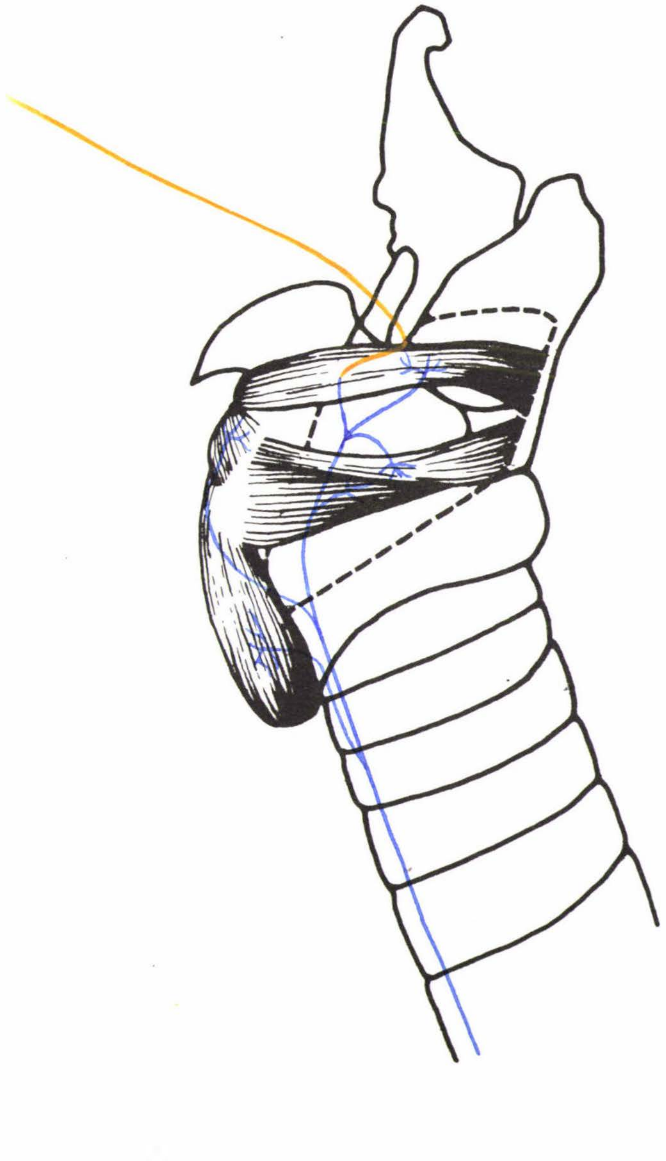


Figure 10. Intrinsic innervation of the larynx

Right lateral aspect of the larynx. Showing the internal distribution of the recurrent laryngeal nerve and cranial laryngeal nerve.

Key:

- 🟦 Recurrent laryngeal nerve
- 🟧 Internal branch of the cranial laryngeal nerve



The cranial laryngeal nerve after entering the larynx from the thyroid foramen immediately divided into numerous fine branches (other than the anastomotic branch described above). These fine fibrils ramified into the mucosa of the aditus laryngis, arytenoid cartilage, aryepiglottic folds and lateral ventricles. There appeared to be no evidence of these fibrils entering muscles. The larger anastomotic branch passed caudally from the thyroid foramen dorsal to the lateral laryngeal ventricle to fuse with the recurrent nerve (Fig. 11).

Histology of the laryngeal nerves

It was found that each nerve examined contained a variable number of perineurial enclosed fascicles of myelineated fibres. The staining technique used did not show unmyelineated fibres. Three distinct types of fibre were identified:

(i) large fibres > 13 µm
(ii) medium fibres < 13 µm > 5 m
(iii) small fibres < 5 µm

There were distinct changes in the distribution and population of the various nerve fibre types depending on the level of transection of the nerve.

At the level of the jugular foramen, the vagus contained many small fibres, and fewer large and medium fibres, randomly distributed throughout the nerve fascicles. The large and medium fibres usually made up 10-15% of the fibre populations (Fig. 12) but in a small number of fascicles, the proportion of the larger fibres was in excess of 50%.

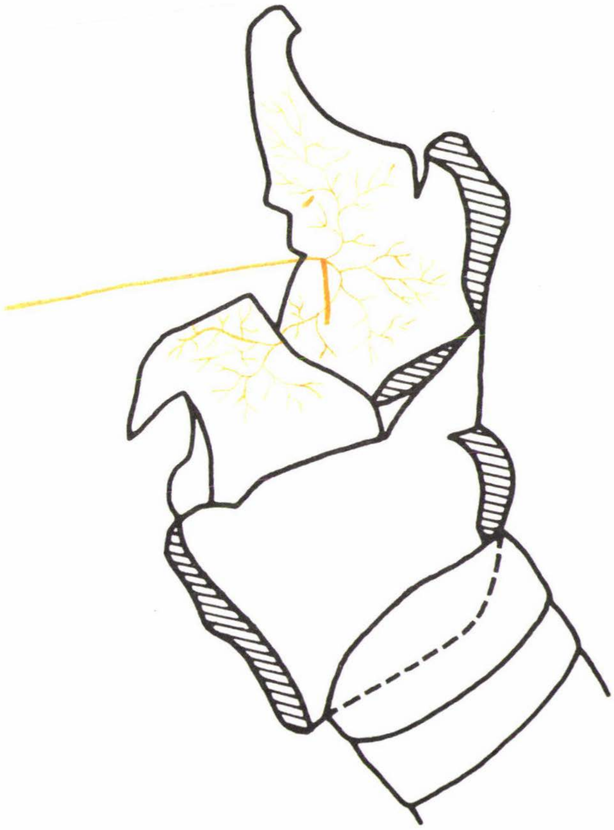
At the thoracic inlet the vagus had fewer fascicles. The fibre population changed within these fascicles. The

Figure 11. Internal branch of the cranial laryngeal nerve

Right lateral aspect of larynx.
Median section through the larynx to show
distribution of nerve within the larynx.

Note anastomotic branch of nerve.

Key: ╲ Internal branch cranial laryngeal nerve



medium sized fibres appeared to coalesce into several fascicles in the nerve trunk. This resulted in some fascicles having the medium sized fibre forming 50-60% of the population. These fascicles lay superficially in the nerve trunk in a distinct area (Fig. 13).

Just prior to the recurrent nerve branching, the fasciclar characteristics of the vagus changed slightly; there were now several fascicles containing solely medium sized fibres. These fascicles lay superficially in the nerve trunk in a distinct group (Fig. 14).

After the recurrent nerve had left the vagus the fasciclar pattern reverted to that seen at the jugular foramen. In all fascicles there was a random distribution of fibre types with a predominance of smaller fibres (Fig. 15).

The recurrent laryngeal nerve was comprised solely of fascicles containing medium sized fibres, although in some horses one fascicle contained small fibres only. This fascicle type was present only in the recurrent nerves as far as the larynx and could not be found in the laryngeal branches (Fig. 16, 17).

Both "adductor" and "abductor" laryngeal branches of the recurrent laryngeal nerve were comprised of fascicles containing medium fibres only (Figs. 18, 19).

The external branch of the cranial laryngeal nerve contained fascicles of medium fibres only (Fig. 20) while the internal branch contained a mixed population similar to that seen in the vagus at the jugular foramen (Fig. 21).

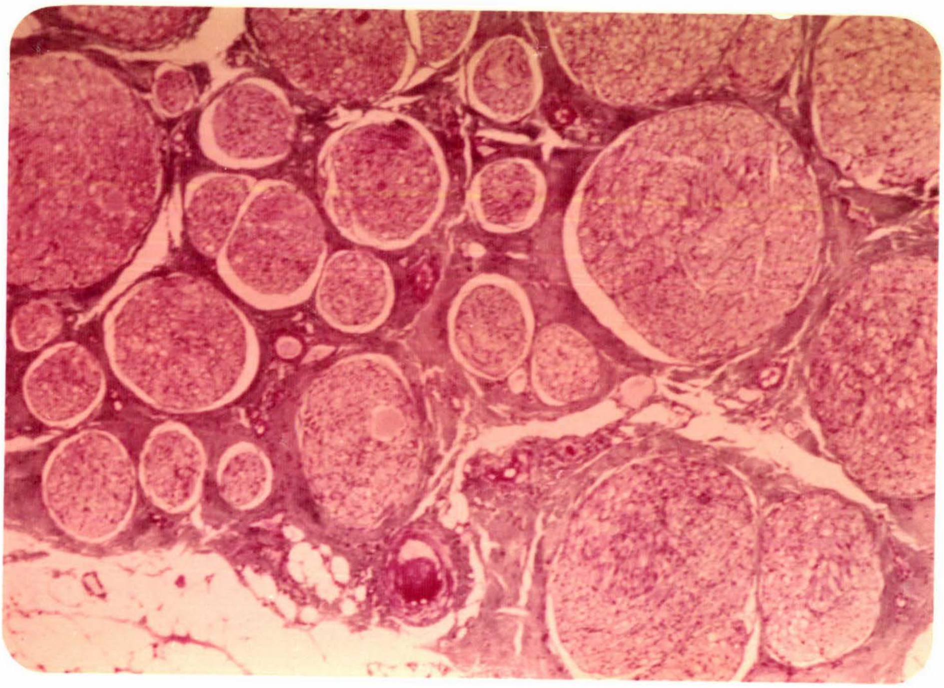
Figure 12. Photomicrograph of transverse section of the vagus at the jugular foramen.

a. Showing distribution of the fascicles present in the vagus.

Trichrome blue 90x.

b. Showing a typical fascicle of the vagus at this point. All types of myelinated fibres can be seen in random distribution.

Trichrome blue 260x.



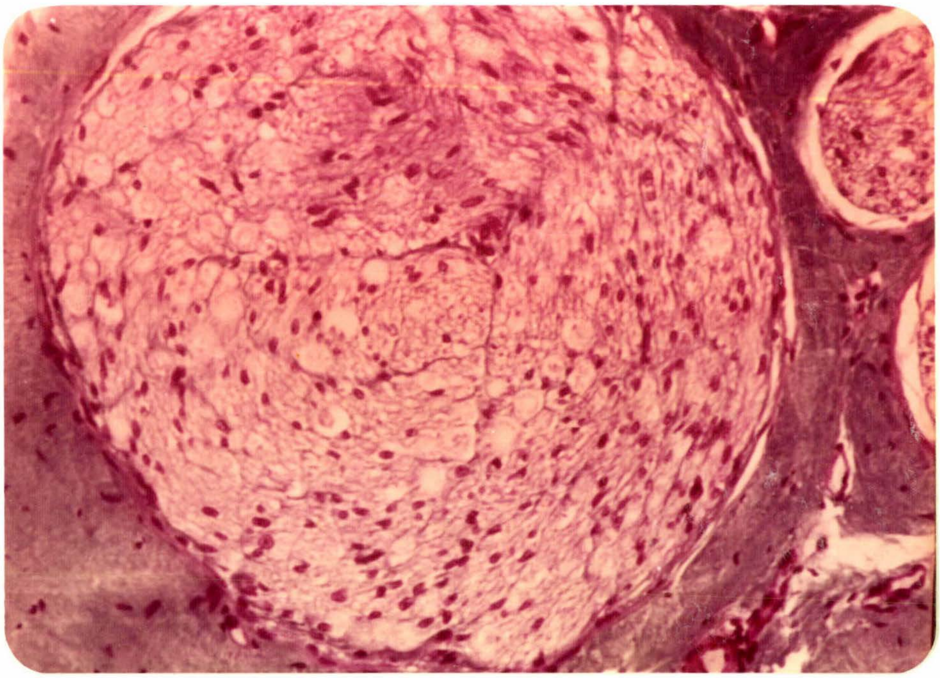


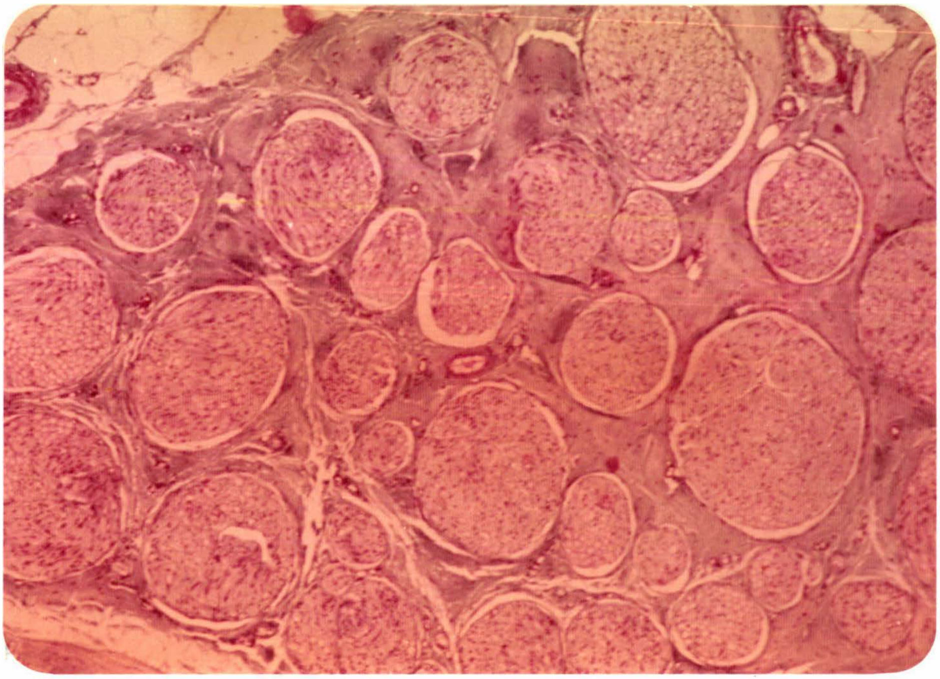
Figure 13. Photomicrograph of transverse section of the vagus at the thoracic inlet.

a. Showing the distribution of fascicles within the vagus. A number of fascicles now show grouping of fibre type.

Trichrome blue 90x.

b. Showing a fascicle where there appears to be a separation of fibre types within the fascicle.

Trichrome blue 260x.



