A STUDY OF SOME MUSCLES OF THE EQUINE LARYNX AND SOFT PALATE

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by

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ABSTRACT

The purpose of this study was to determine the age of onset, incidence and severity of neurogenic disease in the intrinsic laryngeal muscles of a single breed of competitive horse, the New Zealand Thoroughbred. Some palatal muscles from these horses were also studied to ascertain whether neurogenic disease occurred in them.

The left and right dorsal cricoarytenoid, lateral cricoarytenoid, transverse arytenoid, ventricular, vocal, cricothyroid, hyoepiglottic, palatopharyngeal, palatine levator, palatine, and palatine tensor muscles were collected from some or all of 53 Thoroughbred horses. Forty-six of the horses had no history of upper respiratory tract abnormalities, six had suffered from idiopathic laryngeal hemiplegia and one from laryngo-palatal dislocation. For comparative purposes similar muscles from three ponies were also studied.

The muscles were weighed and then frozen sections were prepared from them. Some of these were stained with haematoxylin and eosin and others to demonstrate the activity of myosin adenosine triphosphatase, succinate dehydrogenase and glycogen phosphorylase. These sections were then studied to determine the muscle fibre types present and their proportions. The mean sizes of the groups of myosin adenosine triphosphatase low reacting fibres were measured as were the mean cross sectional areas of the fibres. Abnormal staining characteristics of the fibres were noted along with histological signs of denervation and reinnervation. Where possible this information was analysed to determine the significance of the differences observed between the measured mean values.

A difference in weight between some of the left and right laryngeal muscles was found to be very common in Thoroughbred horses with no history of upper respiratory tract abnormalities. The left lateral cricoarytenoid muscle was lighter than the right in approximately half of these horses. This difference was significant between the muscles of these horses over three years of age and was most obvious in the muscles of the geldings. The left and right dorsal cricoarytenoid muscles showed similar but not such marked differences. These differences were more obvious in the laryngeal hemiplegic horses.

The fibres of the intrinsic laryngeal muscles were predominantly highly reactive for the enzyme myosin adenosine triphosphatase with the ventricular and vocal muscles having the highest proportions of these fibres and the cricothyroid and hyoepiglottic muscles the lowest. Glycogen phosphorylase reactivity in these muscles was again predominantly high, and the fibres were almost exclusively, highly reactive for succinate dehydrogenase. Neurogenic disease appeared to have an influence on the proportions of fibre types present in affected muscles.

The incidence of larger groups of myosin adenosine triphosphatase low reacting fibres in some of the left than right intrinsic laryngeal muscles was also very common in Thoroughbred horses with no history of upper respiratory tract abnormalities. Eighty percent of these horses over three years of age had larger groups in their left than right lateral cricoarytenoid muscles and the youngest horse where this difference was noted was six weeks old. The adductor muscles showed more evidence of this side difference in group size than the abductor muscles.

The mean cross sectional area of the fibres of the intrinsic laryngeal muscles studied increased till approximately the end of the third year of a horse's life. Neurogenic disease eventually reduced the cross sectional area of the fibres of affected muscles but early in its course it may have produced an increase in the mean cross sectional area of fibres. This increase occurred in mildly affected and also in some unaffected muscles. The latter may have been required to increase their activity to compensate for inefficient function in atrophied muscles.

The histological signs of denervation and reinnervation were also very common in the intrinsic laryngeal muscles supplied by the left recurrent laryngeal nerve. These signs were noted in almost 70% of the left lateral cricoarytenoid muscles from horses over one year of age, with no history of upper respiratory tract abnormalities. The incidence of these signs in the dorsal cricoarytenoid muscle was lower but they appeared suddenly and severely in the left muscles of horses during adolescence and early adult life. In the cricothyroid muscle which is not supplied by the recurrent laryngeal nerve, the only histological signs of this nature appeared in the muscles of

a few of the aged horses.

In the palatal muscles examined there was no evidence of a difference in weight between the left and right muscles and most of their fibres were highly reactive for the three enzymes studied. There was no evidence of fibre type grouping resulting from denervation and reinnervation and none of the other histological signs resulting from severe neurogenic disease were noted.

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CHAPTER 1 GENERAL INTRODUCTION

Diseases of the equine upper respiratory tract have for many years been of interest and concern to veterinarians. Two of these conditions which are of particular importance in the racehorse are idiopathic laryngeal hemiplegia and exercise induced laryngo-palatal dislocation. These two conditions may occur concurrently in the same horse and it has been suggested that they are aetiologically related (Quinlan, 1957; Cook, 1976).

The incidence of these two conditions in New Zealand Thoroughbred horses has never been determined but in other parts of the world it has been estimated that five percent of Thoroughbreds become clinically affected with idiopathic laryngeal hemiplegia (Weiss, 1937) and approximately one percent of hunters and racehorses with laryngo-palatal dislocation (Cook, 1965).

Idiopathic laryngeal hemiplegia is known to be related to a neurogenic atrophy of the intrinsic laryngeal muscles supplied by the left recurrent laryngeal nerve (Cole, 1946). This atrophy apparently occurs far more widely in the horse population than is obvious clinically (Cole, 1946; Quinlan *et al.*, 1975). Current methods of therapy for these two conditions are not always successful (Cook, 1981; Appendix 11) and so they are a significant cause of wastage of racehorses.

The purpose of this thesis was to establish the extent and severity of neurogenic damage in the laryngeal muscles of New Zealand Thoroughbred horses and also to determine whether similar neurogenic changes occur in their palatal muscles. For comparative purposes, similar muscles from three ponies were also studied.

1.1 Idiopathic Laryngeal Hemiplegia (Roaring)

In 1825 Bouley first suggested that there was a correlation between the abnormal respiratory noise made by some horses during exercise and atrophy of some of their intrinsic laryngeal muscles (Cook, 1976). The muscles involved were those supplied by the left recurrent laryngeal nerve (Cole, 1946).

The inspiratory dyspnoea and sterterous respiration at exercise which resulted, could be expected to markedly reduce a horse's athletic ability. Cook (1981) estimated that in a situation where only intermediate abduction of the left arytenoid cartilage was possible, the rima glottidis only dilated to 65% of its full capacity. Under these circumstances the rima glottidis became the narrowest point of the horse's airway.

Cole (1946) considered that the dorsal cricoarytenoid muscle must atrophy by 50% or more before the clinical signs of roaring become apparent. If this atrophy develops slowly, then it would be expected that in a population of clinically normal horses there should be a number of subclinical laryngeal hemiplegics. Cole (1946) showed that this occurred when he found that 27% of randomly selected larynges had atrophy of one or more of the left intrinsic laryngeal muscles. This atrophy was thought to be a result of damage to the motor innervation of these muscles.

With the introduction in recent years of the techniques of enzyme histochemistry the early intramuscular changes resulting from neurogenic damage can be clearly demonstrated. For this reason these techniques have been used in studies of equine intrinsic laryngeal muscles (Gunn, 1972 and 1973; Duncan et al., 1974). Using these techniques Gunn (1973) found changes which he considered characteristic of denervation and reinnervation in the left dorsal cricoarytenoid muscle of the foetal larynx. As a result of this finding he concluded that neurogenic disease could commence in equine laryngeal muscles before birth. Duncan et al., (1974) used similar histochemical techniques on a random series of equine intrinsic laryngeal muscles and were able to demonstrate neurogenic changes in some of the muscles from 30% of the horses. In the recurrent laryngeal nerves of these animals they noted a loss of large myelinated nerve fibres which became more severe as the nerve progressed distally.

A number of causal factors of laryngeal hemiplegia have been mentioned in the literature. Such factors as irritant perivascular or perineural injections (Marks et al., 1970), accidents to the neck (Gilbert, 1972) guttural pouch mycosis (Cook, 1976), neoplasia (Hoare, 1915; Cook, 1976),

organophosphate (Rose, 1978), or lead intoxication (Thomassen, 1902; Hoare, 1915), vitamin deficiency (Loew, 1973; Cymbaluk et al., 1977), or plant poisoning (Kainbach and Habersang, 1914; Hoare, 1915; Williams, 1945; Kral, 1951; Frank, 1953; Schebitz, 1964; Neal and Ramsey, 1972) may cause damage to the vagus or recurrent laryngeal nerves. However, according to Goulden and Anderson (Appendix 10) these factors are not apparently involved in the majority of cases of this disease in the New Zealand environment. Many authors consider that the neurogenic muscle damage which occurs in this disorder is related to recurrent laryngeal nerve dysfunction resulting from the effects of bacterial or viral agents (Bouley, 1825; Fergusson, 1838; Argyle, 1934; Neumann-Kleinpaul and Tabbett, 1936; Williams, 1945; Frank, 1953; Quinlan, 1957; Maguire, 1958; Pires, 1958; Schebitz, 1964), mechanical damage to the nerve due to compression (Bouley, 1825; Fergusson, 1838; Haslam, 1893; Vermeulan, 1913; Hoare, 1915; Argyle, 1934; Hutyra et al., 1938) or tension and stretching (Martin, 1887; Narez, 1913; Argyle, 1934; Frank, 1953; Rooney and Delaney, 1970; Marks et al., 1970; Cook, 1970).

Whatever the cause of the neurogenic muscle damage it seems that the condition is widespread. Its frequency is not yet well established and for this reason a survey of the weights, histochemistry and pathology of some laryngeal muscles from New Zealand horses was performed. In addition to establishing the incidence and severity of neurogenic atrophy in equine laryngeal muscles the objectives of this study were to provide a histochemical profile of these muscles so that subtle changes resulting from neurogenic disease could be recognised.

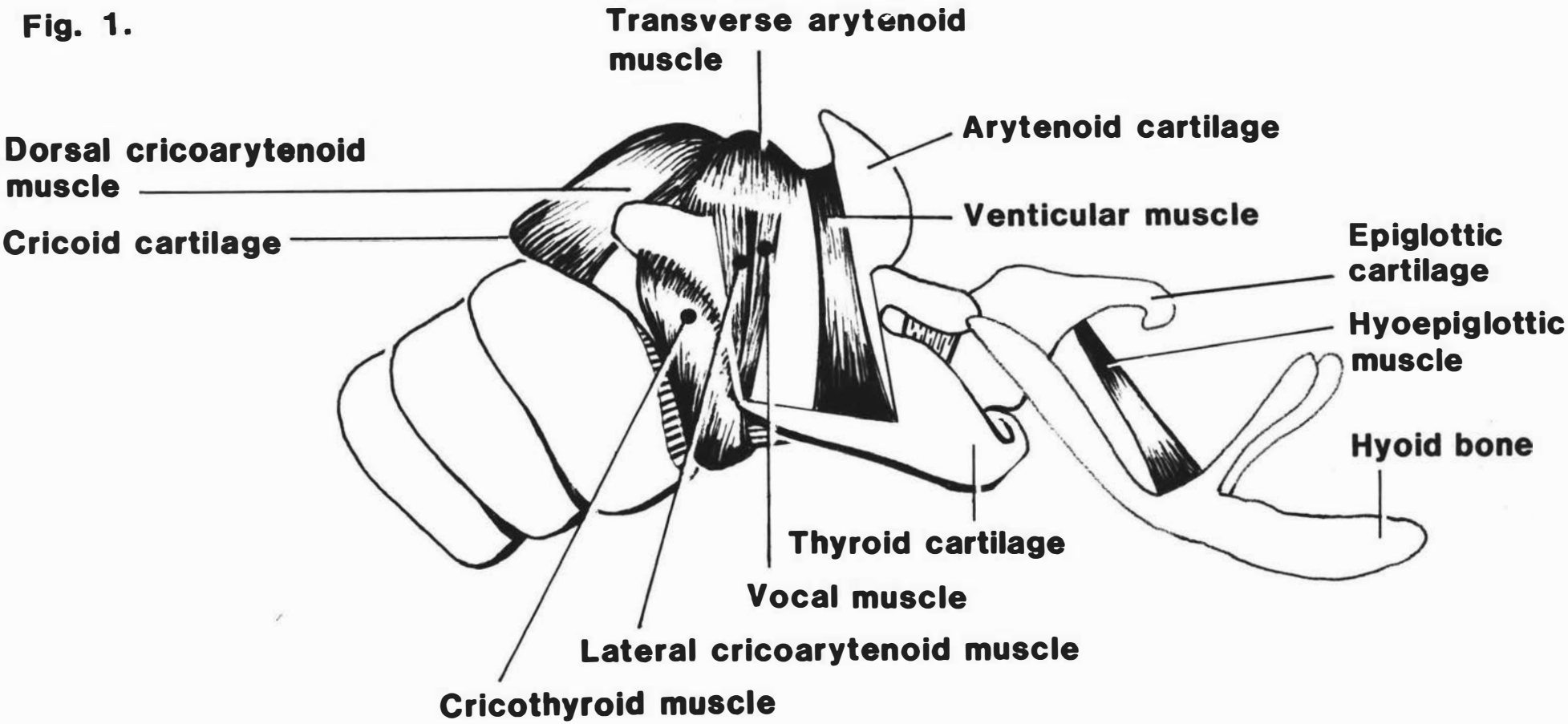
For an understanding of the muscles studied a brief account of their anatomy is included.

1.2 The Anatomy of Some Equine Laryngeal Muscles

The attachments and relationships of the intrinsic laryngeal muscles to the laryngeal cartilages of the horse have been described by Sisson and Grossman (1975), Seiferle (1975) and Quinlan (1976). They are illustrated in Figure 1.

FIGURE 1 The anatomical relationship between the equine intrinsic laryngeal and hyoepiglottic muscles and the laryngeal cartilages and hyoid bone. A section has been removed from the thyroid cartilage to allow visualization of the lateral cricoarytenoid, vocal and ventricular muscles

Fig. 1.


Transverse arytenoid muscle
Arytenoid cartilage
Dorsal cricoarytenoid muscle
Ventricular muscle
Cricoid cartilage
Epiglottic cartilage
Hyoepiglottic muscle
Hyoid bone
Thyroid cartilage
Vocal muscle
Lateral cricoarytenoid muscle
Cricothyroid muscle

1.2.1 Dorsal Cricoarytenoid Muscle

This is a bulky fan-shaped muscle (Figs.1,2) which originates on the dorsal lamina of the cricoid cartilage, and its median ridge. Its fibres converge to insert on the muscular process of the ipsilateral arytenoid cartilage. Grossly the muscle appears to have two parts. Those fibres originating on the median ridge and the caudal border of the lamina of the cricoid cartilage pass anterolaterally in an oblique direction towards the muscular process of the arytenoid cartilage. Those fibres originating on the more lateral aspect of the lamina of the cricoid cartilage have a more anterior to posterior course and attach on the lateral aspect of the muscular process. The dorsal cricoarytenoid muscle is the major abductor of the larynx (Goulden et al., 1976).

1.2.2 Lateral Cricoarytenoid Muscle (Figs.1, 3)

This is a triangular-shaped muscle lying medial to the lamina of the thyroid cartilage. It originates on the anterior border of the lateral part of the arch of the cricoid cartilage and passes dorsally to insert on the ventral aspect of the muscular process of the arytenoid cartilage. The action of this muscle is to adduct the larynx (Goulden et al., 1976).

1.2.3 Transverse Arytenoid Muscle (Figs.1, 4)

This is a relatively thick, fan-shaped muscle which occupies the concave dorsal surface of the arytenoid cartilage. It originates from the muscular process of this cartilage and the ridge which extends in an anterior direction from it. The muscle joins its contralateral partner along a midline fibrous raphé. The muscle acts as a laryngeal adductor (Goulden et al., 1976).

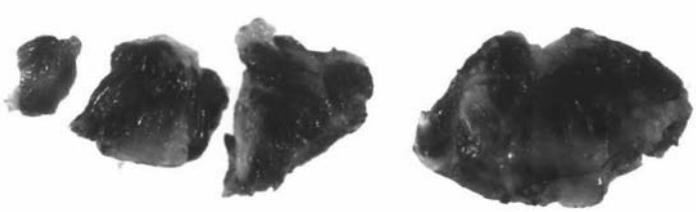
1.2.4 Thyroarytenoid Muscles (Figs.1, 5)

The thyroarytenoid muscles in the horse are present as separate ventricular and vocal muscles. The ventricular muscle is a thin flat sheet of muscle (Fig. 5) lying medial to the thyroid lamina. Along with the ventricular ligament it is contained within the ventricular fold. Its fibres originate from the anterior part of the cricothyroid membrane and the ventral border of the medial aspect of the thyroid

FIGURE 2 The left and right dorsal cricoarytenoid muscles. The sample for processing has been cut from the left muscle

FIGURE 3 The left and right lateral cricoarytenoid muscles. The sample for processing has been cut from the centre of the left muscle

FIGURE 4 The left and right transverse arytenoid muscles. The sample for processing has been cut from the centre of the left muscle



lamina near the midline. The fibres run in a dorsal and posterior direction to end on the anterior border of the muscular process of the arytenoid cartilage. Some of the anterior fibres of this muscle continue over the muscular process and transverse arytenoid muscle to join with similar fibres from the contralateral ventricular muscle.

The ventral extremity of the ventricular muscle is related on its medial aspect to the cuneiform process of the epiglottis and also to the ventricular ligament which arises from the cuneiform process and ends on the lateral surface of the vocal process of the arytenoid cartilage.

The vocal muscle is a flat muscle (Fig. 1) lying medial to the thyroid lamina. It is separated from the ventricular muscle by the laryngeal saccule and along with the vocal ligament occupies the vocal fold. The vocal muscle has its origin on the cricothyroid membrane and the medial aspect of the ventral thyroid lamina. Its fibres pass lateral and posterior to the vocal ligament, and insert on the ventral aspect of the muscular and vocal processes of the arytenoid cartilage. The ventricular and vocal muscles act as laryngeal adductors (Goulden et al., 1976) and control the vocal cords (Sisson and Grossman, 1975).

1.2.5 Cricothyroid Muscle (Figs.1, 6)

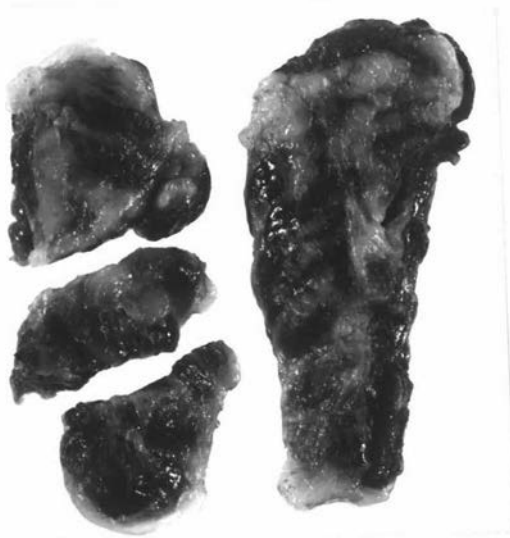
This is a short relatively thick muscle which originates from the ventral and lateral aspect of the cricoid arch and passes anterodorsally to insert on the posterior border and posterolateral aspect of the thyroid lamina as far dorsally as its caudal cornu. This muscle is most active during inspiration and has a laryngeal stabilising function (Goulden et al., 1976).

1.2.6 Hyoepiglottic Muscle (Figs.1, 11)

The hyoepiglottic muscle is an extrinsic muscle of the larynx and was examined in some of the horses in this study. It is a short rounded muscle which originates from the body of the hyoid bone on the midline. It tapers dorsally and inserts on the oral surface of the epiglottis. The muscle is enclosed in an elastic sheath, the hyo-epiglottic ligament. Contraction of this muscle draws the epiglottis down and towards the root of the tongue (Sisson and Grossman, 1975).

FIGURE 5 The left and right ventricular muscles. The sample for processing has been cut from the centre of the left muscle

FIGURE 6 The left and right cricothyroid muscles. The sample for processing has been cut from the centre of the left muscle



1.3 Laryngo-palatal Dislocation

A number of horses with exercise induced respiratory abnormalities exhibit signs such as "tongue swallowing", "gurgling","breath holding" or "choking up". The onset of these signs, often towards the end of a race, is usually associated with a sudden decrease in respiratory efficiency and so the horse is unable to continue racing at its previous pace (Cook, 1965). If these horses are examined endoscopically while the abnormal respiratory noise is still present, the soft palate may be found to be displaced above the epiglottis, thus partially obstructing the nasopharyngeal airway.

This soft palate abnormality was considered to be a functional problem associated with nerve or muscle weakness in which no organic lesion was demonstrable by routine clinical examination, endoscopy or radiography (Cook, 1962). Some authors have stated that in horses exhibiting clinical signs of this syndrome the soft palate was too long (Cook, 1965), thinner than normal or atrophied on its left side (Quinlan, 1957), or sometimes inflammed or ulcerated (Cook, 1965, 1981). It has also been suggested that in some of these cases the epiglottis was shorter than normal (Haynes, 1981).

The disease was originally called "soft palate paresis" as it was thought that the muscles of the soft palate or pharynx may be subjected to a neurogenic myopathy similar to that which occurs on the left side of the larynx (Quinlan et al., 1949; Quinlan, 1957; Cook, 1962; Johnson and Merriam, 1975; Raker, 1975). This idea has been given support by Heffron and Baker (1979b) who found that some horses which had suffered from laryngo-palatal dislocation showed signs of paresis of the muscles of the pharynx or soft palate when they were examined with an endoscope. Signs of neurogenic dysfunction of other structures supplied by the motor nerves of the palatal muscles however have usually not been recorded as part of the laryngo-palatal dislocation syndrome (Heffron and Baker, 1979b; Cook, 1981).

The only information available on the pathology of palatal muscles from horses which had suffered from laryngo-palatal dislocation comes from work carried out by Blythe et al., (1980). These workers observed lesions in palatine muscles which they considered were characteristic of a low grade non-specific myopathy rather than neurogenic disease.

Apart from paresis of the palatal muscles many other factors have been implicated as possible causes of laryngo-palatal dislocation. A study of these factors was not within the scope of this thesis, a purpose of which was to investigate the possibility that neurogenic disease may occur in equine palatal muscles. For this reason some of these muscles were studied using the same horses and techniques as for the laryngeal muscles. A brief account of the anatomy of these muscles is included.

1.4 The Anatomy of Some Equine Palatal Muscles

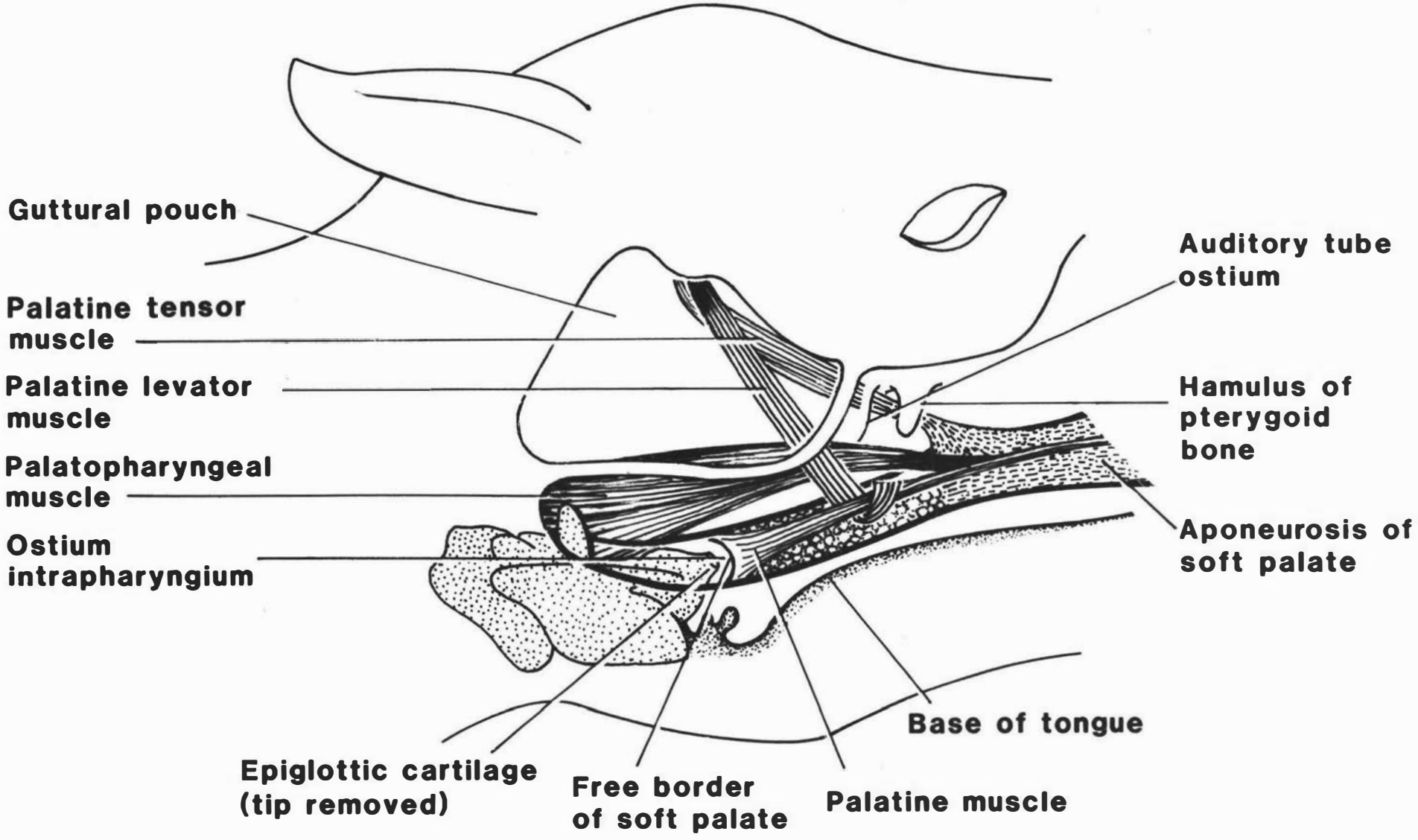
The muscles examined in this study were those which had direct attachments to the soft palate and so could be regarded as intrinsic palatal muscles. They are illustrated in Fig. 7.

1.4.1 Palatopharyngeal Muscle (Figs.7, 8)

This muscle is one of the pharyngeal constrictors and originates by way of the aponeurosis of the soft palate from the palatine and pterygoid bones. Some of its fibres attach to the anterior aspect of the eustachian tube. The muscle passes in a posterior direction in the lateral wall of the pharynx. Some fibres are inserted onto the upper edge of the thyroid cartilage and some turn medially to end at the midline fibrous raphé (Sisson and Grossman, 1975).

When the muscle contracts it shortens the pharynx and pulls the larynx and oesophagus towards the root of the tongue. It also depresses the pharyngeal roof and its action is important in closing of the naso-pharyngeal sphincter (Heffron and Baker, 1979c). It has been proposed that it also plays a part in opening the ostia of the auditory tubes, (Heffron and Baker, 1979a). As the muscle is involved in both increasing the size of the guttural pouches (by depressing the pharyngeal roof) and opening the auditory tube ostia, Heffron and Baker (1979c) have proposed that it may be part of an active pump which ensures air exchange in the guttural pouches. The muscle may also play a part in tensing the soft palate.

FIGURE 7 The anatomical relationships of equine palatal muscles


Guttural pouch
Palatine tensor muscle
Palatine levator muscle
Palatopharyngeal muscle
Ostium intrapharyngium
Auditory tube ostium
Hamulus of pterygoid bone
Aponeurosis of soft palate
Base of tongue
Epiglottic cartilage (tip removed)
Free border of soft palate
Palatine muscle

1.4.2 Palatine Levator Muscle (Figs.7, 9)

This muscle consists of a relatively thin flattened band of muscle fibres which originate from the muscular process of the petrous temporal bone. The muscle passes anteroventrally, deep to the pharyngeal constrictor muscles, and then turns medially into the soft palate above the glandular layer. Here it widens slightly and joins its contralateral partner on the midline of the soft palate (Sisson and Grossman, 1975). The muscle thus forms a sling approximately midway between the hard palate and the free border of the soft palate. Its action is to elevate the soft palate and so it is important in closing the nasopharyngeal sphincter during deglutition (Heffron and Baker, 1979c; Cook, 1981).

1.4.3 Palatine Muscle (Figs.7, 11)

This muscle consists of two circular bundles of muscle fibres which lie together on the midline of the soft palate. It originates by way of the palatine aponeurosis from the palatine bones, passes through the sling of the palatine levator muscle and ends near the free edge of the soft palate. Some bundles of its fibres continue a short distance into the posterior pillars of the soft palate (Sisson and Grossman, 1975). Its action is to shorten the soft palate.

1.4.4 Palatine Tensor Muscle (Figs.7, 10)

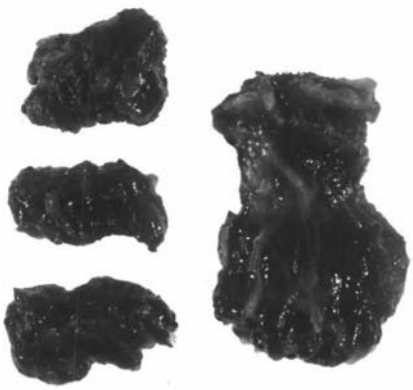
This is a bulky muscle with a flattened cross section. It originates from the muscular process of the petrous temporal bone, the pterygoid bone and the lateral lamina of the eustachian tube and passes anteroventrally, lateral to the palatine levator muscle. It terminates in a tendon which passes around the lateral surface of the hamulus of the pterygoid bone. The tendon is attached to the hamulus by a fibrous band and cushioned by a bursa. After passing the hamulus the tendon is redirected medially and fans out into the aponeurosis of the soft palate (Sisson and Grossman, 1975).

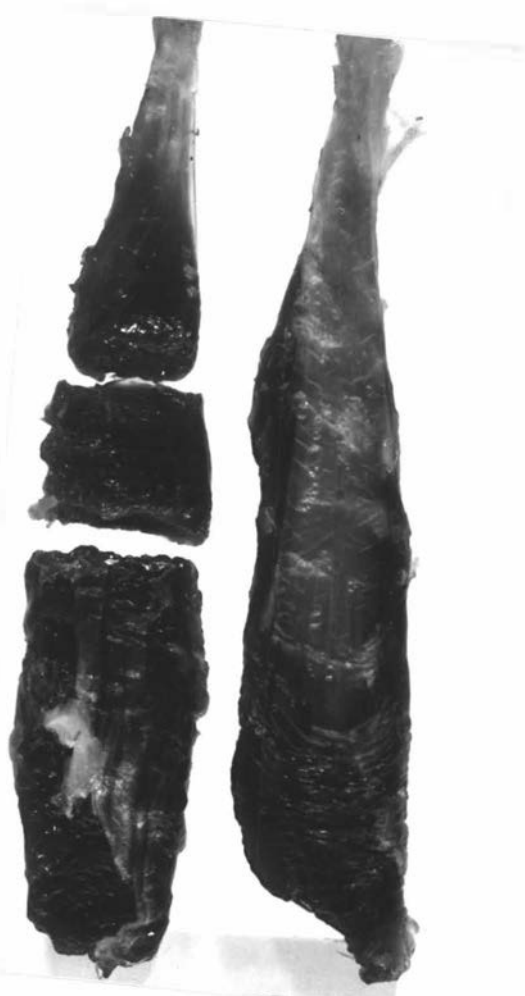
The action of the muscle is to tense and depress the rostral one third of the soft palate. It can thus oppose the negative pressure generated in the nasopharynx during inspiration (Heffron and Baker, 1979c). This stiffening of the anterior aspect of the soft palate is also important

FIGURE 8 The left and right palatopharyngeal muscles. The sample for processing has been cut from the centre of the left muscle

FIGURE 9 The left and right palatine levator muscles. The sample for processing has been cut from the centre of the left muscle

FIGURE 10 The left and right palatine tensor muscles. The medial surface of the left muscle and the lateral surface of the right muscle are facing the reader. The sample for processing has been cut from the centre of the left muscle





in aiding the root of the tongue in propelling a bolus of food into the oropharynx during the first stages of swallowing (Heffron and Baker, 1979c). It has also been proposed that the muscle may play a part in opening the ostia of the auditory tubes during deglutition (Heffron and Baker, 1979a).

CHAPTER 2 MATERIALS AND METHODS

2.1 Horses Used in the Study

During the years 1975-1980 tissues were obtained from 56 horses. Most of these animals were submitted for post mortem examination at the Massey University Veterinary Hospital but a few were destroyed at a local knackery. A foetus was obtained from a mare destroyed in her 10 month of pregnancy. Fifty-three of the horses were Thoroughbred and three were ponies. Their ages ranged from before birth, to older than 20 years.

The laryngeal movements of some of the horses were examined endoscopically but as in the early stages of the study, only a rigid rhinolaryngoscope* was available, this was not always possible. Forty-nine of the horses were selected because they had no history of upper respiratory tract abnormalities and for the purposes of this study they have been classified as the "normal" horses (Table 1). Seven of the horses were selected because they were known to have abnormal upper respiratory tracts. In these seven horses a diagnosis (Table 2) was made after observing their clinical signs and examining their pharynx and larynx with the rigid rhinolaryngoscope or later in the study with a flexible fibreoptic endoscope+. These seven horses, for the purposes of this study, were classified as the abnormal horses.

2.2 Tissue Collection

Each horse was anaesthetised by injecting 10-12 grams of Pentobarbitone sodium* into the jugular vein. When the horse became recumbent it was exsanguinated by transecting the carotid arteries. The head was then removed by disarticulation of the occipital condyles and atlas.

A dissection to expose the left pharynx and larynx was then undertaken.

* Frese's rhinolaryngoscope, Hauptner Instruments, Germany.
+ F8 Panendoscope, American Cystoscope Makers Inc.
* Euthesate, Willows Francis.

The skin from the left lateral aspect of the head ventral to the orbit was excised. The origins and insertions of the left masseter muscle were sectioned and the body of the muscle removed. The left mandible was then removed by sawing through the horizontal ramus caudal to the incisor teeth and through the vertical ramus ventral to the coronoid and condylar processes. The origin of the medial pterygoid muscle on the pterygoid process of the sphenoid and palatine bones (Sisson and Grossman, 1975) was then carefully sectioned to avoid damaging the underlying palatine tensor muscle. The left guttural pouch was by this stage usually open and the stylohyoid bone and muscle exposed and readily removed. The tongue was then divided just anterior to the pharynx and removed. The left lateral wall of the pharynx was then exposed. The palatopharyngeal muscle was identified and a section approximately three centimeters long was removed from its body.

The pterygopharyngeal muscle was then divided and the left palatine levator muscle exposed. Its origins were sectioned and the body of the muscle freed down to the point where it joined its contralateral partner on the midline of the soft palate. The two muscles were divided exactly on the midline.

The double bundle of the palatine muscle could then be identified running longitudinally through the glandular tissue of the soft palate. Approximately three centimeters of the mid section of the body of this muscle were removed.

The left palatine tensor muscle was then sectioned at its origin and the muscle freed to the point where its tendon of insertion was reflected around the hamulus of the pterygoid bone. This tendon was then divided and the whole muscle body removed.

The right lateral wall of the pharynx was then removed and the right guttural pouch opened. The right palatine levator, palatine tensor and palatopharyngeal muscles were then collected.

The left lateral aspect of the larynx was exposed by excising the skin, the linguofacial vein and the remnants of the sternothyrohyoid, omohyoid, sternocephalic and brachiocephalic muscles. The thyropharyngeal and cricopharyngeal muscles were also removed and discarded.

The entire bodies of the left cricothyroid and left dorsal cricoarytenoid muscles were then collected.

The cricothyroid joint was disarticulated and the left lamina of the thyroid cartilage removed. The left lateral cricoarytenoid muscle was then dissected from its cartilagenous attachments.

The left transverse arytenoid muscle was collected by severing its attachments and separating it from its contralateral partner along the midline fibrous raphé. (Sisson and Grossman, 1975).

In some cases (Table 3) the ventricular, vocal and hyoepiglottic muscles were collected.

This completed the dissection of the left laryngeal musculature. The larynx was then separated from the thyroid cornua of the hyoid bone and the right side exposed. A collection technique identical to that described for the left side was performed to obtain the corresponding right muscles. Each muscle after collection was placed on a labelled tray (Fig. 11.).

2.3 Processing of Muscle Samples

As soon as possible after the death of the horse the fresh wet weights of the dorsal and lateral cricoarytenoid, transverse arytenoid, ventricular, cricothyroid, palatine levator and palatine tensor muscles were obtained using a Sartorius top pan balance.

Each individual muscle was then prepared for frozen sectioning. A block approximately one cm square was cut from the centre of the muscle body using a No.20 disposable scalpel blade. Care was taken to ensure that the block came from the same area of the same muscles from each horse (Fig. 12). One face of the block was cut perpendicular to the long axis of the muscle fibres. It was from this face that subsequent transverse sections were cut.

Approximately 100ml. of Isopentrane* were placed in a stainless steel

* 2-Methybutane, Isopentrane, Koch-Light Laboratories Ltd., Coinbrook, Bucks, England.

FIGURE 11 Some of the intrinsic laryngeal and palatal muscles and the hyoepiglottic muscle from a "normal" horse

Lt = left; RT = right
D.C.A.M. = dorsal cricoarytenoid muscle
L.C.A.M. = lateral cricoarytenoid muscle
C.T. = cricothyroid muscle
T.A. = transverse arytenoid muscle
VENT. = ventricular muscle
PAL = palatine muscle
L.P. = palatine levator muscle
T.P. = palatine tensor muscle
P.P. = palatopharyngeal muscle
H.E. = hyoepiglottic muscle

FIGURE 12 The same muscles as in Figure 11 cut to illustrate the sites in the centre of the muscle bodies from which the samples for processing were cut

beaker (Fig. 13). The Isopentrane was cooled to its melting point (-51^{o}C) by gently lowering the beaker into a polystyrene vessel (Fig. 13) containing approximately 500ml. of liquid nitrogen. The muscle block was then mounted in a small wire mesh basket (Fig. 13) so that the face from which sections were to be cut was uppermost. When the Isopentrane was nearing its freezing point (as evidenced by Isopentrane solidifying on the walls of the beaker) the basket containing the muscle block was immersed under its surface and so the muscle rapidly frozen.