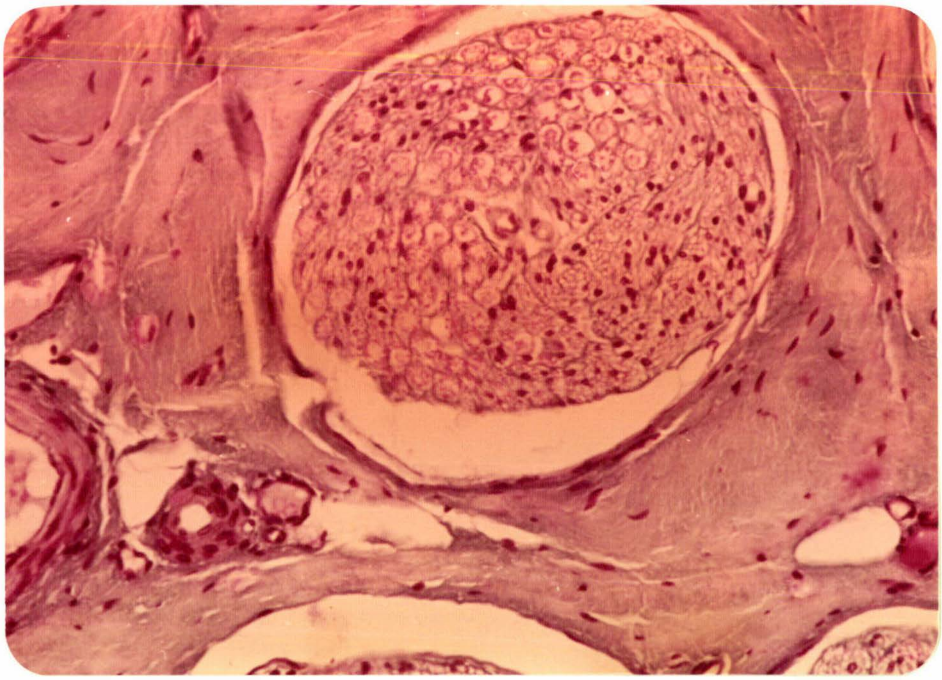


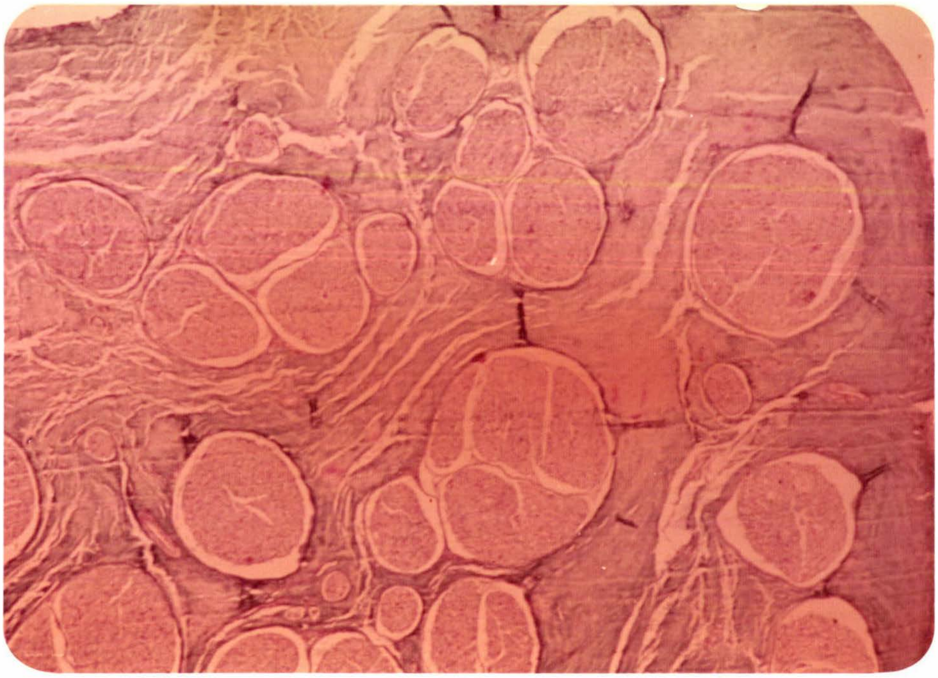
Figure 14. Photomicrograph of transverse section of the vagus before branching of the recurrent laryngeal nerve.

a. Showing distribution of the fascicles in the vagus. Some fascicles now lying on the periphery of the nerve trunk are comprised solely of medium myelinated fibres. See lower right of figure.

Trichrome blue 90x.

b. Showing one of the peripheral fascicles; all the fibres within this fascicle are of the medium myelinated fibre type.

Trichrome blue 260x.



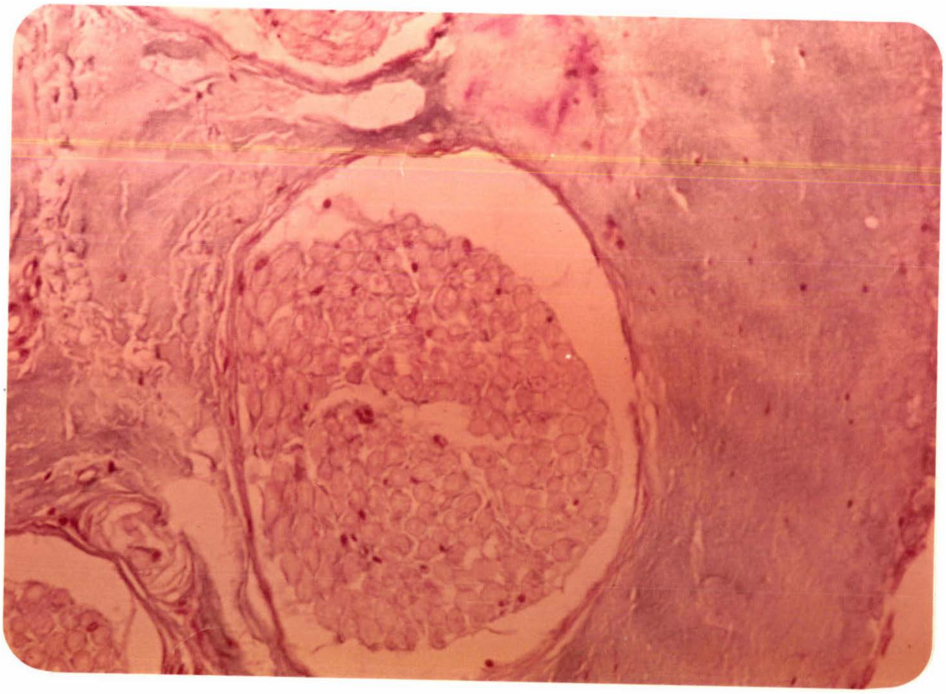


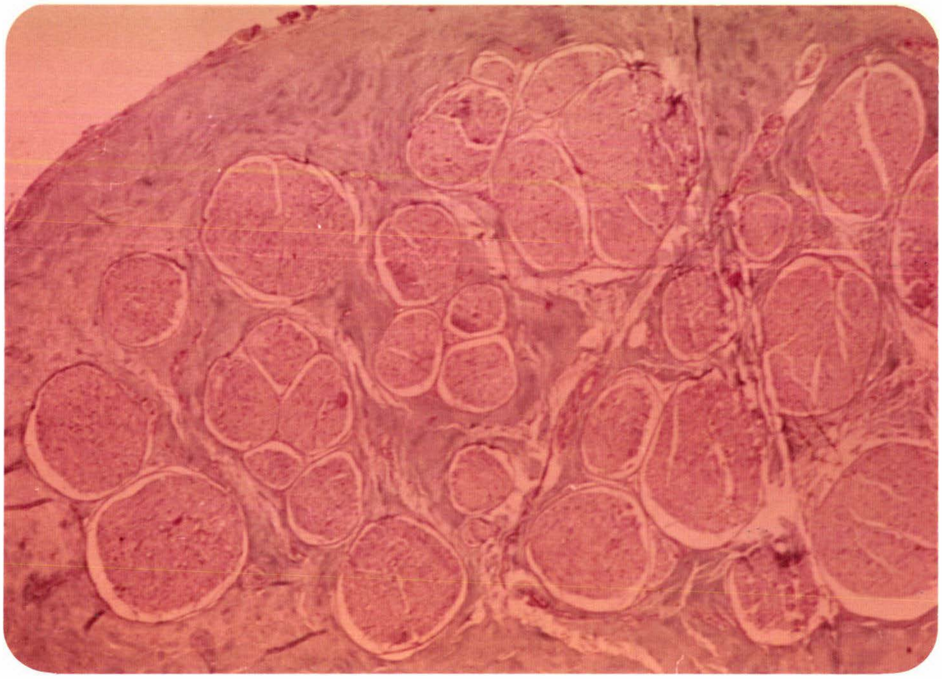
Figure 15. Photomicrograph of transverse section of the vagus after branching of the recurrent laryngeal nerve.

a. Showing pattern of fascicles within the vagus.
The fibre distribution resembles that seen in the vagus at the jugular foramen.

Trichrome blue 90x.

b. Showing a typical fascicle.
There appears to be a random distribution of all fibre types within the fascicle.

Trichrome blue 260x.



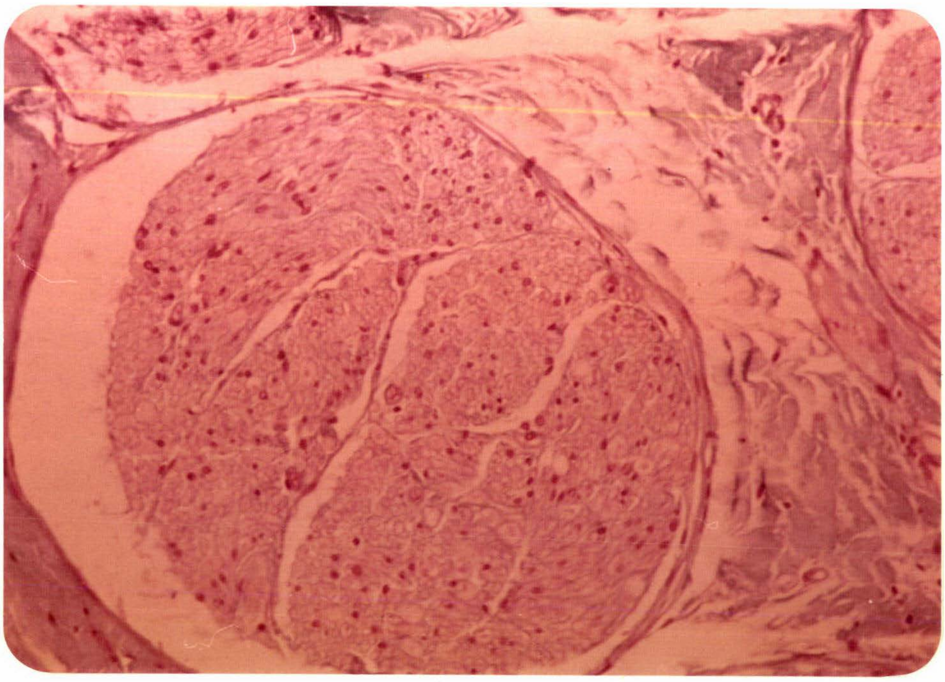


Figure 16. Photomicrograph of transverse section of the recurrent laryngeal nerve at the thoracic inlet.

a. Showing two types of fascicles.
The majority of fascicles are comprised of medium myelinated fibres.
Two fascicles can be seen to the right of the figure which are comprised of finely myelinated fibres.

Trichrome blue 90x.

b. Showing the two types of fibre described above.

Trichrome blue 260x.

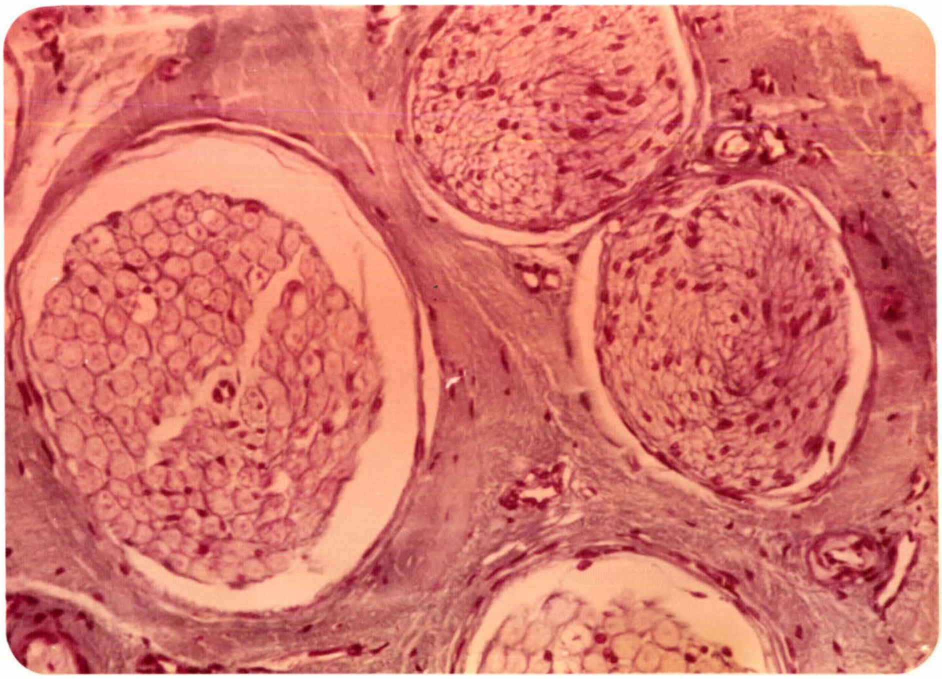


Figure 17. Photomicrograph of the transverse section of the recurrent laryngeal nerve at the larynx.

a. Showing distribution of the fascicles.
The majority of the fascicles are comprised of populations of medium myelinated fibres.
One fascicle of finely myelinated fibres can be seen to the right of the figure stained blue.