The bearing surface of a cryostat chuck (22mm diameter, Fig. 13) which had been precooling on the quick freeze stage of the cryostat* was then coated with embedding medium+. Once the medium had reached a tacky consistency the muscle block was removed from the basket and its appropriate surface fixed to the cryostat chuck.

The chuck and muscle blockwere then mounted in the cryostat which was held at -25^{o}C, and the face of the block transverse to the muscle fibres was trimmed. Once good quality transverse sections were being obtained the cryostat was set to cut sections 10 µm thick. Then, four consecutive sections were cut from the block and mounted on clean, labelled, microscope slides at room temperature.

In this way four serial transverse frozen sections were obtained from each of the muscles included in the study.

One of the serial sections was then stained to demonstrate the activity of the enzyme myosin adenosine triphosphatase (myosin ATPase) using the method of Padykula and Herman (1955) as modified by Davies and Gunn (1972; Appendix 1). The others were stained for succinate dehydrogenase (SDase) activity (Nachlas et al., 1957; Appendix 2), glycogen phosphorylase (GPase) activity (Takeuchi, 1956; Appendix 3) and with haematoxylin and eosin.

* Lipshaw Electric cryotome Model No. 1500.
Lipshaw Manufacturing Co., Detroit, 10 Michigan U.S.A.

\+ Tissue Tek II O.C.T. Compound embedding medium
Lab-Tek Products, Miles Laboratories Inc., Naperville, Illinois 60540 U.S.A.

2.4 Data Collection

The stained sections of the muscles from each horse were then examined using the 10x and 40x objective of a light microscope* to ensure that they were of consistently high quality. Sections were rejected if they were not transverse; not evenly stained; had excessive ice artifact or, in the case of the myosin ATPase sections, excess cobalt sulphide precipitate.

The following features were noted:

1. Presence or absence of fibre type grouping
2. Muscle fibre shape and size variation
3. Density and distribution of nuclei
4. Amount of intramuscular connective tissue
5. Presence or absence of abnormally stained fibres such as targetoid fibres
6. Any other notable staining characteristics.

The sections were then projected onto tracing paper+ using a micro-projector‡ mounted on the tracing table illustrated in Fig. 14.

Areas of the sections were selected so that the same fascicle could be identified in each of the four serial sections. The section which had been stained for the activity of myosin ATPase was then used to make tracings of two individual muscle fascicles (bundle A and bundle B) for each section. Approximately 200 individual muscle fibres per section, were traced in this way.

* American Optical Series 10 Microstar, Scientific Instruments Division, Buffalo, N.Y. 14215, U.S.A.

+ 110g/m^2 surglace, Les Papiers, Canson France, Anciennes Manufacturers, Canson and Montgolfier, France

‡ Leitz, Micro Promar, Ernst Leitz, Wetzlar, Germany.

The 10x objective of the microprojector was used for making the tracings and the apparatus illustrated in Fig. 14 produced a magnification of x400. The system was callibrated using a 0.01mm. objective micrometer (Olympus, Tokyo).

The enzyme level of each fibre, classed as either myosin ATPase high (AH, black-brown stained fibres) or myosin ATPase low (AL, pale, grey-white fibres) was marked on the tracing.

The section stained for the activity of SDase was then back projected onto the tracings of bundle A and bundle B and the level of this enzyme in each fibre marked on the tracings. All those fibres which showed evidence of an overall blue colouration were classed as SDase high (SH). Those fibres which appeared pale in colour or had only slight blue stippling were classed as SDase low (SL).

In a similar manner the section stained for the activity of the enzyme GPase was back projected onto the tracings. All those fibres having evidence of blue colouration were regarded as GPase high (PH) and those without as GPase low (PL). The GPase reaction of each fibre was also marked on the tracings.

Once the tracing was complete the following information was collected for each fascicle:

1. Total number of fibres
2. Histochemical fibre types
3. Size of groups of AL fibres
4. Abnormal fibre types.

The tracings were then cut so that the AH and AL fibres could be separated. The sections of paper representing these two fibre types were then weighed* and this weight converted to the original mean fibre area using a conversion factor calculated with the calibration slide and a known weight and area of tracing paper.

* Mettler Balance, Type H6
E. Mettler, Zurich, Switzerland.

In this way a mean fibre area for AH and AL fibres was obtained for each muscle fascicle traced.

2.5 Data Processing

The information for the muscles from each horse was then compiled as illustrated in Appendix 4.1. All the information collected was arranged in this manner and is presented in Appendix 4.2. The data were typed into a Prime Computer and analysed using a Genstat statistical package*.

The analysis enabled each of the following variables to be examined separately:

1. Muscle weights
2. Proportion of AH to AL fibres
3. Proportion of ALSHGH to ALSHGL fibres
4. Size of groups of AL fibres
5. Area of AH and AL fibres.

Mean values for each of these variables were obtained for each of the following groups:

1. All "normal" horses
2. "Normal" horses three years of age and under
3. "Normal" horses over three years of age
4. All abnormal horses
5. Abnormal horses three years of age and under
6. Abnormal horses over three years of age.

Within each of these groups of horses the analysis provided:

1. An overall mean value of the variable being examined
2. A mean value of the variable for entire males, females and geldings

* Laws Agricultural Trust (1980). Rothamsted Experimental Station, England.

3. A mean value of the variable for the left and right muscle
4. A mean value of the variable for the left and right muscle within each of the sex groups
5. An analysis of variance to determine the significance of the differences of the means between sexes, between sides and between sides within sexes. The analysis also provided information to allow for testing of the significance of the difference of the means between age groups and between "normal" and abnormal horses where numbers were sufficient.

An example of the analysis, in this case the area of AH fibres of the lateral cricoarytenoid muscle is provided in Appendix 5. Occasionally it was necessary to further analyse the data grouped for males and females or to transform the data to natural logarithms for analysis. When the difference between means was significant at the 10% level or less this information has been included in the appropriate results section of this thesis.

TABLE 1 HORSES WITH NO HISTORY OF UPPER RESPIRATORY TRACT ABNORMALITIES ("NORMAL" HORSES)

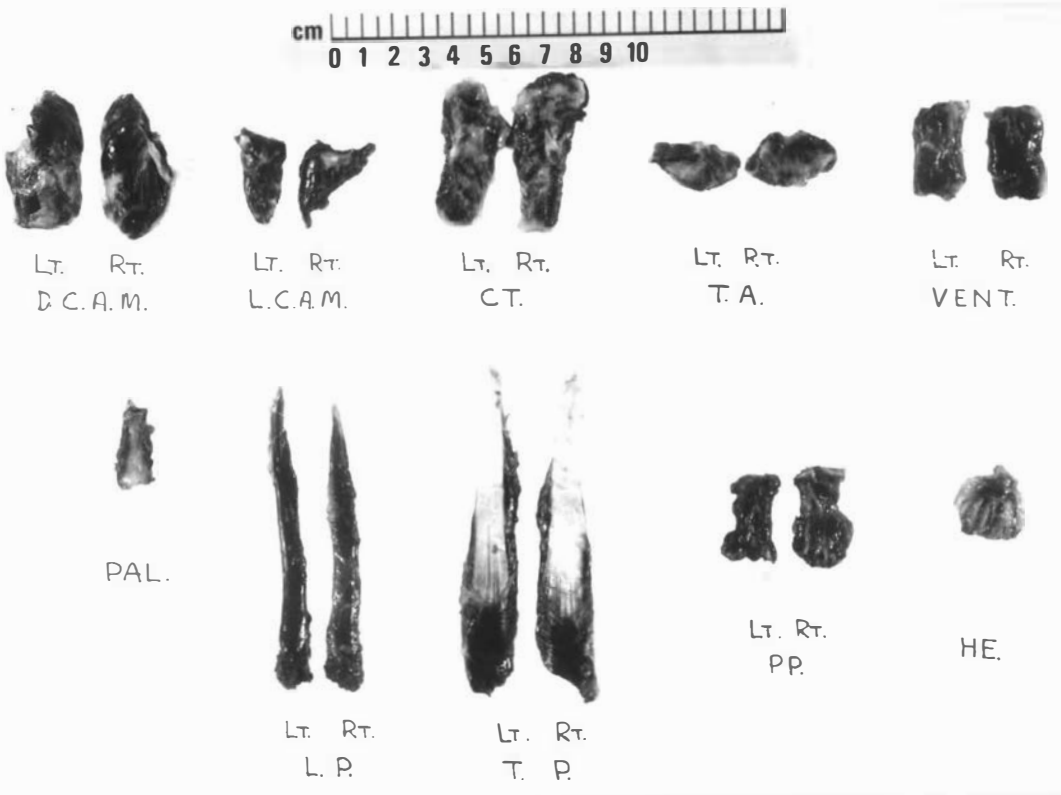
NUMBER	BREED	AGE	SEX	NUMBER	BREED	AGE	SEX
1	TB	before term	F	25	TB	2 years	G
2	TB	2 hours	M	26	TB	2 years	F
3	TB	12 hours	M	28	TB	2.5 years	M
4	TB	1 day	F	31	TB	3.5 years	G
5	TB	1 day	M	32	TB	4 years	G
6	TB	1 day	F	34	TB	4 years	F
7	TB	2 days	F	35	TB	4 years	G
8	TB	2 days	F	36	TB	5 years	G
9	TB	5 days	M	37	TB	5 years	G
10	TB	1 month	NR	38	TB	5 years	G
11	TB	1.5 months	M	39	TB	6 years	G
12	TB	3 months	F	41	TB	7 years	G
13	TB	6 months	F	42	Pony	10 years	F
14	TB	7 months	F	43	TB	12 years	F
15	TB	8 months	F	44	TB	12 years	G
16	TB	8 months	F	45	TB	12 years	F
17	TB	1 year	M	46	Pony	14 years	G
18	TB	1 year	F	48	TB	19 years	G
19	TB	1 year	F	50	TB	over 20 years	F
20	TB	1 year	F	51	TB	"	F
21	TB	1.25 years	M	52	TB	"	F
22	TB	1.5 years	M	53	TB	"	F
23	TB	2 years	M	54	TB	"	F
24	TB	2 years	F	55	TB	"	NR
				56	Pony	10 years	G

TB = Thoroughbred
M = Entire Male
F = Female
G = Gelding
NR = Not recorded

TABLE 2 HORSES WITH CLINICALLY ABNORMAL UPPER RESPIRATORY TRACTS (ABNORMAL HORSES)

NUMBER	BREED	AGE	SEX	CLINICAL DIAGNOSIS
27	TB	2 years	F	I.L.H.
29	TB	3 years	M	L.P.D.
30	TB	3 years	F	I.L.H.
33	TB	4 years	M	I.L.H.
40	TB	6 years	G	I.L.H.
47	TB	15 years	F	I.L.H.
49	TB	20 years	M	I.L.H.

TB = Thoroughbred
M = Entire Male
F = Female
G = Gelding
I.L.H = Idiopathic laryngeal hemiplegia
L.P.D = Laryngo-palatal dislocation


cm
0 1 2 3 4 5 6 7 8 9 10
LT. RT.
D. C. A. M.
LT. RT.
L. C. A. M.
LT. RT.
C. T.
LT. RT.
T. A.
LT. RT.
VENT.
PAL.
LT. RT.
L. P.
LT. RT.
T. P.
LT. RT.
P. P.
HE.

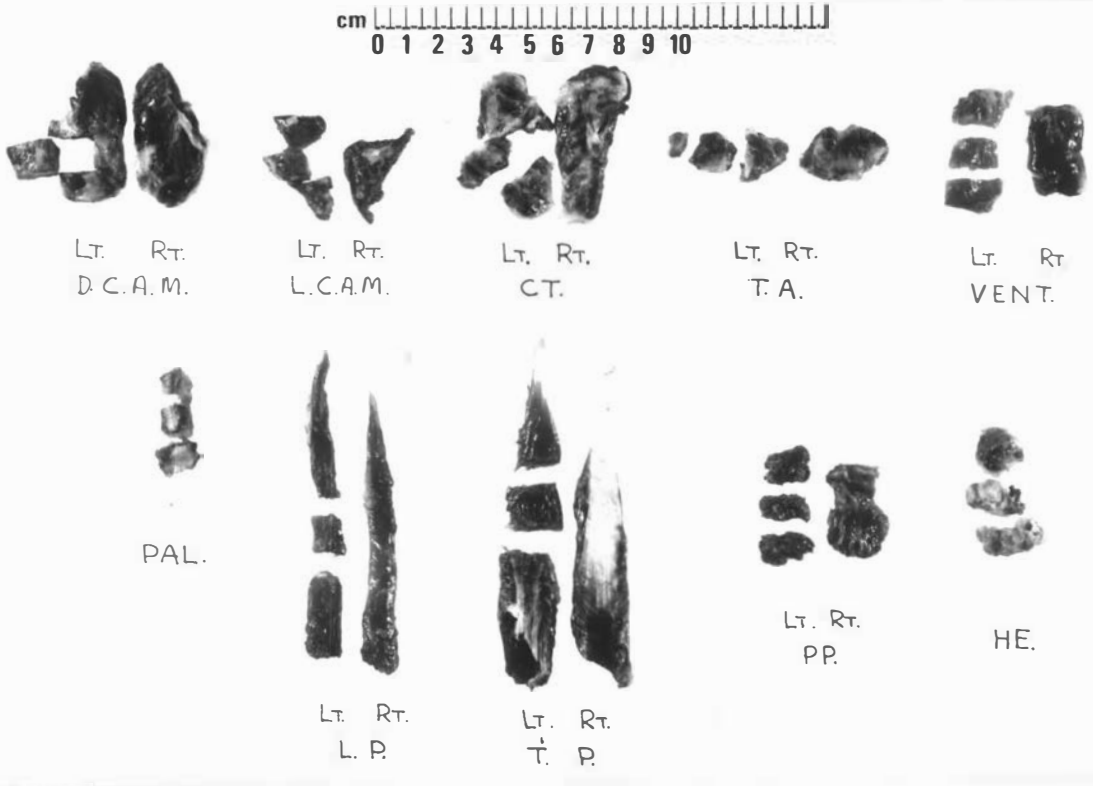

cm
0 1 2 3 4 5 6 7 8 9 10
LT. RT.
D. C. A. M.
LT. RT.
L. C. A. M.
LT. RT.
C. T.
LT. RT.
T. A.
LT. RT
VENT.
PAL.
LT. RT.
L. P.
LT. RT.
T. P.
LT. RT.
P. P.
HE.

FIGURE 13 Some of the equipment used for freezing and mounting the muscle blocks

FIGURE 14 The microprojector and tracing table used during this study


ISOPENTANE

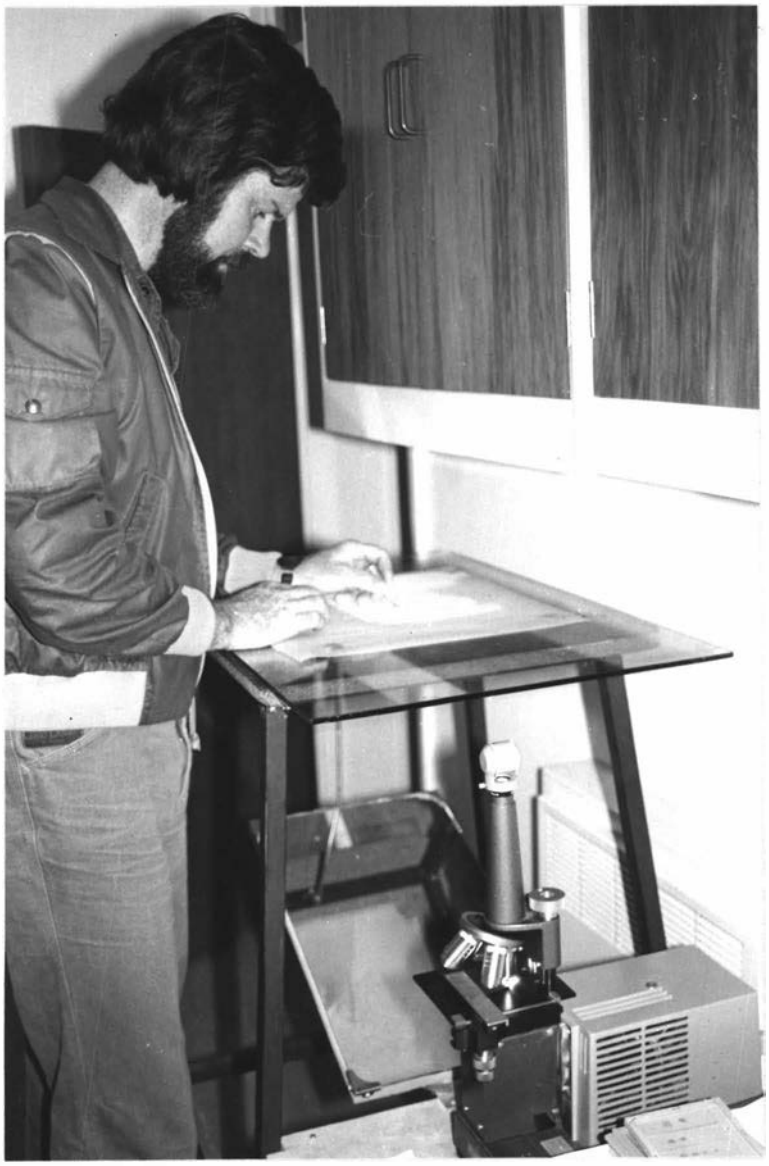


TABLE 3: MUSCLES EXAMINED IN HORSES STUDIED

(* = muscle collected)

Horse No.	Muscle weights	Dorsal cricoarytenoid		Lateral cricoarytenoid		Cricothyroid		Transverse arytenoid		Ventricular		Vocal		Hyoepiglottic	Palatine levator		Palatine tensor		Palatopharyngeal		Palatine
		Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt		Lt	Rt	Lt	Rt	Lt	Rt	
1.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
2.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
3.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	*	*	*	*	*	*	–
4.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
5.	*	*	*	*	*	–	*	–	–	–	–	–	–	–	–	–	*	*	*	*	*
6.	–	*	*	*	*	*	*	*	*	–	*	*	*	*	*	*	*	*	–	–	–
7.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	–	*	*	*	*	*	–
8.	*	*	*	*	*	*	–	–	–	–	–	–	–	–	*	*	*	*	*	*	*
9.	*	*	*	–	*	*	*	–	–	–	–	–	–	–	–	*	*	*	*	*	–
10.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
11.	*	*	–	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	–	–	*
12.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	–	*	*	*	*	*
13.	*	*	*	*	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
15.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
16.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
17.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
18.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
19.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	*	*	*	*	*	*	*
20.	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	–	–	–	–	–
21.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
22.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	*	*	*	–	*	*	*
23.	*	*	*	–	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
24.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	*	*	*	*	*	*	*
25.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
26.	*	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	–	–	–	–	–
27.	*	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*
28.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
29.	–	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*
30.	*	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*
31.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
32.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	–	*	*	*	*
33.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	–	–	*	*	*
34.	*	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	*	*	–	–	–
35.	*	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*
36.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
37.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	–
38.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
39.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
40.	*	–	*	–	*	*	*	–	*	–	*	–	–	*	*	*	*	*	*	*	*
41.	*	–	–	*	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
42.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
43.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	*	*	*	*	*	*	*
44.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
45.	–	–	–	*	*	–	–	–	–	*	*	–	–	*	*	*	–	–	–	–	–
46.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
47.	*	–	*	–	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
48.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
49.	*	–	*	–	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
50.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
51.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
52.	*	*	*	*	*	*	*	–	–	–	–	–	–	–	*	*	*	*	*	*	*
53.	*	*	*	*	*	*	*	–	–	*	*	–	–	*	*	*	*	*	*	*	*
54.	*	*	*	*	*	*	*	–	–	–	–	–	–	*	*	*	*	*	–	–	–
55.	–	–	–	*	*	–	–	–	–	–	–	–	–	–	*	*	–	–	–	–	–
56.	*	*	*	*	*	*	*	*	*	*	*	–	–	*	*	*	*	*	*	*	*

CHAPTER 3 THE WEIGHTS OF INTRINSIC LARYNGEAL MUSCLES IN "NORMAL" AND IDIOPATHIC LARYNGEAL HEMIPLEGIC HORSES

3.1 Introduction

In 1946 when Cole recorded his observations on a series of 174 equine larynges he stated that 27% of them showed atrophy of one or more left intrinsic laryngeal muscles. Of those with atrophy only one quarter had shown clinical signs of roaring. From his findings he concluded that the dorsal cricoarytenoid muscle must atrophy by 50% or more before clinical signs of roaring were exhibited. Cole found that only 4.6% of the larynges had bilateral atrophy and none had unilateral right sided atrophy. He also considered that the left dorsal cricoarytenoid, the left transverse arytenoid, the left lateral cricoarytenoid, the left ventricular and the left vocal muscle were affected in decreasing order of severity. Cole's work thus supported Semon's law (1881) which stated that there was a "proclivity of the abductor fibres of the recurrent laryngeal nerve to become affected sooner than the adductor fibres".

Following Cole's investigation there have been two reports on the weights of equine intrinsic laryngeal muscles (Gunn, 1972; Quinlan et al., 1975). Neither of these studies were carried out on a single breed of horse and their findings were somewhat contradictory. Gunn (1972) weighed the dorsal cricoarytenoid muscles from 12 horses and found no significant difference in weight between left and right sides. Quinlan et al. (1975) weighed the dorsal cricoarytenoid muscles, the adductor muscles (lateral cricoarytenoid, transverse arytenoid, vocal and ventricular) and the cricothyroid muscles from 24 horses. They found a highly significant difference between the weights of the left and the right muscles supplied by the recurrent laryngeal nerve but no significant difference between the weights of the left and right cricothyroid muscles.

The present study was designed to examine this situation and determine the incidence and degree of atrophy of the intrinsic laryngeal muscles in New Zealand Thoroughbred horses. The opportunity was also taken to compare the weights of muscles from these horses with muscle weights from five laryngeal hemiplegic horses.

3.2 Materials and Methods

The details of the horses used in this study and the methods of obtaining and analysing the muscle weights are described in Chapter 2. For each pair of intrinsic laryngeal muscles the difference in weight between the left and right muscles, expressed as a percentage of the heavier muscle, was calculated for "normal" and abnormal horses.

In the case of the transverse arytenoid, the ventricular and all the muscles from the abnormal horses the number of weights available were too small to be confidently analysed. For these muscles it was not possible to establish whether or not differences between the means were statistically significant. The muscle weights from only five of the laryngeal hemiplegic horses were included because the other roarer, a six year old gelding had been subjected to a laryngoplasty operation and had no recognisable left dorsal or lateral cricoarytenoid or transverse arytenoid muscles.

When all the muscle weights collected initially were analysed it was found that inclusion of the lower muscle weights from the ponies influenced the mean weights obtained for those muscles where only small numbers were available. As the primary concern of this study was the competitive horse, the data were re-analysed excluding the muscle weights from the ponies.

3.3 Results

3.3.1 Weight Differences Between Left and Right Intrinsic Laryngeal Muscles

The weights of all the muscles collected are included in Appendix 4.2.

3.3.1.1 Dorsal Cricoarytenoid Muscle

The weights of the dorsal cricoarytenoid muscles were obtained from 41 "normal" and five abnormal horses. In 35% of the "normal" horses the weights of the left dorsal cricoarytenoid muscle averaged 13% less than the right. In the other 65% of "normal" horses

the right dorsal cricoarytenoid muscle weight averaged 5% less than the left.

In the abnormal horses the left dorsal cricoarytenoid muscles were lighter than the right by an average of 55%. The left dorsal cricoarytenoid muscles of the two younger abnormal horses, a two and a three year old female were 24% and 47% lighter than the right muscles. The older abnormal horses, a four year old entire male, a 15 year old female and a 20 year old entire male, had left dorsal cricoarytenoid muscles which were 65%, 64% and 69% lighter than the right muscles.

3.3.1.2 Lateral Cricoarytenoid Muscle

The weights of the lateral cricoarytenoid muscles were obtained from 41 "normal" and five abnormal horses. In 75% of the "normal" horses the weight of the left lateral cricoarytenoid muscle averaged 17% less than the right. Fourty-four percent of these horses had left muscles which were more than 10% lighter than their right muscles. In the other 25% of "normal" horses the right lateral cricoarytenoid muscle weight averaged 10% less than the left.

In the abnormal horses the left lateral cricoarytenoid muscles were lighter than the right by an average of 65%.

3.3.1.3 Transverse Arytenoid Muscle

The weights of the transverse arytenoid muscles were obtained from four "normal" and two abnormal horses. In 75% of the "normal" horses the weight of the left transverse arytenoid muscle averaged 16% less than the right. In the other 25% of "normal" horses the right transverse arytenoid muscles averaged 15% less than the left.

In the abnormal horses the left transverse arytenoid muscles were lighter than the right by an average of 26%.

3.3.1.4 Ventricular Muscle

The weights of the ventricular muscles were obtained from five "normal" and two abnormal horses. In 20% of the "normal" horses the weight of the left ventricular muscle averaged 30% less than the right. In the other 80% of "normal" horses the right ventricular muscle averaged 10% less than the left.

In the abnormal horses the left ventricular muscles were lighter than the right by an average of 40%.

3.3.1.5 Cricothyroid Muscle

The weights of the cricothyroid muscles were obtained from 37 "normal" and five abnormal horses. In 60% of the "normal" horses the weight of the left cricothyroid muscle averaged 7% less than the right. In the other 40% of "normal" horses the right cricothyroid muscle averaged 8% less than the left.

In the abnormal horses the left cricothyroid muscles were lighter than the right by an average of 12%.

3.3.2 The Analysis of the Difference Between the Mean Weights of Some Intrinsic Laryngeal Muscles

3.3.2.1 Dorsal Cricoarytenoid Muscle

The number of horses from which muscles were collected and weighed, their mean muscle weights, and the mean weights of the left and right dorsal cricoarytenoid muscles from the different groups of horses are shown in Table 4.

As was to be expected the weight of the dorsal cricoarytenoid muscle increased with age and this is illustrated in Fig. 15. This increase in weight with age produced significant differences between the mean muscle weights from the different aged groups of horses.

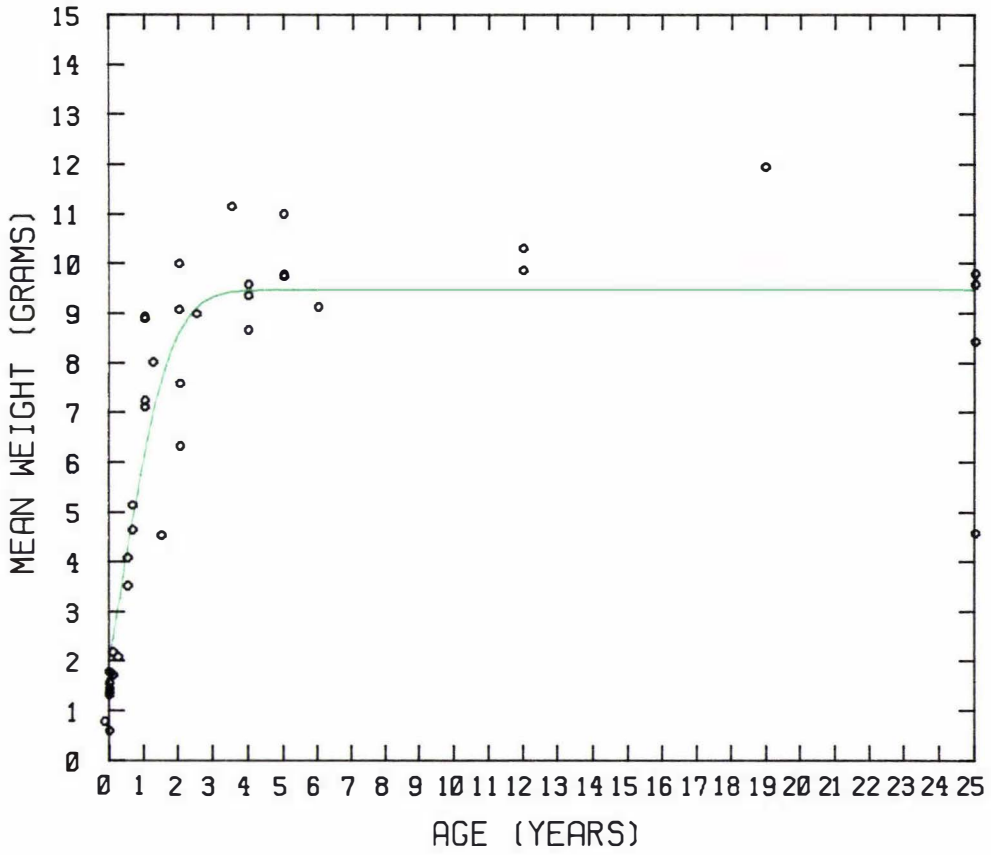
In the "normal" horses there was no significant difference between the weights of the left and right muscles or between the weights of muscles from horses of different sexes. It was common however for the left muscles to be lighter than the right muscles, especially in older horses.

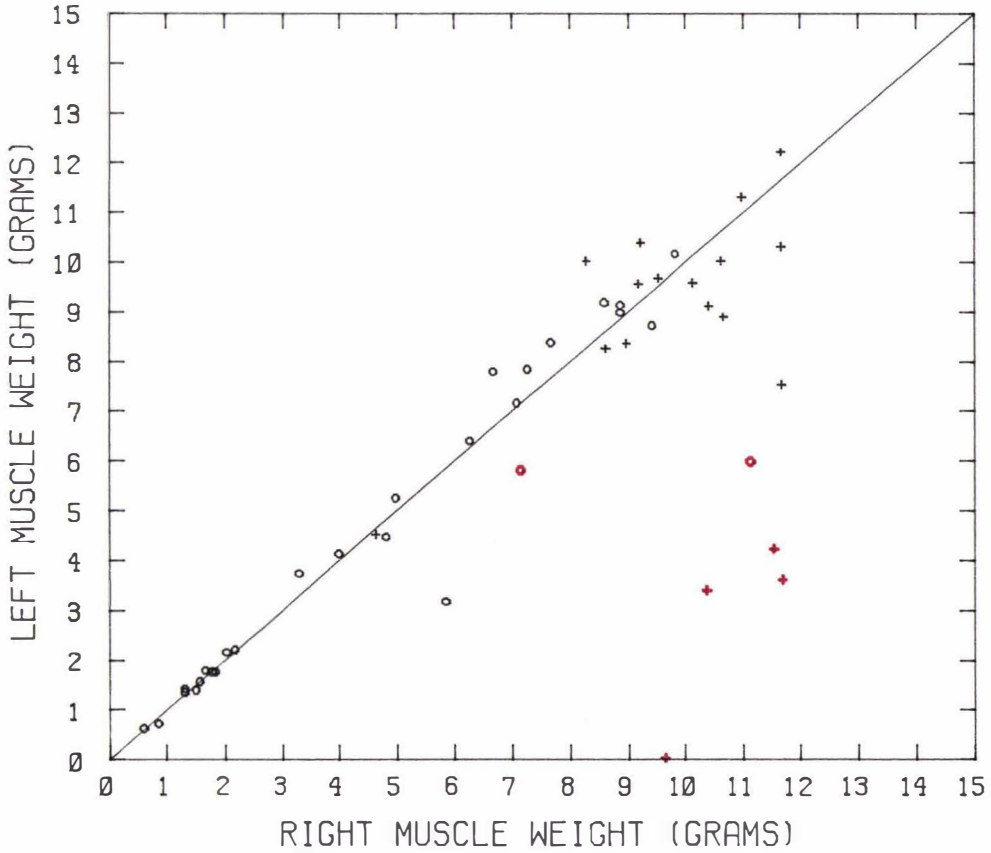
FIGURE 15 The relationship between age (years) and the mean weights of the left and right dorsal cricoarytenoid muscles (grams) of the "normal" horses

FIGURE 16 The weights of the left dorsal cricoarytenoid muscles (grams) plotted against the weights of the right muscles

o = "normal" horses three years and under
\+ = " " over three years

o = abnormal horses three years and under
+ = " " over three years


MEAN WEIGHT (GRAMS)
AGE (YEARS)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25


LEFT MUSCLE WEIGHT (GRAMS)
RIGHT MUSCLE WEIGHT (GRAMS)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

The mean weight of the left muscles was lighter than the mean weight of the right muscles in the abnormal horses especially those over three years of age (Table 4).

This side difference in muscle weights is illustrated in Fig. 16.

TABLE 4 THE MEAN WEIGHTS (GRAMS) OF EQUINE DORSAL CRICOARYTENOID MUSCLES

		Number of horses	Mean Muscle Weights		
			Both sides	Left	Right
"NORMAL" HORSES	all ages	41	6.49	6.44	6.55
	three years and under	27	4.73	4.75	4.71
	over three years	14	8.96	8.79	9.13
ABNORMAL HORSES	all ages	5	7.49	4.60	10.38
	three years and under	2	7.5	5.87	9.14
	over three years	3	7.47	3.72	11.21

3.3.2.2 Lateral Cricoarytenoid Muscle

The number of horses from which muscles were collected and weighed, their mean muscle weights, and the mean weights of the left and right lateral cricoarytenoid muscles from the different groups of horses are shown in Table 5.

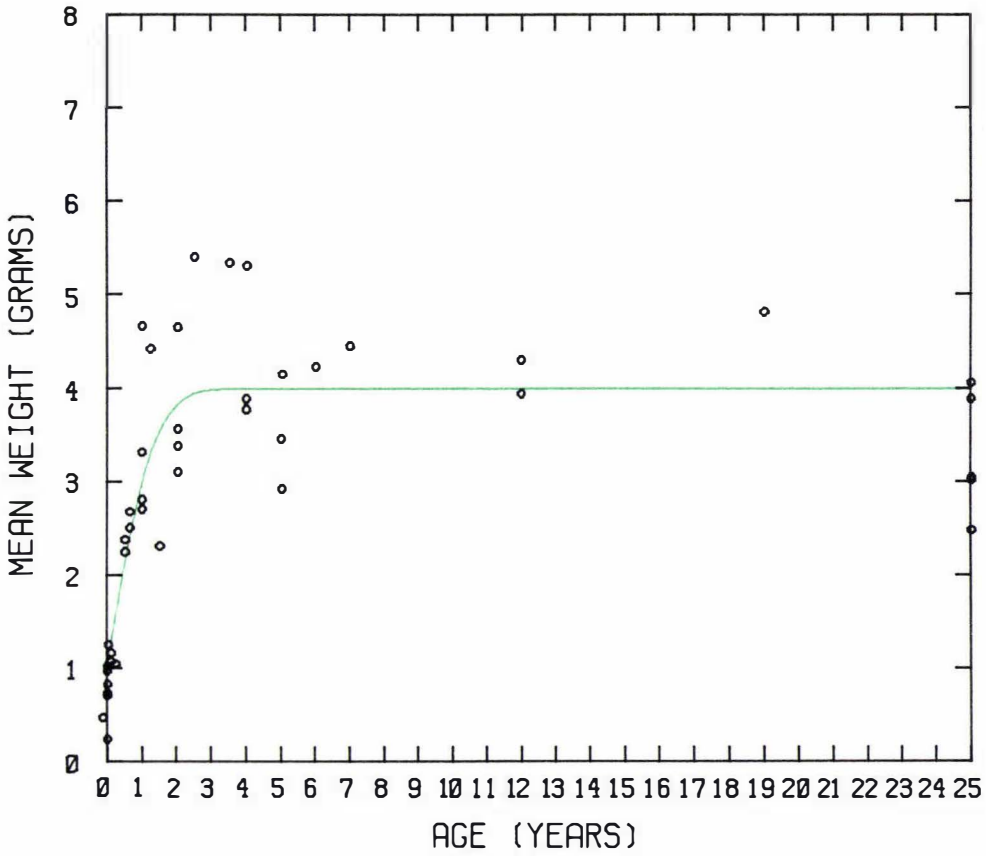
Again the lateral cricoarytenoid muscle increased in weight with age (Fig. 17). For this reason there were also significant differences between the mean lateral cricoarytenoid muscle weights of the different aged groups of horses.

FIGURE 17 The relationship between age (years) and the mean weights of the left and right lateral cricoarytenoid muscles (grams) of the "normal" horses

FIGURE 18 The weights of the left lateral cricoarytenoid muscles (grams) plotted against the weights of the right muscles

o = "normal" horses three years and under
+ = " " over three years

o = abnormal horses three years and under
+ = " " over three years


MEAN WEIGHT (GRAMS)
AGE (YEARS)
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0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

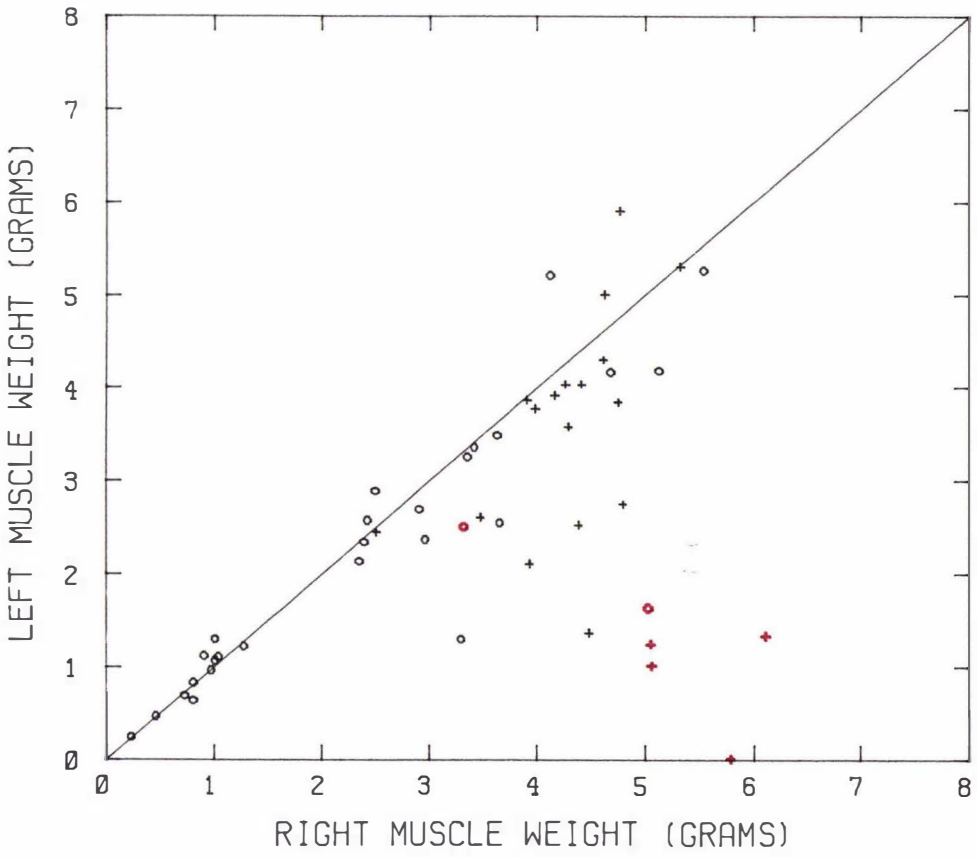

LEFT MUSCLE WEIGHT (GRAMS)
RIGHT MUSCLE WEIGHT (GRAMS)
0 1 2 3 4 5 6 7 8

TABLE 5 THE MEAN WEIGHT (GRAMS) OF EQUINE LATERAL CRICOARYTENOID MUSCLES

		Number of horses	Mean Muscle Weights		
			Both sides	Left	Right
"NORMAL" HORSES	all ages	41	2.99	2.8	3.17
	three years and under	27	2.32	2.23	2.41
	over three years	14	3.92	3.6	4.23
ABNORMAL HORSES	all ages	5	3.27	1.37	5.16
	three years and under	2	3.12	2.06	4.17
	over three years	3	3.30	1.19	5.41

The muscles from abnormal horses over three years of age were lighter than those from the same group of "normal" horses ($P < 0.001$).

The mean weights of the left lateral cricoarytenoid muscles were significantly lighter than those of the right muscles from the "normal" horses over three years of age ($P < 0.025$). When these mean weights were divided according to the sex of the horses the left muscle was significantly lighter than the right in the geldings ($P < 0.02$) but not the mares.

The left muscles were also lighter than the right muscles in all the abnormal horses. These side differences in muscle weights are illustrated in Fig. 18.

3.3.2.3 Transverse Arytenoid Muscle

The number of horses from which muscles were collected and weighed, their mean muscle weights and the mean weights of the left and right transverse arytenoid muscles are shown in Table 6.

TABLE 6 THE MEAN WEIGHTS (GRAMS) OF EQUINE TRANSVERSE ARYTENOID MUSCLES

		Number of horses	Mean Muscle Weights		
			Both sides	Left	Right
"NORMAL" HORSES	all ages	4	2.24	2.15	2.33
	three years and under	2	2.37	2.45	2.29
	over three years	2	2.11	1.85	2.38
ABNORMAL HORSES	three years and under	2	2.41	1.99	2.84

The mean transverse arytenoid muscle weight was less in the older age group of "normal" horses, than in the younger group. The left muscles of the "normal" horses were lighter than the right in the older age group. The left muscles from both the abnormal horses were lighter than the right.

3.3.2.4 Ventricular Muscle

The number of horses from which muscles were collected and weighed, their mean muscle weights and the mean weights of the left and right ventricular muscles are shown in Table 7.

TABLE 7 THE MEAN WEIGHTS (GRAMS) OF EQUINE VENTRICULAR MUSCLES

		Number of horses	Mean Muscle Weights		
			Both sides	Left	Right
"NORMAL" HORSES	all ages	5	2.56	2.56	2.56
	three years and under	2	2.77	2.58	2.98
	over three years	3	2.44	2.55	2.33
ABNORMAL HORSES	three years and under	2	2.54	1.94	3.16

As with the transverse arytenoid muscle, the ventricular muscle was lighter in the older than younger age group of "normal" horses.

In the abnormal horses the left ventricular muscles were lighter than the right.

3.3.2.5 Cricothyroid Muscle

The number of horses from which muscles were collected and weighed, their mean muscle weights and the mean weights of the left and right cricothyroid muscles are shown in Table 8.

TABLE 8 THE MEAN WEIGHTS (GRAMS) OF EQUINE CRICOTHYROID MUSCLES

		Number of horses	Mean Muscle Weights		
			Both sides	Left	Right
"NORMAL" HORSES	all ages	37	4.27	4.17	4.40
	three years and under	26	3.20	3.17	3.24
	over three years	11	5.86	5.79	5.92
ABNORMAL HORSES	all ages	5	6.41	6.00	6.82
	three years and under	2	5.91	5.52	6.30
	over three years	3	6.54	6.12	6.95

The only significant differences between the mean weights of the muscles from the different groups of horses were those which could be attributed to the increase in the weight of the cricothyroid muscle with age. This increase is illustrated in Fig. 19.

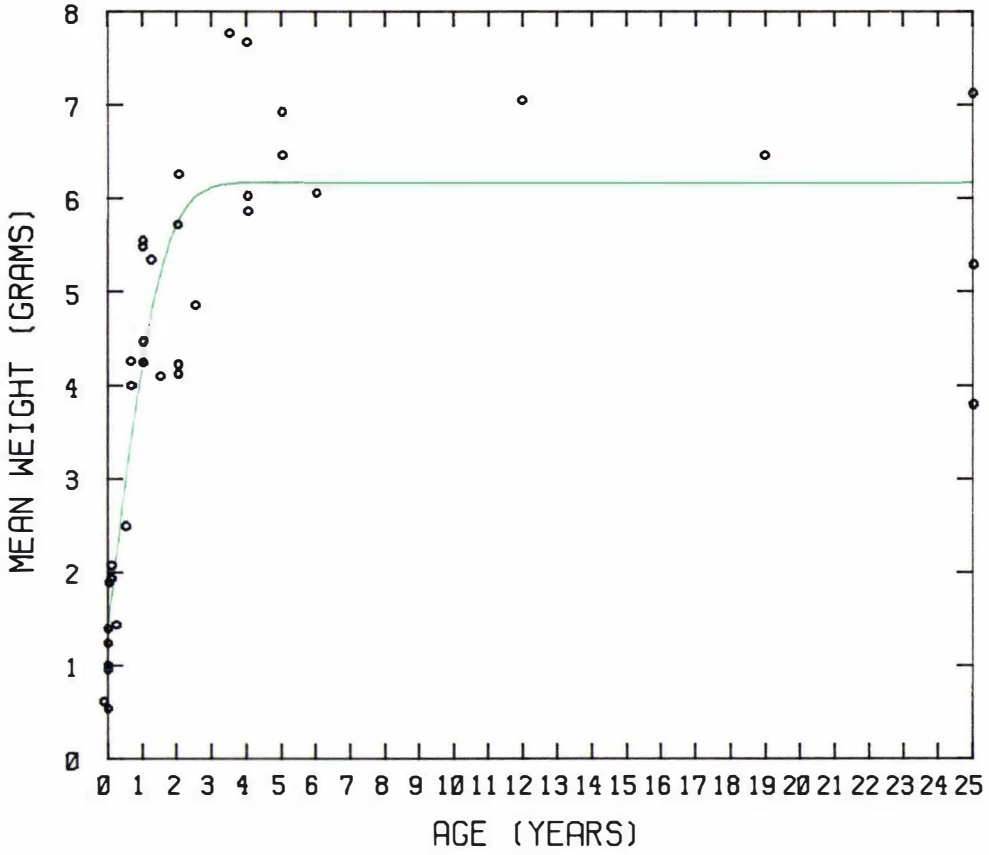
No significant differences in the mean weights of the left and the right cricothyroid muscles were observed. The weight of the left muscles plotted against the weight of the right muscles is illustrated in Fig. 20.

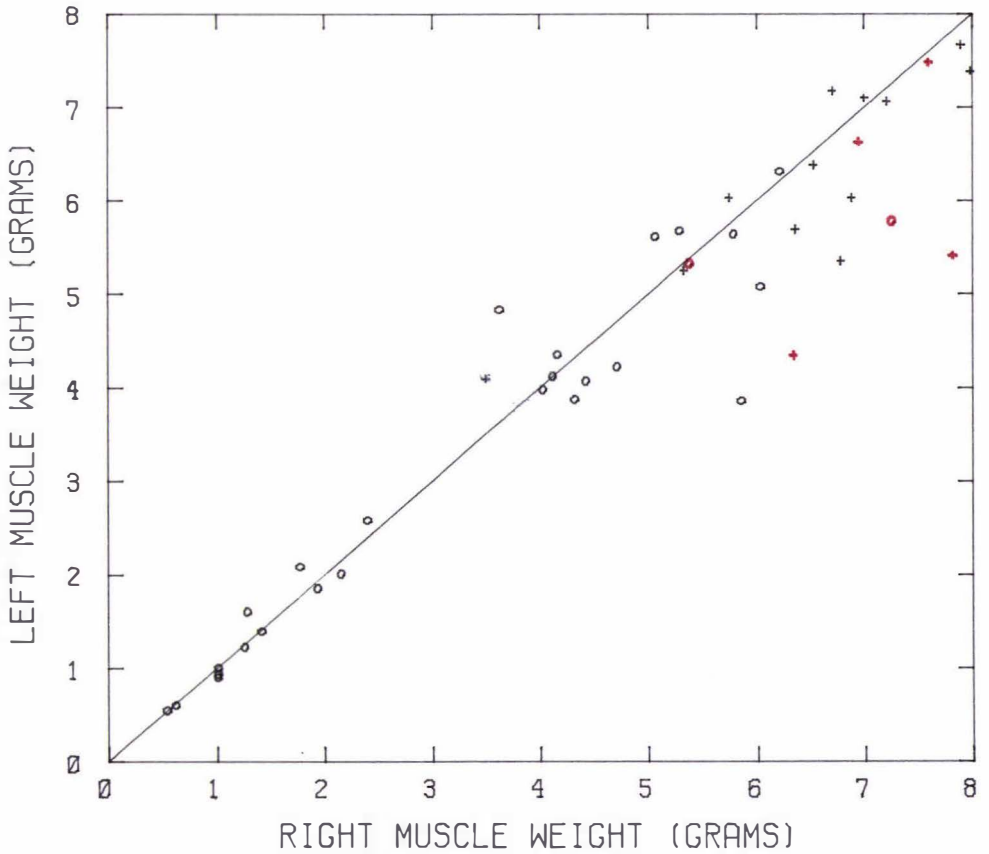
FIGURE 19 The relationship between age (years) and the mean weights of the left and right cricothyroid muscles (grams) of the "normal" horses

FIGURE 20 The weights of the left cricothyroid muscles (grams) plotted against the weights of the right muscles

o = "normal" horses three years and under
\+ = " " over three years

⊙ = abnormal horses three years and under
✚ = " " over three years


MEAN WEIGHT (GRAMS)
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
AGE (YEARS)


LEFT MUSCLE WEIGHT (GRAMS)
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
RIGHT MUSCLE WEIGHT (GRAMS)

3.4 Discussion

The reason it was considered important to measure gross muscle weights in this survey was that although they do not detect the earliest stages of neurogenic atrophy they are a measure of the state of the whole muscle. The histological and histochemical techniques used in the following chapters are much more sensitive indicators of neurogenic disease but involve examination of only small samples of each muscle.

Difference in weights beyond the experimental error between left and right muscles may result from either atrophy or hypertrophy. In the case of the equine intrinsic laryngeal muscles, Cole (1946) and Duncan et al. (1974) have shown that this difference is likely to be due to atrophy of the left muscles.

The present study has demonstrated that atrophy of some of the intrinsic laryngeal muscles innervated by the left recurrent laryngeal nerve is common in New Zealand Thoroughbred horses with no history of laryngeal hemiplegia. This atrophy however is much more severe in the same muscles of horses affected by this disorder. Cole (1946) and Duncan et al. (1974) have demonstrated that the atrophy results from neurogenic disease.

Attempting to measure atrophy by weighing muscles is not particularly accurate as some of the small differences in weight between the left and right muscles could have been due to experimental error. A reasonable estimate of the degree of this error can be obtained from the weights of the cricothyroid muscles of the "normal" horses where neither atrophy nor hypertrophy should occur. In this muscle approximately the same number of left muscles were lighter than the right as were right muscles lighter than the left. The weight difference between sides was also of the same order and averaged less than 10% in both cases. Even allowing for an error of this magnitude, the muscle most affected by atrophy, the left lateral cricoarytenoid, was found to be lighter than its right counterpart in nearly half (44%) of the horses with no history of upper respiratory disease. The difference was significant in "normal" horses over three years of age and when the data from these animals were statistically analysed on the basis of sex it was the

geldings which had significantly lighter left than right muscles. This finding is of interest in light of the statistical evidence provided by Goulden and Anderson (Appendix 9) which indicated that laryngeal hemiplegia more commonly affected males than females.

In addition to the lateral cricoarytenoid muscle two other laryngeal adductor muscles were weighed. These muscles, the transverse arytenoid and ventricular were examined in too few animals to accurately appraise the degree of atrophy present. However in both the abnormal horses studied the left muscles were considerably lighter than the right.

The weight difference between sides of the abductor muscle, the dorsal cricoarytenoid, was not so obvious as in the adductors in either "normal" horses or laryngeal hemiplegics. In the former the older horses tended to have lighter left than right muscles. This possible age relationship to atrophy could explain the apparent confusion in the literature resulting from the observations of Gunn (1972) and Quinlan et al. (1975). Gunn who studied predominantly young horses found no side differences between weights of a series of dorsal cricoarytenoid muscles while Quinlan et al. studied predominantly older horses and did find a significant side difference in the weight of this muscle.

In laryngeal hemiplegic horses the weight difference between left and right dorsal cricoarytenoid muscles was marked. In all cases the left muscle being lighter than the right. It is the inefficient function of this muscle which produces the clinical signs of roaring and it is interesting to note that these clinical signs may occur when the weight difference between left and right sides is as little as 24%. This finding differs somewhat from that of Cole (1946) who considered that at least a 50% atrophy of the left dorsal cricoarytenoid muscle must occur before clinical signs of laryngeal hemiplegia were observed. While Cole did not reveal the ages of the horses he studied it is possible that they were older animals, as in the present study, the older horses with laryngeal hemiplegia had a difference in weight between left and right dorsal cricoarytenoid muscles in excess of 50%.

The finding that in both "normal" and abnormal horses the left sided atrophy was more obvious in the lateral than the dorsal cricoarytenoid

muscle supports Duncan et al's (1974) observation that the adductor muscles showed greater pathological changes than the abductor. In this respect the results of the present study are contrary to those of Cole (1946) and Semon's Law.

The side difference in weights of the cricothyroid muscle differed somewhat from those of the adductor and abductor muscles. Since the cricothyroid muscle is not innervated by the recurrent laryngeal nerve it was not surprising that in "normal" horses the weights of this muscle on each side were similar. Such an observation had previously been made by Cole (1946), Quinlan et al. (1975) and Quinlan (1976). What was unexpected however was that in laryngeal hemiplegic horses the left cricothyroid muscle tended to be lighter than the right. Although, probably because of the small number of animals involved, this difference was not statistically significant, it occurred in all five animals examined. This difference, if real, is not likely to be due to neurogenic atrophy of this muscle. Previous researchers have not demonstrated the presence of neurogenic atrophy in the cricothyroid muscle of laryngeal hemiplegic horses (Cole, 1946; Duncan et al. 1974). The weight difference could perhaps result from the changed mechanics of the abnormal larynx. Possibly right sided cricothyroid hypertrophy occurs in response to the greater requirement for right sided laryngeal action, once left arytenoid abduction becomes ineffective. Further investigation of this muscle in abnormal horses seems indicated.

Another trend which was noted in the abnormal horses was that their right intrinsic laryngeal muscles were heavier than those from similar aged "normal" horses. This may have been because:-

a) laryngeal hemiplegics tend to be larger than normal horses (Appendix 9); or
b) right sided muscular hypertrophy may occur as a compensation for left atrophy; or
c) both of these factors may have contributed to the observed weight differences.

The fact that atrophy of some left intrinsic laryngeal muscles is common could mean that laryngeal functional abnormalities may also occur often. Baker (1983) noted this and considered that asynchronous

movements of the arytenoid cartilages occurred so frequently that they must be normal. The results of the present study however suggest the possibility that the common presence of asynchronous movements of the laryngeal cartilages could be associated with equally common unilateral atrophy of some of the intrinsic laryngeal muscles.

For a number of years percutaneous palpation of the muscular process of the arytenoid cartilage has been used by clinicians as an aid in the diagnosis of left laryngeal hemiplegia (Marks et al., 1970). From the results of the present study it seems likely that the very severe atrophy which occurs in the left lateral and dorsal crico-arytenoid muscles of older roarers could be distinguishable percutaneously. However confusion could arise when attempts are made to differentiate between "normal" horses and young roarers early in the course of the disease.

Apart from indicating the degree of muscle atrophy which occurs in some left intrinsic laryngeal muscles in both "normal" and laryngeal hemiplegic horses this study has provided information on the normal increase in weight with age of most of the intrinsic laryngeal muscles of the Thoroughbred. The results show that these muscles reach their adult weight when horses are between three and four years of age.

CHAPTER 4 THE HISTOCHEMISTRY OF SOME EQUINE LARYNGEAL MUSCLES

4.1 Introduction

Various staining procedures have been used to demonstrate the levels of a number of different enzymes in individual muscle fibres. Some of these procedures form the basis of classification systems for describing different types of fibres present in a muscle. These fibre types can be classified according to the level of a particular enzyme they contain. It is possible using one or a combination of enzyme stains to define for a muscle the staining characteristics of its individual fibres, the normal fibre types present, and their proportions and spatial distributions. Once this information is available for normal muscle it is possible to use these histochemical techniques to investigate various disease processes which may occur in that muscle.

The information which was available when this study was undertaken suggested that histochemical techniques (particularly that for demonstrating the activity of myosin adenosine triphosphatase) were useful for detecting the changes resulting from denervation and reinnervation in equine laryngeal muscle. Gunn (1972, 1973) and Duncan et al., (1974) had applied these techniques to some intrinsic laryngeal muscles from clinically unaffected and laryngeal hemiplegic horses and they described the fibre types and their proportions which occurred. It appeared that neurogenic changes were common in some of the muscles from both groups of horses. Histochemical techniques were also shown to demonstrate changes in fibre architecture which resulted from denervation and reinnervation occurring during idiopathic laryngeal hemiplegia (Gunn, 1973; Duncan, 1975).

Part of the present study involved an investigation of the fibre types and their proportions present in the intrinsic laryngeal muscles of a large number of "normal" horses of a single breed, the New Zealand Thoroughbred and a small number of ponies. It was then possible to compare these with the fibre types and proportions present in the muscles of idiopathic laryngeal hemiplegic horses. In order to understand the concept of histochemical fibre type it is helpful to have

some knowledge of the motor unit. For this reason a brief review of the subject is included.

4.1.1 The Motor or Muscle Unit Concept

The number of axons making up the efferent supply to a muscle depends on the size and function of that muscle and on the number of muscle fibres innervated by each large motor axon (innervation ratio). The innervation ratio of equine laryngeal muscles is not recorded but in human laryngeal muscles 100-150 muscle fibres are innervated by each motor axon (Buchthal and Schmalbruch, 1980).

As a peripheral nerve approaches the muscle it innervates, the axons begin to branch and do so at a greater rate with increasing proximity to the muscle. Most of the axonal branching takes place within the muscle body. The axons travel into the muscle along connective tissue septa and those travelling together tend to innervate adjacent areas of the muscle (Burke, 1980).

The intramuscular nerve trunks form an intricate plexus and there is much mixing of nerve fibres during their distribution within the muscle. The main trunks give off a number of secondary branches which form a complex interweaving pattern. These secondary branches in turn produce a number of tertiary branches each of which ends in a fine spray of nerve fibres. Each twig of these sprays comes into intimate contact with an individual muscle fibre at the motor end plate (Feindel et al., 1952).

The end plates of adjacent muscle fibres tend to be grouped together but they are usually not supplied by twigs of the same axon (Feindel et al., 1952). As a general rule, a single muscle fibre has a single end plate. When a muscle fibre has more than one end plate it is usual for them to be supplied by the same axon (Feindel et al., 1952). This is often the case in laryngeal muscles (Rossi and Cortesina, 1965).

A single axon provides the motor supply to a variable number of muscle fibres which are not located adjacent to each other within the body of the muscle. The spatial distribution of these muscle fibres is best

understood in terms of the functional unit, the so-called "motor unit" a term introduced by Sherrington in 1929 (Burke, 1980) to designate the combination of a motor neurone and the set of muscle fibres innervated by it. This set of muscle fibres is sometimes referred to as the "muscle unit" (Burke, 1967).

Each muscle unit occupies an area or territory within the body of a muscle. All muscle fibres within this territory do not belong to the same muscle unit. The fibres of a single muscle unit have a relatively random distribution throughout this territory (Burke and Tsairis, 1973) although its fibres do tend to be more numerous at the centre of the territory than at the periphery. This arrangement ensures that adjacent muscle fibres usually belong to different muscle units although fibres of the same muscle unit have been observed in groups of two, four and occasionally six fibres (Buchthal and Schmalbruch, 1980).

A primary fascicle of the cat gastrocnemius muscle contains 15-90 individual muscle fibres. These fascicles usually contain two to five fibres of the same muscle unit (Burke and Tsairis, 1973). If, as in this muscle the number of muscle fibres in a muscle unit is within the range of 570-760 (Burke and Tsairis, 1973), then the muscle fibres of a single muscle unit must be relatively widely dispersed throughout the body of a muscle. In fact, in the medial gastrocnemics muscles of the cat the territory of a single muscle unit can occupy 15-20% of the total muscle volume (Burke and Tsairis, 1973). In human laryngeal muscles the territorial arrangements of the muscle units are essentially similar but information on the territorial arrangement of muscle units in equine intrinsic laryngeal muscles is not available.

In an individual muscle unit the fibres tend to become more closely related to each other as an animal ages. This may be due to death of some motor neurones and re-organisation of the muscle unit (Burke, 1980). Changes in the spatial relationships of muscle fibres within a muscle unit so that they come to lie closer together, are much more profound however, as a consequence of denervation and reinnervation (Chapter 5).

The muscle fibres of an individual muscle unit have the same physiological

properties with respect to their speed of contraction, their resistance to fatigue and their tension output. They also have the same histochemical profile (Burke, 1980).

4.1.2 Fibre Types

4.1.2.1 Some Systems of Typing Muscle Fibres

Certain skeletal muscles and parts of the same muscle differ from each other in their gross colouration and this difference is a reflection of the predominant fibre type they contain. A few muscles are homogenous in their fibre type, but these are unusual. Most mammalian muscles are made up of a number of different types of muscle fibres and although the fibres of the same type have similar physiological and biochemical properties, there is a wide variation of properties between types.

Muscle fibres can be classified in many ways and it is rationalising these different classification systems which has lead to much confusion. A system of classification for a muscle of one species is not necessarily suitable for other muscles of the same species, or the same muscle of another species. Histochemical classification has also suffered from problems of nom enclature as this has differed depending on which enzyme system was being used (Buchthal and Schmalbruch, 1980). The fact remains however that there are certain fundamental differences between muscle fibre types and these differences are reflected in many of the properties.

Skeletal muscles are basically mixtures of two types of fibres -

i) The "red fibres", so called because of their abundant myoglobin, are of smaller diameter than,

ii) the "white fibres" which have a relatively low content of myoglobin.

Fibres intermediate between these two do occur (Ham, 1974; Barnard et al., 1971; Edgerton and Simpson, 1969).

It has been known for many years (Ranvier, 1874) that there is a correlation between muscle colour and contraction speed, red fibres having lower contraction speeds than white. The two types also differ in their resistance to fatigue and the twitch tension they are capable of developing. The fast white fibres can develop greater twitch tensions

but fatigue more readily than the slower, red fibres (Buchthal and Schmalbruch, 1980; Burke, 1980).

The physiological differences are reflected in different histological properties of the fibres. The red fibres are abundantly supplied with capillaries (Romanul, 1965) and their sarcoplasm is heavily populated with mitochondria which are found along the periphery of the fibres and packed longitudinally between the myofibrils (Ham, 1974). White fibres on the other hand are less abundantly supplied with capillaries and have relatively few mitochondria.

Mitochondria have been assigned the function of housing the enzymes of oxidative metabolism (Ham, 1974). If a histochemical stain which localises one of these enzymes (e.g. succinate dehydrogenase) is used, its distribution within a muscle cell reflects the distribution of mitochondria (Romanul, 1965).

The red fibres have a well developed blood supply and a small diameter so that there is efficient diffusion to the centre of the fibre (Davies, 1973). They are well supplied with the oxygen carrying pigment myoglobin and the enzymes of aerobic metabolism. Again, using histochemical methods, it is possible to demonstrate the activity of the enzymes of anaerobic metabolism in muscle fibres, one of these enzymes is glycogen phosphopylase. As a general rule the white fibres have a higher level of these enzymes than red fibres (Table 9).

The energy for driving the contractile process of skeletal muscle is produced by enzymatic splitting of the high energy phosphate, adenosine triphosphate (ATP). One of the enzymes involved is myosin ATPase and again the levels of this enzyme can be demonstrated histochemically in muscle fibres. It is generally accepted that the level of activity of this enzyme in a muscle fibre is directly proportional to the speed with which that fibre can contract (Bárány, 1967), the fast contracting white fibres having higher levels of this enzyme than the slower contracting red fibres (Table 9). Production of ATP can take place in a muscle cell utilising either aerobic or anaerobic metabolism. The former process is approximately 12 times as efficient but requires immediate oxygen and is a relatively slower process than the latter. Anaerobic metabolism produces ATP quickly without the immediate need

TABLE 9 SUMMARY OF THE PROPERTIES OF MUSCLE FIBRES (AFTER BURKE, 1980; BUCHTHAL AND SCHMALBRUCH, 1980)

	RED	WHITE
Muscle fibre types	High levels of myoglobin and mitochondria	Less myoglobin and mitochondria
	Well supplied with capillaries	Poorer capillary supply
	Best suited for aerobic metabolism	Best suited for anaerobic metabolism
	Slow contraction time	Fast contraction time
	Low tension output	High tension output

	TYPE I	Type II*		
		A		B
Motor unit types*	Slow	Fast	Fast	Fast
	fatigue resistant	fatigue resistant	moderately fatigue resistant	fatigue susceptible
Histochemical profile	myofibrillar ATPase alkaline preincubation (pH 9.4) low acid preincubation (pH 4.3) high	high low	high low	high low
	oxidative enzymes (e.g. succinate dehydrogenase) high	medium-high	medium	low
	enzymes of anaerobic metabolism (e.g. glycogen phosphorylase) low	high	high	high

* Dubowitz and Brooke, 1973.

* Burke et al., 1973

for oxygen but an oxygen debt develops and so the process cannot be continuous and the muscle fibre fatigues (Bradley, 1981).

In its simplest interpretation a muscle which is composed predominantly of red fibres, is slow contracting, has a relatively low tension output, is best suited to aerobic metabolism and so is capable of sustained activity without fatigue. These are the properties required of postural muscles. A muscle which has a high proportion of white fibres, is fast contracting, generates high tension outputs, is best suited to anaerobic metabolism and so is susceptible to fatigue. These muscles are best suited for short duration powerful contractions such as propelling muscles of a shot putter (Bradley, 1981).

4.1.2.2 Physiological Influences on Fibre Type

Muscle fibres have certain predictable properties and these can be used to define a particular type. A problem arises however in that most of these properties cover a range of values and there is often overlapping between different fibre types (Burke, 1980). This applies particularly to the type of metabolism predominant within a muscle as this can change within certain limits in response to environmental factors. For example, in endurance training where sustained activity without fatigue is required, muscle cells can respond by increasing their aerobic capacity. Red fibres which already function with this type of metabolism respond by increasing their mitochondrial fraction and the quantity of aerobic enzymes within them. White fibres which were anaerobic can, to a limited extent, develop an aerobic metabolism. Muscle cell size increases moderately by an increase in myofibrillar volume (Bradley, 1981). Thus the type of metabolism and fatigue resistance of a particular muscle fibre are not inherently static properties. The intermediate fibre types (Table 9) which have been observed may well be fibres involved in this transition (Burke, 1980).

The evidence concerning the stability of the inherent twitch contraction time and myosin ATPase staining characteristics of a muscle fibre appears to be somewhat contradictory. Burke (1980) stated that there is no change in twitch contraction time of a muscle unit with hypertrophy, immobilisation atrophy or training. Buchthal and Schmalbruch (1980) also have stated that there is no change in myofibrillar ATPase

staining of fibres with endurance training. Burke (1980) suggested that physiological overload does not cause interconversion of fast and slow twitch muscle units. Davies (1973) however, stated that the contractile properties of a muscle can change without direct interference with the nerve supply. He also quoted work (Gutmann and Hajek, 1971; Guth and Yellin, 1971) which indicated that when the amount of isometric exercise performed by a muscle was increased, for example by eliminating the effect of its synergists, there was a decrease in its speed of contraction and a reduction in its level of myosin ATPase activity. Guy and Snow (1977) found that after 10 weeks training certain limb muscles of the horse underwent changes in the proportion of fibre types observed so that there was an increase in myosin ATPase high fibres at the expense of myosin ATPase low fibres.

Evidence is also available which indicates that changes within subgroups of myosin ATPase high fibres do occur with endurance training. Myosin ATPase high fibres can be divided into two subgroups A and B by acid preincubation (Table 9). The fibres of the A subgroup have greater levels of oxidative enzymes than those of the B subgroup. Endurance training increases A fibres at the expense of B fibres (Buchthal and Schmalbruch, 1980; Burke, 1980). Thus there appears to be some evidence that the contraction speed of a muscle fibre and its level of myosin ATPase are also not static properties even within the physiological range of functional demands.

There are changes in the proportion of myosin ATPase high and low fibres as a muscle ages. Davies (1973) cited evidence indicating an increase in the proportion of ATPase low fibres in certain muscles as an animal grows. He proposed that this histochemical change occurs in response to a demand on a muscle for prolonged isometric contraction proportional to the weight it must support. It appears that the relationship between speed of contraction and level of myosin ATPase observed in adult muscle may not be true for immature muscle. Neonatal muscle fibres are initially slow contracting and their contraction times quicken with age (Denny Brown, 1929; Buller et al., 1960). Some of these muscles however have higher proportions of myosin ATPase high fibres than their adult counterparts (Davies, 1973; Buchthal and Schmalbruch, 1980). This anomaly is thought to be due to the fact that the intense ATPase

reaction of some immature fibres results from the staining of mitochondrial ATPase as these fibres are densely populated with mitochondria (Guth, 1973). The ATPase activity of actomyosin isolated from immature muscle is in fact similar to that of the corresponding adult muscle (Guth and Samaha, 1972).

The preceding comments apply to physiological demands. Pathological or experimentally induced changes involving the motor neurones will dramatically alter the physiological and histochemical profiles of muscle units. Experimental nerve cross union, chronic stimulation with various pulse patterns and the reinnervation which occurs after pathological denervation will all result in restructuring of muscle units (Burke, 1980; Chapter 5).

4.1.3 Histochemical Profiles

To classify a muscle fibre using its histochemical properties it has become customary to construct a "histochemical profile" by applying different stains to the same fibre in a number of serial sections. The stains usually used are myosin ATPase to define the fibre's contraction speed, one of the oxidative enzymes (e.g. succinate dehydrogenase) to indicate the level of the fibre's aerobic metabolism and one of the glycolytic enzymes (e.g. glycogen phosphorylase) to indicate its anaerobic metabolism (Buchthal and Schmalbruch, 1980; Davies, 1973).

4.1.3.1 Myosin Adenosine Triphosphatase (Myosin ATPase)

Myosin ATPase is one of at least three ATPases which have been isolated from skeletal muscle fibres. ATPases are also found in association with the mitochondria and the sarcotubular system (Padykula and Gauthier, 1963). Myosin isolates from many different muscles have an ATPase activity which is actin and calcium activated (Bárány, 1967) and it is this ATPase which is thought to be responsible for releasing the chemical energy of ATP, thus making it available for mechanical work (Perry, 1954).

The histochemical procedure for demonstrating ATPase activity in a muscle fibre is based on Gomori's metal salt procedure for localising

phosphatase (Pearse, 1961; Culling, 1974). The dephosphorylation of ATPase releases orthophosphate which acts as the primary reaction product. Under alkaline conditions calcium ion can be used to trap the released orthophosphate. The calcium ion can then be displaced by cobalt and the resulting cobalt phosphate treated with ammonium sulphide to produce black cobalt sulphide, the visible final reaction product. When the method of Padykula and Herman (1955) is used, this dark final reaction product has a purely myofibrillar distribution (Brooke, 1973; Guth, 1973) and the interfibrillar network of mitochondria, transverse tubular system and sarcoplasmic reticulum is not visible. For this reason it is considered that the histochemical method is specific for myosin ATPase (Padkula and Herman, 1955; Guth and Samaha, 1969). When the incubating medium used in the reaction is alkaline most skeletal muscles are seen to have two distinct populations of fibres. Those fibres designated myosin ATPase high (AH, Type 11) stain intensely with a dense dark brown to black reaction product. These are distinct from myosin ATPase low fibres (AL, Type 1) which have much lower levels of reaction product and so are light brown to pale grey.

Barany (1967) showed that the ATPase activity of myosins isolated from a number of different muscles was directly proportional to the intrinsic speed of contraction of that muscle. Guth and Samaha (1969) demonstrated that the ATPase activity of actomyosin isolated from fast muscle of the cat was three times greater than that of actomyosin isolated from slow muscle. This evidence has been used to justify the designation of AH, mammalian extrafusal fibres as fast twitch and AL fibres as slow twitch (Davies and Gunn, 1972).

The difference in staining characteristics of fast and slow fibres using this histochemical reaction is a reflection of the difference in pH stability of myosin ATPase from fast and slow fibres. Fast myosin ATPase is alkali stable and acid labile while slow myosin ATPase is acid stable and alkali labile (Guth and Samaha, 1969). If the pH of the incubating medium is acid rather than alkaline the colouration taken up by most of the fibres is the reverse of that seen with alkaline incubation. This probably indicates a difference in the biochemical nature of the myosin ATPase of fast and slow muscle (Guth and Samaha, 1969). Indeed, the myosins of fast and slow muscle have been shown to

have different heavy chain configurations and it is possible to prepare specific antibody for two distinctly different myosins (Arndt and Pepe, 1975; Snow et al., 1981).

This difference in pH stability of myosin ATPase has been used in the classification systems of muscle fibre types. Most commonly these systems involve subdividing Type II fibres into a number of sub groups and there is some correlation here with the enzymes of oxidative metabolism (Brooke and Kaiser, 1970. Table 9). Type II fibres have also been subdivided on the basis of their formaldalyde sensitivity but the significance of this difference is not understood (Guth and Samaha, 1969). The systems of fibre typing then are clouded by inconsistencies in the definition of different types and lack of correlation between different systems of nomenclature (Brooke and Kaiser, 1970).

A fundamental question concerning the histochemical method for demonstrating myosin ATPase is that regarding its specificity (Padykula and Herman, 1955). Skeletal muscle mitochondria have been shown to have a high ATPase activity under the conditions prevailing during the histochemical procedure for myofibrillar ATPase (Guth, 1973). In fact Perry (1954) believed that 20-25% of the ATPase activity of muscle was associated with mitochondrial ATPase. Guth (1973) stated that the phosphate released by mitochondrial ATPase was localised on the myofibrils and not in the mitochondria, so that the reaction product demonstrated by the histochemical procedure for myosin ATPase can result from the activity of the ATPase of myofibrils, mitochondria or both. Mitochondrial ATPase is both alkali and acid stable (Samaha and Yuris, 1973) and so will appear in both Type I and Type II fibres depending on the number of mitochondria and their ATPase activity. For these reasons Guth (1973) contended that the intensity of the reaction product demonstrated by the histochemical procedure for ATPase may not be as directly related to the speed of contraction of a muscle fibre as the ATPase activity of its myosin.

The fact remains however that the muscle fibres of a muscle unit have the same histochemical profile (Edström and Kugelberg, 1968; Burke et al., 1973) and that the method for myosin ATPase provides a useful technique for visualising different fibre types. The main reason

for using this histochemical procedure in this study was to investigate the process of denervation and reinnervation in equine laryngeal muscles (Chapter 5). In this context the possible limitations of the procedure could be accepted and no attempt was made to classify fibres as anything other than Type I or Type II under conditions of alkaline preincubation.