Trichrome blue 90x.

b. Showing a fascicle of medium myelinated fibres.
The fascicle appears to be splitting into separate branches.

Trichrome blue 260x.

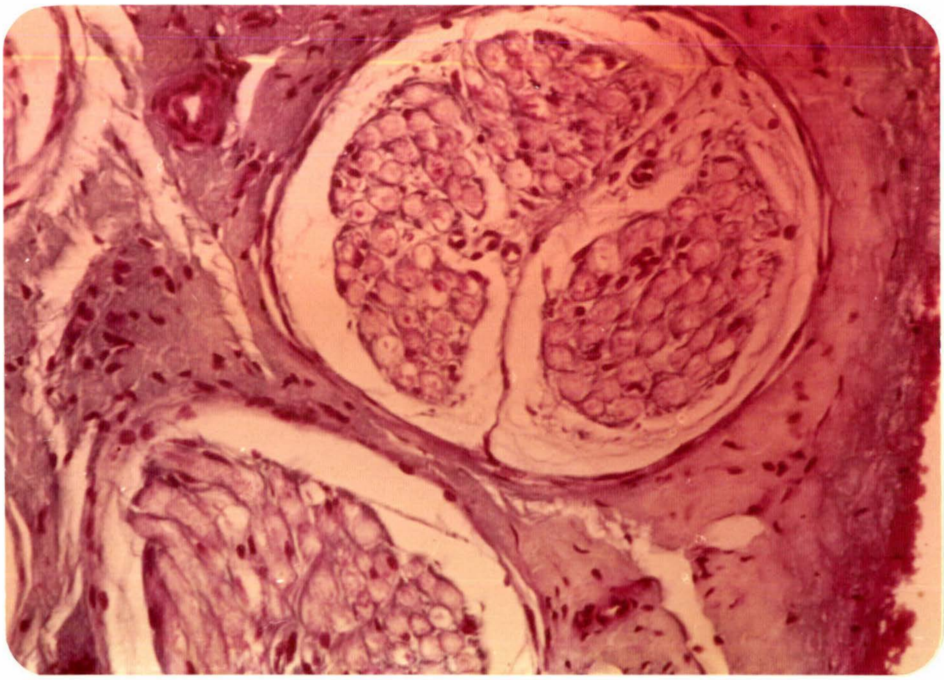


Figure 18. Photomicrograph of transverse section of the recurrent laryngeal nerve.

Abductor branch to the dorsal cricoarytenoid muscle.

a. Showing fascicles comprised predominantly of fibres of medium myelination.

Trichrome blue 260x.

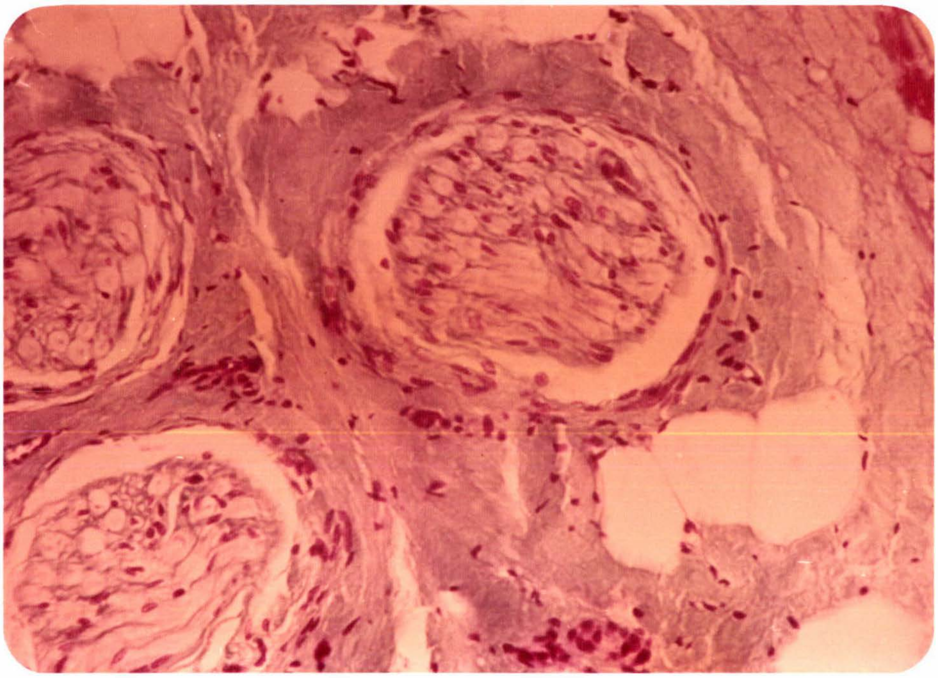


Figure 19. Photomicrograph of transverse section of the recurrent laryngeal nerve.

Adductor branch to all intrinsic laryngeal muscles supplied by the recurrent laryngeal nerve other than the dorsal cricoarytenoid muscle.

a. Showing distribution of fascicles in the nerve. All fascicles are comprised of fibres of medium myelination.

Trichrome blue 90x.

b. Showing fascicle comprised of medium myelination.

Trichrome blue 260x.

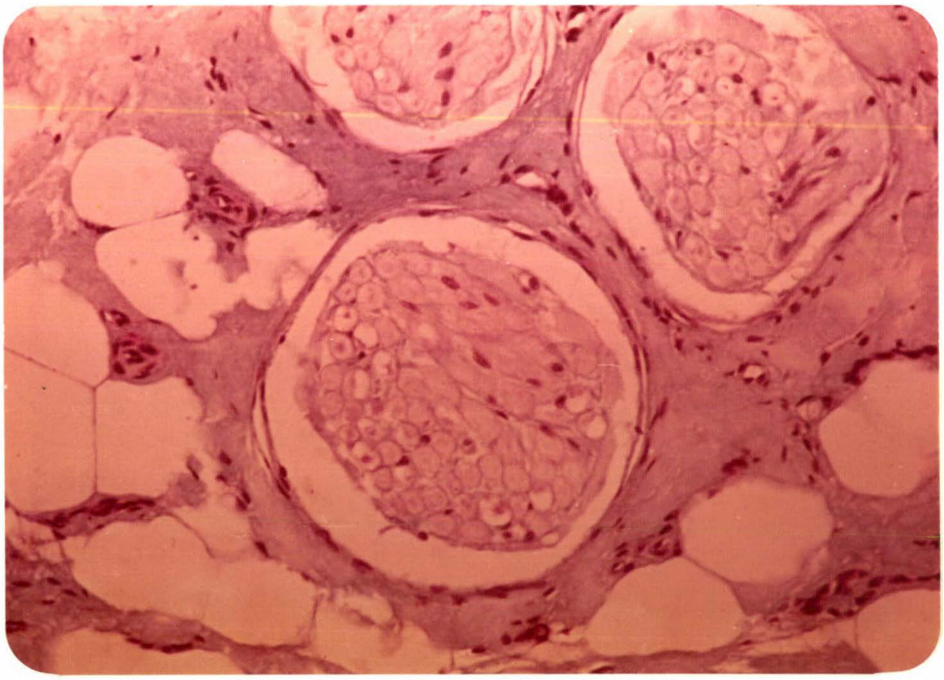


Figure 20. Photomicrograph of transverse section of the external branch of the cranial laryngeal nerve

a. Showing fascicles which have a majority of the medium myelinated fibre type.

Trichrome blue 260x.

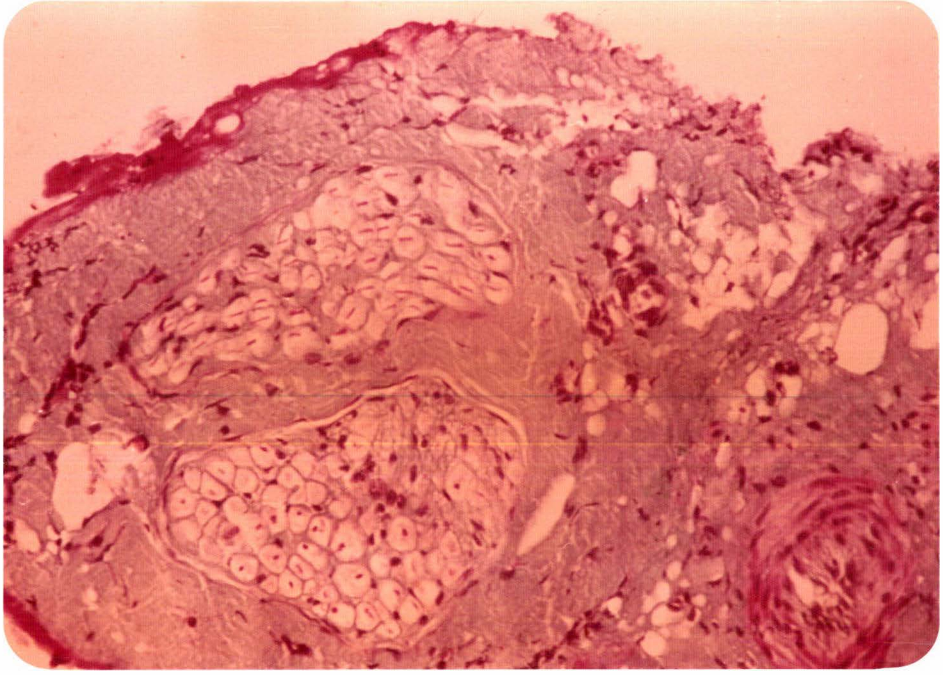


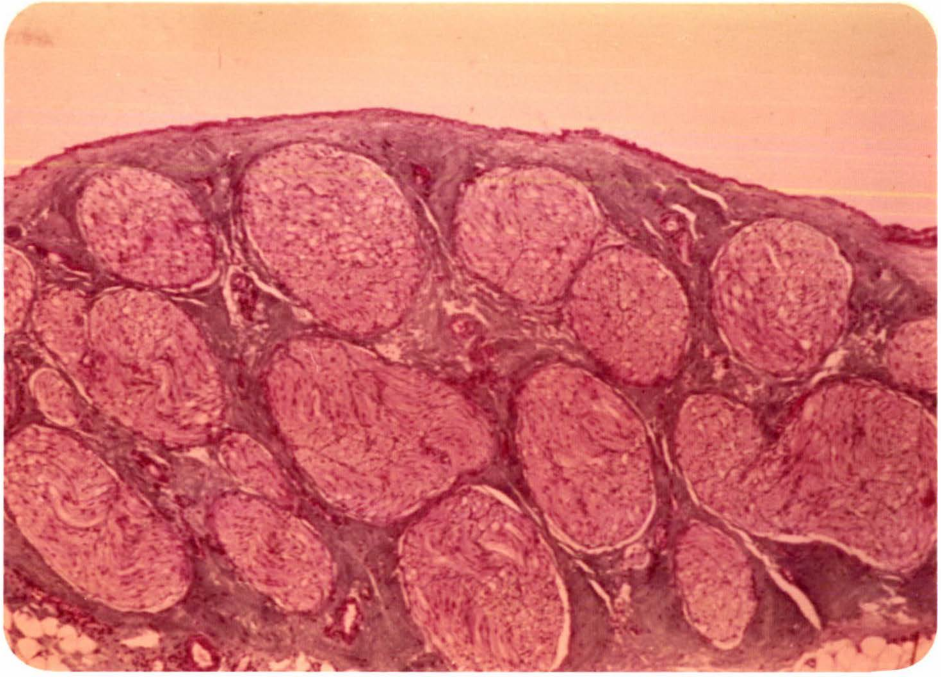
Figure 21. Photomicrograph of transverse section of the internal branch of the cranial laryngeal nerve.

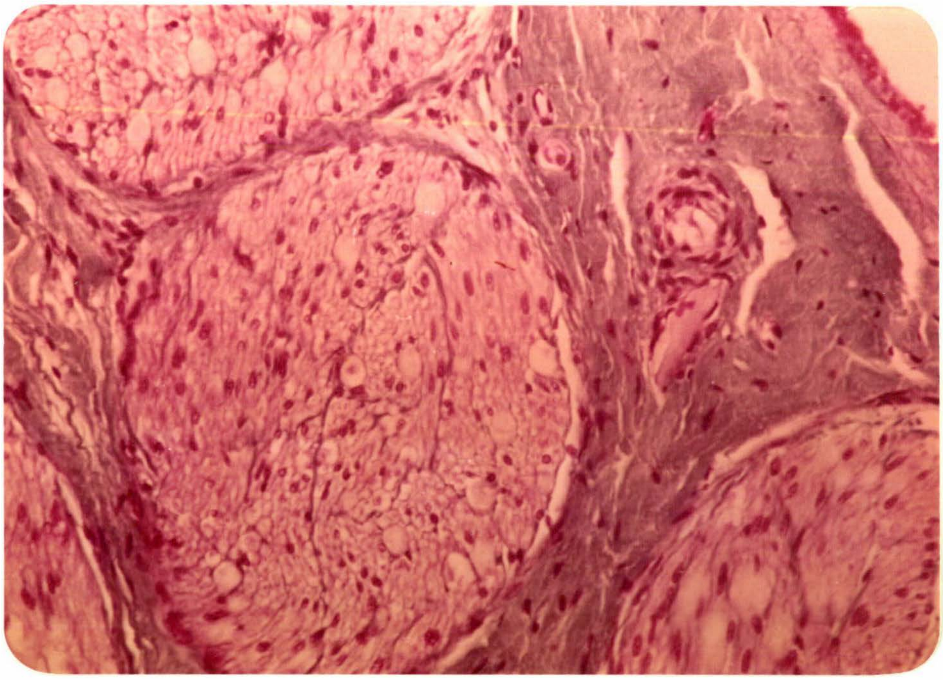
a. Showing fibre type distribution in the fascicles, similar to that seen in the vagus at the jugular foramen.

Trichrome blue 90x.

b. One fascicle containing a random distribution of large, medium and small fibres.

Trichrome blue 260x.