4.1.3.2 Succinate Dehydrogenase (SDase)

Succinate dehydrogenase is one of the citric acid cycle enzymes and is involved in the conversion of succinate to fumarate which results in the production of two hydrogen ions i.e. it is an oxidation step (Pearse, 1961). In the normal reaction in the cell the hydrogen ions produced pass into the respiratory chain and become involved in the production of ATP (Davies, 1973). SDase is believed to be a purely intramitochondrial enzyme (Roodyn, 1967).

Colourless tetrazolium salts when introduced to this system may act as hydrogen ion acceptors and in the process are converted to blue diformazan, which is the final reaction product in the procedures using these salts. This conversion to diformazan may take place at the site of action of any dehydrogenase but SDase is the only dehydrogenase which does not require a coenzyme (Pearse, 1961). Under the conditions prevailing in the histochemical procedure used in this study, SDase should be the only enzyme demonstrated.

Individual mitochondria in a muscle cell may vary in their SDase activity but the overall density of diformazan deposited in a muscle fibre is thought to be a reflection of mitochondrial density rather than the actual level of activity of the enzyme (Stein and Padykula, 1962; Romanul, 1965; Davies and Gunn, 1972). The available evidence suggests however that there is a relationship between the biochemically determined level of SDase in a muscle and the density of its mitochondria (Holloszy, 1967). This evidence has been used to justify the assumption that the histochemical procedure for demonstrating the activity of SDase of a muscle fibre will give an indication of the aerobic capacity of that fibre (Davies and Gunn, 1972).

As with myosin ATPase a number of questions have been raised about the

specificity of the histochemical reaction. Hitzeman (1963) noted that a small amount of diformazan may be deposited away from the site of SDase activity (e.g. in lipid droplets). Seligman et al., (1967) reported an all or none deposition of diformazan on some individual mitochondria, an observation which could affect the interpretation of the SDase reaction. Again, as with myosin ATPase it has been shown that the intensity of SDase activity is very similar for the fibres of a single motor unit (Edström and Kugelberg, 1968).

The intensity of staining of a muscle fibre with diformazan and the pattern of its distribution have been used in some of the systems of fibre typing. Stein and Padykula (1962) have described three fibre types using this system. The tendency is for fibres high in myosin ATPase activity to be lower in SDase activity but this is not always consistent (Davies and Gunn, 1972).

The aerobic capacity of a muscle fibre as reflected by its SDase activity is not static but depends upon the amount and type of work performed by the muscle (Bradley, 1981). In muscles such as the equine intrinsic laryngeal muscles the level of SDase activity of their fibres probably varies with the state of training of the horse. The process of denervation and reinnervation which was thought to be common in some equine intrinsic laryngeal muscles when this study was undertaken will also influence the level of SDase activity of laryngeal muscle fibres (Chapter 5). As horses both in work and not in work were used in this study and, as it was expected that the processes of denervation and reinnervation would probably be relatively common in their muscles, it seemed that using the more complex systems of SDase fibre typing for these muscles would probably not be very meaningful. For these reasons a simple high (SH) or low (SL) classification was used for the activity of this enzyme.

4.1.3.3 Glycogen Phosphorylase (GPase)

Glycogen is the major source of energy for muscle contraction in the absence of oxygen and phosphorylation is the first step in glycolysis (Davies and Gunn, 1972). Of the two basic fibre types the larger diameter fibres with their lower content of oxidative enzymes tend to have higher levels of glycogen (Stein and Padykula, 1962)

and phosphorylase (Dubowitz and Pearse, 1960). Phosphorylase is one of the enzymes of the Embden-Meyerhof pathway, the metabolic route by which these fibres utilise glycogen during the short periods of rapid activity for which they are best suited (Dubowitz and Pearse, 1960).

Glycogen occurs as aggregates in the sarcoplasm and is concentrated near the myofibrillar bands of striated muscle (Stein and Padykula, 1962). The histochemical method for demonstrating phosphorylase in striated muscle indicates a similar distribution of this enzyme (Takeuchi and Kuriaki, 1955).

Takeuchi and Kuriaki (1955) showed that their histochemical method for phosphorylase was specific for the enzyme catalysing the successive phosphorylation of the terminal glucose units of the glycogen chain to produce glucose-1-phosphate. Insulin and adenosine-5-monophosphoric acid were added as activators for the reaction and it was found that with muscle it was not necessary to add glycogen as a primer. The method utilises the fact that the reaction is reversible to produce a polyglucose of the amylose type which becomes the reaction product. This product is demonstrated by iodine staining so that it becomes blue or blue black (Takeuchi and Kuriaki, 1955; Pearse, 1961). The synthesised polyglucose is distinguished from any native glycogen remaining after incubation by differences in the iodine staining characteristics, native glycogen stains red to red brown. The intensity of the blue colouration varies between and within fibres (Takeuchi and Kuriaki, 1955) and is used to indicate different levels of phosphorylase activity of individual fibres (Davies and Gunn, 1972).

The blue colouration fades with time and so the iodine staining must be repeated before each use of the sections. Pearse (1961) noted that thin sections (as used in this study) allow greater precision in localising the site of enzyme activity but staining can be patchy. The method will also demonstrate the activity of amylo-1,4-1,6-transglycosylase, but the inclusion of ethanol in the medium suppresses this enzyme (Pearse, 1961).

Phosphorylase is present in muscle as active phosphorylase A or its inactive precursor phosphorylase B. In resting muscle most phosphorylase is present in the B form but the A form increases rapidly with stimulation

(Bocek and Beatty, 1966) and also with freezing (Dubowitz and Pearse, 1960). There is some debate as to whether or not the histochemical method demonstrates the total or only the active phosphorylase content of muscle (Kugelberg and Edström, 1968) but the larger body of opinion supports the idea that it demonstrates a muscle's total content (Dubowitz and Pearse, 1960; Bocek and Beatty, 1966).

It appears reasonable to assume that the intensity of the blue colouration demonstrated by this histochemical method is directly related to the phosphorylase activity of an individual fibre and is a measure of the rate at which it can derive energy for contraction anaerobically (Davies and Gunn, 1972).

In the present study individual fibres were designated GPase high or low (PH,PL) depending on the relative intensity of their blue colouration.

4.1.4 Fibre Types in Equine Muscles and Laryngeal Muscles of Other Species

The discussion to this point has centred around the concepts of fibre type derived largely from the study of limb muscles (Table 9). The fibre types observed in those equine limb muscles which have been examined histochemically are similar to those described in other species. Guy and Snow (1976, 1977) and Essen et al., (1980) have studied a number of the upper limb muscles of both Thoroughbred and Standardbred horses. While the proportion of fibre types varied with age (Essen et al., 1980), state of training (Guy and Snow, 1977), and between muscles (Guy and Snow, 1977; Essen et al., 1980); and the two groups of workers used different classification systems, there was overall agreement as to the predominant fibre types present (Table 10).

TABLE 10 THE FIBRE TYPES IN SOME EQUINE LIMB MUSCLES

Fibre type	Guy and Snow,(1977)	Essen et al., (1980)
ALSHPL	20.0 ± 1.9%	13-31%
AHSHPH	44.9 ± 2.1%	69-87% predominantly AHSH
AHSLPH	35.1 ± 1.3%	

Studies on laryngeal muscles have indicated that their fibre type profiles are somewhat different to those observed in other skeletal muscles. Laryngeal muscles are in general fast contracting (Mårtensson and Skoglund, 1964; Hast, 1969) and those with the fastest contraction times have the highest proportions of AH fibres (Sahgal and Hast, 1974). Some, for example, the lateral cricoarytenoid and transverse arytenoid muscles of the primate larynx have almost exclusively AH fibres (Sahgal and Hast, 1974).

Laryngeal muscles also tend to have a high content of oxidative enzymes. Some are exclusively SH (Hall-Craggs,1968; Malmgrem and Gacek, 1981) while others demonstrate a checker board pattern when stained for SDase activity (Sahgal and Hast, 1974).

There appears to be some variation in anaerobic metabolism between the fibres of laryngeal muscles. Malmgrem and Gacek (1981) found that in the human posterior cricoarytenoid muscle the Type IIA fibres tended to have a higher level of GPase than the Type I fibres. In view of the work performed by the intrinsic laryngeal muscles it would be expected that their fibre types would allow them to contract rapidly, continuously, and efficiently without fatigue. The fibre types observed support these expectations.

The literature on fibre types in equine laryngeal muscles is by no means extensive. In fact only the reports of Gunn (1972, 1973) and Duncan (1975) and his co-workers (1974) were available prior to the commencement of this study. Gunn (1972) stated that of the possible combinations of fibre types, only four are found in normal equine muscle. These were AHSLPH,AHSHPH, ALSHPL and ALSHPH. In one horse, a two-year-old Thoroughbred filly, Gunn counted the fibre types present in the left dorsal cricoarytenoid muscle. He found that 72% of the fibres were AHSHPH; 27% of the fibres were ALSHPH and 1% of the fibres were ALSHPL. He concluded that fast twitch, low oxidative fibres (AHSLPH) did not occur in normal equine intrinsic laryngeal muscles. In a later publication Gunn (1973) reported on the fibre types in the laryngeal muscles of a full term equine foetus. In this animal he found similar fibre types to those present in normal adults although the overall density of the reaction products of the SDase and GPase reactions were less in foetal than in adult muscles.

Following Gunn's work, Duncan (1975) in a study of the pathology of equine intrinsic laryngeal muscles commented on the fibre types present. He found that a mosaic pattern was obtained with the myosin ATPase and GPase reactions. This mosaic pattern was not so obvious with the SDase reaction as all fibres tended to have a relatively high level of this enzyme. Duncan also found that most AH fibres were PH but that there was no consistent relationship so far as their SDase reaction was concerned. Although he did not quantitate fibre types he stated that there were approximately 60% AH and 40% AL fibres present. These findings indicated that most of the fibres of equine intrinsic laryngeal muscles, like those of other species have a well developed aerobic and anaerobic metabolism. In this way laryngeal muscles differ from most other skeletal muscles.

4.1.5 The Effect of Denervation on Fibre Type and Fibre Architecture

The common equine respiratory disease, idiopathic laryngeal hemiplegia, is a result of a continuous process of denervation and reinnervation of muscle fibres supplied by the recurrent laryngeal nerves (Duncan, 1975). As has been mentioned, histochemical techniques, because they can be used to demonstrate the metabolism of a muscle fibre, the distribution of fibres of a similar type, and some aspects of muscle fibre architecture, are useful for investigation of this disease.

The most obvious histochemical change which occurs in muscle early in the course of denervation and reinnervation is fibre type grouping. This phenomenon is particularly well demonstrated by the myosin ATPase reaction and will be discussed in Chapter 5. In addition to fibre type grouping a number of workers have noted that there is an increase in the number of intermediate fibres demonstrated by this reaction and have suggested that these are fibres in the early stages of reinnervation (Morris, 1970; Warszawski et al., 1975).

Experimental denervation has been shown to reduce oxidative and glycolytic activity of muscle fibres (Romanul and Hogan, 1965), although severely atrophied fibres may stain intensely for oxidative enzymes and lack phosphorylase activity (Engel et al., 1966; Dorman, 1973; Brooke, 1973). Abnormal fibre types resulting from reduced levels of activity of such enzymes have been noted in equine intrinsic laryngeal muscles.

Gunn (1972) observed the abnormal fibre types AHSHPL; ALSLPH and ALSLPL in the laryngeal muscles of a horse which was clinically unaffected with laryngeal hemiplegia. Fibre type grouping was also present in these muscles. He later reported his findings in the muscles of a horse suffering from laryngeal hemiplegia (Gunn, 1973). In the left dorsal cricoarytenoid muscle he found that 13% of the AH fibres were SL in contrast to normal muscle which contains no such fibres. Furthermore AL fibres which were SL (70%) and PL (29%) were much more numerous than fibres of these types seen in normal muscles. In the left transverse arytenoid muscle of the same horse all the fibres observed were AH while the corresponding right muscle contained some AL fibres.

Changes in fibre architecture which occur as a result of denervation are best demonstrated in sections stained for oxidative enzymes. Some of the affected fibres take up these stains in an abnormal manner so that there is a relatively unstained central zone, a heavily stained intermediate zone and a relatively normal outer zone. Such fibres have been referred to as "target fibres" because the unevenly stained zones resemble the rings of a target. The staining characteristics of these fibres correspond to changes in mitochondrial density; the central zone has disorganised myofibrillar material with no mitochondria and the intermediate zone has increased numbers of mitochondria (Buxton, 1980). These fibres are most common in muscles recovering from denervation (Brooke, 1973). They are however, not observed in completely denervated muscle (Tomonaga, 1969). Duncan et al. (1974) noted the presence of occasional target or target-like fibres in the laryngeal muscles of laryngeal hemiplegic horses. They also noted the presence of fibres with central areas of high oxidative activity and stated that they had seen these in completely denervated equine and canine laryngeal muscles. Fibres with a similar high activity of SDase centrally had been reported in a laryngeal hemiplegic horse by Gunn (1973).

Another abnormality of oxidative enzyme staining of muscle fibres in neurogenic disease is the "moth-eaten-whorled fibre". Here the regular ordered network between myofibrils stains irregularly so that the fibre assumes a moth-eaten appearance (Brooke, 1973). These fibres have not been recorded in equine laryngeal muscles.

4.2 Materials and Methods

The details of the muscles collected, the methods of preparation and the histochemical techniques used are described in Chapter 2 and Appendices 1-3.

The number of horses from which muscles were collected and studied are shown in Table 11. The vocal muscles from 2 horses (numbers 6 and 20) were also examined.

4.3 Results

Serial sections of a dorsal cricoarytenoid and cricothyroid muscle showing staining patterns characteristic of those obtained with each of the enzyme reactions when used on "normal" muscle during this study are shown in Figures 21 and 22.

4.3.1 The Predominant Fibre Types Found and Their Proportions

4.3.1.1 Dorsal Cricoarytenoid Muscle

4.3.1.1.1 "Normal" Horses

The fibre types and their proportions observed in the dorsal cricoarytenoid muscles from all the "normal" horses are shown in Table 12. The proportion of these fibre types did not vary significantly in the muscles from the left and right sides or in those from groups of horses of different ages and sexes.

4.3.1.1.2 Abnormal Horses

When the muscles of the abnormal horses were taken as a group there appeared to be a reduction ($P < 0.1$) in the proportion of AH:AL fibres (60%:40%) when compared with the "normal" horses. This was particularly noticeable in the left muscles of the younger horses where the proportion was 50%:50%. It was however, not present in the older abnormal horses where an increase in the proportion of AH:AL fibres (79%:21%) occurred.

The difference between the phosphorylase activity of the muscles from the two age groups of abnormal horses appeared to be opposite to that seen with the myosin ATPase reaction. The young abnormal horses had

TABLE 11 THE NUMBER OF HORSES FROM WHICH MUSCLES WERE STUDIED HISTOCHEMICALLY

	"Normal" horses three years of age and under	"Normal" horses over three years of age	Abnormal horses three years of age and under	Abnormal horses over three years of age	Total number of horses studied
Dorsal cricoarytenoid muscle	28	18	3	4	53
Lateral cricoarytenoid muscle	28	21	3	4	56
Transverse arytenoid muscle	3	3	3	1	10
Ventricular muscle	3	5	3	1	12
Hyoepiglottic muscle	8	7	3	1	19
Cricothyroid muscle	27	18	3	4	52

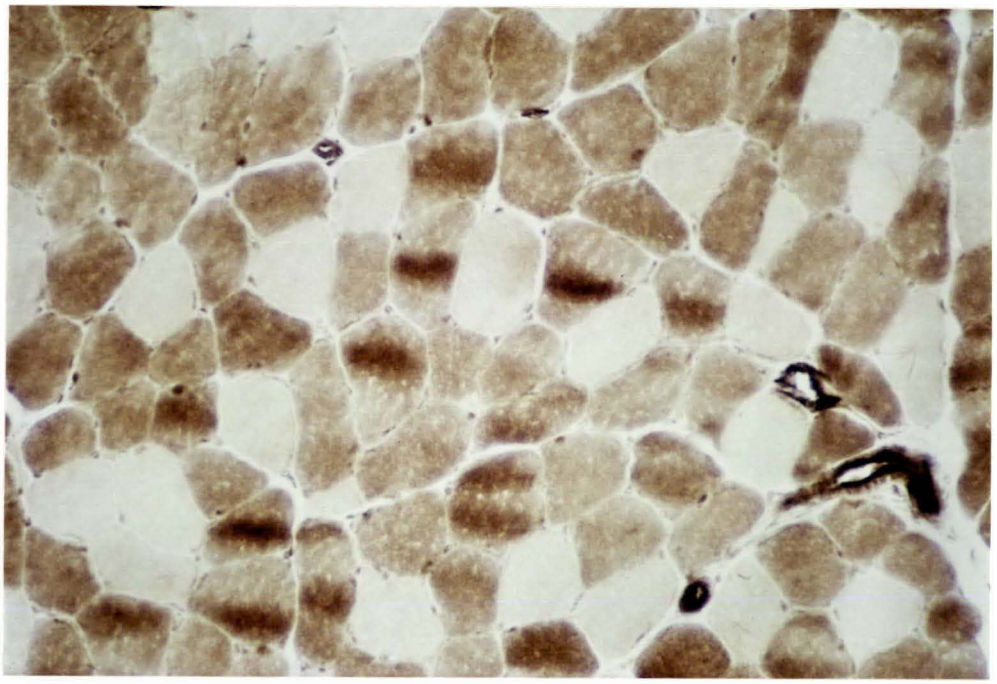
FIGURE 21 Transverse serial sections of the right dorsal cricoarytenoid muscle from a "normal" horse (x260). Sections stained to demonstrate the activity of:-

a. Myosin ATPase

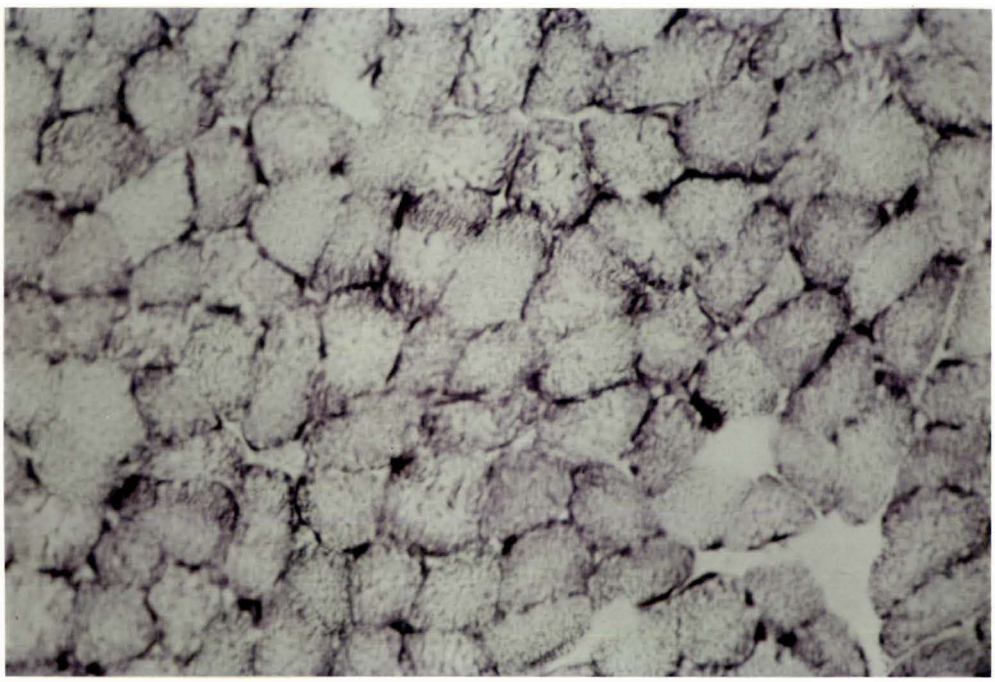
b. SDase

c. GPase

a



b



c

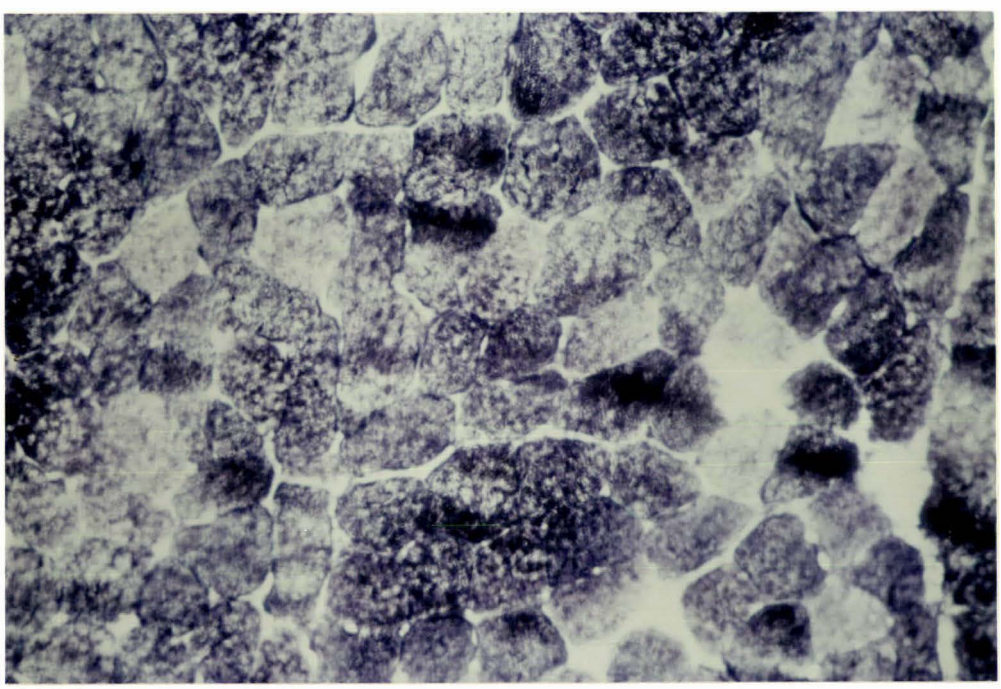


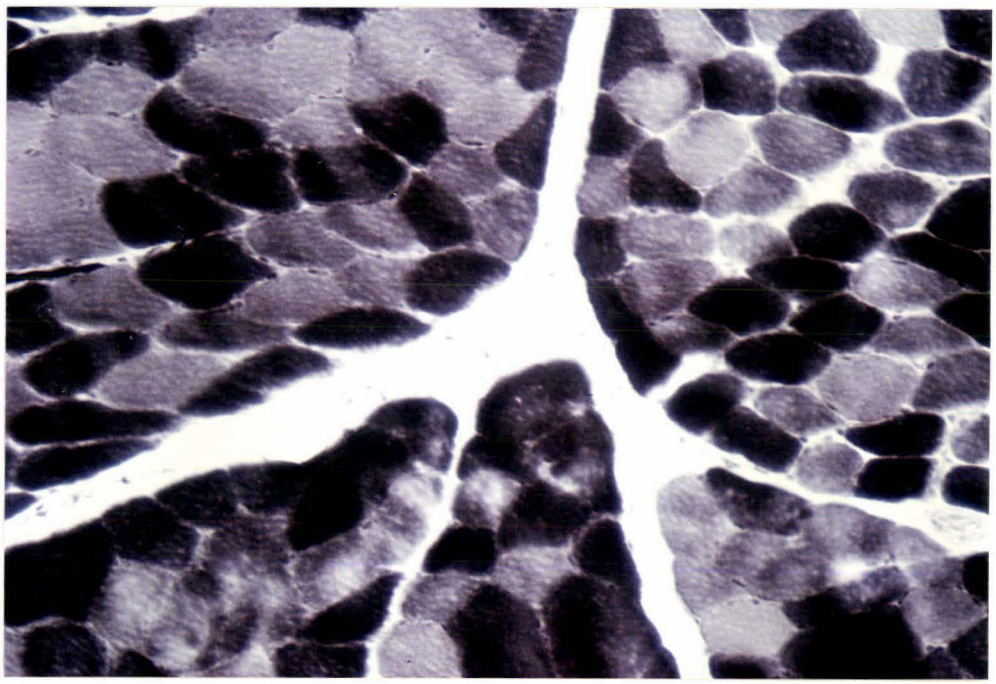
FIGURE 22 Transverse serial sections of the left cricothyroid muscle from a "normal" horse (x260). Sections stained to demonstrate the activity of:-

a. Myosin ATPase

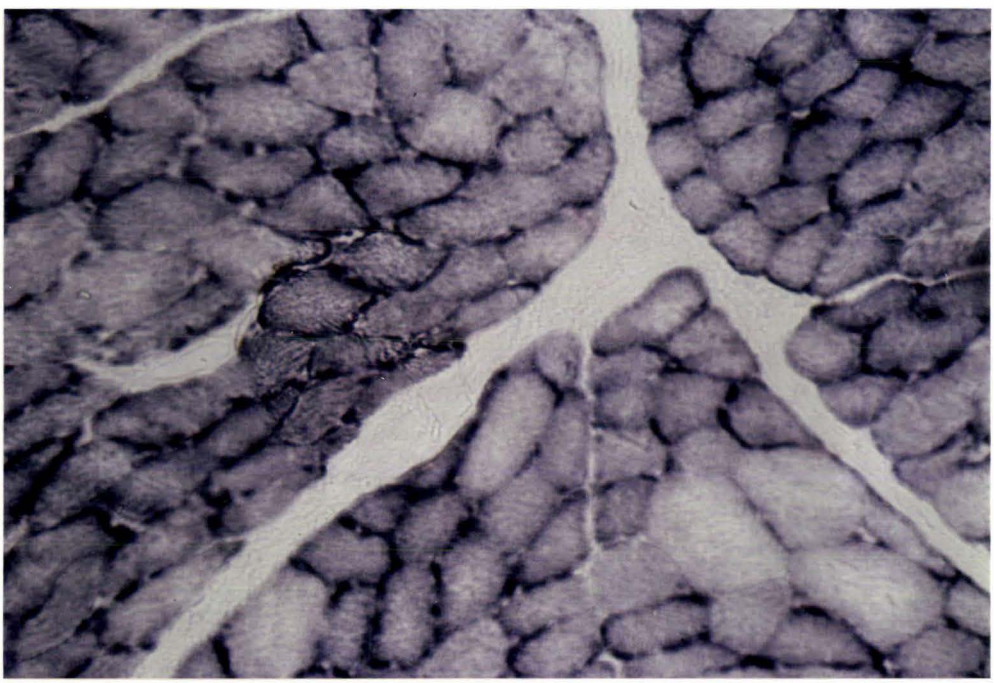
b. SDase

c. GPase

a



b



c

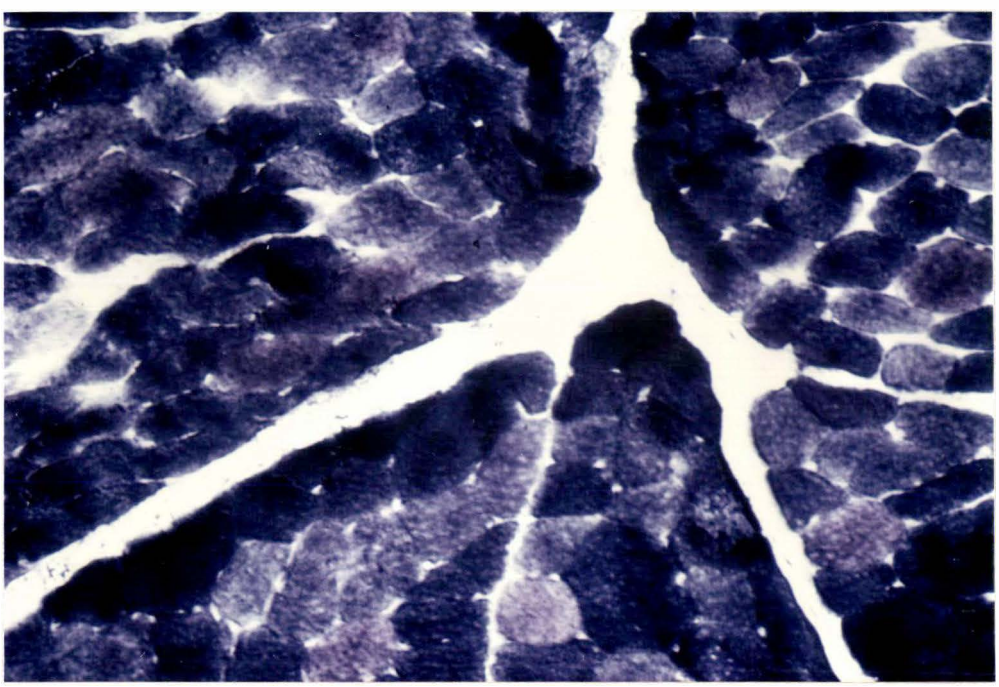


TABLE 12 FIBRE TYPE PROFILES OF LARYNGEAL MUSCLES FROM HORSES WITH NO HISTORY OF UPPER RESPIRATORY TRACT ABNORMALITIES

Muscle	Number of horses studied	Fibre types %		
		AH:SH:PH	AL:SH:PH	AL:SH:PL
Dorsal cricoarytenoid	46	69	23	8
Lateral cricoarytenoid	49	70	25	5
Transverse arytenoid	6	74	22	4
Ventricular	8	89	10.8	0.2
Vocal	2	77.5	22	0.5
Cricothyroid	46	64	29	7
Hyoepiglottic	15	46	42	12

a significantly ($P < 0.01$) higher proportion of PH:PL fibres (98%:2%) than the "normal" horses (75%:25%) and the older abnormal group had a significantly lower ($P < 0.02$) proportion (61%:39%) than their "normal" counterparts. These changes were also more marked in the left than the right muscles.

4.3.1.2 Lateral Cricoarytenoid Muscle

4.3.1.2.1 "Normal" Horses

Although the fibre types and their proportions observed in this muscle were very similar to those found in the dorsal cricoarytenoid muscle (Table 12), the older age group had a significantly ($P < 0.1$) lower proportion of AH:AL fibres (67%:33%) than the younger group (72%:28%). These changes were more marked in the left muscles.

4.3.1.2.2 Abnormal Horses

The changes in the proportions of AH:AL and PH:PL fibres in the lateral cricoarytenoid muscles were similar to those observed in the dorsal cricoarytenoid muscles. It was interesting to note that in the young abnormal horses no PL fibres were observed whereas in the older group PH:PL was 68%:32% compared to 84%:16% in the older "normal" horses ($P < 0.02$).

4.3.1.3 Transverse Arytenoid Muscle

4.3.1.3.1 "Normal" Horses

Again the fibre types and their proportions were similar in the transverse arytenoid muscles to the lateral cricoarytenoid muscles (Table 12).

Changes in the proportions of fibre types present in the different age groups were observed and were opposite to those seen in the lateral cricoarytenoid muscles. The ratio of AH:AL fibres (81%:19%) was significantly higher ($P < 0.02$) in the older horses when compared with the younger group (67%:33%). In addition, in the older group there were significantly fewer ($P < 0.05$) AH fibres in the left muscle (AH:AL, 79%:21%) when compared with the right (83%:17%).

There were also changes between the age groups in the proportion of PH:PL fibres. This proportion was significantly less ($P < 0.001$) in the older (73%:27%) than the younger group (95%:5%).

4.3.1.3.2 Abnormal Horses

The proportions of fibre types in the muscles from abnormal horses were not significantly different to those from the "normal" horses. There was a tendency however for the AL fibres from the abnormal horses to have a higher proportion of PH fibres than the "normal" horses. The muscles studied were from a small number of younger horses as the only older roarer available had a completely atrophied left transverse arytenoid muscle which was not suitable for histochemistry.

4.3.1.4 Ventricular Muscles

4.3.1.4.1 "Normal" Horses

The most notable features of the ventricular muscles were that the fibres were predominantly AH and PH (Table 12). As was the case in the transverse arytenoid muscle there was a significant increase ($P < 0.001$) in the proportion of AH fibres in the muscles from older horses (AH:AL, 96%:4%) when compared with those from the younger group (AH:AL, 75%:25%).

4.3.1.4.2 Abnormal Horses

The abnormal horses examined were again all in the young age group as the only older roarer from which ventricular muscles were collected had a left muscle which was so severely atrophied that it was not suitable for histochemical examination. In the muscles examined there was a significant increase ($P < 0.02$) in the proportion of AH:AL fibres (88%:12%) when compared to those from "normal" horses (75%:25%) and all the AL fibres were PH.

4.3.1.5 Vocal Muscles

The vocal muscles from two "normal" horses (six and 20) were examined histochemically. The fibre types observed and their proportions are shown in Table 12.

4.3.1.6 Cricothyroid Muscles

4.3.1.6.1 "Normal" Horses

The fibre types and their proportions in the muscles from the "normal" horses are shown in Table 12. The proportion of AH fibres appeared to be less ($P < 0.1$) in the older horses (AH:AL, 59%:41%) than in the younger group (68%:32%).

4.3.1.6.2 Abnormal Horses

The significant differences between the proportions of fibres present in muscles of the "normal" and abnormal horses were:-

i. The young abnormal horses had a significantly ($P < 0.001$) lower proportion of AH fibres (AH:AL, 57%:43%) than the young "normal" horses (68%:32%)

ii The young abnormal horses had a signifcantly higher ($P < 0.01$) proportion of PH fibres (PH:PL, 95%:5%) than the young "normal" horses (79%:21%).

4.3.1.7 Hyoepiglottic muscle

The proportion of AH fibres was less in the hyoepiglottic muscle than in the other laryngeal muscles examined. The fibre types and their proportions observed in the "normal" horses are shown in Table 12. With one exception these proportions were similar in the hyoepiglottic muscles of all the horses examined. This exception was the horse suffering from laryngo-palatal dislocation (29) where a higher proportion of AH fibres was observed (AH:AL, 63%:37%).

The AL fibres of the abnormal horses tended to have a higher proportion of PH fibres (PH:PL, 95%:5%) than the "normal" horses (PH:PL, 78%:22%).

4.3.2 Other Fibre Types

The only other fibre type which occurred with any frequency was the AH:SH:PL type. This fibre type tended to occur much more regularly in the very young horses. It was observed in at least one

of the muscles of the youngest 14 horses examined, but only infrequently in older animals. Each of the intrinsic laryngeal muscles in this study occasionally contained this fibre type but when it did occur it accounted for less than five percent of the fibres counted in that particular muscle.

4.3.3 Other Staining Characteristics and Changes in Fibre Architecture

All the fibres of the muscles studied took up the blue diformazan deposited during the SDase reaction, but there was often some variation in the intensity of the blue colouration between fibres (Figs. 21b and 22b) within a section. This variation was more noticeable in the cricothyroid and hyoepiglottic muscles than in the other muscles studied. The difference between fibres however was usually not distinct enough to allow for reliable separation of fibre types using this reaction. Only those very rare fibres with an obvious reduction in blue colouration were designated SL. There was a tendency for the SDase reaction to be inversely related to the myosin ATPase reaction but this inverse relationship was not always consistent.

Most of the fibres in the muscles studied took up a blue colouration in a manner which indicated a high activity of the enzyme GPase. As with the SDase reaction there was some variation in the intensity of the colour between fibres within a section (Figs. 21c and 22c). Only those fibres which were much paler than neighbouring fibres were classed as PL. The AL fibres tended to be paler when stained for GPase than the AH fibres but not all AL fibres were PL. The muscles from the very young foals in this study, when stained to demonstrate the activity of SDase and GPase showed a similar but less intense reaction to that seen in the older horses.

In the muscles supplied by the left recurrent laryngeal nerve there were changes in the staining characteristics exhibited by the SDase and GPase reactions which coincided with other signs of denervation. The most subtle of these signs was "fibre type grouping" of AL fibres (Chapter 5). In muscles showing this grouping, there was often a reduction in the intensity of the SDase and GPase reactions. The groups of AL fibres tended to be visible also in the sections stained

to demonstrate the activity of GPase, as groups of fibres with a less intense GPase reaction. In muscles where this pathology had progressed to the stage where fibre atrophy was evident this reduction in intensity of the SDase and GPase reactions was even more pronounced and usually there was an obvious difference in the intensity of these reactions between the left and right muscles (Fig. 23).

At this late stage in the development of neurogenic atrophy the SDase reaction also demonstrated changes in fibre architecture. Sometimes fibres developed a dark staining peripheral ring while their centre was pale (Fig. 24). Target-like fibres were common in these muscles (Fig. 25a and 25b). True target fibres appeared to be rare but these targetoid fibres with alternating light and dark concentric areas were often present in muscles with fibre atrophy. Mottled fibres which had features consistent with "moth-eaten whorled fibres" were also occasionally noted in these muscles (Fig. 26). In the older abnormal horses with severe pathology of the left muscles, these mottled fibres were sometimes seen in the right muscles.

Another feature noted in muscles with severe pathology was that the majority of the small diameter muscle fibres remaining stained intensely with myosin ATPase. This meant that often in severely affected muscle the fibres were predominantly AH (Fig. 27).

The above changes were not observed in the cricothyroid muscles from either "normal" or abnormal horses except for the occasional fibres which resembled the mottled or moth-eaten fibres already described. These were seen in the left and right muscles of some of the aged horses (Fig. 28).