FUNCTIONAL STUDY OF THE LARYNGEAL NERVE SUPPLY

Normal electromyographic activity of intrinsic laryngeal muscles

Electromyographic activity of the dorsal cricoarytenoid muscle:

A total of 38 recordings were obtained from the 14 horses examined. In all 38 recordings there was an increased rate of firing of the electrical activity of a motor unit during inspiration. In most recordings a greater number of motor units were active during this phase of the cycle (Fig. 22). In 34 of the 38 recordings there was considerable motor unit activity during the rest phase between respiratory excursions.

In the remaining 4 recordings, motor unit activity was not discernible during this phase of the respiratory cycle. However, these 4 recordings were obtained from animals, which, on repeated examination, showed motor units that were active during this phase of the cycle. In the majority of the recordings (23 out of 34) in which activity during the resting phase of the respiratory cycle was present there was a variable interval on commencement of expiration in which little activity could be observed. In the other 9 recordings motor unit activity was noticed throughout the entire respiratory cycle.

Electromyographic activity of the cricothyroid muscle

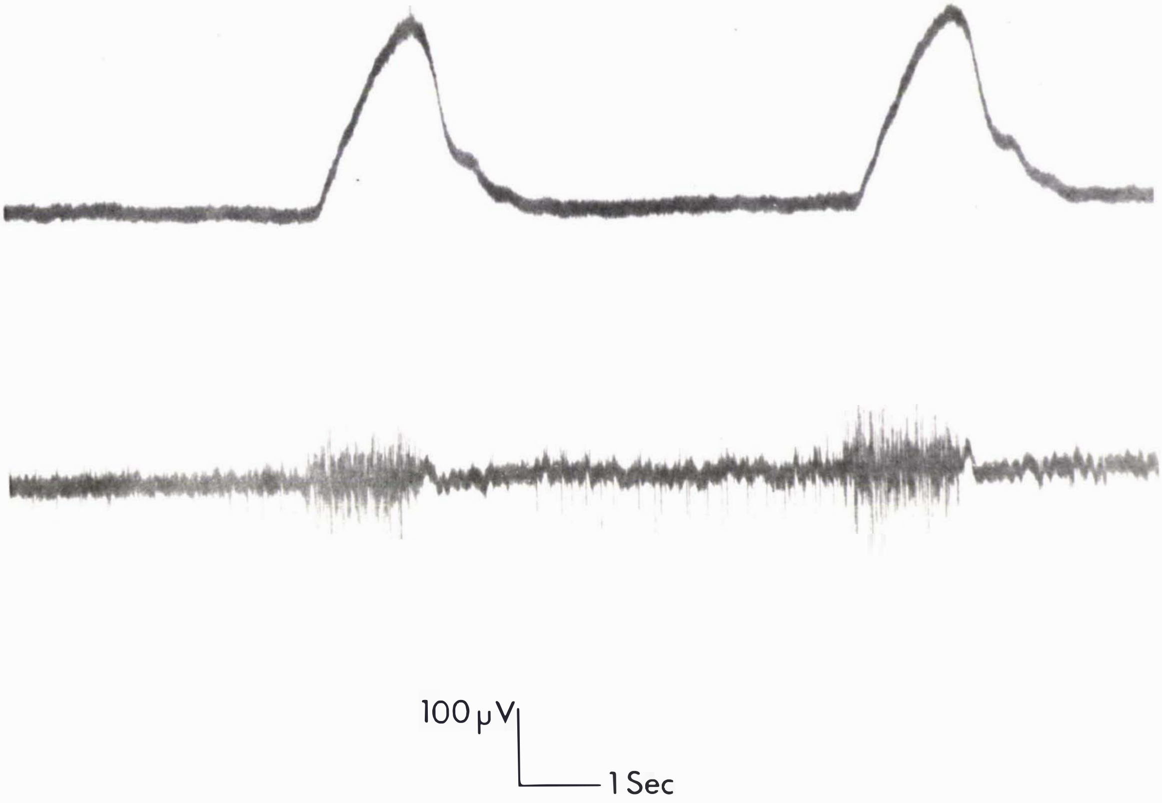
The right cricothyroid muscle was electromyographically examined in 13 horses and a total of 32 recordings were obtained.

In all traces there was tonic activity between respiratory

Figure 22. Abductor activity in the dorsal cricoarytenoid muscle.

Two examples of inspiratory firing in the dorsal cricoarytenoid muscle.

The upper traces show respiration.
Lower traces show tonic firing during the rest phase with an increase in firing during inspiration and a short period of silence during expiration.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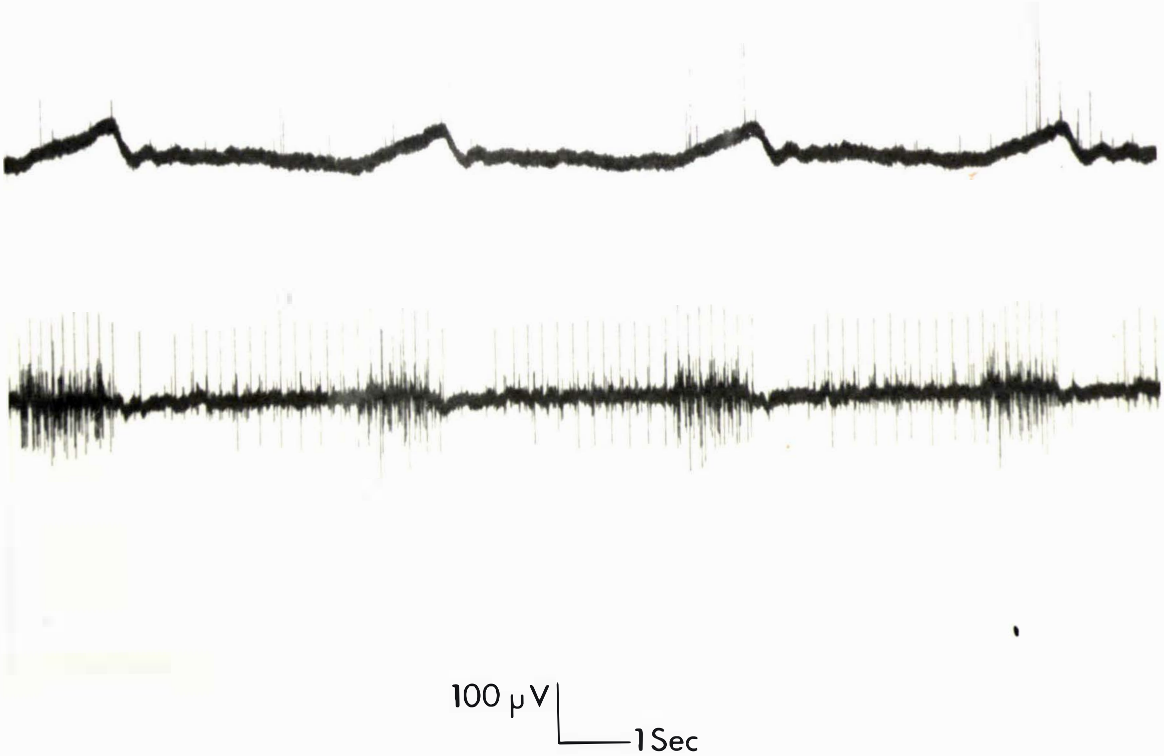
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excursions. In approximately half the animals (7) in which 17 recordings were observed the tonic firing was accompanied by an increase in rate of motor unit activity during inspiration. In some cases an increase in the number of active motor units was also observed.

In a further 4 animals the rate of electrical activity was not apparently related to the respiration as in all these animals a regular rate of firing was observed throughout the entire respiratory cycle. However, in a repeated recording in one such animal, although the rate of activity was constant throughout the respiratory rest period, it actually decreased and almost ceased during both inspiration and expiration.

In the remaining two animals a slightly different pattern of electrical activity was seen. The rate of motor unit activity decreased during inspiration, accelerated during expiration but was most rapid during the respiratory rest period. In one of the animals there occurred sudden bursts of firing apparently unrelated to the respiratory cycle. During these bursts an increased number of motor units and an increased amplitude and rate of discharge was observed.

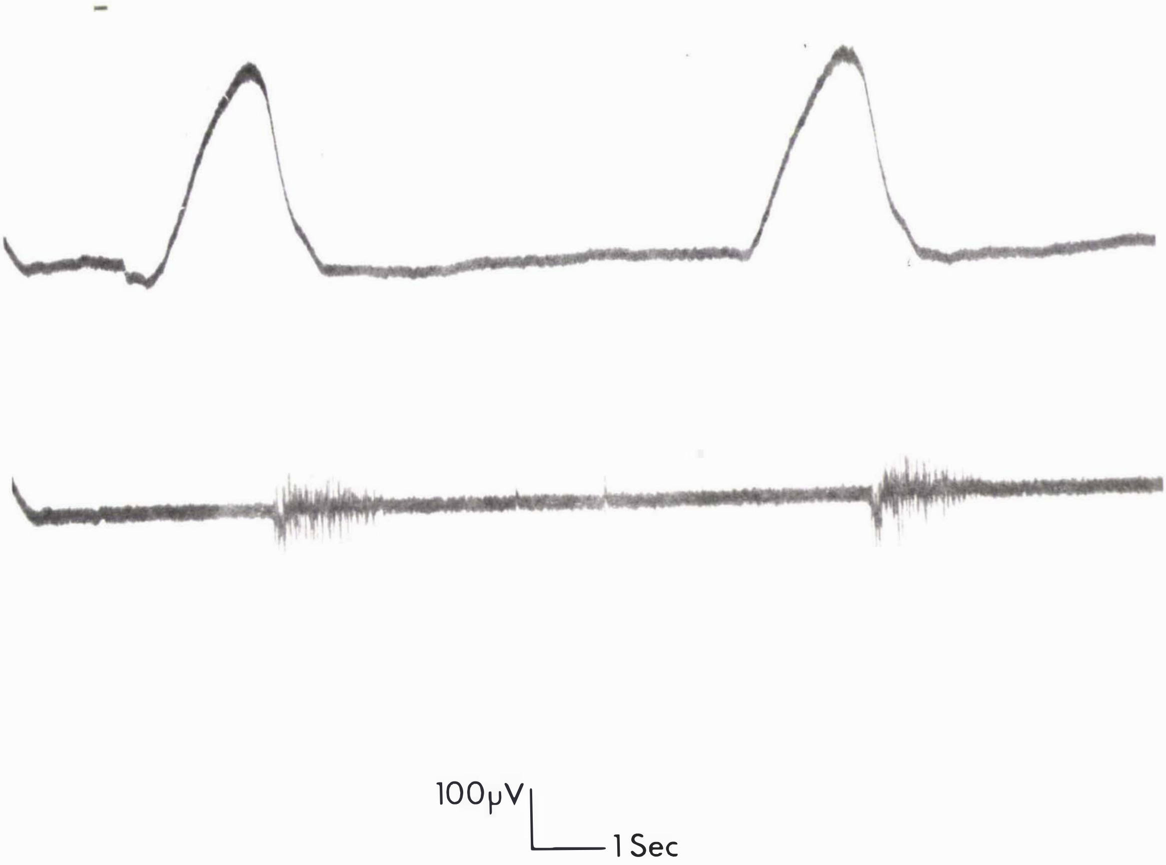
Electromyographic activity of the lateral cricoarytenoid muscle

Twenty-four recordings were obtained from 12 different horses. Four different patterns of motor unit activity were observed. The most common electromyographic pattern, which was seen in 5 of the 12 animals, was that in which a burst of electrical activity of varying duration occurred during expiration (Fig. 23). In 1 of these 5 animals

Figure 23. Adductor activity in the lateral cricoarytenoid muscle

Two examples of activity in the lateral cricoarytenoid muscle.

Upper traces show respiration.
Lower traces show electrical activity in the muscles during expiration and a variation in the length of variable time during the rest phase.
There is no activity discernible during inspiration.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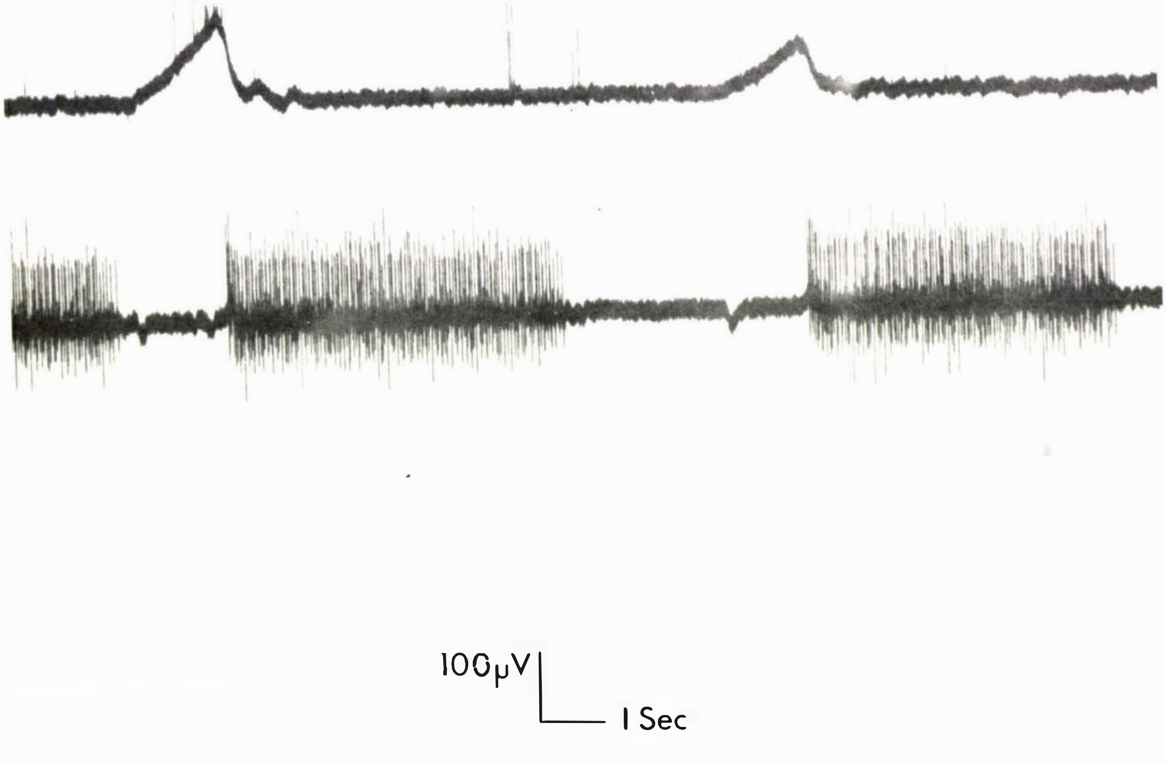
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considerable activity was observed throughout the respiratory rest period.