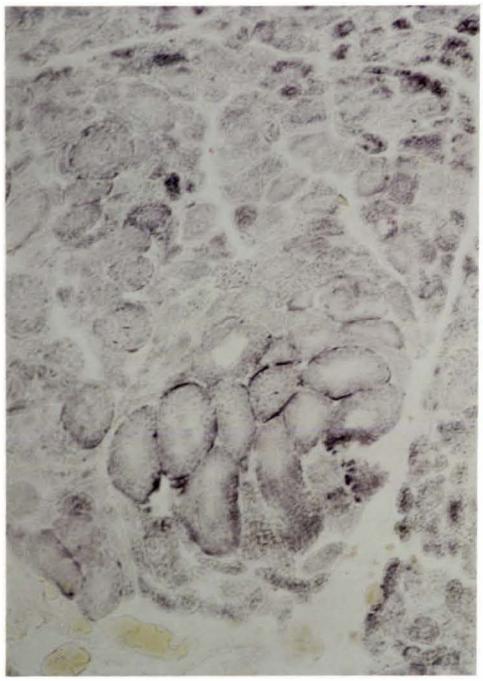
4.4 Discussion

4.4.1 Preparation of Muscle Blocks

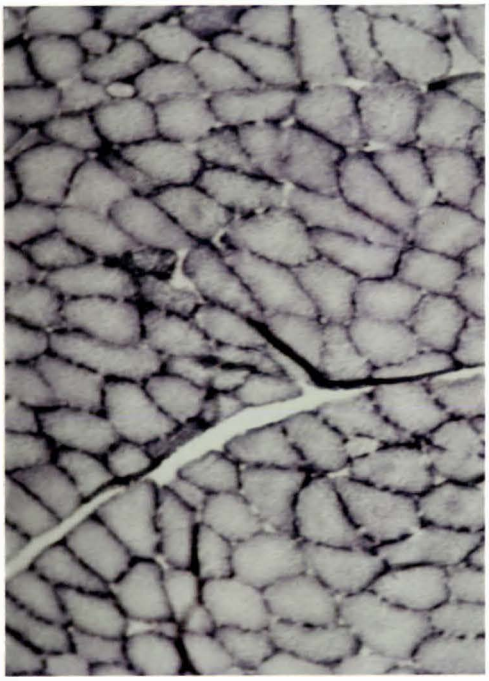
It was found in the early stages of the study that consistently high quality histochemical sections could only be obtained if the muscle blocks were prepared as soon as possible after the death of the horse. Deep freezing the muscles and then thawing them later to cut and refreeze the blocks produced large amounts of

FIGURE 23, a and b — Transverse sections of the left (a) and right (b) lateral cricoarytenoid muscles from a laryngeal hemiplegic horse (x100). Sections stained to demonstrate the activity of SDase. The atrophied fibres in the left muscle show a less intense reaction for this enzyme than the normal sized fibres in the right muscle

FIGURE 23, c and d — Transverse sections of the left (c) and right (d) transverse arytenoid muscles from a laryngeal hemiplegic horse (x100). Sections stained to demonstrate the activity of GPase. The majority of the atrophied fibres in the left muscle show a less intense reaction for this enzyme than the normal sized fibres in the right muscle



a



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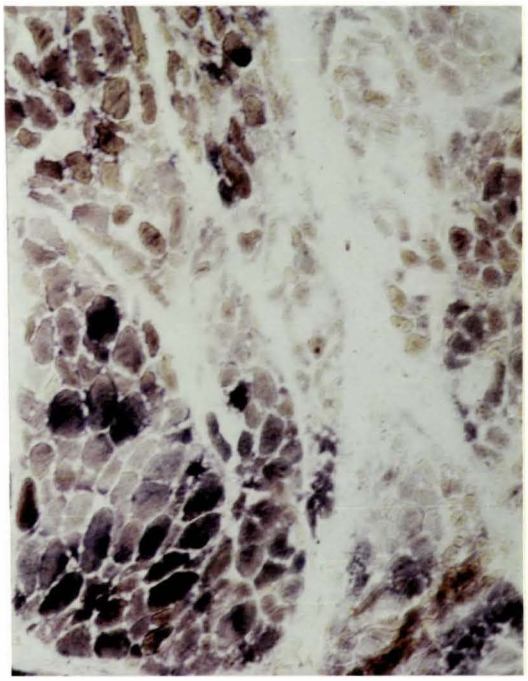
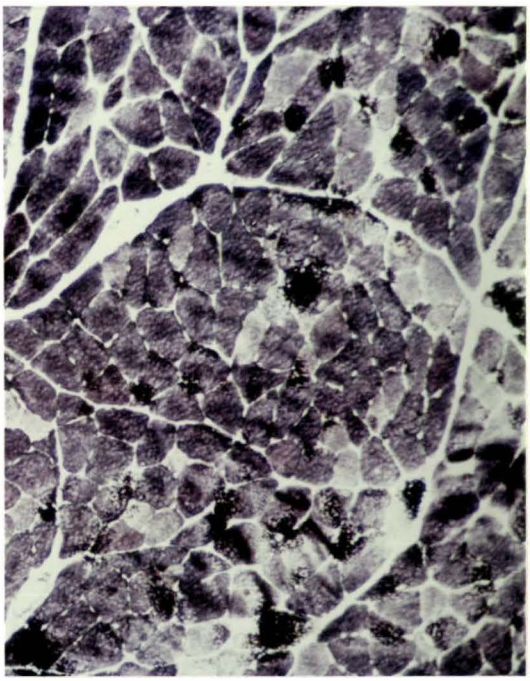


c

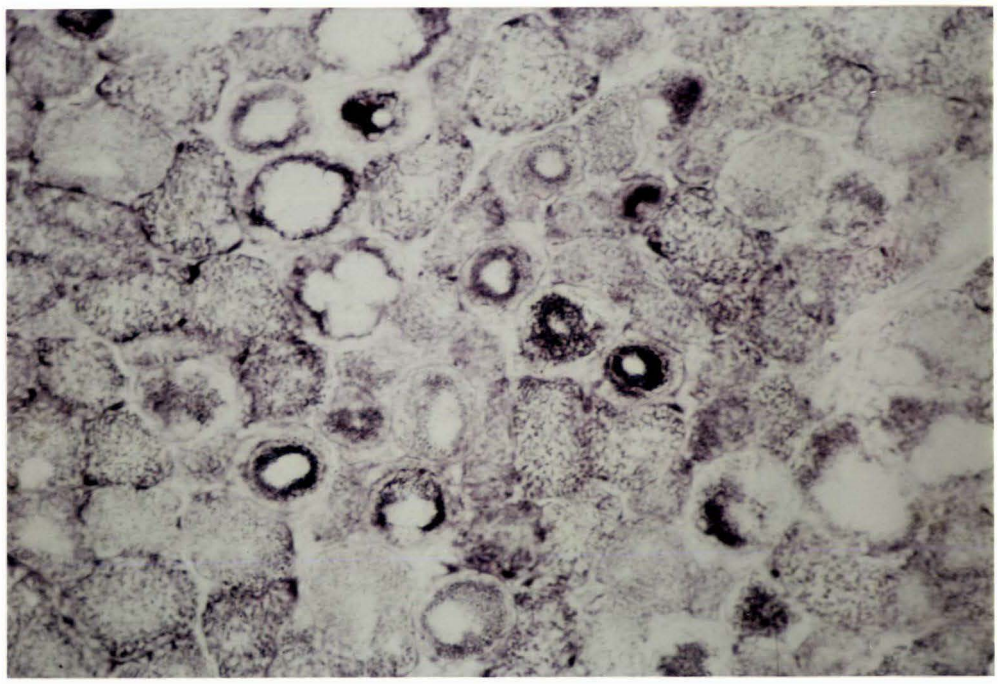


d

FIGURE 24 A transverse section of the left dorsal cricoarytenoid muscle from a laryngeal hemiplegic horse (x260). Section stained to demonstrate the activity of SDase. Some of the fibres have a dark staining peripheral ring and a pale centre

FIGURE 25 a. A transverse section of the left ventricular muscle from a laryngeal hemiplegic horse (x260). Section stained to demonstrate the activity of SDase. Targetoid fibres are apparent

b. A greater magnification (x650) of the larger targetoid fibre visible in Fig. 25 a



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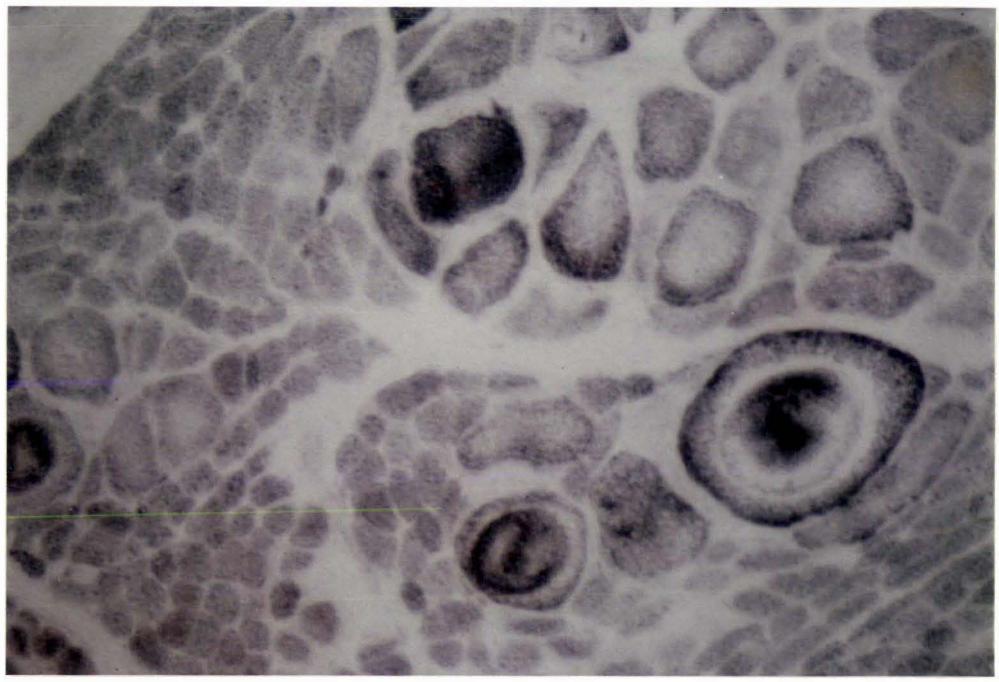


b

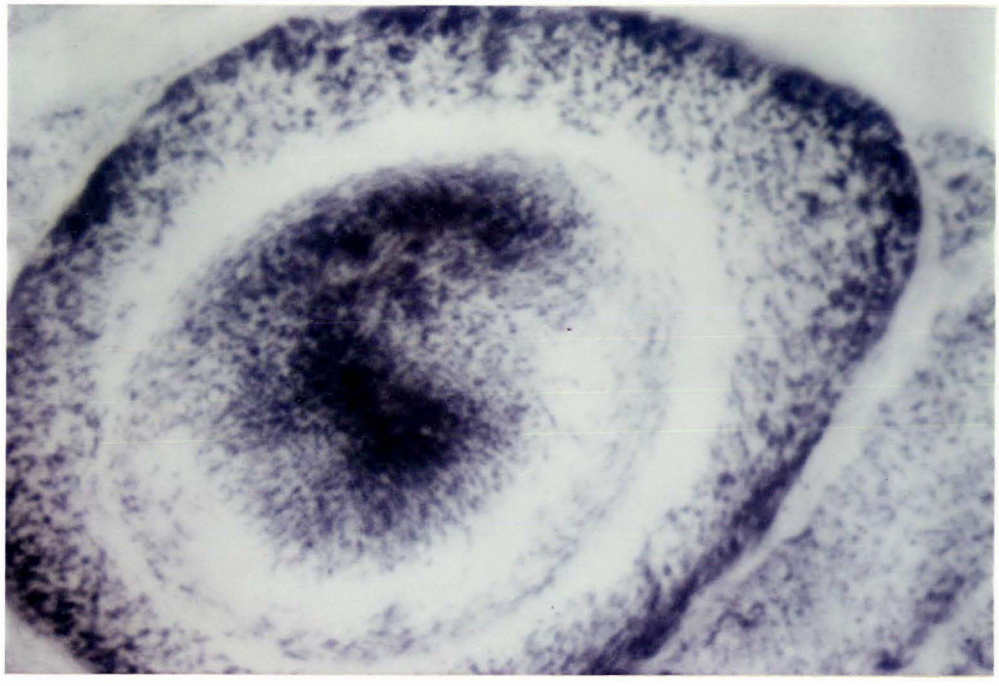
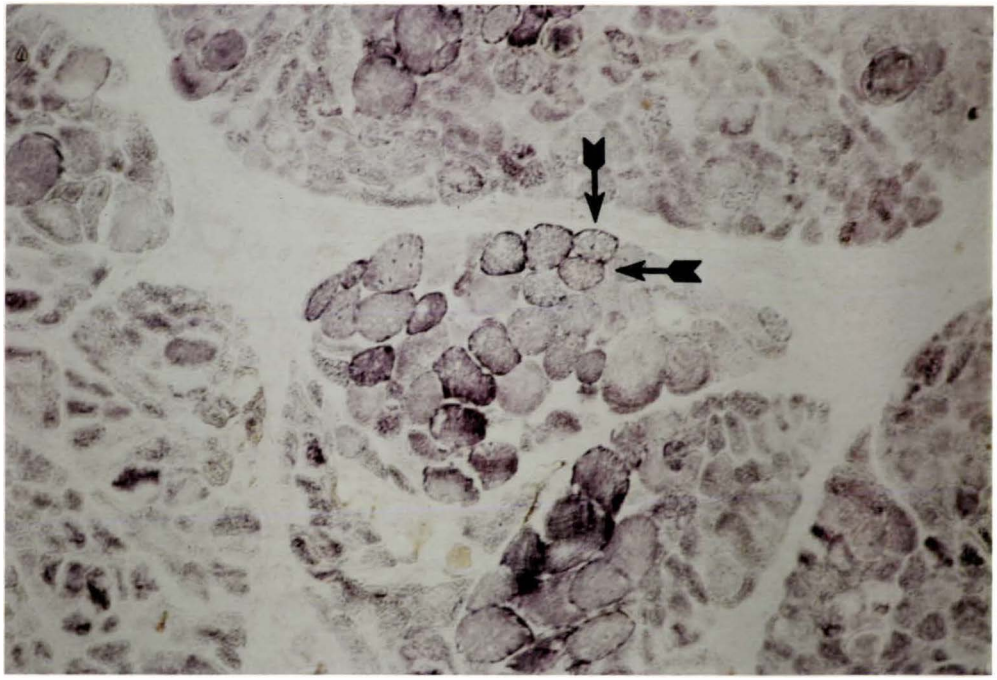
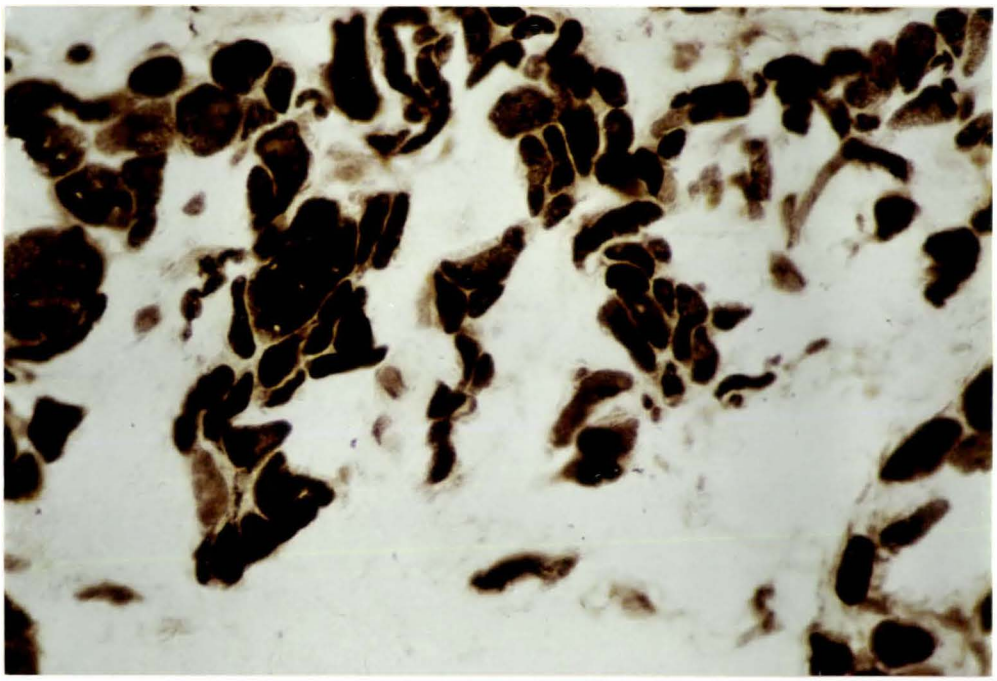


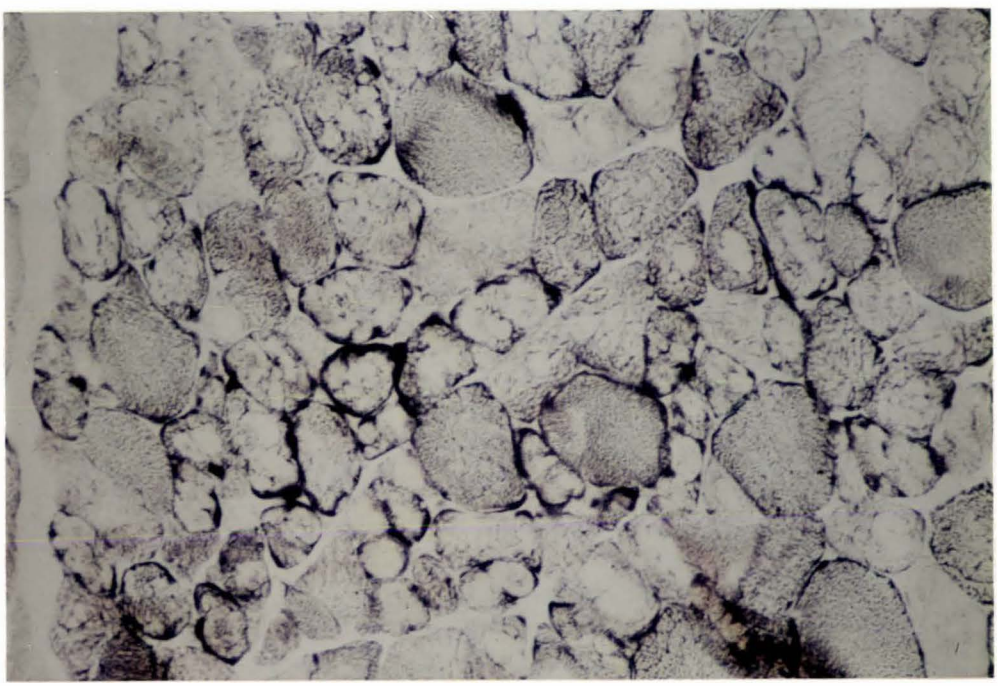
FIGURE 26 A transverse section of the left transverse arytenoid muscle of a two year old laryngeal hemiplegic horse (x100). Section stained to demonstrate the activity of SDase. Mottled fibres with features similar to "moth-eaten whorled" fibres are present (arrows)

FIGURE 27 A transverse section of the left dorsal cricoarytenoid muscle of a laryngeal hemiplegic horse (x260). Section stained to demonstrate the activity of myosin ATPase. Most of the fibres in this severely atrophied muscle react in a manner consistent with AH fibres

FIGURE 28 A transverse section of the left cricothyroid muscle of an aged "normal" horse (x260). Section stained to demonstrate the activity of SDase. Fibres which have mottled or "moth-eaten whorled" characteristics are present







ice artefact in sections and inconsistent enzyme stains. If muscles were left for more than two hours after the death of the horse, before cutting and freezing the blocks, the results of enzyme staining were also inconsistent.

Once the blocks were frozen care was taken to ensure that thawing did not occur at any stage before the mounting of sections. If thawing did occur ice artefact in the sections was common.

4.4.2 Fibre Types in Equine Laryngeal Muscles

This study is the first to have typed and counted the fibres of the intrinsic laryngeal muscles of a large number of horses of a single breed, in this case, the New Zealand Thoroughbred. It has provided more comprehensive information on the fibre types and their proportions in these muscles than was previously available.

The results of this study show that the abductors and adductors of the arytenoid cartilage have fibres which are approximately 70% or more AH, almost exclusively SH and predominantly PH. This proportion of AH fibres is a little higher than that estimated by Duncan (1975) but is very similar to that found by Gunn (1972).

The predominant fibre types observed in the laryngeal muscles of the "normal" horses in this study were the same as those reported by Gunn (1972), so this work provides support for his suggestion that the fast twitch low oxidative type does not occur in normal equine laryngeal muscle. The fibre types seen in the arytenoid abductors and adductors were those which would be expected in respiratory muscles which must contract rapidly and repeatedly. There was a high proportion of fast twitch fibres (AH) with the capacity for both anaerobic and aerobic metabolism (SH and predominantly PH).

Prior to this study no information was available on the proportion of fibre types present in such muscles as the equine vocal, ventricular, cricothyroid and hyoepiglottic muscles. The vocal and ventricular muscles are involved primarily in the control of the vocal cords and the production of voice, functions which require very rapid movements. Not surprisingly therefore it was found that these muscles had the

highest proportions of AH fibres observed. The cricothyroid muscle has been shown by Goulden et al., (1976) to function both on inspiration and expiration and to have a major laryngeal stabilizing component, a function which requires sustained contractions. The proportion of AL fibres observed in the cricothyroid muscle was higher than was seen in the arytenoid abductor and adductor muscles. Of the muscles studied, the requirement for sustained tonic contraction would be expected to be highest in the hyoepiglottic muscle which must keep the epiglottis depressed during respiration. The hyoepiglottic muscle did in fact contain the highest proportion of AL fibres of the laryngeal muscles studied.

In other species the differences in proportion of AH fibres are related to differences in contraction speeds of the muscles (Sahgal and Hast, 1974). It would be of interest to know if this occurs in the horse. The proportions of AH fibres observed during this study and their relationship to the expected function of the muscles suggests that it probably does.

4.4.3 Changes in the Proportion of Fibre Types Observed in the Different Groups of Horses

The changes in the proportions of fibre types which were observed in this study probably represent a complex series of inter actions. It is possible that there were a number of factors operating together to produce the changes observed and the influence of these factors could not be separated. It was only possible therefore to suggest what the causes of these changes may have been.

4.4.3.1 The Proportion of AH:AL Fibres

As mentioned previously there is evidence that muscle fibres can respond to demands for different types of work output by changing their fibre type proportions to most effectively meet those demands. The proportion of AH fibres decreases in some postural muscles as an animal ages and becomes heavier (Davies, 1973). On the other hand, some of the upper limb muscles of the horse respond to periods of training by increasing the proportion of AH fibres they contain, probably to be better able to contract rapidly during fast work (Guy and Snow, 1977).

There appears to be little information available on the effect of denervation and reinnervation on the proportion of AH:AL fibres present in a muscle. It has been stated that the process of denervation in a muscle initially causes specific atrophy of AH fibres (Engel et al., 1966). It would be expected that when denervation began, some of the AH fibres would undergo a reduction in their cross sectional area while some or all of those retaining their innervation and perhaps also the AL fibres would increase their cross sectional area during compensatory hypertrophy. It is difficult to predict what the nett effect of these changes would be on the proportion of fibres present in a given area of a muscle as this would depend on the extent to which each of the changes occurs.

If AH fibres begin to atrophy first then it is also possible that they may be the first fibres denervated. Early in the course of denervation it is likely that denervated AH fibres would become reinnervated from other unaffected AH fibres because these are the most numerous type. This transient loss of a nerve supply may bring about a temporary reduction in the level of ATPase activity of a muscle fibre and Morris (1967) and Warszawski et al. (1975) have suggested that intermediate fibres are fibres undergoing this change. When denervation becomes widespread however it is possible that a higher proportion of denervated AH fibres would become reinnervated by sprouting from axons supplying AL fibres. If this process does in fact occur in this way, then the nett effect on the proportion of fibre types could be a reduction in the number of AH fibres and an increase in the number of AL fibres.

The observation made during the present study that in severely affected muscle the fibres were predominantly AH was similar to that made by Gunn (1973) when he noted that the left transverse arytenoid muscle of a roarer contained only AH fibres. The reason for this is not clear, but possibly in severely atrophied muscle fibres, myofibrillar and perhaps mitochondrial ATPase becomes more concentrated and so the histochemical reaction no longer distinguishes fibre types.

It is possible then, that as neurogenic atrophy progresses in a muscle there may be first a reduction in the proportion of fibres with a high level of myosin ATPase as demonstrated by the histochemical

reaction used in this study; followed by an increase in the proportion of these fibres. Many of the changes in the proportion of AH:AL fibres observed in this study can be explained in these terms.

In the dorsal cricoarytenoid and lateral cricoarytenoid muscles of the laryngeal hemiplegic horses where the process of denervation and reinnervation was occurring, the muscles from the younger horses had a lower proportion of AH fibres than those from the older horses. In terms of the hypothesis outlined above this may have been because pathology was at an earlier stage in the younger group. In the lateral cricoarytenoid muscle where less severe pathology was found to be relatively common in the "normal" horses especially the older ones, there was a drop in the proportion of AH fibres when compared with the younger group. These changes were most marked in the muscles from the left side of the larynx, as would be expected because the left recurrent laryngeal nerve is primarily involved.

In the transverse arytenoid and ventricular muscles of the abnormal horses there was only an increase in the proportion of AH fibres when compared to the same muscles from "normal" horses. This may indicate that pathology is well advanced in these muscles when compared with the dorsal cricoarytenoid muscle.

The situation in the cricothyroid muscle was different as here there was a decrease in the proportion of AH fibres in the older "normal" horses and the young abnormal horses when compared with the young "normal" horses. As a horse matures and comesinto work it would be expected that the laryngeal stabilizing activity of the cricothyroid muscle would become more developed and so the proportion of AL fibres may increase. This same change could have come about in the abnormal horses because they were in work and older on average than the young "normal" horses.

In the hyoepiglottic muscle of the horse which had suffered from laryngo-palatal dislocation there was a higher proportion of AH fibres than was seen in the other hyoepiglottic muscles. AH fibres tend to be more susceptible to fatigue than AL fibres (Table 9) and consequently it is possible that the hyoepiglottic muscle of this horse may have

been less effective at depressing the epiglottis than that of a "normal" horse, thus fascilitating laryngo-palatal dislocation. Further histochemical investigation of the hyoepiglottic muscles from such horses is thus indicated.

4.4.3.2 The Proportions of SH and PH Fibres

It has been suggested that the levels of SDase and GPase observed in the fibres of a muscle may increase with age (Gunn, 1973) and exercise (Bradley, 1981) and decrease when the process of denervation begins in a muscle (Romanul and Hogan, 1965). These changes are a reflection of the type and level of work performed by the muscle in question.

The fibres of the equine intrinsic laryngeal muscles examined in this study were predominantly SH and PH, a finding which is in agreement with those of previous workers (Gunn, 1972; Duncan, 1975). The fibres of the cricothyroid and hyoepiglottic muscles showed a greater variation in their levels of SDase than the other muscles studied although the majority of the fibres still took up the blue deformazan deposited during the reaction. While there was not a consistent inverse relationship between the levels of ATPase and SDase in the fibres of the muscles in this study, this greater variation of the SDase reaction in these two muscles probably reflects their higher levels of AL fibres.

The fact that the only other fibre type observed with any frequency in this study (AH:SH:PL) was much more common in the younger horses is probably a reflection of the increase in the level of GPase in a muscle as it ages and performs more work. It was noted during this study and had been previously suggested by Gunn (1973) that the SDase and GPase reactions in the muscles of the foals were less intense than in the muscles of adults horses. The reduction in the levels of the SDase and GPase reactions in the fibres of muscles showing other signs of neurogenic atrophy was well demonstrated in this study. As the level of these enzymes can increase in a muscle as its level of work increases, it is not surprising that they are reduced when it becomes denervated. This reduction in the level of these enzymes with denervation is the most likely reason for Gunn's (1972) observation of the abnormal fibre types AH:SH:PL, AL:SL:PH and AL:SL:PL in muscles showing fibre type grouping.

4.4.4 Changes in Fibre Architecture

The decrease in the intensity of the SDase and GPase reactions in muscle where denervation was occurring was accompanied by architectural changes in individual fibres in these muscles. The pale fibres, mottled fibres and targetoid fibres all appeared to be variations of the form the enzyme reactions took as the levels of the enzyme in question varied in a muscle fibre as it became denervated and then reinnervated. The mottled fibres observed in this study were similar in appearance to the "moth-eaten whorled" fibres described by Brooke (1973). These mottled fibres were also observed occasionally in muscles such as the right cricothyroid of aged horses where it would not be expected that denervation and reinnervation as it occurs in laryngeal hemiplegia, would be taking place. Braund (1982) has cited evidence which indicates that in the peripheral nerves of people of increasing age a reduction in the number and density of myelinated fibres occurs. It is possible that the appearance of these "moth-eaten, whorled" fibres in the muscles of aged horses results from a similar change in their peripheral nerves.

4.4.5 Concluding Comments

This histochemical study of equine laryngeal muscles has provided new information which adds to an understanding of their function, metabolism and the changes brought about by the process of denervation and reinnervation which occurs in some of them. Physiological studies to determine the contraction times of these muscles and biochemical assays to determine their actual enzyme content would add further to this understanding.

The results of this study suggest that neurogenic disease produces significant changes in the fibre type profiles of these muscles and further studies to separate the influence of age, state of training and denervation and reinnervation on fibre type are needed to more fully illucidate this situation.

CHAPTER 5 THE HISTOPATHOLOGY OF SOME EQUINE LARYNGEAL MUSCLES

5.1 Introduction

The work of Gunn (1972 and 1973) and Duncan et al., (1974), in which the techniques of enzyme histochemistry were applied to equine laryngeal muscles indicated that histopathology was common in the muscles of clinically normal horses but more severe in those of laryngeal hemiplegics. These workers considered that the histopathology they observed was characteristic of neurogenic disease. The signs which are observed in an affected muscle are firstly, grouping of fibres of similar histochemical type and then fibre atrophy and hypertrophy. As neurogenic disease becomes more severe, widespread atrophy of fibres and their replacement by the connective tissue elements of muscle occurs. This chapter is concerned with a study of these changes in some equine laryngeal muscles. For an understanding of neurogenic muscle disease, knowledge of fibre type grouping, variation in muscle fibre cross sectional area and some aspects of muscle histopathology is helpful, so a brief review of these subjects follows.

5.1.1 Fibre Type Grouping in Equine Intrinsic Laryngeal Muscles

When cross sections of most equine intrinsic laryngeal muscles are stained to demonstrate the activity of the enzyme myosin ATPase after alkaline preincubation a mosaic pattern of AH and AL fibres results. The spatial relationship of the two fibre types depends on which type is numerically dominant in the section. Since AH fibres are more numerous in equine intrinsic laryngeal muscles (Chapter 4) the sections made from these muscles are mostly dark stained, but randomly distributed throughout are either individual or small groups of AL fibres (Fig. 21a). The spatial distribution and the size of the groups of these light stained AL fibres depend on the territorial arrangement of the motor units in the muscle examined.

In human skeletal muscles studied by Johnson et al., (1973) the observed distribution of AH and AL fibres was the same as that predicted by a statistical model which assumed a random distribution of fibres. The only exception was the short digital extensor muscle where grouping of fibres of the same type was often observed. This was considered to occur as a result of damage to its motor nerve caused by ill-fitting

shoes (Jennekens et al., 1972)

In spite of this random distribution of fibres however, some degree of fibre type grouping is frequently observed in normal muscles. Muscle fibres of the same motor unit can occasionally occur in small groups of up to six, (Buchthal and Schmalbruch, 1980) and adjacent fibres of the same type may belong to different motor units. In the left dorsal cricoarytenoid muscle of horses without neurogenic disease, Gunn (1972) found that 85% of AL fibres occurred in groups of less than ten. He defined a group as a number of AL fibres in the same fascicle, whose sides rather than angles were in contact, and normal muscles as those which exhibited little difference between the left and right sides either in fibre type or distribution.

Abnormally large groups of AL fibres have been reported in the laryngeal muscles of horses by both Gunn (1972) and Duncan et al., (1974). In the dorsal cricoarytenoid muscles of three of 12 normal horses which Gunn (1972) examined, abnormally large groups of AL fibres occurred. Although the groups of AL fibres were much larger in the left muscles, larger than normal groups were also present in the right muscles. Gunn (1973) also showed that the mean group size of AL fibres was greater in the left than the right dorsal cricoarytenoid muscle of one of three foetal horses and an aged roarer. In a series of normal horses of various breeds Duncan et al., (1974) noted that large groups of AL fibres occurred commonly in some of the muscles supplied by the left recurrent laryngeal nerve especially the lateral cricoarytenoid muscle. In the muscles of a roarer they found that fibre type grouping also occurred in the right adductor and abductor muscles.

Duncan (1975) also examined some equine cricothyroid muscles and distal limb muscles and said that in these muscles he saw no evidence of fibre type grouping. From the work of these authors it appears that fibre type grouping was common both in the laryngeal muscles of roarers and clinically unaffected horses. It was most obvious in the muscles supplied by the left recurrent laryngeal nerve, particularly the lateral cricoarytenoid, but sometimes occurred in the right muscles, particularly those of roarers.

5.1.2 The Significance of Fibre Type Grouping

Metabolic properties and hence histochemical profile appear to be conferred on a muscle fibre by the characteristics of its motor nerve. Buller, Eccles and Eccles (1960) demonstrated that the speed of contraction of a slow muscle could be increased by replacing its motor innervation with the motor nerve from a fast muscle and vice versa. Dubowitz (1967) demonstrated that this change in contraction speed was accompanied by a change in fibre type; a muscle which increased its speed of contraction also increased its content of myosin ATPase. This change (slow twitch to fast twitch) can also be accomplished by chronically stimulating a slow twitch muscle fibre at a firing frequency characteristic of a fast twitch motor neurone. Changes in the reverse direction are also possible (Buchthal and Schmalbruch, 1980).

Fast twitch muscle fibres are innervated by motor neurones which have higher conduction velocities and firing frequencies than those innervating slow twitch muscle fibres. The firing frequency of its motor neurone appears to be the major factor determining the contraction time of a muscle fibre and this may be mediated by a "trophic" substance acting on the muscle cell membrane (Buchthal and Schmalbruch, 1980; Harris, 1980).

If the motor nerve to a muscle is experimentally damaged by crushing, or sectioning and resuturing, fibre type grouping can be demonstrated by the myosin ATPase reaction in that muscle some months later (Karpati and Engel, 1968; Edström and Kugelberg, 1969). If however the nerve is damaged in such a way that regeneration cannot occur, then type grouping does not take place in the muscle which was originally supplied by that nerve (Karpati and Engel, 1968).

It has also been shown that after reinnervation of a muscle there is a marked rearrangement of the fibres of a motor unit, with groups of contiguous fibres belonging to the same motor unit (Kugelberg *et al.*, 1970; Kugelberg, 1973). This rearrangement is a consequence of the process generally known as collateral or terminal sprouting.

When the motor neurone of a motor unit is damaged the first response

is atrophy of the individual muscle fibres of that motor unit. These widely dispersed small atrophic muscle fibres can persist as such for months or years (Dorman, 1973) but if there are surviving neurones in the vicinity a response is elicited from them, perhaps as a result of a mediator which is released from degenerating fibres or their end plates (Hoffman, 1957). This mediator supposedly stimulates the growth of reactive sprouts from surviving axons either from one of the terminal expansions of an end plate or from nerve fibres within an intramuscular nerve bundle (Cöers and Woolf, 1959). At the same time regeneration of the axon proximal to the site of damage begins and both the regenerating axons and the collateral sprouts are guided to denervated end plates by the Schwann cell sheaths which remain after degeneration of axis cylinders (Burke, 1980).

Collateral sprouting is a rapid process and denervated fibres can be reinnervated within a month, usually well before interrupted fibres can regenerate back into a muscle (Hoffman, 1957). Innervation at the old end plates is thus re-established and once a fibre is reinnervated it will usually not accept further innervation, although occasionally during this process it is possible for a number of end plates to become established on a single muscle fibre (Buchthal and Schmalbruch, 1980). When regenerating axons arrive they can also reinnervate the end plates so persistent double innervation is possible (Hoffman, 1957).

The reinnervated muscle fibres re-establish their original size and take on the histochemical and physiological characteristics dictated by the properties of the new neurone. Histochemical conversion takes approximately two weeks after functional neuromuscular connections have been re-established (Burke, 1980).

As denervated fibres are likely to be reinnervated by collateral sprouting from adjacent surviving neurones it is likely that groups of histochemically similar fibres will result. Morris (1969) provided evidence to support this concept when he demonstrated collateral sprouting to groups of fibres of the same histochemical type.

If fibre type grouping is to occur it is necessary to damage the motor neurones of a number of motor units whose muscle fibres are in a

similar territory within a muscle. It is then necessary to reinnervate those muscle fibres with a single neurone, or neurones of the same type, otherwise a mosaic pattern of different muscle fibre types will again occur (Johnson et al., 1973). It is possible that these conditions will not always be met and so the process of denervation and reinnervation may be more widespread in a muscle than is indicated by the incidence of fibre type grouping in that muscle.

As indicated in Gunn's work (1973) the groups of muscle fibres of the same histochemical type which result can be very large. A motor neurone can support approximately four times its normal number of terminal end plates but perimysial connective tissue boundaries impose limits on the size of groups (Burke, 1980). If the motor neurone or neurones supplying one of these large groups also becomes involved in the denervation process then atrophy of the whole group (grouped atrophy or fascicular atrophy) is the result. Duncan et al., (1974) noted that this change was commonly seen in severely damaged equine laryngeal muscles.

Thus the occurrence of fibre type grouping in a muscle while not necessarily giving a direct measure of the extent of the process of denervation and reinnervation, is a reliable indicator of its presence. Moreover in most muscles which normally display a mosaic pattern of myosin ATPase activity, its presence concurrent with group or fascicular atrophy indicates that severe motor nerve damage has occurred.

5.1.3 Fibre Size

5.1.3.1 Factors Influencing the Size of Muscle Fibres

The cross sectional area of individual muscle fibres is a useful parameter to measure when investigating neurogenic muscle pathology as it is profoundly influenced by denervation. The area of a muscle fibre varies within certain limits under the influence of a number of normal physiologic processes and it is against this background that the influence of pathology must be interpreted. Factors such as the age, body size, work output and the sex of an animal may influence the cross sectional area of its muscle fibres.

Individual muscle fibres increase in area in proportion to the increase in total cross section of a muscle which occurs as an animal grows

(Davies, 1973). In man it has been shown that the average fibre diameter in a range of muscles increases till approximately 40 years of age and then slowly diminishes through middle and old age (Moore, Rebeiz and Holden, 1971).

Gauthier and Padykula (1966) in a study of the muscle fibres of the diaphragm of 13 mammalian species suggested that there was a direct relationship between fibre diameter and body size although Davies and Gunn (1972) in a similar study were not able to substantiate this.

The increase in the work output of a muscle which occurs during training will result in an increase in the diameter of its fibres, particularly its Type II fibres (Brooke, 1973; Bradley, 1981). Conversely, lack of use of a muscle will lead to a gradual reduction in the size of all muscle fibres (Adams, Denny-Brown and Pearson, 1967) although Type II fibres are often the first to be involved (Dorman, 1973). The changes in fibre shape and size resulting from disuse are very similar to those noted early in the course of neurogenic atrophy (Dorman, 1973). Disuse atrophy however, is not as profound as that produced by denervation (Buxton, 1980). Moreover the degenerative changes which are a feature of denervated muscle are not observed in disuse atrophy (Tower, 1939).

In man a sex difference in fibre diameter has been recorded. In normal children of both sexes under the age of 14 years, Type I and Type II fibres are approximately equal in size. In males over this age however, Type II fibres are larger than Type I fibres and the reverse is true in females. The Type I fibres of both sexes are approximately the same size (Brooke, 1973). This difference in fibre size may represent an inate difference between male and female muscle or it may reflect Type II fibre hypertrophy resulting from greater physical activity by males than females (Brooke, 1973).

Adams, Denny-Brown and Pearson (1967) described the changes which occur in large limb muscles after removal of their motor nerve. They found that during the first two weeks after denervation there was a loss of muscle weight but at that stage a reduction in size of individual muscle

fibres was not obvious. Soon after denervation, affected fibres were observed to undergo a transitory hypertrophy (Zak, Grove and Rabinowitz, 1969) and to assume a more sharply angulated contour (Dorman, 1973). A month after denervation there was a noticeable reduction in the size of muscle fibres which also began to lose their polygonal shape and became rounded in cross section. Furthermore an increased variation in fibre size became evident. After two months most of the fibres were reduced to one third or one half of their normal diameter although these changes were more pronounced in some fascicles than in others. After four months the atrophy progressed less rapidly. The rate of muscle fibre atrophy after denervation, may however, differ between muscles as Adams, Denny-Brown and Pearson (1967) cited the work of De Buch and De Moor (1903) who found that after section of the recurrent laryngeal nerve the small muscles of the larynx reached an advanced state of atrophy in two weeks.

In denervated muscle it is common to see larger than normal fibres as well as atrophic fibres. This is thought to be a result of hypertrophy of healthy fibres to compensate for the functional inadequacies of denervated fibres (Dorman, 1973).

5.1.3.2 Fibre Size in Equine Laryngeal Muscles

The denervation of the intrinsic laryngeal muscles which occurs in idiopathic laryngeal hemiplegia is partial rather than complete, in that not all the motor axons of the recurrent laryngeal nerve are involved at once (Duncan et al., 1974). As a consequence the initial distribution of atrophic fibres is random, in accordance with the normal anatomy of the motor unit. Small groups of atrophic fibres may be seen when adjacent fibres belong to the same motor unit or when a number of adjacent motor units are affected simultaneously. Large group atrophy is observed either when denervation is widespread or when a reinnervating neurone is damaged.

Cole (1946) in his study of equine intrinsic laryngeal muscles noted that the major microscopic change observed in these muscles was a reduction in the diameter of individual muscle fibres without a change in their architecture. Fibre atrophy was also a prominant feature

noted in the muscles studied by Duncan et al., (1974). There was angulation and atrophy of fibres and marked variation in fibre size in some of the laryngeal muscles of many of the horses they described as subclinical cases. Fascicular atrophy was seen in the more severely affected muscles and here nearly every fascicle was involved to a greater or lesser extent. Hypertrophy of muscle fibres was also a common feature. Duncan et al., (1974) also considered that, in all cases the lateral cricoarytenoid muscles were more severely affected than the dorsal cricoarytenoid. In the muscles of the laryngeal hemiplegic horses which were included in their study the atrophy of muscle fibres was more severe with the majority being affected and only occasional normal fibres remaining.

Duncan (1975) further reported observing variation in fibre size in the intrinsic laryngeal muscles and in some limb muscles of a number of aged ponies and cited Jennekens (1971) who had suggested that similar changes may result from a vascular insufficiency which develops with age. Braund (1982) also noted this variation in fibre size in the muscles of older dogs but suggested that this may result from an increasing loss of myelinated fibres in peripheral nerves with age as occurs in man.

Gunn (1972) provided quantitative information on the areas of the muscle fibres in the dorsal cricoarytenoid muscles from a 14 year old Thoroughbred mare which was a clinical roarer. The mean transverse sectional area of the AH and AL fibres was less in the left muscles (738 μm^2 and 99 μm^2 respectively) than in the right (5735 μm^2 and 5330 μm^2).

The most comprehensive information available to date on the cross sectional area of the muscle fibres of equine intrinsic laryngeal muscles has been provided by Duncan (1975). He calculated an overall mean fibre area for the left and right dorsal and lateral cricoarytenoid muscles and the left and right cricothyroid muscle from a small number of individual horses of different breeds. Some of these horses were clinical roarers and some were subclinical cases. Duncan did not separate Type I and Type II fibres. He attempted to differentiate the amount of fibre atrophy and hypertrophy occurring in each muscle by analysing the variance of their fibre areas. His results are

summarised as follows:-

The variance in fibre cross sectional area was:-

a) greater in the left than the right lateral and dorsal cricoarytenoid muscles;

b) greater in the left lateral cricoarytenoid than in the left dorsal cricoarytenoid muscle;

c) greater in the right lateral cricoarytenoid muscle than in the right dorsal cricoarytenoid muscle.

Duncan (1975) also measured the mean fibre cross sectional area in some equine laryngeal muscles and the results can be summarised as follows:-

a) In subclinical cases the mean fibre area was greater in the left and right lateral cricoarytenoid muscles than in the left and right dorsal cricoarytenoid muscles.

b) In some of the subclinical cases the fibres from the left cricothyroid muscles had a greater cross sectional area than the fibres from the right muscles.

c) In a clinical roarer the mean fibre area was less in the left lateral than in the left dorsal cricoarytenoid muscle.

He concluded that:-

a) The left muscles were more severely affected than the right muscles.

b) The lateral cricoarytenoid muscle was more severely affected than the dorsal cricoarytenoid muscle on both the left and the right side.

c) The lateral cricoarytenoid muscle may initially have fibres of greater cross sectional area than the dorsal cricoarytenoid muscle.

d) The fibres of the left cricothyroid muscle may undergo compensatory hypertrophy in clinical cases of roaring.

5.1.4 Histological Features of Normal and Denervated Muscle

5.1.4.1 The Normal Histological Features Observed in Transverse Sections of Skeletal Muscle

Skeletal muscles such as the intrinsic laryngeal

muscles are made up of closely packed fascicles of polygonal muscle fibres of approximately equal diameter. The bulk of each individual muscle fibre consists of myofibrils which are just visible with the light microscope (Ham, 1974). These myofibrils are surrounded by a thin layer of sarcoplasm which is more abundant at the periphery of the muscle cell, beneath the sarcolemma.

Striated muscle cells are multinucleate and in some muscle fibres there may be several hundred nuclei (Bloom and Fawcett, 1964). The nuclei are elongated in the direction of the long axis of the fibre and in transverse section they appear oval and flattened. In most striated muscle, including the intrinsic laryngeal muscles, they occupy a peripheral position in the muscle cell and lie just beneath the sarcolemma (Ham, 1974). In a representative transverse section two or three are visible per muscle cell (Fig. 29). Occasionally nuclei may be seen within fibres although in normal human muscle not more than three percent of fibres have these internal nuclei (Greenfield et al., 1957).

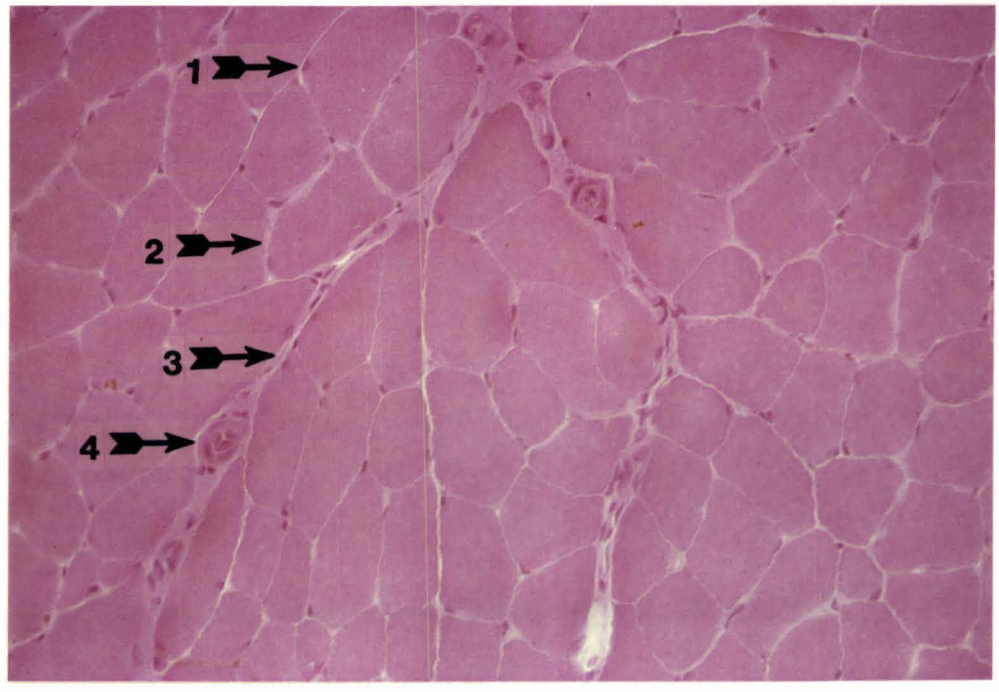
With the light microscope it is difficult to differentiate the muscle cell nuclei from those of the satellite cells which share the same basement membrane. These satellite cells are actively involved in growth and regeneration of muscle fibres so that their nuclei are capable of division, unlike those of the muscle cells (Ham, 1974).

Each individual muscle fibre is surrounded by a thin layer of connective tissue, the endomysium. This endomysium consists of a fine network of reticular fibres and contains fibroblasts and fixed macrophages. Groups of muscle fibres are bound together in bundles or fascicles by a septum of connective tissue the perimysium, the elements of which are continuous with the endomysium. The perimysium consists of irregularly arranged collagenous, reticular and elastic fibres and a variety of connective tissue cells and fat cells. The perimysial septa continue to the surface of the muscle to join with the epimysium, a thick layer of connective tissue at the periphery (Ham, 1974, Fig. 29).

This connective tissue stroma serves to conduct elements of the vascular and nervous system into the body of the muscle to supply individual

FIGURE 29 A transverse section of the left lateral cricoarytenoid muscle from a "normal" horse (x260). Section stained with haematoxylin and eosin. The histological features of normal equine intrinsic laryngeal muscle are illustrated

1. Sarcolemmal nucleus
2. Endomysium
3. Perimysium
4. Blood vessel


1
2
3
4

muscle fibres. The arterial branches subdivide into arterioles and then capillaries as they progress into the substance of the muscle. The capillaries form a rich network around individual muscle fibres (Romanul, 1965; Ham, 1974). The lymphatic system of striated muscle forms a similar branching network but it is confined to the thicker connective tissue septa (Ham, 1974).

5.1.4.2 The Histology of Denervated Muscle

When skeletal muscle is deprived of its motor nerve supply a number of changes take place in the muscle fibre, its nuclei, its surrounding stroma and the intramuscular nerves and blood vessels. These changes are additional to the changes in fibre size, shape and distribution already described.

One of the first histological changes to be noted in denervated muscle involves the sarcolemmal nuclei. They lose their flattened compressed appearance and become enlarged and rounded. Their chromatin stains more darkly than usual and becomes stippled so that they become 'tigroid' in appearance (Brooke, 1973). These changes have been noted as early as one week after experimental denervation (Adams, Denny-Brown and Pearson, 1967). Many of the sarcolemmal nuclei appear to migrate from their peripheral position and become more numerous among the myofibrils (Brooke, 1973). In experimental denervation it has been noted that these central nuclei are surrounded by an accumulation of sarcoplasm so that they appear in a halo or cleft (Adams, Denny-Brown and Pearson, 1967).

One month after experimental denervation some of the subsarcolemmal nuclei enlarge still further and become vesicular but at this time there is also evidence of small scattered dark staining nuclei which appear to be regressing (Adams, Denny-Brown and Pearson, 1967). Brooke (1973) stated that these vesicular nuclei were more common in myopathic conditions whereas 'tigroid' nuclei tended to be more a feature of neurogenic conditions. A section of denervated muscle examined at this time appears to contain an increased number of sarcolemmal nuclei, however mitoses involving these nuclei are not seen. It would appear that the apparent increase in numbers of nuclei is relative rather than absolute and is due to the decrease in fibre size which is occurring

at the same time. There is, however, no general agreement on this point (Adams, Denny-Brown and Pearson, 1967).

Once fibre atrophy becomes widespread, the sarcolemmal nuclei remain as pyknotic nuclear clumps inside atrophic fibres, and eventually, when the sarcolemma and sarcoplasm degenerate, the nuclear clumps remain in connective tissue. These nuclear clumps are characteristic of denervation but may also occur in myopathic disorders (Dorman, 1973). The initial responsesin a muscle to partial denervation are individual fibre atrophy and compensatory hypertrophy of fibres retaining their motor nerve supply. As the process continues, rounding and atrophy of fibres becomes more widespread and grouped atrophy becomes evident. In peripheral neuropathies partial splitting of individual fibres is occasionally seen (Brook, 1973).

Two months after experimental denervation, degeneration of individual fibres begins (Adams, Denny-Brown and Pearson, 1967). The fibres become granular and basophilic and fat vacuoles and necrosis of fibres with or without phagocytosis become evident (Dorman, 1973). As fibre atrophy progresses the endomysial and periphysial connective tissue elements become more prominant but it is doubtful that there is any increase in number and size of fibroblasts. It is likely that the apparent increase in endomysial and perimysial connective tissue is relative and due to condensation of the pre-existing fibrous network (Adams, Denny-Brown and Pearson, 1967; Dorman, 1973).

In experimental denervation the intramuscular blood vessels, particularly the arterioles and capillaries become congested and their walls become thickened with an apparent increase in endothelial and adventitial cells (Adams, Denny-Brown and Pearson, 1967). Intramuscular nerve bundles may show a decrease in their axon component and an increase in their collagen content (Dorman, 1973).

Denervated muscle finally comes to consist of nuclear clumps, very atrophic and degenerate fibres, fat and connective tissue. The final stages of denervation and severe myopathic degeneration produce muscle sections which appear similar histologically. In fact Dorman (1973) suggested that myopathic disease may prove to be neurogenic in origin

as it could result from abnormalities of the trophic mechanism exerted by nerves on muscles.

5.1.4.3 Other Histological Changes Observed in Equine Intrinsic Laryngeal Muscles

The process of repetitive denervation and reinnervation which occurs in idiopathic laryngeal hemiplegia apparently causes the changes described in the immediately preceding section to develop more slowly and allows the full spectrum to be seen in a single muscle (Duncan, 1975). These changes have been described by Cole (1946), Gunn (1973), Duncan et al., (1974) and Duncan (1975). They include, the presence of central nuclei (Gunn, 1973; Duncan et al., (1974); atrophy and degeneration of fibres with consequent fibrous and/or fatty replacement (Cole, 1946; Gunn, 1973); an increase in endomysial and periphysial connective tissue; and occasional necrotic and split fibres and lack of myelinated nerve fibres (Duncan et al., 1974; Duncan, 1975). The pathology noted by Duncan et al., (1974) was more severe in the left dorsal and lateral cricoarytenoid muscles of laryngeal hemiplegic horses. In those muscles intramuscular nerve bundles were very rare and when present had obvious endoneurial fibrosis with only a few axons remaining. These workers commonly saw myelinated intramuscular nerves in sections of cricothyroid muscles and right laryngeal muscles and suggested that this observation confirmed that the muscle pathology they described was neurogenic rather than myopathic in origin.

The histological changes observed by these workers (Duncan et al., 1974; Duncan, 1975) were more severe in the adductor than the abductor muscles; were only observed in the right muscles of one clinically affected horse and on no occasion were they present in the cricothyroid muscles.

This was the state of the information available on the histopathology of equine laryngeal muscles at the time this study commenced. Many questions were still unanswered. For example, although some evidence suggested that the earliest recognisable neurogenic changes were present in the laryngeal muscles of very young horses (Gunn, 1973) the age of onset of these changes in the majority of horses was still unknown. It was important to attempt to clarify this situation in

order to gain further insight into possible aetiological factors in idiopathic laryngeal hemiplegia. It was also desirable to know the normal variation in fibre size and the normal size of groups of fibres of the same type as this information is necessary before the early changes associated with neurogenic disease can be confidently recognised. It was thought that once an accurate distinction could be made between normal and abnormal muscle it may be possible, using biopsy techniques to categorise horses into neurogenically affected and unaffected groups. For these reasons a histochemical and histological survey was carried out on the muscles from horses of varying ages and sexes. Neurogenic laryngeal disease is clinically and economically most important in the competitive horse so the survey concentrated on a single competitive horse breed, the New Zealand Thoroughbred, although the muscles from three ponies were included for comparative purposes.

5.2 Materials and Methods

The muscles examined were the same as those used for the histochemical study described in Chapter 4. Tables 1 and 2 provide details of the ages and sexes of the horses used and Table 11, the number of horses from which each of the muscles were collected. The methods used to collect and analyse the information presented in this Chapter are detailed in Chapter 2. In addition to measuring the mean size of groups of AL fibres and the mean cross sectional area of AH and AL fibres, each muscle section was examined and any histopathology present was graded and recorded. The grading system adopted was as follows:-

Subtle pathology (Fig. 30 a and b) included: 1. Rounding of, and an apparent increase in the number of sarcolemmal nuclei. 2. The occurrence of a higher than normal number of nuclei within the body of muscle fibres. 3. Minor variation in fibre size. 4. Fibre type grouping.

Moderate pathology (Fig. 31 a and b) included: 1. Fibre atrophy and hypertrophy. 2. An apparent increase in the amount of endomysial and perimysial connective tissue. 3. The appearance of grouped atrophy.

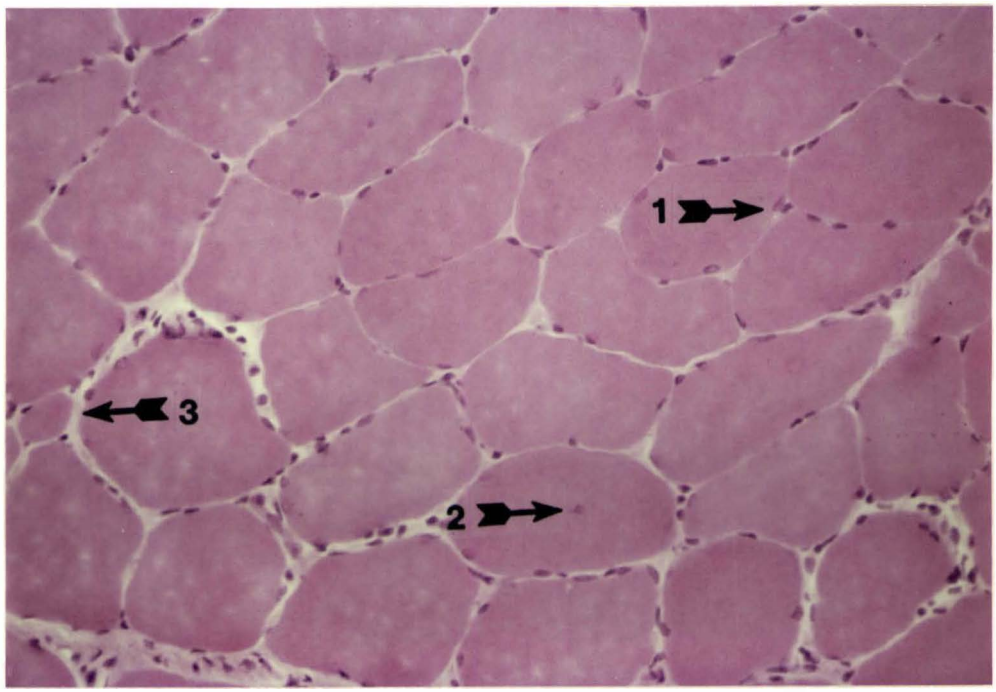
FIGURE 30 Subtle pathology. Transverse sections of the right dorsal cricoarytenoid muscle of a laryngeal hemiplegic horse.

a. Section stained with haematoxylin and eosin (x260)

b. Section stained to demonstrate the activity of myosin ATPase (x100)

1. Rounding of, and an apparent increase in the number of sarcolemmal nuclei
2. Internal nucleus
3. Variation in fibre size
4. Fibre type grouping

a



b

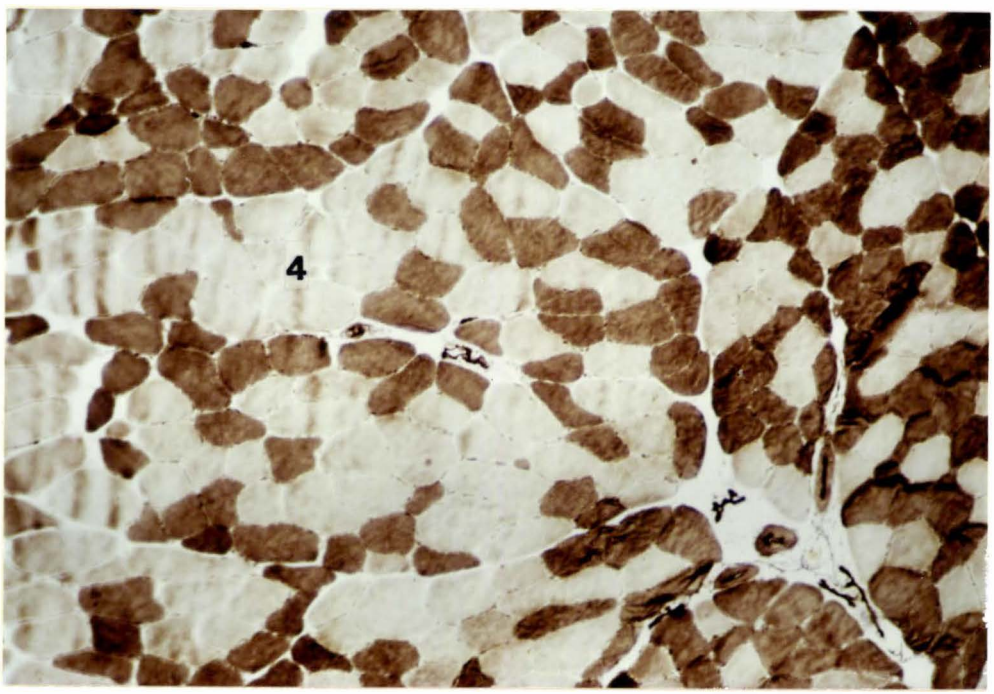


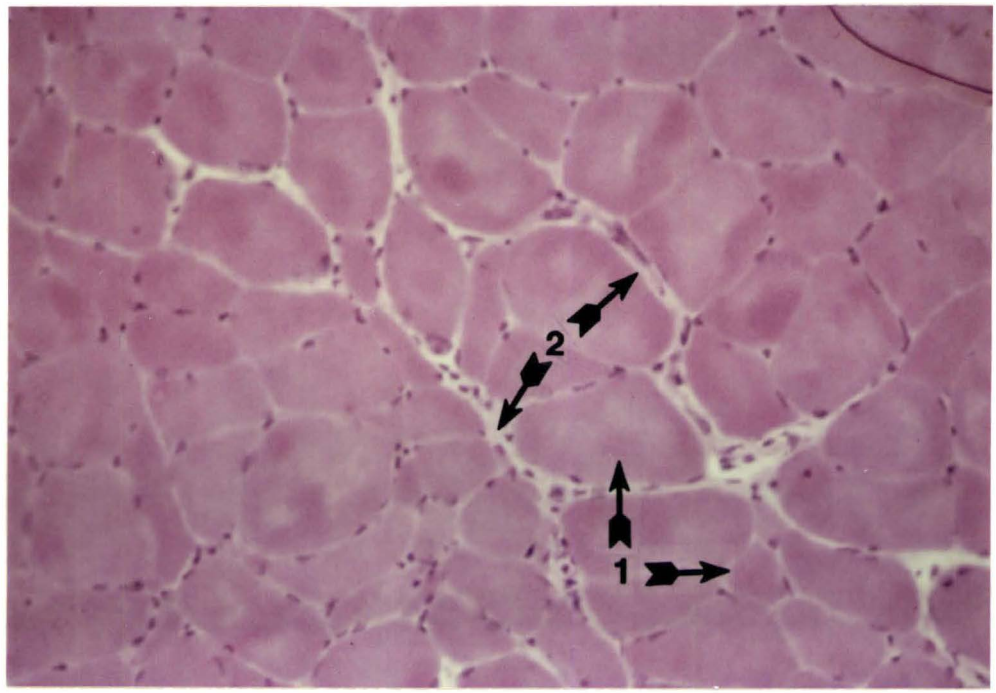
FIGURE 31 Moderate pathology. Transverse sections of the right lateral cricoarytenoid muscle of a laryngeal hemiplegic horse

a. Section stained with haematoxylin and eosin (x260)

b. Section stained to demonstrate the activity of myosin ATPase (x100)

1. Fibre atrophy and hypertrophy
2. An apparent increase in the amount of endomysial and perimysial connective tissue
3. Grouped atrophy

a



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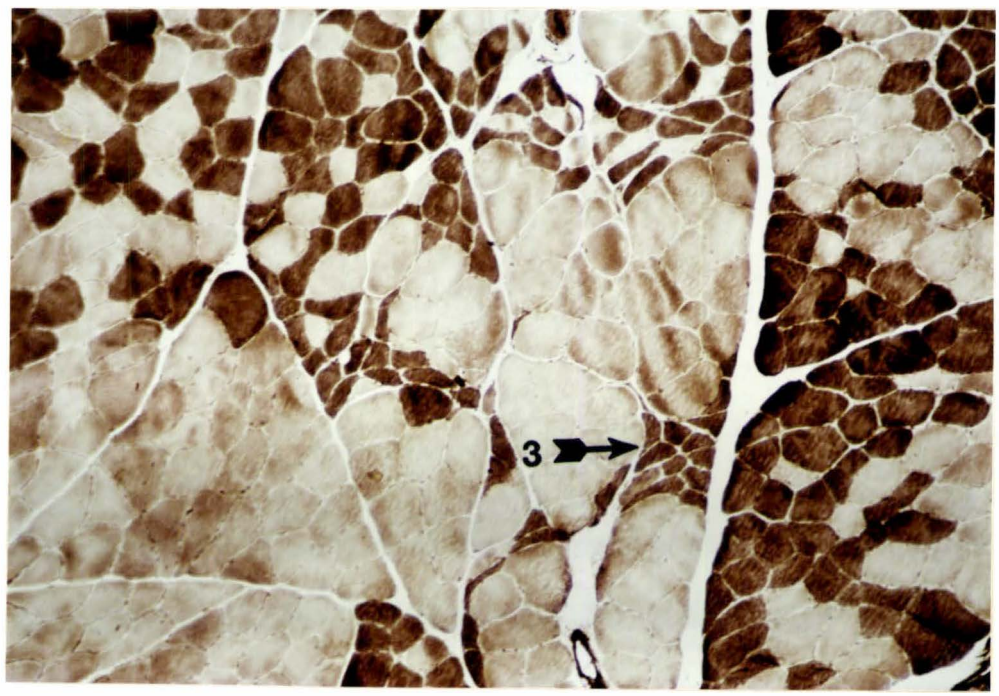


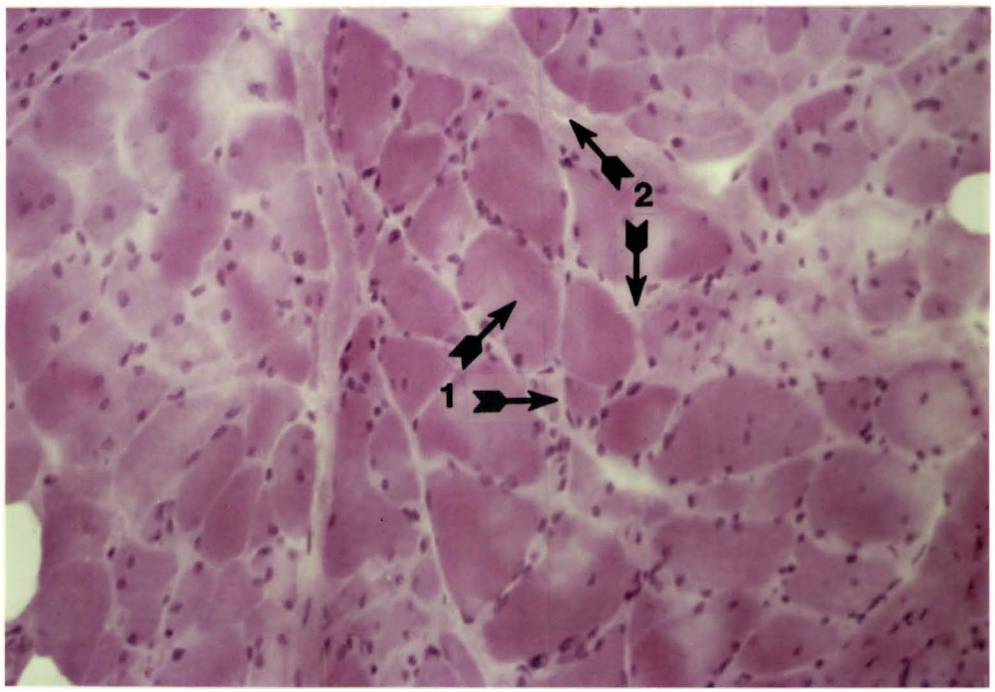
FIGURE 32 Marked pathology. Transverse sections of the left lateral cricoarytenoid muscle of a laryngeal hemiplegic horse

a. The section is stained with haematoxylin and eosin (x260)

b. The section is stained to demonstrate the activity of myosin ATPase (x100)

1. Widespread atrophy and hypertrophy of fibres
2. Marked endomysial and perimysial fibrosis

a



b

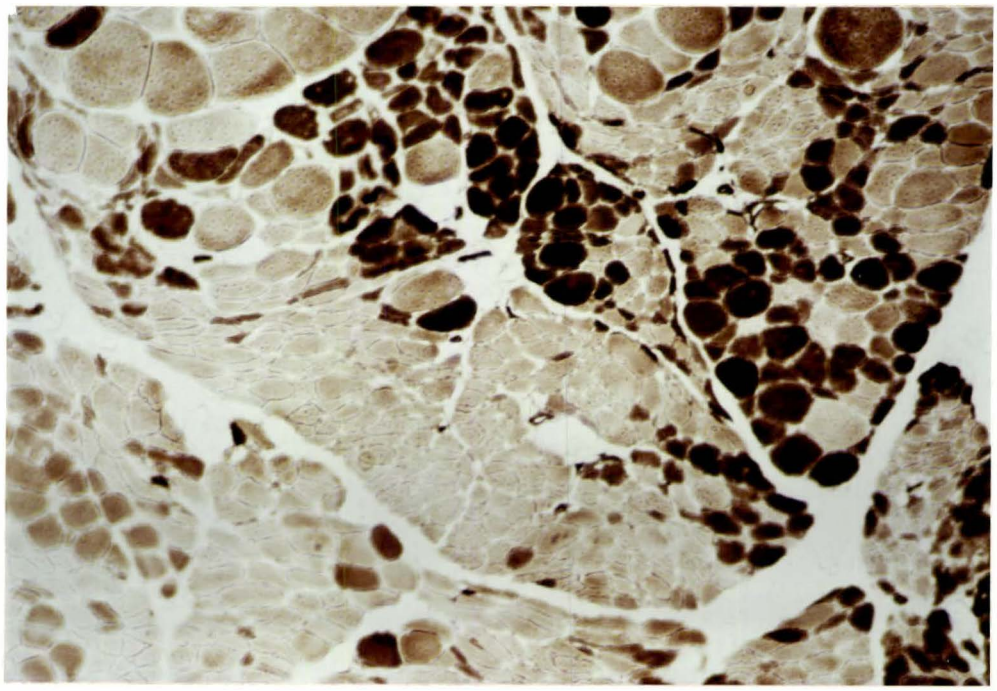


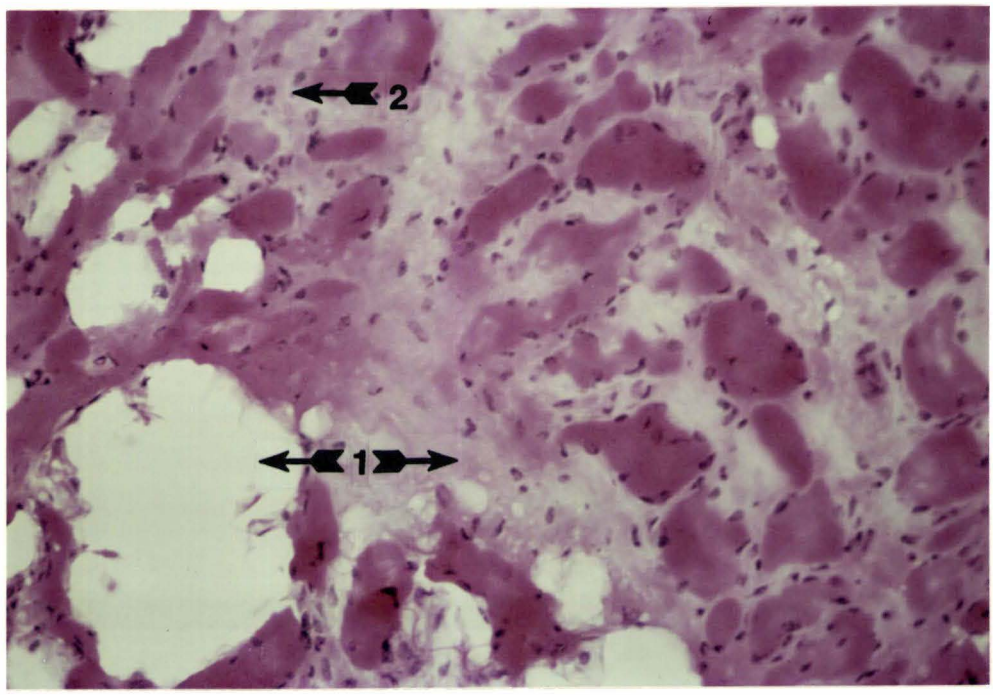
FIGURE 33 Severe pathology. Transverse sections of the left dorsal cricoarytenoid muscle of a laryngeal hemiplegic horse

a. The section is stained with haematoxylin and eosin (x260)

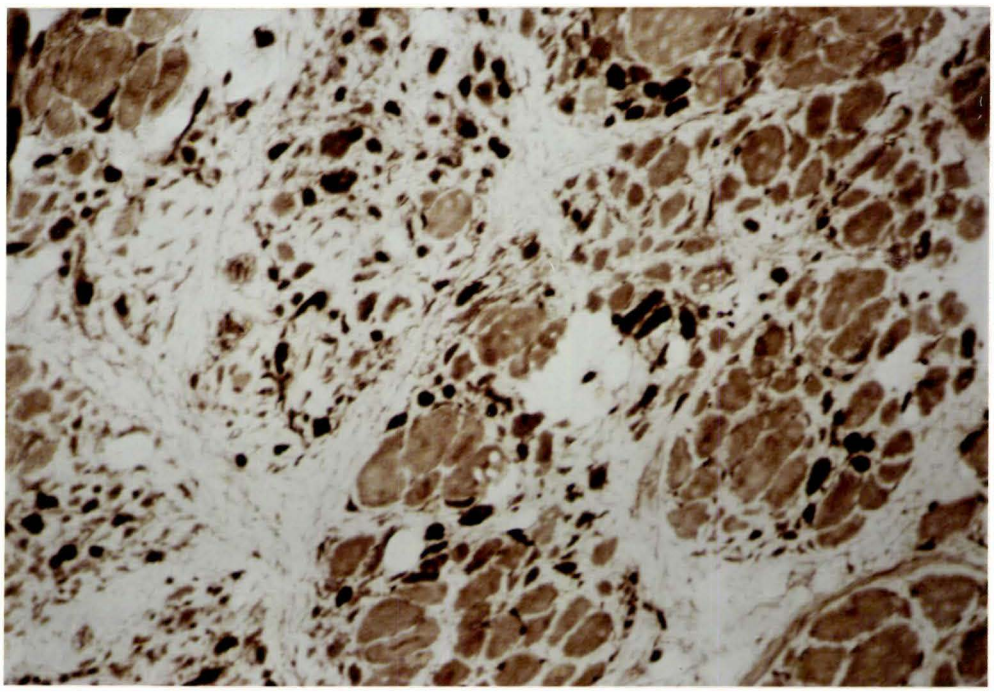
b. The section is stained to demonstrate the activity of myosin ATPase (x100)

1. Fatty and fibrous replacement of muscle fibres
2. Pyknotic nuclear clumps

a



b



Marked pathology (Fig. 32 a and b) included: 1. Widespread atrophy and hypertrophy of fibres. 2. Marked endomysial and perimysial fibrosis.