In a further 3 animals activity occurred throughout the entire respiratory cycle without a detectable respiratory rhythm. In another animal, increased electric activity occurred in bursts sometimes during inspiration and sometimes during expiration.

Finally in 3 animals, although repeated attempts were made to obtain electromyograms, no activity was recorded.

Electromyographic activity of the transverse arytenoid muscle.

Attempts were made to obtain electromyograms of this muscle in 10 horses. However, a total of 13 recordings were obtained in 6 animals only.

In 4 of the 6 animals, increased electrical activity occurred during expiration with occasional activity throughout the period between respiratory excursions. In the remaining 2 animals increased activity commenced at the start of expiration and continued until several seconds after expiration had ceased.

Electromyographic activity in the ventricularis and vocalis muscles

Attempts were made to obtain recordings from 12 horses but electrical activity was obtained in only 9 recordings from 6 animals.

In 4 of these 6 animals, bursts of firing were seen during expiration but no activity was seen other than this. In another the burst of firing during expiration continued into the period between respiratory excursions. The recordings from the remaining animal contained an increased burst of firing during inspiration. Motor unit activity throughout the respiratory rest period was observed in only one of the nine recordings obtained (Fig. 24).

Denervation experiments

Experiment I - Abductor activity

When the abductor branch of the right recurrent laryngeal nerve was severed there was a resultant violent increase in electrical activity of the right dorsal crico-arytenoid muscle followed by electrical silence (Fig. 25). However, there were no obvious changes in the electrical activity of all other intrinsic laryngeal muscles. Rhino-laryngoscopic examination during section revealed a violent abduction of the arytenoid cartilages followed by adduction after which there was no respiratory movement of the right arytenoid cartilage. Normal respiratory movement of the left arytenoid cartilage continued.

Experiment II - Adductor activity

When the adductor branches of the right recurrent laryngeal nerve were cut brief violent electrical activity was recorded from the lateral cricoarytenoid muscle. Rhino-laryngoscopic examination during nerve section revealed a vigorous adduction of the arytenoid cartilages followed by apparently normal abductor movements during consequent respirations.

When the animals were re-examined six weeks after nerve section a fibrillation pattern in the right intrinsic laryngeal muscles with the exception of the cricothyroid and dorsal cricoarytenoid was recorded (Fig. 26).

Figure 24. Adductor activity in the ventricularis muscle

Upper trace shows respiration.
Lower trace shows a burst of electrical activity in expiration that continues through part of the rest period.

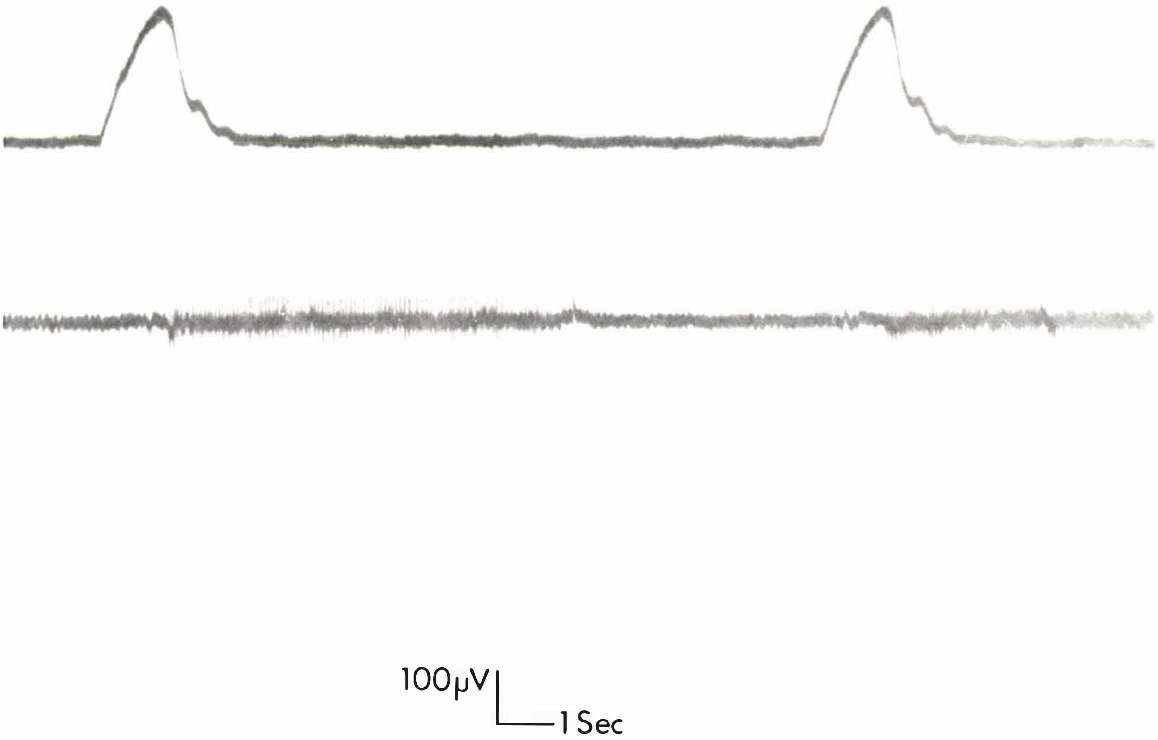
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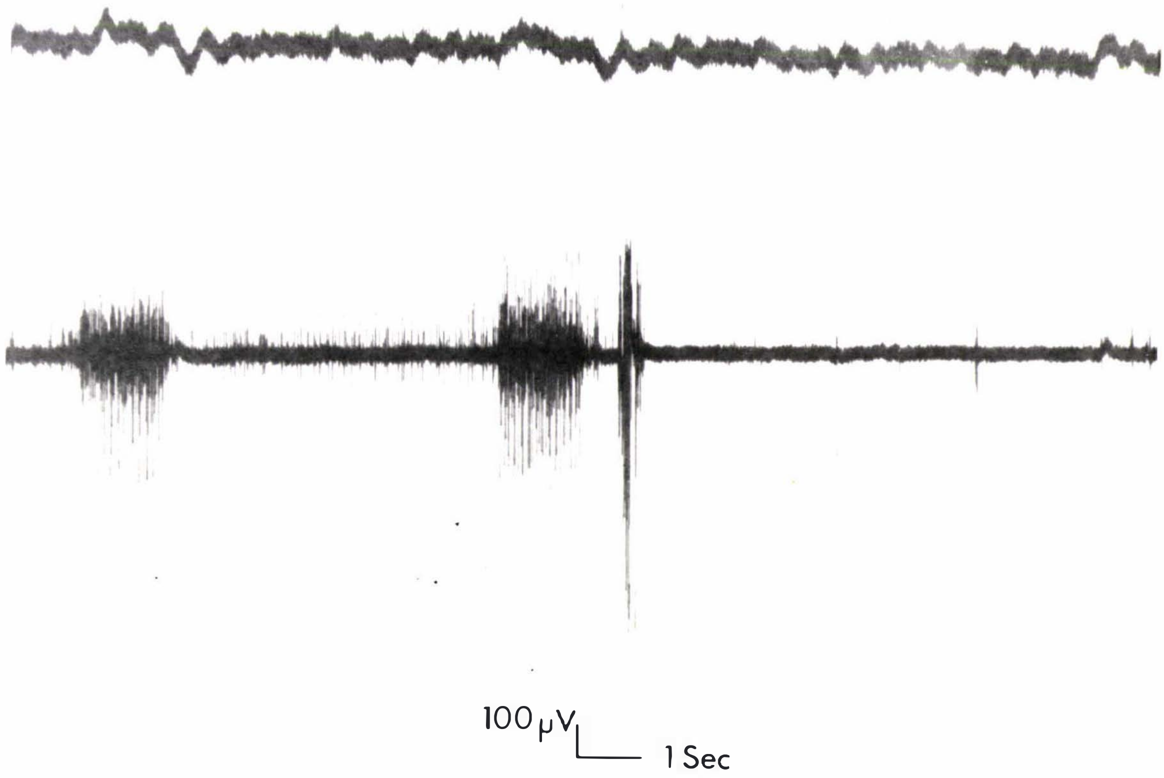
Figure 25. Activity in the dorsal cricoarytenoid muscle during nerve section.

a. Upper trace shows respiration.
Lower trace shows tonic activity throughout the respiratory cycle, with a short burst of activity during inspiration.
After nerve section there was no further electrical activity.

b. Upper trace shows respiration.
Lower trace shows electromyogram in the same muscle as in a., six weeks post section.
There is constant random electrical activity "fibrillation", no respiratory pattern is discernible.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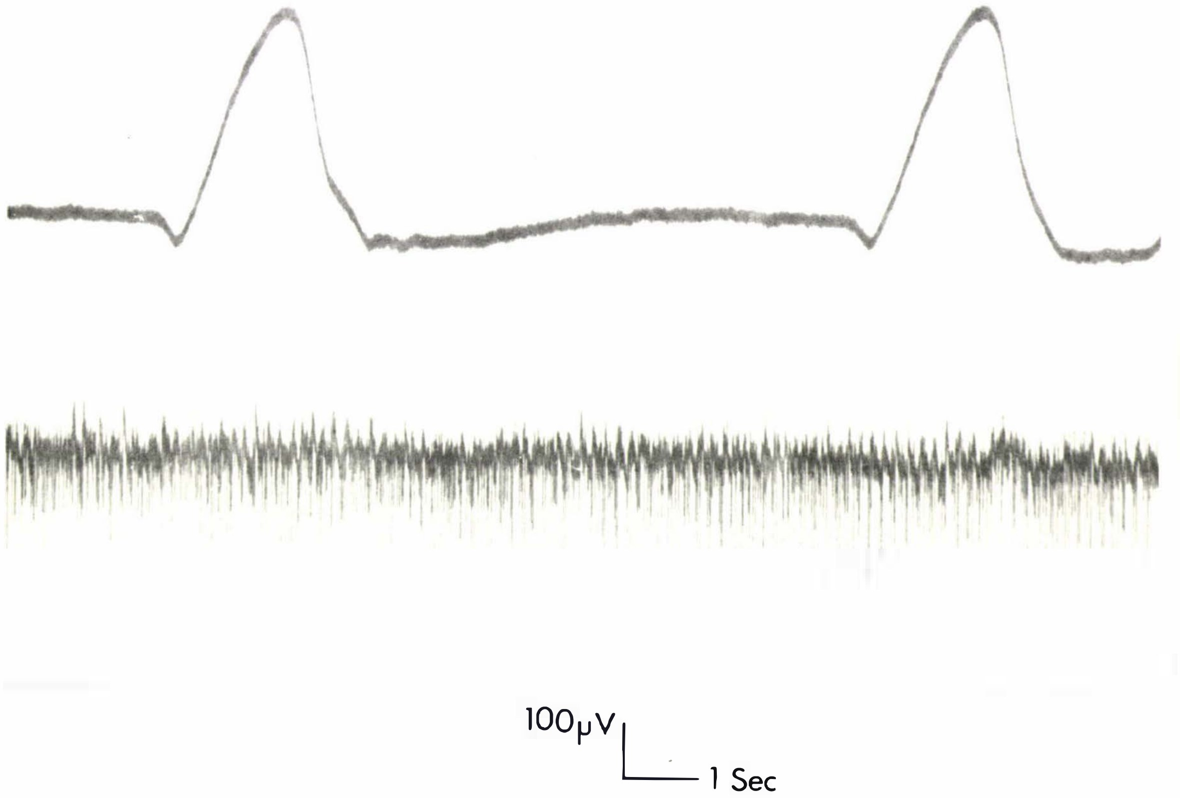
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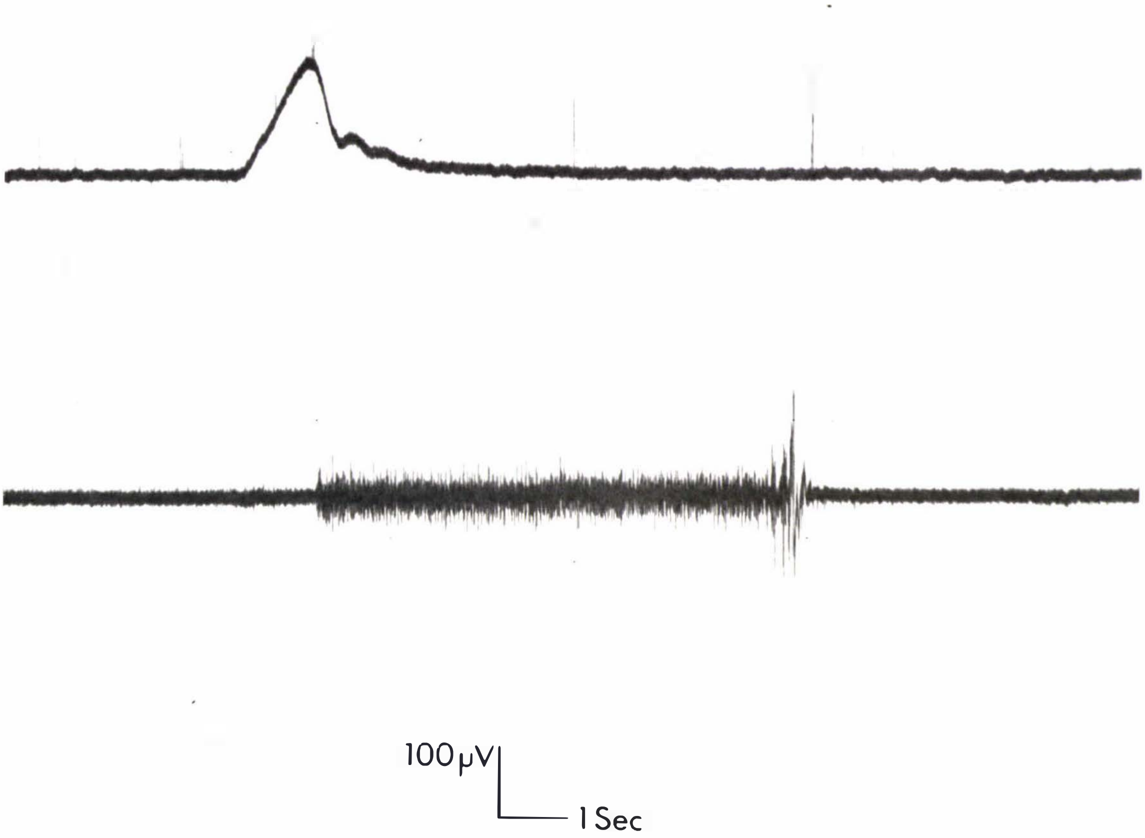
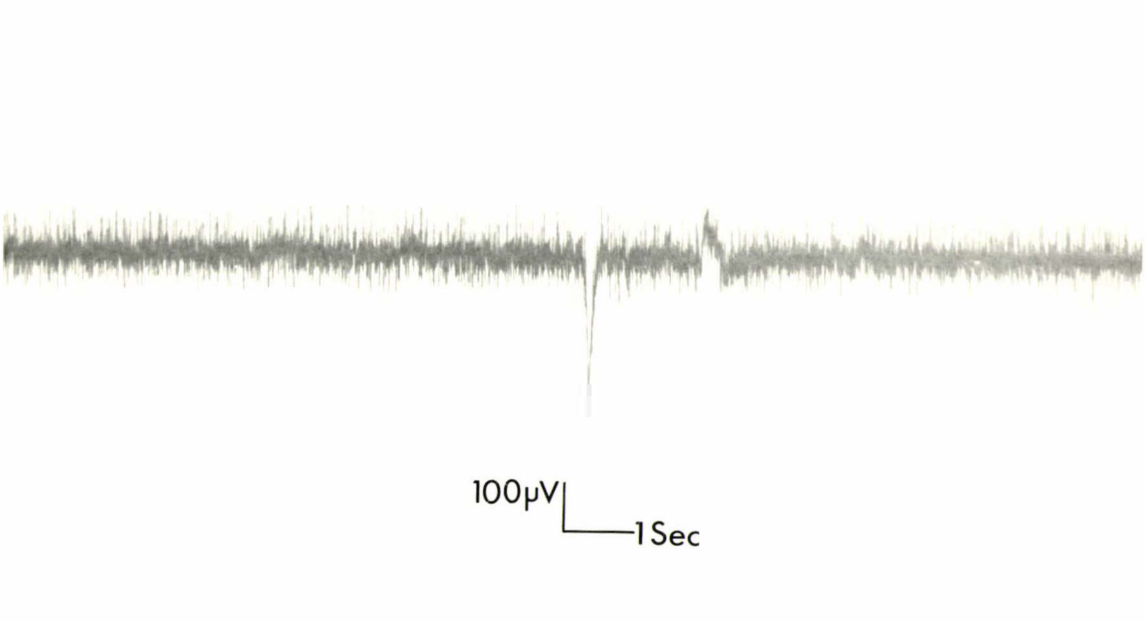
Figure 26. Activity in the ventricularis muscle during nerve section.

a. Upper trace shows respiration.
Lower trace shows electromyogram from the ventricularis muscle.
The nerve is sectioned during contraction of the muscle.
After section there was no further activity.

b. Upper trace shows respiration.
Lower trace shows electromyogram of the ventricularis muscle six weeks post section.
There is constant random electrical activity "fibrillation".
No respiratory pattern can be seen.


100µV
1 Sec


100µV
1 Sec

Experiment III - Cricothyroid activity

During section of the right cranial laryngeal nerve there was a violent increase in electrical activity in the cricothyroid muscle followed by electrical silence.

After six weeks the cricothyroid muscle showed fibrillation activity (Fig. 27). Electrical activity similar to that observed prior to nerve section was seen in all other intrinsic laryngeal muscles on the right side and all the intrinsic laryngeal muscles on the left.

Experiment IV - Cranial laryngeal activity

Following section of the internal branch of the right cranial laryngeal nerve no change in electrical activity was seen in any of the muscles on the right side. After six weeks, intrinsic laryngeal muscle activity on both sides of the larynx was similar to that recorded from the muscle on the right side prior to nerve section.

WEIGHTS OF INTRINSIC LARYNGEAL MUSCLES

a. The wet and dry weights of muscles from both sides of the larynges are shown in Table 3. The dry weights of the abductors, adductors supplied by the recurrent laryngeal nerve, and the cricothyroid muscles were plotted on graphs to illustrate the relationship between the left and right sides (Figs. 28, 29 and 30).

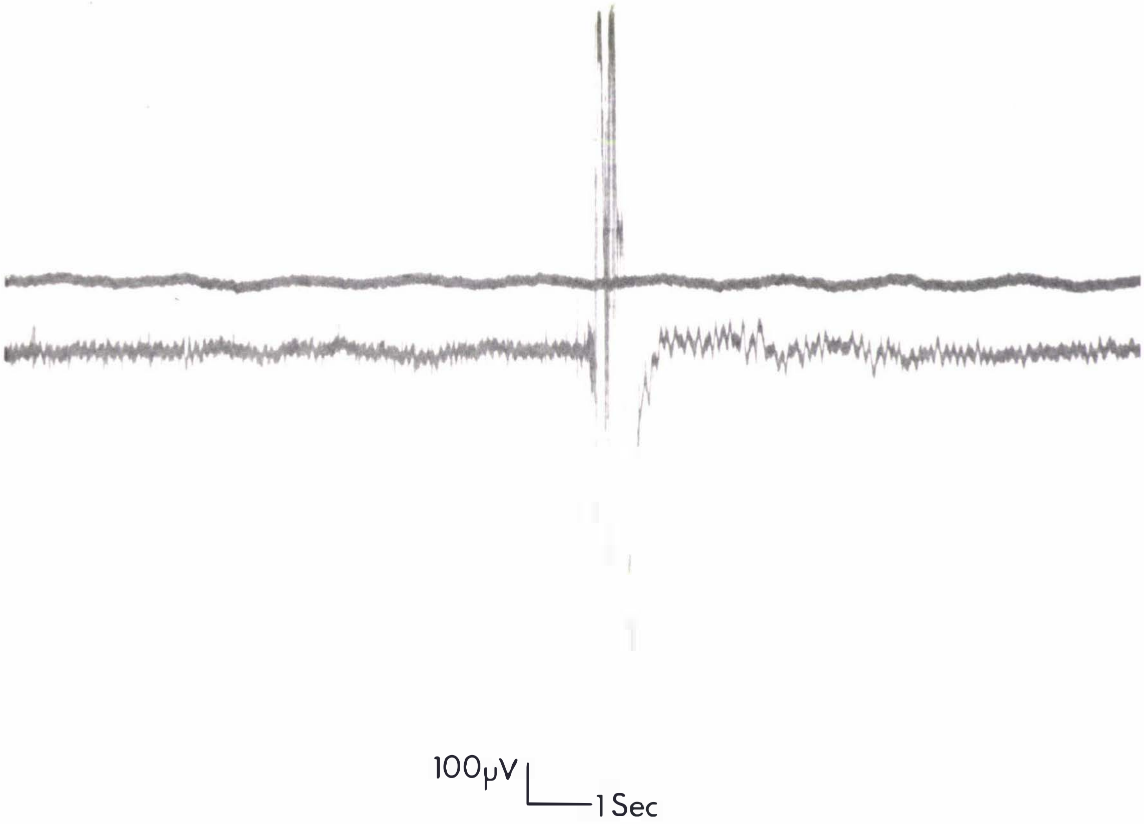
Statistical analysis on both wet and dry weights demonstrated a highly significant difference in weight between sides of abductors and adductors supplied by the recurrent laryngeal nerve ($P < 0.001$). In both cases the muscles on the left side were significantly lighter than those on the right. In contrast there was no significant difference in

Figure 27. Cricothyroid activity during nerve section.

a. Electromyogram of tonic type of cricothyroid activity on lower trace.
During nerve section there are a number of giant potentials followed by electrical silence.

b. The upper trace shows respiration.
The lower trace shows constant tonic activity of the cricothyroid muscle.
Nerve section occurred during respiration.

Note giant potentials at this time followed by electrical silence.


100µV
1Sec

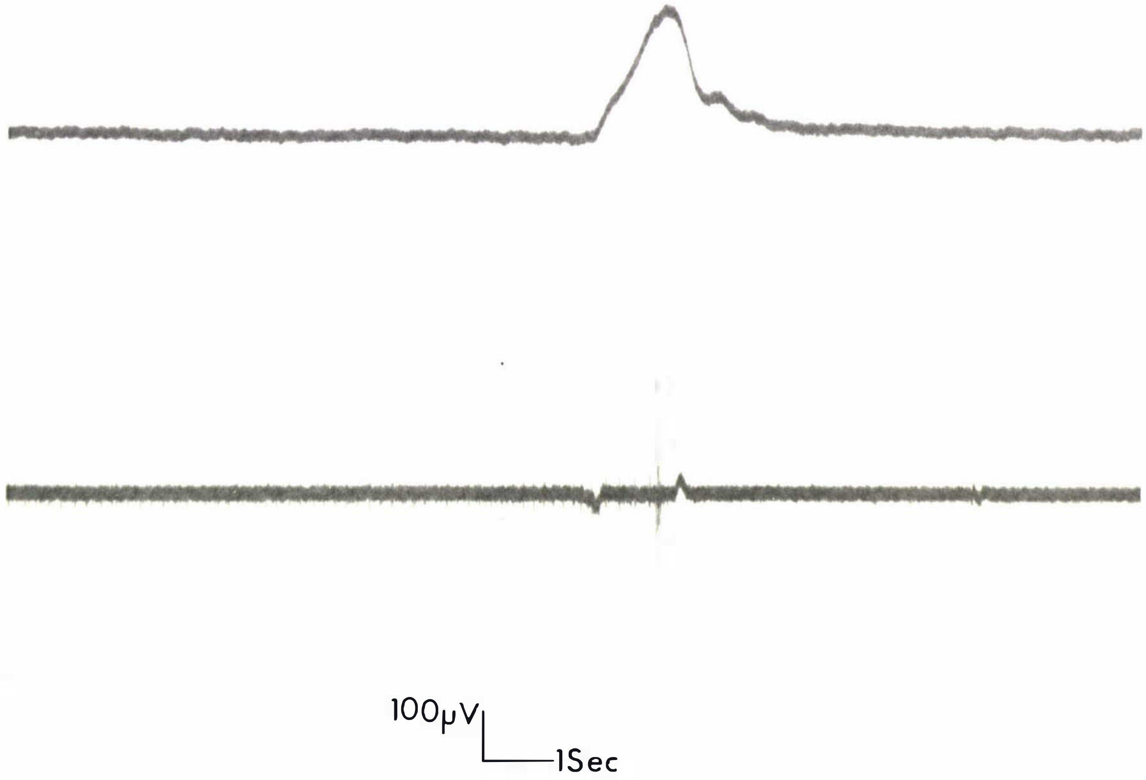

100µV
1Sec

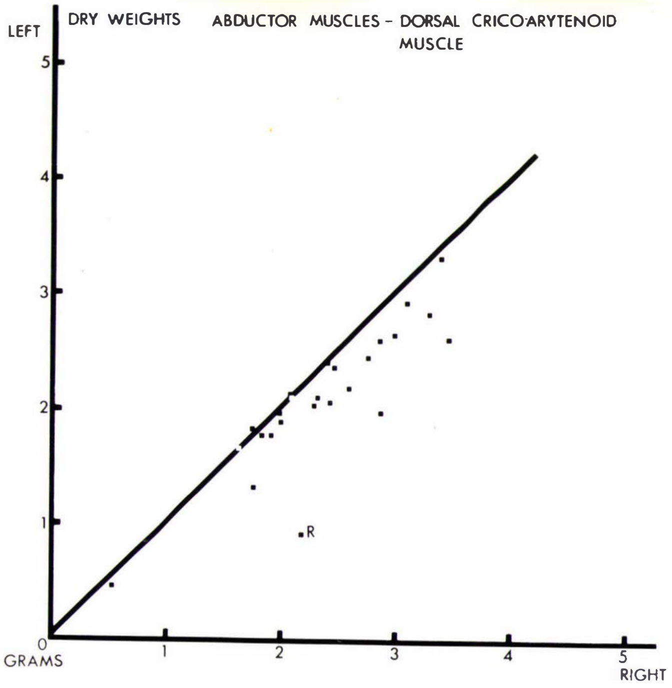
Figure 28. The freeze dried weights of the abductor muscles. The dorsal cricoarytenoid muscles.

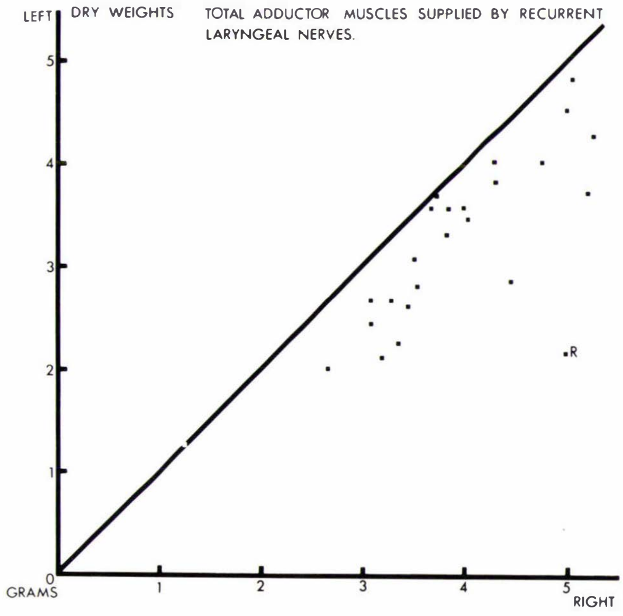
R. - A clinical roarer.