Severe pathology (Fig. 33 a and b) included: 1. Widespread fatty and fibrous replacement of muscle fibres. 2. Pyknotic nuclear clumps.

In the left dorsal and lateral cricoarytenoid muscles from the older age group of abnormal horses the pathology was so severe that few of the sections were suitable for histochemistry. Consequently mean numbers of AL fibres per group and mean fibre sizes could not be calculated for these muscles.

The numbers of transverse arytenoid and ventricular muscles, and of all muscles from abnormal horses were too small to statistically analyse the differences between the means found in the different groups of horses.

5.3 Results

5.3.1 Size of Groups of AL Fibres

The mean size of groups of AL fibres (as defined by Gunn, 1972) was calculated in all the muscles studied.

5.3.1.1 Dorsal Cricoarytenoid Muscle

The number of horses from which muscles were collected and the mean number of AL fibres per group observed in both the left and right muscles of all the "normal" horses and abnormal horses three years of age and under, are shown in Table 13.

In "normal" horses the mean number of AL fibres per group was larger in the older than the younger group ($P < 0.1$).

When the means were separated according to the sex of the horses there was a tendency for the geldings to have larger groups than either the mares or the entire males. When sides were considered the

tendency was for the groups of AL fibres to be larger in the left muscles, particularly those of the geldings. For example, the mean number of AL fibres per group, in the dorsal cricoarytenoid muscles of "normal" mares over three years of age was 3.28 in the left and 2.66 in the right muscles. The equivalent figures for the same group of geldings were 6.32 and 3.23, moreover, the mean age of this group of mares was greater than the mean age of the geldings.

TABLE 13 THE MEAN NUMBER OF AL FIBRES PER GROUP OBSERVED IN THE DORSAL CRICOARYTENOID MUSCLES

			Mean number of AL fibres per group		
		Number of horses	Both sides	Left	Right
"Normal" horses	all ages	46	3.39	3.8	2.98
	three years and under	28	2.93	2.89	2.97
	over three years	18	4.01	5.04	2.99
Abnormal horses	three years and under	3	17.42	30.59	4.26

When the sections were examined and the largest groups of AL fibres observed in the left muscles were compared with the largest groups observed in the right muscles the following conclusions were reached:-

1. There was no convincing evidence that the groups of AL fibres were larger in the left muscles than the right muscles of the horses less than 1.5 years of age. Groups of 15-20 AL fibres were not uncommon (even in foals as young as one day old) but they were just as likely to be seen in the right muscles as the left.
2. The youngest horse with marked fibre type grouping in the left muscle was a 1.5 year old entire male. The fibre type grouping was accompanied by other histological signs of relatively advanced neurogenic disease. The corresponding right muscle was normal.

3. Of the "normal" Thoroughbred horses over 1.5 years of age 42.9% had fibre type grouping in the left muscles and had larger groups of AL fibres in the left muscles than in the right. In the "normal" Thoroughbred horses greater than five years of age this figure became 63.6%.

4. The abnormal horses with left muscles suitable for histochemistry had marked fibre type grouping in these muscles along with other histological signs of neurogenic disease.

5. Of the six horses with laryngeal hemiplegia, two had marked grouping of AL fibres in the right muscle.

5.3.1.2 Lateral Cricoarytenoid Muscle

The mean numbers of AL fibres per group observed in the left and right muscles of the "normal" horses and the abnormal horses three years of age and under are shown in Table 14.

TABLE 14 THE MEAN NUMBER OF AL FIBRES PER GROUP OBSERVED IN THE LATERAL CRICOARYTENOID MUSCLES

			Mean number of AL fibres per group		
		Number of horses	Both sides	Left	Right
"Normal" horses	all ages	49	3.36	4.25	2.55
	three years and under	28	2.79	3.45	2.20
	over three years	21	4.11	5.28	2.95
Abnormal horses	three years and under	3	5.20	6.15	4.25

The mean number of AL fibres per group in the muscles of "normal" horses over three years of age and abnormal horses less than three years of age were significantly larger than in the muscles of the younger group of "normal" horses ($P < 0.05$ and $P < 0.001$, respectively).

In both the younger and older group of "normal" horses the mean number of AL fibres per group was larger in the left muscles than the right ($P < 0.1$ and $P < 0.05$ for the younger group and older group respectively).

When the mean group sizes for the left and right muscles were divided according to the sex of the horses it was again in the geldings where most of the significant differences occurred. For example, the mean number of AL fibres per group in the left lateral cricoarytenoid muscle of all the "normal" geldings was 6.03 and 2.97 in the right muscle (significantly different $P < 0.02$).

When the muscle sections were examined to assess the level of fibre type grouping in the lateral cricoarytenoid muscle the following conclusions were reached:-

1. Relatively large groups of AL fibres (up to 25) were not uncommon in both the left and right lateral cricoarytenoid muscles of foals.
2. The youngest horse with substantially larger groups of AL fibres in the left muscle than in the right muscle was a six week old entire male and the next youngest was a seven month old female.
3. Of the "normal" Thoroughbred horses over six months of age, 65.5% had fibre type grouping in the left muscles and had larger groups of AL fibres in the left muscle than the right. Almost all of these, with the exception of a few of the younger horses, had other histological signs of neurogenic disease in the left muscles. In this group of horses 22% had large groups of AL fibres in the right muscle. All but one of the horses with grouping in the right muscle, were over six years of age. If only the older group of horses (over three years) was considered, almost 80% of them had larger groups of AL fibres in their left than right muscles.
4. Of the abnormal horses, the three younger ones had marked fibre type grouping in the left muscle and two of these had obvious grouping in the right muscle. The left muscles of the older horses were so severely atrophied that measurement of fibre group size was not possible but fibre type grouping was observed in the right muscles of two of these animals.

5.3.1.3 Transverse Arytenoid Muscle

The number of horses from which muscles were collected and the mean numbers of AL fibres per group in the left and right transverse arytenoid muscles of the "normal" and the abnormal horses are shown in Table 15.

TABLE 15 THE MEAN NUMBER OF AL FIBRES PER GROUP OBSERVED IN THE TRANSVERSE ARYTENOID MUSCLES

		Mean number of AL fibres per group		
	Number of horses	Both sides	Left	Right
"Normal" horses	6	2.17	2.58	1.76
Abnormal horses	4	2.46	2.63	2.28

There was a tendency for the groups of AL fibres to be bigger in the muscles from abnormal horses than "normal" horses and bigger in the left muscles than the right muscles of both groups of horses.

When the muscle sections from individual horses were examined it was noted that there were larger groups of AL fibres in the left muscles than the right muscles of the four "normal" Thoroughbred horses greater than two years of age. In the left muscles of these four horses this grouping was always accompanied by other histological signs of neurogenic disease. The left muscles of all the abnormal horses had larger groups of AL fibres than the right muscles except in the case of the oldest 'roarer' where the left muscle was so severely atrophied that fibre typing was not possible.

5.3.1.4 Ventricular Muscle

The mean number of AL fibres per group in the muscles from all the "normal" horses was 1.56 and that in the muscles from all the abnormal horses was 1.23. The means obtained when the

horses were divided according to age and sex and when the left and right muscles were compared, did not vary significantly from these values although the number of muscles collected was small.

When the sections were examined individually there was no evidence of larger groups of AL fibres in the left or right muscles of the "normal" or abnormal horses although other histological signs of neurogenic disease were present.

5.3.1.5 Cricothyroid Muscle

The number of horses from which muscles were collected and the mean numbers of AL fibres per group observed in the left and right cricothyroid muscles from the different age groups of "normal" and abnormal horses are shown in Table 16.

TABLE 16 THE MEAN NUMBER OF AL FIBRES PER GROUP OBSERVED IN THE CRICOTHYROID MUSCLES

			Mean number of AL fibres per group		
		Number of horses	Both sides	Left	Right
"Normal" horses	all ages	45	4.24	4.38	4.08
	three years and under	27	3.19	3.11	3.48
	over three years	18	5.41	5.98	4.85
Abnormal horses	all ages	7	6.17	7.80	4.53
	three years and under	3	5.32	6.09	4.56
	over three years	4	6.80	9.09	4.51

The mean number of AL fibres per group was significantly greater in the muscles from the older than younger "normal" horses ($P < 0.02$). The group sizes also tended to be larger in the left muscles of the older "normal" horses than in the right

muscles. This difference was significant for the "normal" geldings (left 5.98, right 4.26, $P < 0.02$) and was marked in the muscles of the abnormal entire males (left 10.90 right 3.51).

The cricothyroid muscle was found to have a higher proportion of AL fibres (AH:AL, 64%:36%) than most of the other intrinsic laryngeal muscles. It was also noted that the proportion of AL fibres tended to be greater in the older than in the younger horses (Chapter 4).

In the cricothyroid muscle the AL fibres were often present in relatively large groups. In fact, groups of up to 22 AL fibres occurred in both the left and right cricothyroid muscles of most of the horses under one year of age. With increasing age the group size in both left and right cricothyroid muscles also increased. Groups of up to 40 AL fibres were common in both the left and right muscles from the older age groups of both "normal" and abnormal horses.

5.3.1.6 Hyoepiglottic Muscle

The mean number of AL fibres per group in the muscles of all the "normal" horses was 10.73. There was no significant variation from this in the muscles of the other groups of horses including the abnormal horses with the exception of the one horse examined which had suffered from laryngo-palatal dislocation. In the hyoepiglottic muscle from this horse the mean group size of AL fibres was 2.41.

When the muscle sections were examined individually it was noted that the AL fibres were all in large groups. This occurred in the muscles of all the different groups of horses including the abnormal horses. The hyoepiglottic muscle, unlike the other laryngeal muscles examined, had more AL than AH fibres (AH:AL, 46%:54%, Chapter 4).

5.3.2 Fibre Cross Sectional Area

5.3.2.1 Dorsal Cricoarytenoid Muscle

The number of horses from which muscles were collected and the mean cross sectional areas of the AH and AL fibres are shown in Table 17.

TABLE 17 THE MEAN CROSS SECTIONAL AREAS (μm^2) OF AH AND AL FIBRES IN THE DORSAL CRICOARYTENOID MUSCLES

		Number of horses	Fibre type	Mean cross sectional area		
				Both sides	left	right
"Normal" horses	all ages	46	AH	2006.07	2097.52	1914.62
			AL	1945.78	2097.21	1772.71
	three years and under	28	AH	1448.28	1490.54	1406.03
			AL	1337.29	1450.27	1224.31
	over three years	18	AH	2769.34	2928.13	2610.55
			AL	2778.34	3189.87	2366.82
Abnormal horses	three years and under	3	AH	2527.83	1868.67	3187.00
			AL	2674.92	2381.33	2968.50

There was no significant difference between the mean cross sectional area of AH and AL fibres in the muscles of any of the horses examined.

As expected the mean cross sectional area of both AH and AL fibres was greater in the muscle of the older than younger horses. This increase in cross sectional area of muscle fibres with age is shown in Fig. 34 and it produced significant differences between the mean muscle fibre areas from the different age groups of horses.

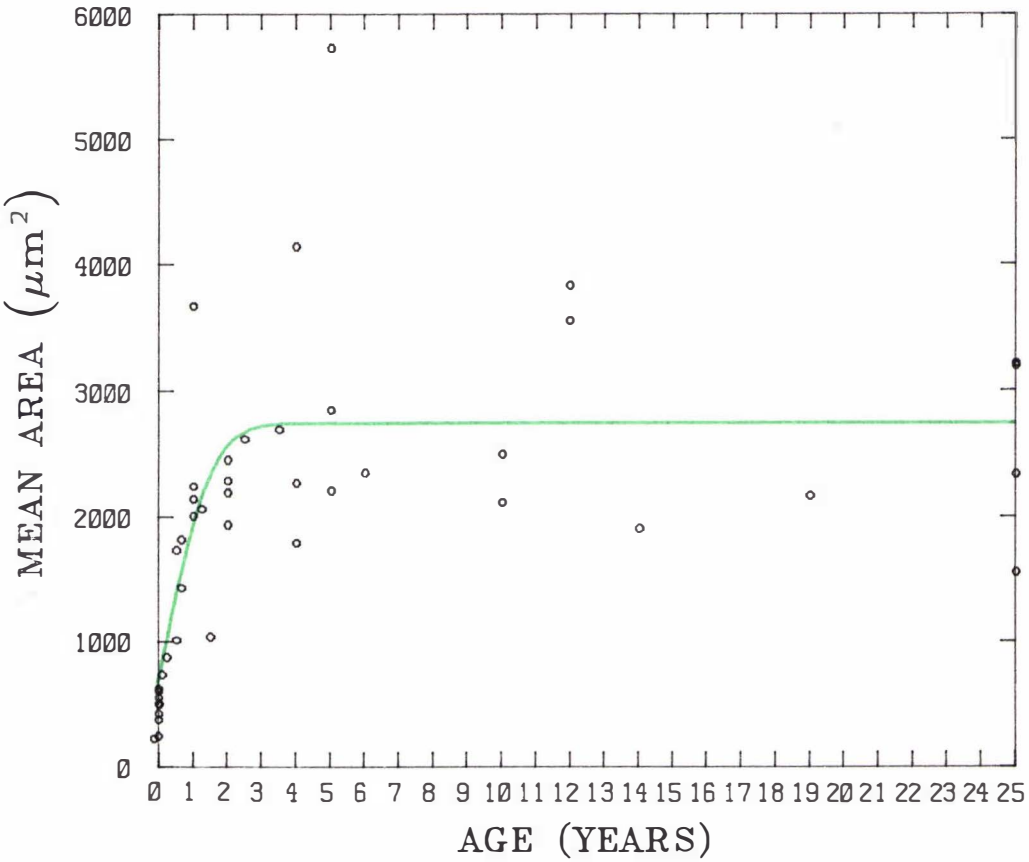
In the "normal" mares and geldings the AH fibres had greater cross sectional areas in the left muscles than the right ($P < 0.05$). This was also true for the AL fibres of the "normal" mares ($P < 0.05$) and geldings ($P < 0.01$).

In the abnormal horses the number of fibre area measurements available were too small for statistical analysis however the AL and particularly the AH fibres in the muscles of the younger group were smaller on the left side than the right.

Pathology was so severe in the older group of abnormal horses that only

FIGURE 34 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the dorsal cricoarytenoid muscles from the "normal" horses

FIGURE 35 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the lateral cricoarytenoid muscles from the "normal" horses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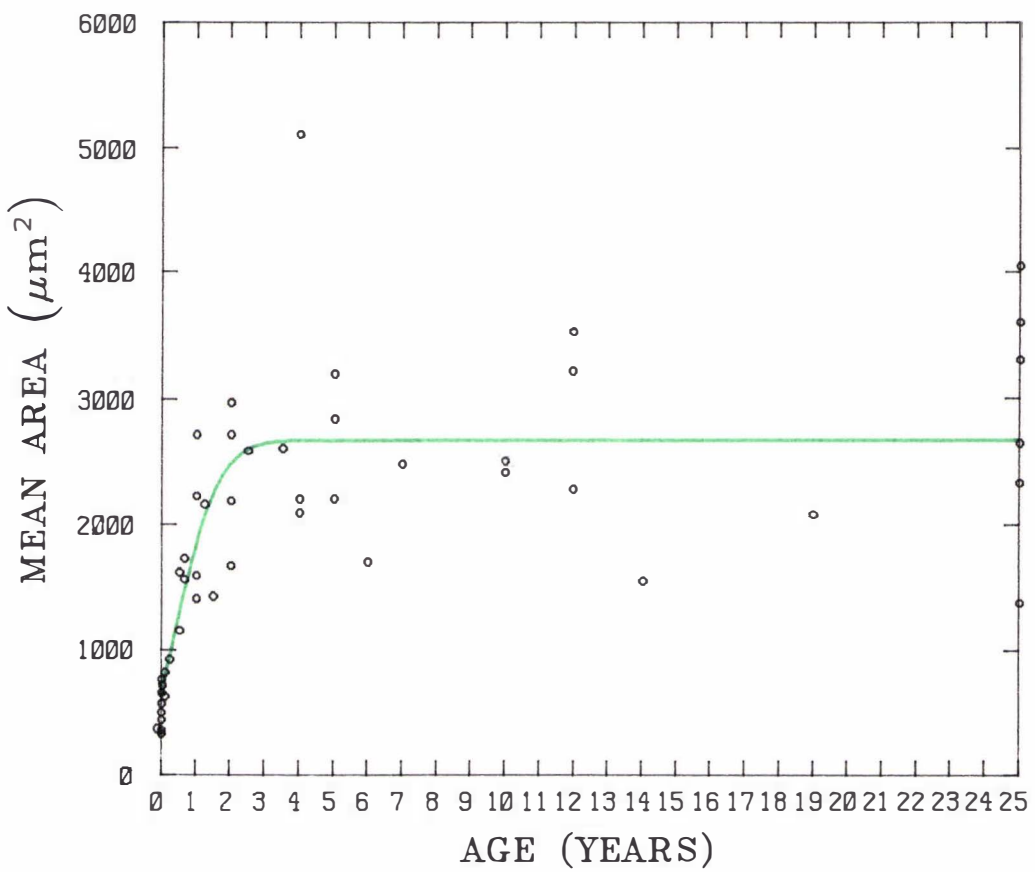
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one had a left muscle suitable for histochemistry. In this muscle there was hypertrophy (7543 μm^2) and atrophy (908 μm^2) of AH fibres and atrophy (1430 and 703 μm^2) of AL fibres. The mean cross sectional fibre areas in the right muscles from this group of horses were 3167.12 μm^2 for AH and 2667.0 μm^2 for AL fibres.

5.3.2.2 Lateral Cricoarytenoid Muscle

The number of horses from which muscles were collected and the mean cross sectional areas of the AH and AL fibres from the lateral cricoarytenoid muscles are shown in Table 18.

TABLE 18 THE MEAN CROSS SECTIONAL AREAS (μm^2) IN THE LATERAL CRICOARYTENOID MUSCLES

				Mean cross sectional area		
		Number of horses	Fibre type	Both sides	left	right
"Normal" horses	all ages	49	AH	2077.64	2214.2	1941.09
			AL	1788.42	2028.3	1548.53
	three years and under	28	AH	1532.15	1493.3	1571.00
			AL	1229.35	1239.02	1195.13
	over three years	21	AH	2824.04	3200.55	2447.53
			AL	2553.84	3075.55	2032.13
Abnormal horses	three years and under	3	AH	2801.00	2973.5	2628.5
			AL	3261.42	4523.67	1999.17

There was a notable difference between the mean cross sectional area of the AH and AL fibres in the lateral cricoarytenoid muscles of only one of the groups of horses examined. In the muscles of the older group of "normal" horses the AL fibres were smaller than the AH fibres ($P<0.1$). As was the case with the dorsal cricoarytenoid muscle there was an increase in the mean cross sectional area of both fibre types with age. This is shown in Fig. 35 and was responsible for significant differences between the mean cross sectional area of muscle fibres from the different

age groups of horses.

In the lateral cricoarytenoid muscle also, the mean cross sectional area of the fibres of some of the left muscles were larger than those of the right muscles. This difference was significant in the muscles of the "normal" horses greater than three years of age ($P < 0.02$ for AH fibres, and $P < 0.01$ for AL fibres) and was most pronounced in the geldings ($P < 0.05$ for AH fibres and $P < 0.02$ for AL fibres).

Pathology was again severe in the left muscles of the older group of abnormal horses and in the one muscle where the myosin ATPase reaction worked all the fibres appeared to be AH. Both the mean cross sectional area measurements of these fibres were less (1583 μm^2 and 414 μm^2) than those from the right muscles (AH, 2632.5 μm^2 and AL, 2309.5 μm^2).

5.3.2.3 Transverse Arytenoid Muscle

The mean cross sectional areas of the AH and AL fibres (1782.29 and 991.21 μm^2) of the transverse arytenoid muscles from the 'normal' horses were smaller than those of the fibres of the other two muscles previously discussed. Both the AH and AL fibres were larger in the older than the younger "normal" horses (Fig. 36). There was a tendency for the AL fibres to be smaller than the AH fibres in the "normal" horses.

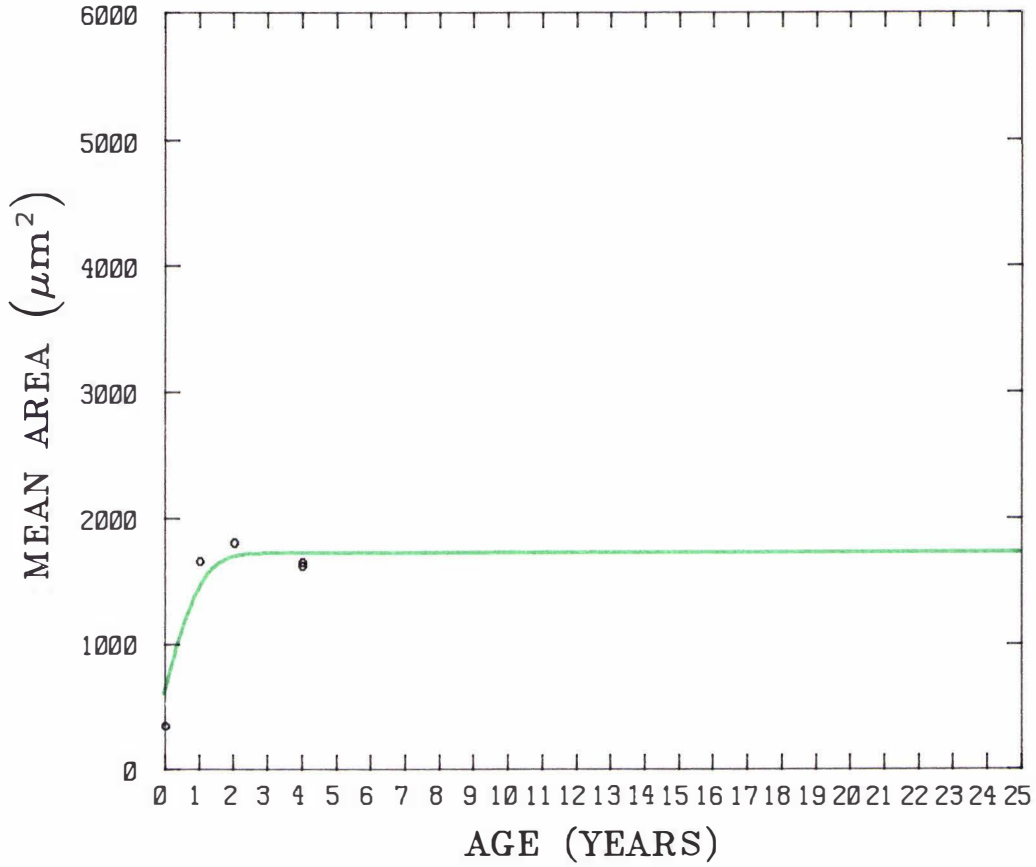
The AH and AL fibres were smaller in the left transverse arytenoid muscles than in the corresponding right muscles in the younger age group of "normal" and abnormal horses. In the one abnormal horse over three years of age from which muscles were collected, the left muscle was too severely affected for histochemistry to be carried out. The AH and AL fibres in the right muscle from this horse were larger than those from the right muscles of similar aged "normal" horses.

5.3.2.4 Ventricular Muscle

The mean cross sectional areas of the AH and AL fibres (1598.71 and 1252.8 μm^2) of the ventricular muscles of "normal" horses were also smaller than those in the dorsal and lateral cricoarytenoid muscles.

FIGURE 36 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the transverse arytenoid muscles from the "normal" horses

FIGURE 37 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the ventricular muscles from the "normal" horses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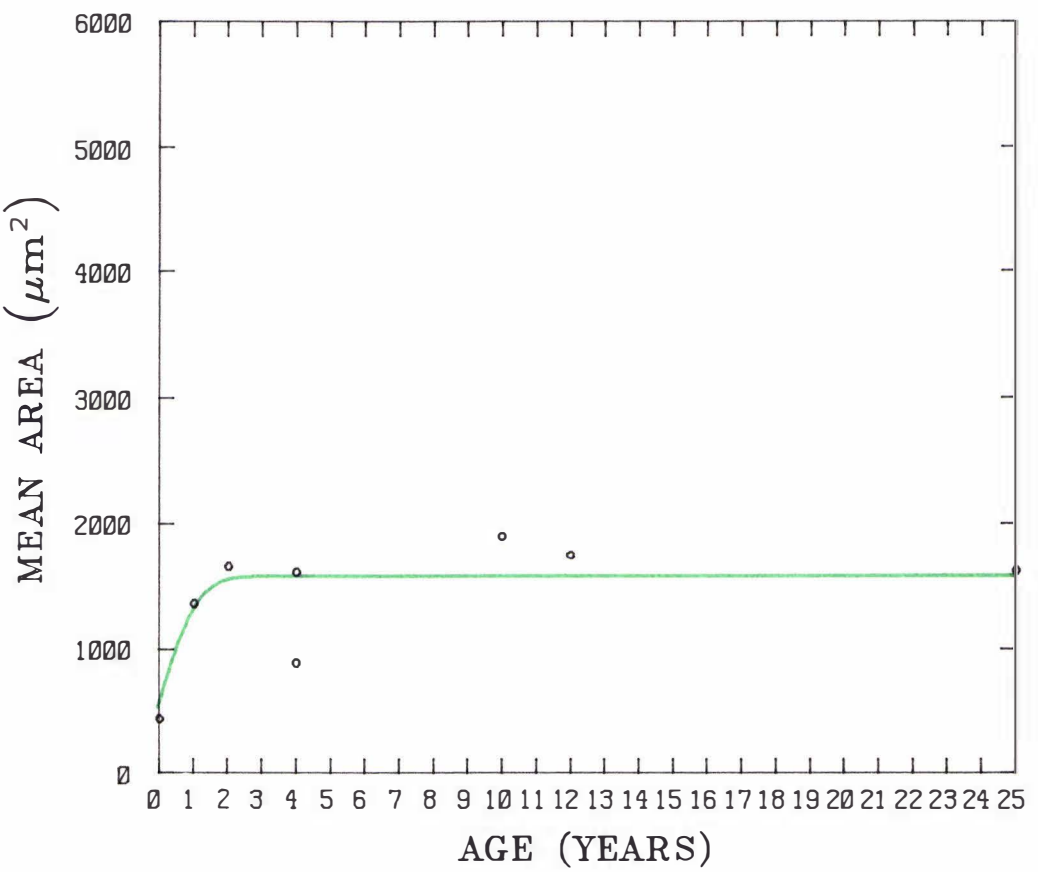
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Again the mean cross sectional areas of AH and AL fibres were greater in the muscles of the older than those of the younger "normal" horses (Fig. 37).

The muscles of the younger group of abnormal horses had smaller AH fibres than those of similar aged "normal" horses and the AH fibres from their left muscles were smaller than those from their right muscles. The AL fibres from the left muscles of this group of horses were larger than those from the right.

5.3.2.5 Cricothyroid Muscle

The number of horses from which muscles were collected and the mean cross section area of AH and AL fibres from all the groups of horses are shown in Table 19.

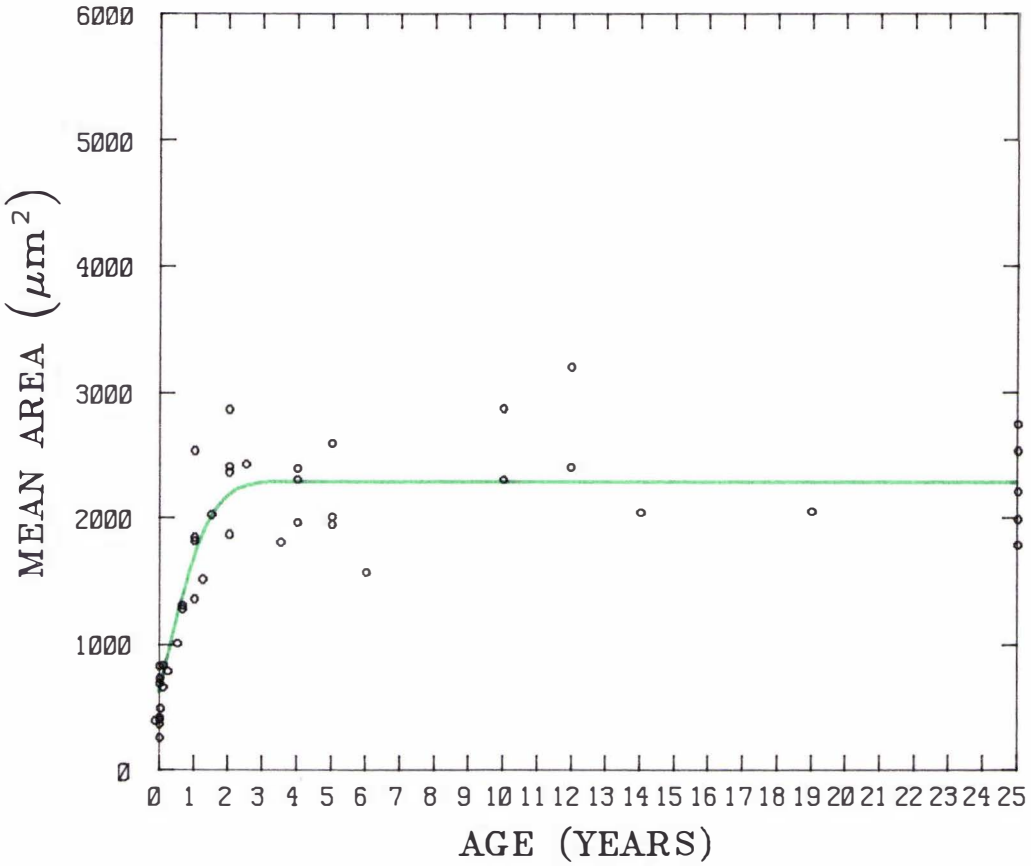
TABLE 19 THE MEAN CROSS SECTIONAL AREAS (μm^2) OF AH AND AL FIBRES IN THE CRICOTHYROID MUSCLES

		Number of horses	Fibre type	Mean cross sectional area
"Normal" horses	all ages	45	AH	1736.96
			AL	1676.66
	three years and under	27	AH	1407.43
			AL	1187.66
	over three years	18	AH	2167.57
			AL	2319.80
Abnormal horses	three years and under	3	AH	2334.42
			AL	2572.83
	over three years	4	AH	2636.81
			AL	2374.00

In this muscle the mean cross sectional area of the AH and AL fibres was greater in the muscles from the older group of "normal" horses

FIGURE 38 The mean reslationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the cricothyroid muscles from the "normal" horses, plotted against their ages (years)

FIGURE 39 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the hyoepiglottic muscles from the "normal" horses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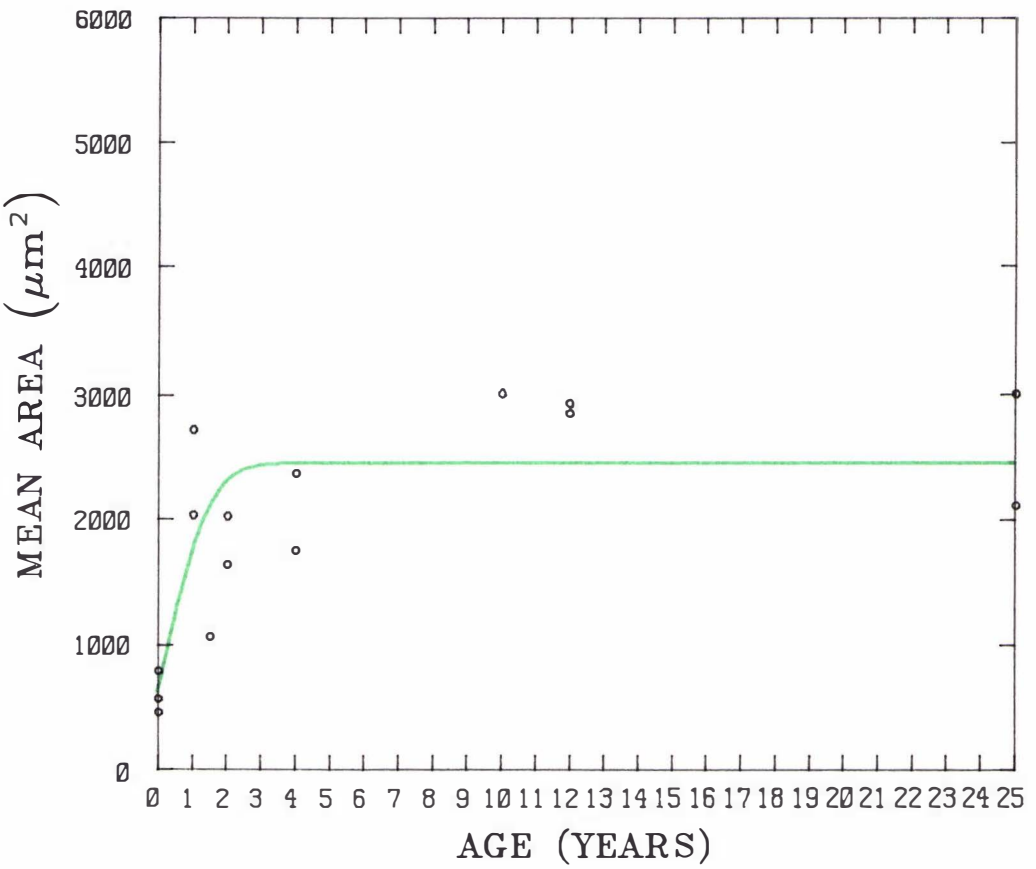
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than in those from the younger group. This is shown in Fig. 38 and produced significant differences between the mean fibre sizes from the different age groups of horses.

One significant interaction which did not appear to be due to this increase in fibre size with age was noted in the muscles from the older group of abnormal horses where the AH and AL fibres were larger than those in the muscles of the same group of "normal" horses ($P < 0.05$).

In most of the groups examined the sex of the horse or the side from which the muscle came did not appear to influence the mean size of AH or AL fibres. There was however a tendency for the AH and AL fibres from the left muscles of "normal" and abnormal horses to be larger than those from the right. This trend was most marked in the muscles from the geldings and was significant for the AL fibres from the older "normal" horses ($P < 0.02$).

5.3.2.6 Hyoepiglottic Muscle

The numbers of horses from which muscles were collected and the mean cross sectional areas of the AH and AL fibres from all the groups of "normal" horses and the abnormal horses are shown in Table 20.

TABLE 20 THE MEAN CROSS SECTIONAL AREAS (μm^2) OF AH AND AL FIBRES IN THE HYOEPIGLOTTIC MUSCLES

		Number of horses	Fibre type	Mean cross sectional area
"Normal" horses	all ages	15	AH	1928.30
			AL	1986.03
	three years and under	8	AH	1385.44
			AL	1428.50
	over three years	7	AH	2548.70
			AL	2623.21
All abnormal horses		4	AH	2150.50
			AL	2107.13

Again in the hyoepiglottic muscle the AH and AL fibres were larger in the muscles from the older than those from the younger horses (Fig. 39). This age increase again caused significant differences between the mean cross sectional areas of the fibres from the muscles of the different age groups of horses.

The sex of the horses appeared to have no effect on fibre cross sectional area.

5.3.3 Histological Features Noted in This Study

5.3.3.1 Histological Features of Juvenile Muscle

The histological characteristics of the muscles from foals appeared to be somewhat different from those of the adult horses (Fig. 40). The fibres from juvenile muscle were round in cross section rather than polygonal and there was more variation in fibre size than was usually seen in adult muscle. The round cross section of the fibres gave the endomysial and perimysial connective tissue septa a more prominant appearance so that in juvenile muscle the connective tissue components appeared to occupy a higher proportion of the total cross sectional area than in the muscle of adults.

The population of sarcolemmal nuclei appeared higher in juvenile muscle than in adult muscle and the occurrence of these nuclei within the body of the fibres was not uncommon.

There was no apparent side difference in the histological features of the muscles from horses less than one year of age. By the time the horses reached approximately one year of age the histological features of their muscles were assuming adult characteristics. The apparent number of sarcolemmal nuclei was decreasing and the connective tissue components were less prominant and present only as thin bands between polygonal fibres.