Figure 29. The freeze dried weights of the total adductor muscles supplied by the recurrent laryngeal nerves.

R. - A clinical roarer.

Figure 30. The freeze dried weights of the cricothyroid muscles.


DRY WEIGHTS
ABDUCTOR MUSCLES – DORSAL CRICO-ARYTENOID MUSCLE
LEFT
RIGHT
GRAMS
0
1
2
3
4
5
R


DRY WEIGHTS
TOTAL ADDUCTOR MUSCLES SUPPLIED BY RECURRENT LARYNGEAL NERVES.
LEFT
RIGHT
GRAMS
0
1
2
3
4
5
R

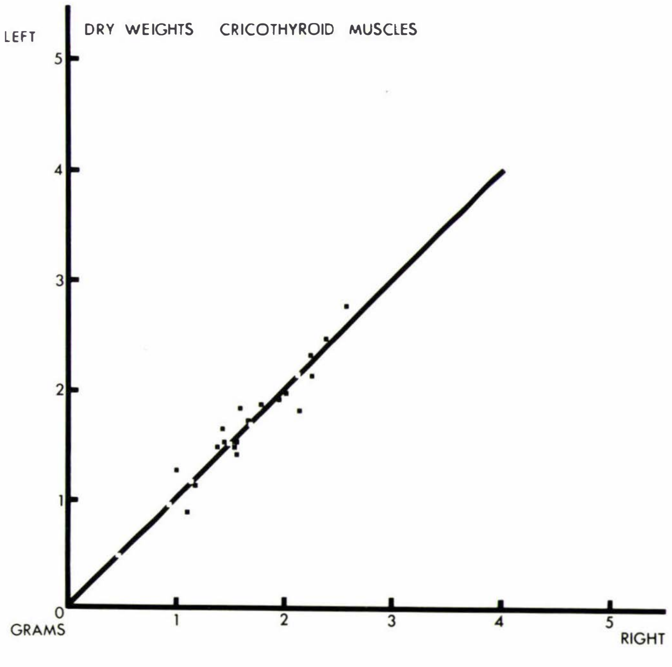

DRY WEIGHTS
CRICOTHYROID MUSCLES
LEFT
RIGHT
GRAMS
0
1
2
3
4
5

TABLE 3

WEIGHTS OF INTRINSIC LARYNGEAL MUSCLES OF RANDOMLY SELECTED HORSES

Muscle		Wet Weight (Grams)		Freeze Dried Weight (Grams)	
		Left	Right	Left	Right
Dorsal crico-arytenoid	$\bar{x}$	8.17	8.70	2.12	2.36
	S.D.	2.16	2.36	0.57	0.67
	C.V.	26	27	27	28
Lateral crico-arytenoid	$\bar{x}$	3.04	3.79	0.78	0.97
	S.D.	0.98	0.91	0.25	0.24
	C.V.	32	24	32	25
Transverse arytenoid	$\bar{x}$	2.38	2.72	0.60	0.67
	S.D.	0.81	0.73	0.19	0.19
	C.V.	34	27	32	28
Vocalis	$\bar{x}$	5.10	5.73	1.29	1.46
	S.D.	1.71	1.45	0.36	0.40
	C.V.	34	25	28	27
Ventricularis	$\bar{x}$	2.23	2.65	0.54	0.62
	S.D.	0.60	0.51	0.11	0.13
	C.V.	27	19	20	21
Cricothyroid	$\bar{x}$	5.76	5.74	1.62	1.58
	S.D.	1.74	1.72	0.58	0.50
	C.V.	30	30	36	32

$\bar{x}$ Mean: S.D. Standard Deviation: C.V. Coefficient of Variance the weights of the left and right cricothyroid muscles. Since the coefficient of variation of muscle weights are similar for both wet and dry muscles the experimental error was similar for each. Variation was generally greater in the weight of muscles from the left side.

b. The mean muscle weights of the denervated right cricoarytenoid muscles were heavier than those of the undenervated left muscles, with the exception of the cricothyroid muscles (Table 4). However, insufficient results were available for accurate statistical analysis.

HISTOLOGY OF INTRINSIC LARYNGEAL MUSCLES

There were no distinct changes seen in the denervated muscle when compared with its contralateral equivalent. A loss of cytoplasm apparent in the sections of denervated muscle (Fig. 31, 32), was attributed to preparation artifact. However, a small number of muscle cells in the denervated muscle showed centrally placed nuclei which are found in atrophied muscle. These could not be found in the control muscle.

TABLE 4

COMPARISON OF DENERVATED AND NORMAL MUSCLE WEIGHTS

		Mean muscle weights (grams)	
	Muscle	Normal	Denervated
Expt. I	Dorsal crico-arytenoid	9.08	9.80
Expt. II	Total adductor muscles supplied by the recurrent laryngeal nerve	13.03	14.61
Expt. III	Cricothyroid	5.13	4.45

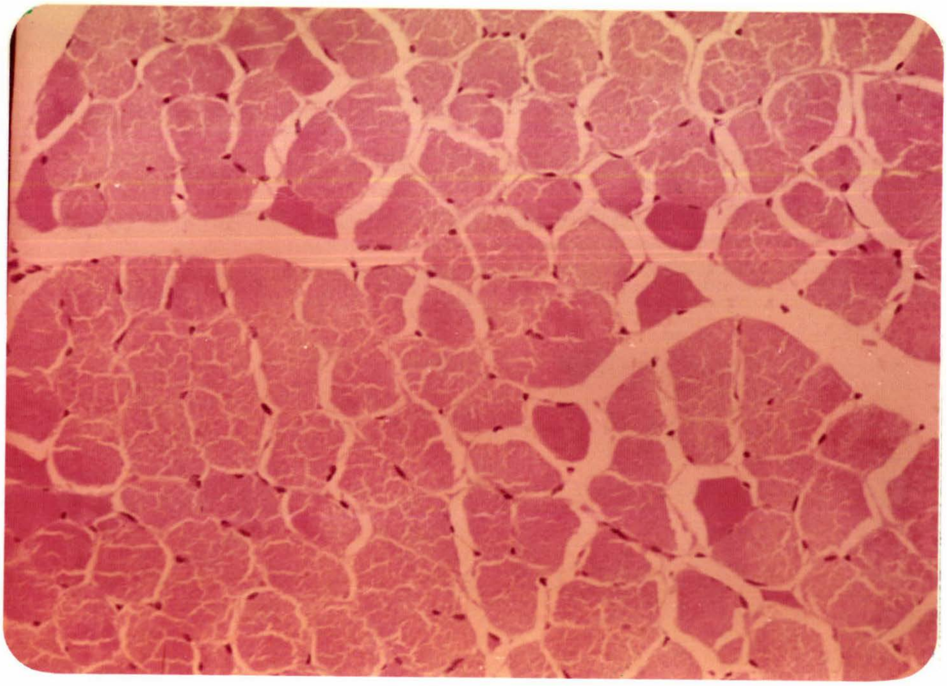
Figure 31. Photomicrograph of transverse section of dorsal cricoarytenoid muscle.

a. Left muscle.
Showing normal pattern of muscle fibres.

H & E. 260x.

b. Right muscle.
Showing artifactual shrinkage of muscle fascicles. There is some evidence of atrophy depicted by cellular invasion of the muscle fascicles seen as centrally placed nuclei in the substance of the myofibrils.

H & E. 260x.



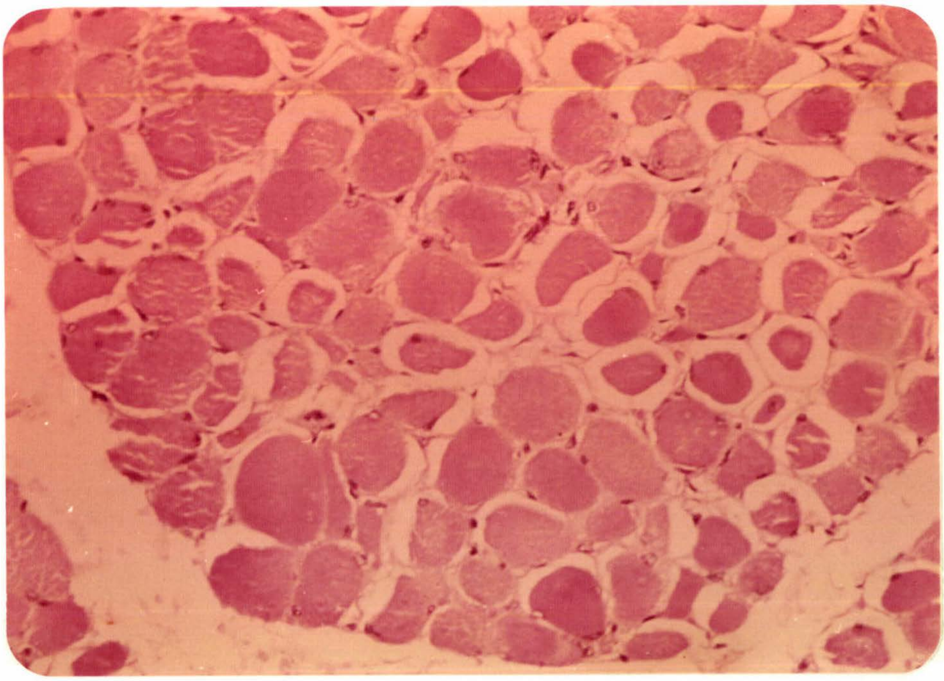


Figure 32. Photomicrograph of transverse section of lateral cricoarytenoid muscle.

a. Left muscle.

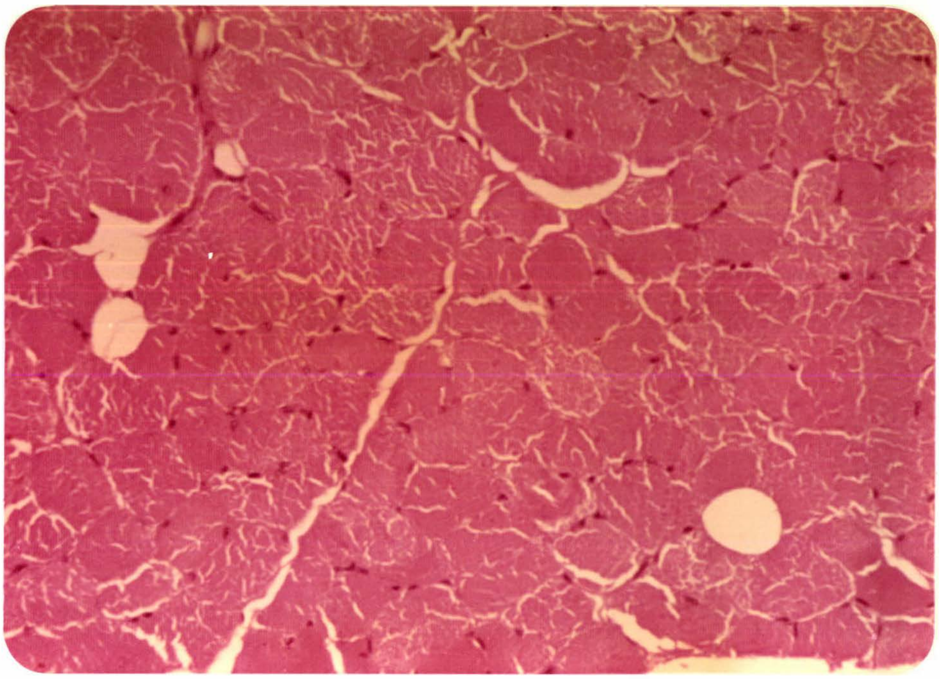
Showing normal pattern of muscle fibres.

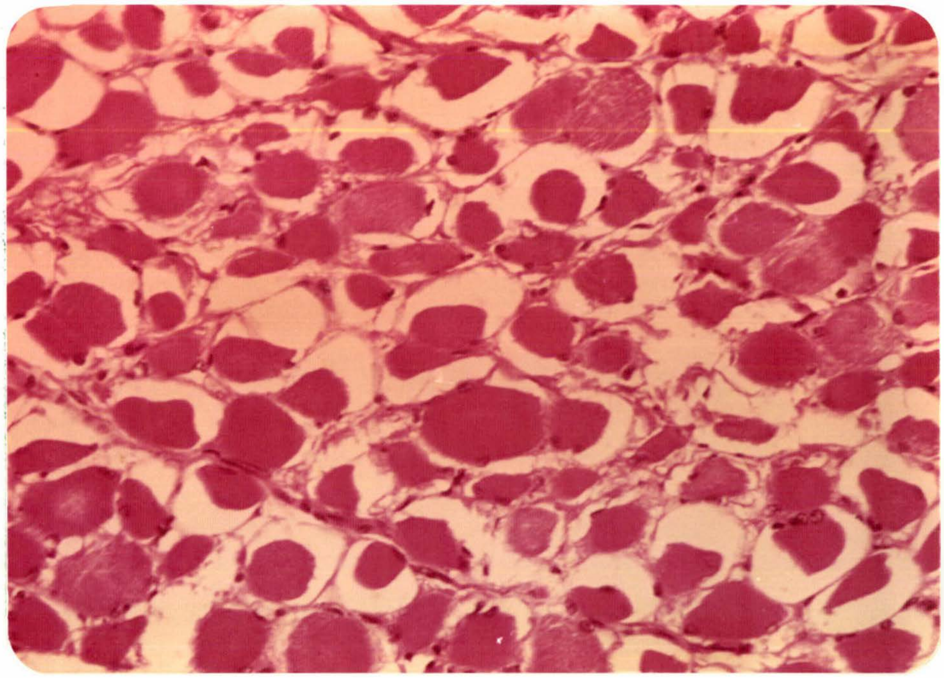
H & E. 260x.

b. Right muscle.

Showing artifactual shrinkage of muscle fascicles. There is some evidence of atrophy depicted by cellular invasion of the muscle fascicles seen as centrally placed nuclei in the substance of the myofibrils.

H & E. 260x.





DISCUSSION

GROSS ANATOMY OF THE LARYNGEAL NERVES

The gross anatomy of the nerves to the larynx was not at variance with previous reports (Sisson and Grossman, 1953; Mason, 1973; Nickel et al., 1973) and therefore needs no further comment. However, the course of the laryngeal nerves within the larynx had not previously been described in detail. A distinct unilateral pattern was evident; in no case could nerve fibres be traced across the median plane into the contra-lateral side of the larynx. This finding was confirmed by the electromyographic results and the dissections of two horses affected with laryngeal hemiplegia. It appears that, as with vascular and lymphatic system (Pressman and Kelemen, 1955), the motor nerve supply to the larynx is unilaterally independent and separate.

HISTOLOGY OF LARYNGEAL NERVES

The structural pattern of the vagus and recurrent laryngeal nerves corresponded to that already described in the cat (Dubois and Foley, 1936) and dog (Chase and Ranson, 1914). However, the different sizes of fibres (large, medium and small) within the recurrent laryngeal nerve reported by Murtagh and Campbell (1951, 1952) and Cole (1946) were not seen in this study. The only type of myelinated fibre in the recurrent laryngeal nerve at the level of the larynx was the medium sized fibre. This was seen in both abductor and adductor branches of the nerve. Murtagh and Campbell (1951) attributed differing functions to small fibres and large fibres, the former being abductor and the latter being adductor. They claimed that in cases of damage to the nerve these small fibres would be more susceptible, resulting in abductor malfunction. In the vagus fascicles appeared to diverge and fuse in a complex manner along the length of the nerves. When the medium and large fibres together were traced from the cranium peripherally there was a reorganisation of these fibres until fascicles had populations between 50 and 100% of large and medium fibres. Such an observation was made by Dubois and Foley (1936) in the cat and by Sunderland and Swaney (1952) in man. The claim of Russell (1892) and Murtagh and Campbell (1951) that nerve fibres to adductor and abductor muscles can be recognised in discrete bundles does not therefore appear valid in the horse.

The single fascicle composed of small fibres seen in some horses in the recurrent nerve which could not be found peripherally in the larynx most probably contains sensory fibres to the oesophagus and trachea as fine branches of the nerve were detected passing into these structures. This finding agrees with the observations of Dubois and Foley (1936) in the cat.

The external branch of the cranial laryngeal nerve was also found to consist mainly of medium and large fibres and is therefore expected to be motor to the muscles it supplies. This is contrary to the finding of Dubois and Foley (1936) who claimed that the nerve fibres in the external branch in the cat were of small fibre diameter. The term "external branch of the cranial laryngeal nerve" is misleading, for in all horses dissected, this small branch originated from the oesophageal branch of the pharyngeal nerve of the vagus and not the cranial laryngeal.

The internal branch of the cranial laryngeal nerve was found to contain a mixed population of medium and small fibres randomly spread throughout the bundles. Dubois and Foley (1936), and Chase and Ranson (1914) also found this and claimed that these were sensory fibres. Electromyographic results confirm that this nerve contains no motor fibres to the intrinsic laryngeal muscles.

FUNCTIONAL STUDY OF LARYNGEAL NERVES

Normal electromyographic activity of intrinsic laryngeal muscle

The dorsal cricoarytenoid muscle

Although there are many similarities in the electrical activity of the dorsal cricoarytenoid during quiet respiration between the horse and other species, there are also some marked differences. As with the electrical activity in the equivalent human muscle (Faaborg-Andersen, 1957) there is a pronounced degree of electrical activity during the respiratory rest phase. However, in contrast to Faaborg-Andersen's human findings, the electrical activity in the horse increased during inspiration. Similar electrical behaviour during quiet respiration in this muscle has been observed in anaesthetised cats (Green and Neil, 1955; Rex, 1969; Suzuki and Kirchner, 1969), dogs (Nakamura *et al.*, 1958; Siribodhi *et al.*, 1963) and goats (Murtagh and Campbell, 1954) but in these species the activity ceased during expiration. In the horse, however, the electrical activity was inhibited in most subjects during some or all of the expiratory phase, while others did not show electrical silence at any time during the respiratory cycle.

The degree of motor unit activity during the rest phase of the respiratory cycle indicates that the dorsal crico-arytenoid muscle plays an important role in maintaining the arytenoid cartilages in their normal resting position during the period between respiratory excursions. In addition, the degree of activity during inspiration confirms that the main function of the muscle is to abduct the arytenoid cartilages during this phase of the respiratory cycle.

The cricothyroid muscle

Considerable confusion exists in the literature regarding the activity of the cricothyroid muscles during quiet respiration. Numerous authors consider it to be most active during expiration (Tschiassny, 1944; Murtagh and Campbell, 1954; Nakamura et al., 1958; Siribodhi et al., 1963; Shin et al., 1969; Suzuki et al., 1970) whilst others consider it to be most active during inspiration (Andrew, 1955; Armstrong and Smith, 1955; Faaborg-Andersen, 1957; Hiroto et al., 1964).

In the present study, whilst approximately half the horses had increased electrical activity during inspiration, there were no recordings in which the greatest degree of electrical activity occurred during expiration. This finding is difficult to equate with the opinion of most authors that the cricothyroid muscle is an adductor muscle of the larynx (Murtagh, 1945; Seymour and Henry, 1954; Faaborg-Andersen, 1957; Nakamura et al., 1958; Siribodhi et al., 1963). Rather it is more in accord with the opinion of Hiroto et al., (1964) and Suzuki et al. (1970) who considered that the cricothyroid assisted the dorsal cricoarytenoid muscle in opening the glottis during inspiration. Suzuki et al. (1970) believed that such an hypothesis was supported by the work of Konrad and Rattenborg (1969) who recorded laryngeal airway resistance during electrical stimulation of the cricothyroid and dorsal cricoarytenoid in various combinations. These research workers found that simultaneous stimulation of both the cricothyroid and dorsal cricoarytenoid muscles produced less resistance to air flow than did stimulation of the dorsal cricoarytenoid alone.

The tonic activity of the cricothyroid muscle in the horse is apparently different from that observed in other species. For example, in the anaesthetised dog, Nakamura et al. (1958) reported that the cricothyroid and the adductor muscles remained silent throughout the rest phase of the respiratory cycle. Suzuki et al. (1970) reported that the cricothyroid activity during quiet respiration in cats under general anaesthesia was so weak that it could be observed only when the anaesthesia was very light. In the anaesthetised horse, during the present study, the amplitude of motor unit activity in the cricothyroid was significantly larger than in any of the intrinsic laryngeal muscles with the exception of the dorsal cricoarytenoid. This finding indicates that the tonic activity of the cricothyroid muscle is important during quiet respiration. This muscle apparently assists in stabilising the diameter of the glottis by tensing the vocal folds during this phase of the cycle.

The variation in the pattern of electrical activity found in the recordings obtained from the cricothyroid indicates that whilst the majority of motor units in this muscle fire phasically others are not apparently influenced by the rhythm of breathing.

The adductor muscles

The increased electrical activity observed during expiration in the majority of animals supports the findings of researchers in other species (Green and Neil, 1955; Portmann, 1957; Siribodhi _et al_., 1963; Rex, 1969). However in the present study no electrical activity was found in a significant number of adductor muscles. One or more of the following possibilities could account for this finding. It could perhaps have been the result of large numbers of motor units in these muscles being inactive during quiet breathing. It may also have resulted from misplacement of the needle electrode, although this is unlikely as ready access to these muscles was obtained in the experimental procedure used. Finally it may have been a manifestation of the depths of anaesthesia. In this connection it is interesting to note the observations of Suzuki and Kirchner (1969) who found that in anaesthetised cats the depth of anaesthesia influenced spontaneous electromyographic activity in laryngeal muscles. They considered that with deepening anaesthesia, activity disappeared initially in the adductor muscles and finally in the abductors.

The absence of electrical activity in some adductor muscles has been previously noted during quiet respiration in conscious humans (Knutson _et al_., 1969) and in anaesthetised dogs (Nakamura _et al_., 1958).

The observation that electrical activity occurred throughout the respiratory rest period in some recordings suggests that slight tonic activity may occur in all these muscles between respiratory excursions.

Electromyographic activity following denervation

In all denervation experiments there was complete electrical silence immediately after nerve section. After eight weeks there appeared, in the denervated muscle, a constant random electrical activity which was not related to the respiratory cycle. This random activity has been identified as intrinsic fibrillation of the muscle cells (Faaborg-Andersen, 1957).

Fibrillation voltage is characterised by the intrinsic activity of the muscle and arises from three days to three weeks after injury. Hiroto et al. (1964) claimed that this activity remains until reinnervation or marked atrophy takes place.

The results of the denervation experiments supported the hypothesis that the recurrent laryngeal nerve supplies motor fibres unilaterally to the dorsal cricoarytenoid muscle and all the adductor muscles with the exception of the cricothyroid. This muscle receives its motor supply from the external branch of the cranial laryngeal which does not supply any other intrinsic laryngeal muscle.

The experiments also demonstrated that the internal branch of the cranial laryngeal nerve did not carry motor fibres to the intrinsic laryngeal muscles.

WEIGHTS OF INTRINSIC LARYNGEAL MUSCLE

a. Assuming that the muscles on the left and right sides of the larynx share similar embryological development, and that the nature and amount of work performed by the laryngeal muscles is equal on both sides, the intrinsic laryngeal musculature of the normal animal should be bilaterally symmetrical. This has been shown to be the case with respect to the weights of human laryngeal muscles (Bowden and Scheuer, 1960). In the horse, however, many authors (Cole, 1946; Gunn, 1972, 1973; Duncan and Griffiths, 1973; Duncan, et al., 1974) have reported differences in the histological structure of intrinsic laryngeal muscles between each side of the larynx. They have shown that muscle fibres in the adductors and abductors on the left side frequently show atrophic changes, whilst such changes are only rarely observed in most muscles on the right side of the larynx (Duncan and Griffiths, 1973). In the opinion of many authors (Cole, 1946; Gunn, 1972, 1973; Duncan and Griffiths, 1973; Duncan, et al., 1974) the atrophy follows degenerative changes in the left recurrent laryngeal nerve. This nerve unilaterally supplies motor fibres to all the intrinsic laryngeal muscles with the exception of the cricothyroid (Sisson and Grossman, 1953).

In the equine larynges examined the marked weight difference between the muscles supplied by the recurrent laryngeal nerve contrasted with the bilaterally symmetrical weights of the cricothyroid muscles. The difference in the weight of affected muscles could result therefore from degenerative changes in the recurrent laryngeal nerve.

The only previous author to report on the weight of equine intrinsic laryngeal muscles was Gunn (1972). His findings showed no significant differences to exist between the dorsal cricoarytenoid muscles on each side of the larynx in each of 12 animals. Moreover, in one case a markedly histologically atrophied left muscle was found to be heavier than its partner. These findings are at variance with those of the present study in which, however, histological examinations were not made.

The weight differences found in the clinically diagnosed case of recurrent laryngeal nerve paralysis affected similar muscles but the wasting was more extensive. It is possible, therefore, that the other animals in which gross weight difference existed between right and left muscles are the subclinical "roarers" referred to by Duncan et al. (1974). In the opinion of Cole (1946) horses required a 50% atrophy of the left dorsal cricoarytenoid muscle to show symptoms of laryngeal hemiplegia at exercise.

When discussing this condition, Gunn (1973) suggested that it was congenital in character and progressive in development. The source of the animals studied in the present series tended to select a disproportionately high percentage of aged animals. If Gunn's theory is correct, this may have accentuated the weight differences recorded.

b. The weighing of muscles from the denervated larynges was attempted to determine whether there was a muscle weight loss due to denervation. The results show that no significant changes were seen, and also showed that the muscles of the left or "control" side were lighter than the contralateral denervated muscle.

Sola and Martin (1953) denervated the hemidiaphragm of the white rat, and found an initial increase of weight in the affected muscle during the first six days. Atrophy slowly occurred to control levels at 23-38 days. Obvious atrophy, i.e. 60% was only evident after 492 days. Therefore the weight "increase" seen the present experiments could be either denervation hypertrophy or the result of the normal discrepancy in unilateral laryngeal muscle weights shown in experiment A.

HISTOLOGY OF INTRINSIC LARYNGEAL MUSCLES

In the sections of denervated muscle the only visible changes were the occurrence of centrally placed nuclei in some fibres. This illustrates the failure of normal histological techniques to indicate pathological changes in muscle following denervation or in early neurogenic atrophy. The histochemical techniques used by Gunn (1972) may have been of more value in this experiment.

CONCLUSIONS:

1. The motor nerve supply to the intrinsic laryngeal muscles is completely unilateral.
2. Within the recurrent laryngeal nerve and external cranial laryngeal nerve which are the motor supply to the intrinsic laryngeal muscles, there was a common type of nerve fibre. This was the medium myelinated type.
3. In the vagus, recurrent nerve fibres could not be found in distinct bundles.
4. There was continual decussation and coalescence of fibres along the length of the vagus and gradual formation of recurrent nerve bundles distally.
5. In the anaesthetised horse, all intrinsic laryngeal muscles show a respiratory rhythm which is manifest electromyographically as a characteristic and identifiable firing pattern.

6. The dorsal cricoarytenoid and cricothyroid muscles showed tonic activity throughout respiration with increased activity during inspiration. It is thought that the cricothyroid as well as the dorsal cricoarytenoid muscle has some function in abduction during inspiration.

7. The transverse arytenoid, lateral cricoarytenoid and thyroarytenoid muscles all showed adductor activity during expiration.

8. Electrical silence was seen immediately after denervation of muscles. After six to eight weeks fibrillation was seen which has been described in cases of neurogenic atrophy.

9. Weights of the left intrinsic muscles supplied by the recurrent laryngeal nerve in apparently normal horses were significantly lighter than the right. This may possibly be due to degenerative changes in the left recurrent laryngeal nerve.

10. Conventional histopathology fails to identify early neurogenic atrophy.

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