5.3.3.2 Histopathological Changes Characteristic of Denervation Noted in This Study

All the histological features already described as being characteristic of denervation and reinnervation were noted in some of the muscles collected during this study. An attempt was

FIGURE 40 Transverse sections of the left dorsal cricoarytenoid muscles from foals, (a) two hours and (b) two days old (x260). Sections stained with haematoxylin and eosin. The histological features of juvenile equine intrinsic laryngeal muscle are illustrated

1. Nuclear population greater than that observed in normal mature muscles
2. Internal nucleus
3. Fibres round in cross section and varying in cross sectional area
4. Prominant endomysium
5. Prominant perimysium

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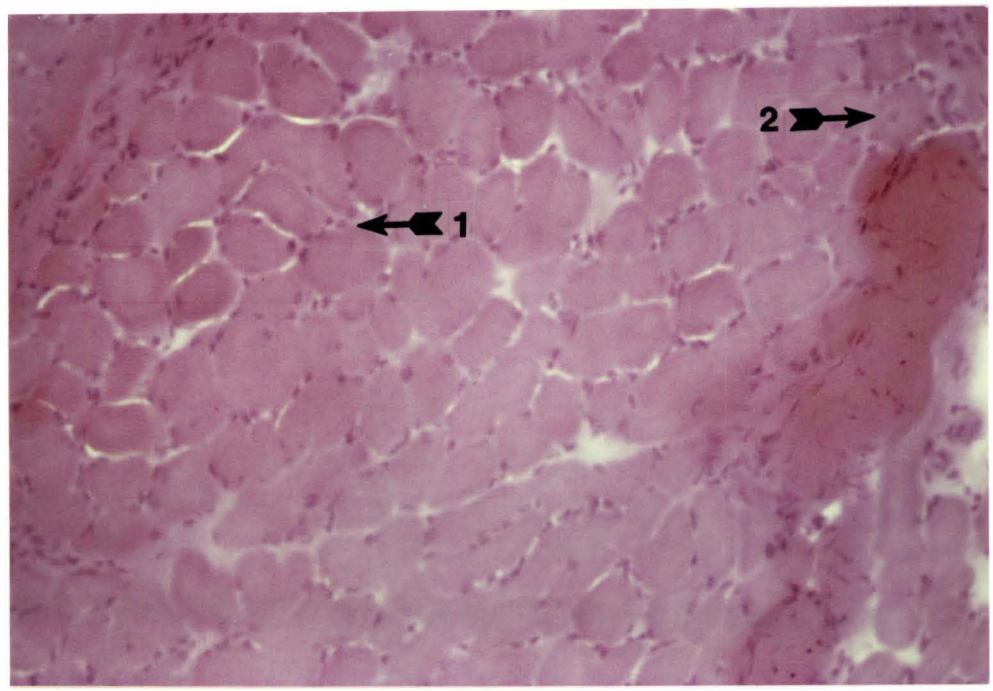

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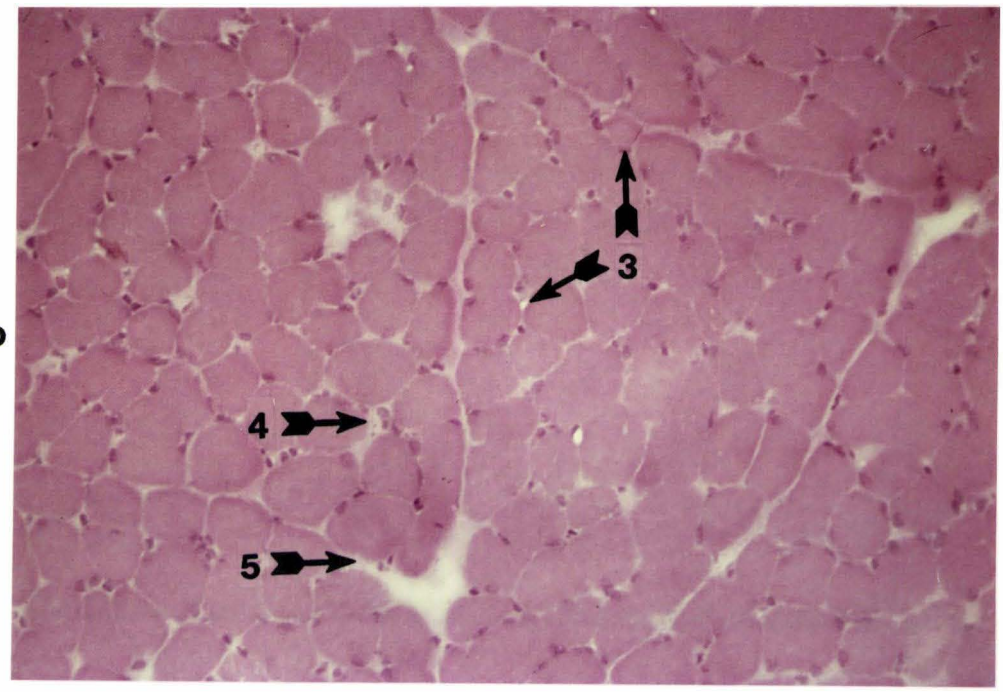


made to grade these features noted in the individual muscles of adult horses according to the severity of the pathological process present so that some within and between horse comparisons could be made.

5.3.3.2.1 Dorsal Cricoarytenoid Muscle

In the muscles from horses up to 1.5 years of age there was no convincing evidence of histological changes characteristic of denervation and reinnervation. The youngest horse with obvious pathology in its dorsal cricoarytenoid muscle was a 1.5 year old entire male. The changes noted were classed as marked and the left muscle only was involved. Thirty-eight percent of the "normal" Thoroughbred horses over 1.5 years of age had subtle to marked pathology in their left dorsal cricoarytenoid muscles. Only one horse (0.05%) from this group had evidence of histopathology in the right dorsal cricoarytenoid muscle and this was classed as subtle.

The histological changes observed in the "normal" horses were not as well developed as those in the laryngeal hemiplegic horses. It appeared that there may have been an age after which there was a decrease in the percentage of horses showing histopathology in their left dorsal cricoarytenoid muscles. There were 13 "normal" Thoroughbred horses between 1.5 and six years of age. Of these, 10 were males and histopathology was observed in the left dorsal cricoarytenoid muscle of seven of these horses. The muscles from the three females in this group showed no signs of histopathology. There were eight "normal" Thoroughbred horses over six years of age, three geldings and five mares. The only horse in this group with histopathology in its left dorsal cricoarytenoid muscle was a 12 year old gelding and in this animal the pathology was classed as marked.

Of the laryngeal hemiplegic horses the youngest had moderate changes and the others had marked to severe changes in their left dorsal cricoarytenoid muscles. They all had subtle to moderate changes in their right dorsal cricoarytenoid muscles except the oldest where the changes were marked. The horse which had suffered from laryngo-palatal dislocation had subtle changes in its left dorsal cricoarytenoid muscle and its right muscle was normal.

None of the histopathology associated with denervation and reinnervation was observed in the muscles of the three ponies.

5.3.3.2.2 Lateral Cricoarytenoid Muscle

Apart from fibre type grouping, histological evidence of denervation and reinnervation was not apparent in the muscles from horses less than one year of age. A one year old entire male was the youngest horse in which histopathological changes were observed in the left lateral cricoarytenoid muscle. The changes in the left muscle were classed as moderate and the right muscle was normal. Of the "normal" horses over this age 68% had subtle to severe histopathological changes in their left muscles and 24% had subtle or moderate changes in their right muscles. It appeared that the changes observed were more severe and occurred more frequently than in the dorsal cricoarytenoid muscles.

In the lateral cricoarytenoid muscle there did not appear to be an age at which the incidence of pathology was greatest as the majority of horses over one year of age were affected. Most of the "normal" males between one and six years of age exhibited pathology in their left muscles whereas only one of the six females in this group was affected. The pathology observed in this group was often more severe than that seen in the horses over six years of age.

The laryngeal hemiplegic horses all had marked or severe changes in their left muscles and 66.6% of their right muscles had subtle or moderate changes. The horse which had suffered from laryngo-palatal dislocation had marked changes in its left muscle whereas the right muscle was normal.

No histopathological changes were noted in the muscles of the ponies.

5.3.3.2.3 Transverse Arytenoid Muscle

Only a relatively small number of transverse arytenoid muscles were available for histopathological examination (Table 11). The one foal included (24 hours old) had normal left and right transverse arytenoid muscles.

The muscles from four "normal" Thoroughbred horses over one year of age (one - four years) were available. The left transverse arytenoid muscle from the youngest horse in this group was normal and the other three had subtle to marked histopathological changes. The right muscles from these horses were all normal.

The left transverse arytenoid muscles from three laryngeal hemiplegic horses were examined and all had evidence of moderate to marked histopathological change. Their right muscles all exhibited subtle changes.

The muscles from the horse which had suffered from laryngo-palatal dislocation showed marked change on the left side and subtle change on the right side.

5.3.3.2.4 Ventricular Muscle

The ventricular muscles from the only foal (24 hours) examined were found to be normal. Seven other pairs of ventricular muscles from "normal" Thoroughbred horses over one year of age (one year to greater than 20 years) were available for examination. Three (42%) of the left muscles from these horses showed subtle to marked changes and only one (14%) of the right muscles (from an aged mare) showed any histopathological changes and these were graded as subtle. All the left ventricular muscles of the laryngeal hemiplegic horses showed mild to marked histopathological changes but 75% of their right muscles were normal, the remainder showing only subtle changes.

The horse with laryngo-palatal dislocation had normal left and right ventricular muscles.

5.3.3.2.5 Cricothyroid Muscle

The cricothyroid muscles from all the horses were similar in their histological features and there was no evidence of any side difference or differences between the muscles from normal and abnormal horses.

The only histological changes noted were in the muscles from some of

the older horses where there were occasional isolated areas with an increased nuclear population, endomysial fibrosis and some central nuclei and split fibres. These were just as likely to occur in the left or right muscles of "normal" or abnormal horses.

5.3.3.2.6 Hyoepiglottic Muscle

The histological features of the hyoepiglottic muscle from adult horses appeared to be somewhat different from the other adult muscles so far described. The muscle fibres had a round rather than polygonal cross section and there was more variation in fibre size than was usual in the other adult muscles. The population of sarcolemmal nuclei appeared higher in this muscle than in the other adult muscles examined, as did the proportion of the total muscle cross sectional area occupied by fibrous tissue elements so that the endomysial and perimysial connective tissue septa were prominant (Fig. 41).

The muscles of all the horses, including the foals, the laryngeal hemiplegics and the horse with laryngo-palataldislocation were similar in appearance and, with one possible exception, histopathological changes characteristic of denervation and reinnervation were not seen.

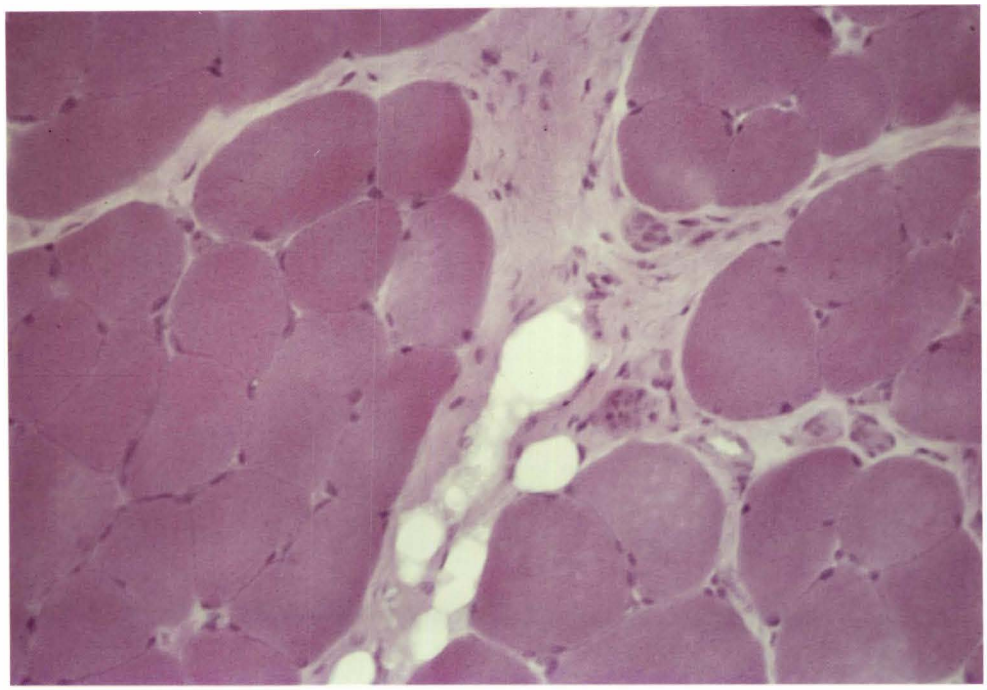
The muscle from an aged Thoroughbred mare (horse 54), had a bigger variation in fibre size than the other hyoepiglottic muscles and the presence of internal nuclei and some fibre splitting was noted.

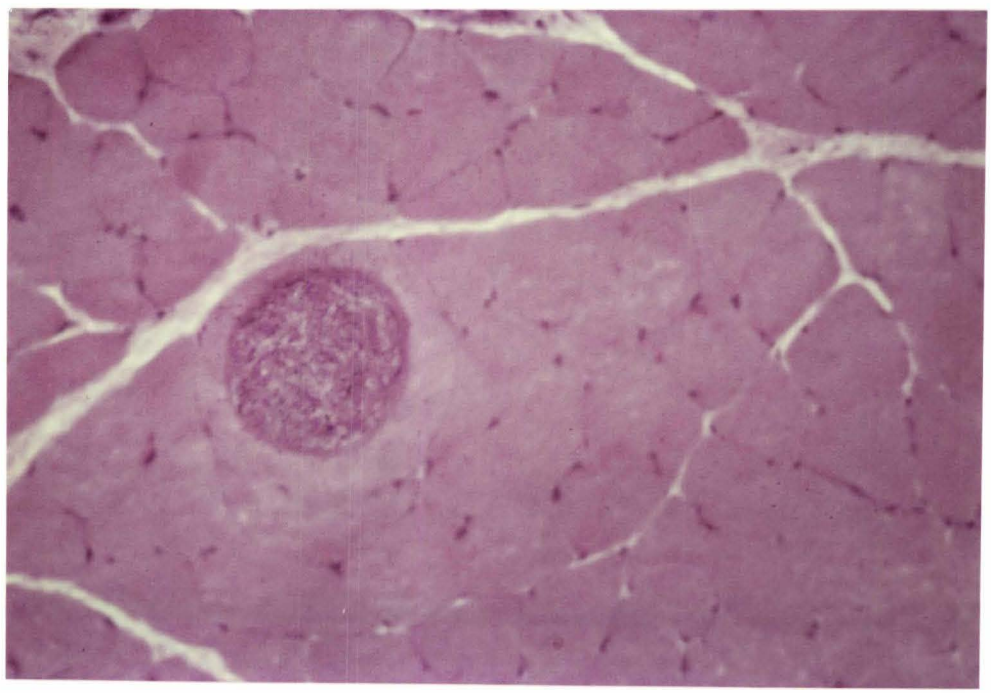
5.3.3.3 Other Features Noted During Histological Examination of Muscles

The only other histological feature which was noted during individual examination of the muscle sections was the occasional appearance of sarcocysts. When these occurred there was normally only one per section and they appeared as circular membranous capsules within or between the muscle fibres (Fig. 42). They were seen in the left and right dorsal cricoarytenoid, lateral cricoarytenoid, cricothyroid and the hyoepiglottic muscles of both "normal" and laryngeal hemiplegic horses over three years of age. They were not observed in the muscles of the younger group of horses. In some horses subtle to marked histopathological changes were evident in the same muscles as the sarcocysts, but other muscles where sarcocysts occurred were histologically normal.

FIGURE 41 A transverse section of the hyoepiglottic muscle from a "normal" horse. Section stained with haematoxylin and eosin (x260)

FIGURE 42 A transverse section of the cricothyroid muscle from a "normal" horse. Section stained with haematoxylin and eosin (x260). A sarcocyst is evident





5.4 Discussion

5.4.1 Size of Groups of AL Fibres

The presence of larger than normal groups of AL fibres in some equine laryngeal muscles has been considered to provide evidence of the presence of denervation and reinnervation in that muscle. The results of the present study however indicate that there are other factors which may influence the size of groups of AL fibres in laryngeal muscles. In addition to the presence of disease which results in denervation and reinnervation, fibre type grouping appears to be influenced by the proportion of AL to AH fibres, age, sex and breed.

An important influence on the size of groups of AL fibres in the muscles of "normal" horses is the proportion of AL to AH fibres present. In muscles which have a high proportion of AL fibres the average group size of these fibres would be expected to be larger. Such is the case in the hyoepiglottic and cricothyroid muscles. The hyoepiglottic was the only muscle studied in "normal" horses which had a fibre population consisting of predominantly AL fibres and the average group size (10.73) was much larger than that from the other muscles examined. In the cricothyroid muscle where the percentage of AL fibres was higher than in other intrinsic laryngeal muscles, it was common to observe groups of 20-40 fibres in both left and right muscles. This was not the case in other intrinsic laryngeal muscles of "normal" adult horses. Conversely in muscles such as the ventricular which contained comparatively few AL fibres, no large groups were observed in any of the horses examined.

Age apparently had an important effect on the size of groups of the fibres in some intrinsic laryngeal muscles. In immature animals relatively large groups (15 or more) of AL fibres were observed in some intrinsic laryngeal muscles from both the left and right sides. Gunn (1973) had observed relatively large groups of AL fibres in foals and considered that they could indicate the presence of denervation and reinnervation. However as Duncan (1975) noted, Gunn's observations were made on a small number of animals and the apparent difference in group size on the left and right sides was not evaluated statistically. Moreover Guth and Samaha (1972) questioned the relationship between the histochemical reaction for myosin ATPase and the biochemical level of this enzyme in very young muscle fibres. They considered that the relationship may be

different from that in the adult. If this is the case, relatively large groups of AL fibres could occur in immature muscle without necessarily being associated with denervation and reinnervation. In the present study the frequent observance of large groups of AL fibres in some immature intrinsic laryngeal muscles (including the cricothyroid) with approximately equal incidence on left and right sides seems to indicate that these groups are not necessarily associated with the process of denervation and reinnervation. Their significance in very young animals is therefore conjectural.

Notwithstanding this, the observation of larger groups of AL fibres in the left than in the right lateral cricoarytenoid muscles of three of the 16 horses less than one year of age, could indicate the occasional occurrence of denervation and reinnervation in these muscles. The findings in very young animals however did not provide evidence in support of Gunn's (1973) theory that denervation and reinnervation were present in the laryngeal muscles of the horse at birth. In fact larger left than right AL fibre groups were not observed in any of the 10 animals less than six weeks of age.

Age also appeared to have some effect on groups of AL fibres in some intrinsic laryngeal muscles of more adult animals. The average group size of these fibres was larger in the dorsal cricoarytenoid, lateral cricoarytenoid and cricothyroid muscles of the older horses. In the case of the latter muscles this is consistent with the increase in proportion of AL fibres with age discussed in Chapter 4. In the other two muscles the larger group size was probably related to the presence of denervation and reinnervation as these groups were more obvious on the left.

Sex seemingly influenced the size of groups of AL fibres in some of the laryngeal muscles studied. On these occasions it was in the dorsal cricoarytenoid, lateral cricoarytenoid and cricothyroid muscles of the geldings where the larger groups of AL fibres occurred. In the dorsal and lateral cricoarytenoid muscles the large AL fibre groups were probably related to denervation and reinnervation as they were larger on the left side and affected muscles contained other evidence of neurogenic disease. The finding of larger groups of AL fibres in the cricothyroid muscles of geldings than in those of mares was surprising.

These groups were also larger on the left side but were not associated with other histopathological evidence of neurogenic disease. A possible explanation of this occurrence was provided by Davies (1973) who considered that AL fibre group size increased as a muscle performed more work. If the intrinsic laryngeal muscles supplied by the left recurrent laryngeal nerves became damaged by neurogenic disease, and if this occurred more often in geldings, then in these animals the left cricothyroid muscle activity may be increased in an attempt to stabilise the left side of the larynx.

Breed may also influence AL fibre group size in horses. As only a small number of ponies were included in the present study no accurate comparison could be made between the size of AL fibre groups in their muscles and those of Thoroughbreds. It was interesting to note, however, that abnormally large groups of AL fibres were not found in any of the muscles from the ponies. This could suggest that the process of denervation and reinnervation which was apparently occurring in some of the "normal" Thoroughbreds was not obvious in the intrinsic laryngeal muscles of the smaller breed of horses.

The presence of abnormally large groups of AL fibres in some intrinsic laryngeal muscles of clinically normal horses was noted by Duncan et al. (1974) who attributed their presence to the influence of denervation and reinnervation. These authors found grouping to be most obvious in the left muscles although it was occasionally observed on the right and approximately 30% of the horses they examined were affected. The present study however has revealed a much higher incidence of abnormal AL fibre group size. In fact the majority of adult horses had evidence of the presence of abnormally large groups of AL fibres in some of their left intrinsic laryngeal muscles. This was most obvious in the lateral cricoarytenoid muscles where almost 80% of animals over three years of age had evidence of larger AL fibre groups in their left than in their right muscles. The dorsal cricoarytenoid muscle appeared to be not quite so commonly affected although 63.6% of horses over five years of age had larger groups on the left side. As discussed earlier in this Chapter the level of fibre type grouping may well underestimate the incidence of denervation and reinnervation which is actually occurring in a muscle. Consequently it could be postulated that almost, if not all, adult Thoroughbreds may have the effects of

denervation and reinnervation in one or more of their left intrinsic laryngeal muscles.

Duncan et al. (1974) found that when fibre type grouping occurred, it most often involved the left intrinsic laryngeal muscles. Occasionally however, it appeared to a lesser extent in the right muscles of affected animals. A similar situation existed in the present study where abnormally large AL fibre groups were detected in some right intrinsic laryngeal muscles of abnormal horses and in 22% of the right lateral cricoarytenoid muscles of "normal" horses over 6 months of age. When fibre type grouping of AL fibres was observed in a right muscle there was always more obvious grouping in the left.

The fibre type grouping measurements conducted during this present study have provided support for the observations of earlier workers (Duncan et al., 1974; Duncan, 1975) that the adductors of the larynx are affected with abnormally large AL groups sooner and to a greater extent than the abductors. In the lateral cricoarytenoid muscles examined, fibre type grouping was observed at an earlier age, in more horses, and more often in the right muscle, than was the case in the dorsal cricoarytenoid muscle. The numbers of transverse arytenoid muscles examined in this study were too small to allow accurate comparisons of the incidence of fibre type grouping in this muscle to be made with others. The impression gained however was that denervation and reinnervation occurs commonly in the left transverse arytenoid muscle of horses with no history of upper respiratory disease.

5.4.2 Fibre Cross Sectional Area

This study did not produce any evidence to suggest that there was a difference between the mean cross sectional areas of AH and AL fibres in the intrinsic laryngeal muscles of "normal" Thoroughbred horses. The difference between the sexes in the cross sectional area of Type I and Type II fibres which is seen in human skeletal muscles (Brooke, 1973) was not observed in equine laryngeal muscles. It has been postulated that this difference in fibre area in humans may result from males being more physically active than females (Brooke, 1973). This possible sex difference in activity seems unlikely to occur in the almost continuously moving intrinsic laryngeal muscles of horses.

In all the muscles studied the mean cross sectional area of the fibres was greater in the older than the younger group of "normal" horses. This is consistent with the increase in cross sectional area of individual fibres and the whole muscle which occurs as an animal grows.

The mean cross sectional areas of the fibres of the different intrinsic laryngeal muscles included in this study were approximately the same magnitude, except for the transverse arytenoid and ventricular muscles where the fibres were smaller. In the case of the transverse arytenoid muscle this may have been due to the fact this muscle was collected mainly from young horses. The ventricular muscles of young and old horses however, were included, so it is possible that the fibres of this muscle may in fact be smaller than those of the other intrinsic laryngeal muscles studied.

Most of the differences which were noted between the mean cross sectional areas of fibres of different groups of horses and different muscles could be explained in terms of the high incidence of neurogenic atrophy which was occurring in some of these muscles. When denervation is initiated in a muscle there will only be a relatively small number of fibres involved and they will begin to atrophy. The majority of the fibres in that muscle will initially retain their normal innervation and may increase their cross sectional area to compensate for the loss of function of the denervated fibres.

The present study has shown that denervation of some fibres of the left lateral and dorsal cricoarytenoid muscle is common in Thoroughbred horses. Compensatory hypertrophy of the remaining fibres possibly explains the observation that the fibres of these left muscles had a greater cross sectional area than those in the right muscles of "normal" horses.

The fact that the lateral cricoarytenoid muscle has a higher incidence of denervation and reinnervation than the dorsal cricoarytenoid muscle as evidenced by the level of fibre type grouping and gross pathology noted in this study, could indicate that compensatory hypertrophy is more marked in this muscle. This may have been the reason for Duncan's (1975) observation that the fibres of the lateral cricoarytenoid muscle

were larger than those of the dorsal cricoarytenoid muscle, a finding not supported by the present study.

Where a difference occurred between the size of fibres in the left and right muscle it was often in the geldings where this was most marked, again perhaps a reflection of the higher incidence of denervation and reinnervation in geldings. Hypertrophy of all the fibres in a muscle to compensate for lack of function of its contralateral partner may also occur. Changes of this nature were noted in the right dorsal cricoarytenoid and transverse arytenoid muscles of abnormal horses. In the cricothyroid muscles it was interesting to note that the fibres from the older group of abnormal horses were larger than those from the same group of "normal" horses and that they tended to be larger in all the left cricothyroid muscles than the right. Duncan (1975) also noted this difference. This may occur if the cricothyroid muscle either increases its stabilizing activity or becomes to some extent involved in widening the laryngeal aditus when the left dorsal cricoarytenoid muscle fails.

As the process of denervation and reinnervation becomes more widespread in a muscle it would be expected that fibre atrophy would be more pronounced. During the present study this change was observed. The fibres from the left muscles of abnormal horses were smaller than those from the right muscles but it was not possible to be certain whether or not the specific atrophy of Type II fibres observed in man (Brooke, 1973) was occurring.

Duncan (1975) stated that when measurements of fibre area were made in sections from different blocks obtained from the same muscle, large variations could result. When denervation and reinnervation were occurring in a muscle, Duncan (1975) suggested that counting relatively small numbers of fibres in a localised area of a section made from that muscle could give results which were not representative of the section as a whole. The small number of fibres counted could be innervated by the same motor neurone, and so they could all be larger or smaller than fibres from another area of the section. For this reason Duncan (1975) suggested that either a large number of fibres (approximately 1500) should be counted per section, or that a smaller number of fibres should be counted from a large number of different areas of the section, these areas being selected in a random fashion.

In the present study the blocks were always cut from the same part of the body of each of the muscles and initially attempts were made to use a random system to select the areas of a section to be counted. However, the need to find an area suitable for tracing in three serial sections, each stained with a different histochemical stain, placed severe limitations on the number of areas which could be counted. It was decided to limit the number of areas counted to two, to select these from different areas of the section, and to count approximately 200 fibres in total. When a muscle was obviously involved in the process of denervation and reinnervation, an affected area and a relatively normal area were counted. This had the effect of levelling the mean values measured and so on occasions not giving a true indication of the extent of pathology present. For this reason the whole section was also examined and an attempt made to grade the pathology observed. It was considered that the inclusion of a large number of horses in the survey would, to a certain extent, overcome the inherrent errors in this sampling system.

5.4.3 Histological Features of Juvenile Muscle

The histological features of juvenile equine muscle noted during this study were similar to those described by other workers in young muscles of other species. Braund (1982) noted that neonatal canine muscle had loose facicles, prominant endomysial and perimysial connective tissue and fibres which were round or oval in cross section. In the present study variation in the cross sectional area of muscle fibres was noted, however the large central fibres surrounded by smaller peripheral fibres which are a feature of some human (Adams et al., 1977) and canine (Braund, 1982) neonatal muscle were not observed in juvenile equine laryngeal muscles. During the development of human muscle the muscle cell nuclei migrate from a central position in the muscle fibre to occupy their final subsarcolemmal position so that, in the newborn, central nuclei as well as subsarcolemmal nuclei, are relatively more numerous than in later life. In fact it has been stated that neonatal muscle fibres resemble those of a mature muscle undergoing atrophy (Adams et al., 1967).

The present investigation has shown that histological evidence of neurogenic muscle damage is relatively rare in the intrinsic laryngeal muscles

of horses less than one year of age. Indeed, apart from the three animals in which larger AL fibre groups were observed in the left than in the right lateral cricoarytenoid muscle, no clear evidence of muscle damage was found in any of the 16 foals studied.

5.4.4 Histopathology

This study has provided evidence that histopathological damage related to nerve dysfunction is extremely common in some intrinsic laryngeal muscles of adult New Zealand Thoroughbred horses which have no history of upper respiratory tract disease. This damage was not observed in 16 horses less than one year of age although, as mentioned, fibre type grouping was noticed in three. The incidence of histopathological damage in the intrinsic laryngeal muscles appears to change dramatically in early adolescent life as the majority of horses (68%) over one year of age exhibited signs of this damage in the left lateral cricoarytenoid muscle. Both Cole (1946) and Duncan et al. (1974) had previously reported that a proportion of "normal" horses had evidence of pathological changes in their left intrinsic laryngeal muscles. However in neither report were the majority of animals affected. The higher incidence of muscle pathology observed in adolescent and adult animals in the present study possibly results from the main breed of horses investigated. In Cole's (1946) and Duncan et al.'s (1974) series various breeds were examined, whereas in the present study, with the exception of three ponies, all the animals were Thoroughbred.

As reported by Duncan et al. (1974) the left lateral cricoarytenoid muscle appeared to be affected more commonly and more severely than the dorsal cricoarytenoid. It was also involved at a slightly earlier age. In adddition to these muscles the damage was also observed in all the other intrinsic laryngeal muscles supplied by the left recurrent laryngeal nerve.

Duncan et al. (1974) found no evidence of damage to the cricothyroid, the only intrinsic laryngeal muscle not supplied by the recurrent laryngeal nerve. The present study supports this finding as the few pathological changes observed in these muscles occurred in old horses and appeared to be related to age.

In affected animals, as in the "normal" horses, the left lateral cricoarytenoid muscle was most severely involved. In the laryngeal hemiplegic horses some neurogenic muscle damage was observed in all the muscles innervated by the left recurrent laryngeal nerve. Moreover similar changes, albeit less severe, were present in the right lateral cricoarytenoid, dorsal cricoarytenoid and transverse arytenoid muscles. As considerable damage to the left dorsal cricoarytenoid muscle is required before clinical signs of laryngeal hemiplegia become obvious, horses showing these signs represent the "tip of the iceberg".

This study provides the first histopathological evidence that the disease process which initiates the neurogenic muscle damage occurs more frequently in males than females. In the muscle most commonly affected, the left lateral cricoarytenoid, most adolescent and young adult males exhibited pathology whereas the females in this age group were not so commonly affected.

Some evidence is also provided in support of the belief that the neurogenic muscle damage more commonly affects the larger breeds of horses as in the three adult ponies examined none of the signs of denervation and reinnervation were noted. Goulden and Anderson (in Press) in a preliminary endoscopic survey of New Zealand Clydesdale horses, commonly found (50% of horses examined) reduced or absent abduction of the left arytenoid cartilage during quiet respiration. This finding may reflect a very high incidence of idiopathic laryngeal hemiplegia in this breed. Other factors, however, such as breed influences could be responsible for these apparent differences in incidence of the disease in horses of different stature.

The occurrence of neurogenic disease in some of the left intrinsic laryngeal muscles in the three year old entire male with laryngo-palatal dislocation is of interest in view of Cook's (1981) hypothesis that this disorder may occur as a result of inefficient abductor function. Cook postulated that in affected horses the airtight seal normally present at the ostium intrapharyngium is not kept under tension by bilateral abduction of the arytenoid cartilages, and consequently air is permitted to leak into the oropharynx. When this happens the soft palate may become elevated and hence laryngo-palatal dislocation can occur.

5.4.5 Sarcosporidiosis

It appeared that sarcocysts were an incidental finding in the equine laryngeal muscles examined during this survey. They were not particularly common and did not appear to be associated with a local reaction in the muscles where they occurred. In man they have been associated with muscle weakness, impaired tendon reflexes and periarteritis (Adams, Denny-Brown and Pearson, 1967). In the muscle sections examined in this survey they did not necessarily occur in the muscles exhibiting histopathological changes.

5.4.6 Concluding Comments

During this study the presence of fibre type grouping and other histopathological evidence of denervation and reinnervation were examined separately. Nevertheless there was a very close relationship between the two. Although fibre type grouping did occur in the left lateral cricoarytenoid muscle at a younger age than other histological signs of denervation and reinnervation this was not the case in the other muscles. In these, fibre type grouping and the other histopathological signs of neurogenic disease most often occurred concurrently. In this study conventional histological techniques such as haematoxylin and eosin preparations revealed the presence of significant neurogenic disease in laryngeal muscles almost as accurately as the more sophisticated techniques of enzyme histochemistry. The latter techniques, particularly myosin ATPase preparations, did however provide useful additional and supporting information.

One of the features of this study was that the dorsal cricoarytenoid muscle, the inefficient function of which is responsible for the clinical signs of roaring, appears to become suddenly and dramatically involved in neurogenic disease during the adolescent or early adult life of Thoroughbred horses. This finding corresponds closely to the clinical observations on the age incidence of idiopathic laryngeal hemiplegia reported in these animals by Goulden and Anderson (Appendix 9).

Another interesting observation on neurogenic pathology in the dorsal cricoarytenoid muscle was that, whilst the incidence of fibre type grouping increased with age, the severity of the other histopathological

signs of neurogenic disease was greatest in "normal" horses between 1.5 and 5 years of age. This may have been related to the disproportionately high number of geldings in this age group or it could indicate that if a horse is to develop severe neurogenic disease it is likely to do so in its adolescent or young adult life.

The disparity between the clinical and pathological incidence of dorsal cricoarytenoid muscle disease indicates that the damage to this muscle is insufficient to produce overt clinical signs of laryngeal dysfunction in many horses. Nevertheless it is possible that the competitive performances of some of these animals may be compromised because neurogenically damaged muscle is probably not capable of sustained, efficient abduction. Consequently this disease may be far more important in restricting performance, especially over long distances, than is generally appreciated.

This study has demonstrated that the effects of severe left sided muscle atrophy can lead to hypertrophic changes of the right muscles. This could cause hyperabduction of the right arytenoid cartilage in laryngeal hemiplegic horses. Movements of this nature are commonly observed during endoscopic examination of these animals.

The cause of the neurogenic damage which, when severe, is responsible for laryngeal hemiplegia, appears to exert its initial influence early in the life of a horse. The muscle changes which result appear to increase in severity during adolescence and early adult life. They occur more often and more severely in some of the left muscles of geldings. The nerve supplying the severely affected muscles, the left recurrent laryngeal, is the longest in the horse's body (Duncan and Griffiths, 1973). Its right counterpart, although shorter, is the second longest equine nerve. The muscles it supplies are affected but less often and less severely. As it also appears that the larger horses are more susceptible, at least to the more severe muscle pathology related to recurrent laryngeal nerve damage, it seems likely that the disease is in some way related to nerve lengths. For this reason further examination of the recurrent laryngeal nerves and other long peripheral nerves of young geldings, particularly those suffering from laryngeal hemiplegia, seems indicated. This approach would appear to offer the best opportunity of providing useful information about the

nature of the nerve damage responsible and perhaps eventually the cause of the disease. Whatever the process which leads to the muscle damage described it occurs in the majority of Thoroughbred horses and must be one of the commonest pathological syndromes yet described in this breed of horse.

CHAPTER 6 THE PALATAL MUSCLES

6.1 Introduction

One of the early hypotheses concerning the aetiology of laryngo-palatal dislocation was that the musculature of the soft palate is involved in a neurogenic myopathy similar to that which occurs in the muscles of the larynx (Quinlan et al. 1949; Cook, 1962; Johnson and Merriam, 1975; Raker, 1975). This suggestion resulted from the belief that the major motor nerve supplying the palatal muscles of the horse was the pharyngeal branch of the vagus (Quinlan, 1957; Cook, 1962). The idea was supported by Quinlan (1957) who had described unilateral left sided atrophy of the soft palate in horses with laryngo-palatal dislocation. He also found that some animals with this condition had concurrent idiopathic laryngeal hemiplegia and so suggested that both the pharyngeal and recurrent branches of the vagus could be involved in the same degenerative process. Cook (1965) also observed the simultaneous occurrence of laryngo-palatal dislocation (or soft palate paresis as he then called the condition) and idiopathic laryngeal hemiplegia in horses.

Thus it appears that there is some association between the two conditions but basing this association on the fact that the palatal and laryngeal muscles are supplied by the same cranial nerve is in fact erroneous. There is still confusion in the literature regarding the motor nerve supply to the palatal muscles of the horse but cranial nerves other than the vagus are involved.

The palatine tensor muscle of the Vervet monkey (Cleaton-Jones, 1977), man (Dominech-Ratto, 1977), the cat (Keller et al. 1983), and the horse (Heffron and Baker, 1976b; Sha Ban, pers. comm.), is supplied by the mandibular division of the trigeminal nerve.

In man the palatine levator muscle is supplied by the glossopharyngeal nerve (Dominech-Ratto, 1977) but in the horse Heffron and Baker (1976b) consider that the pharyngeal branch of the vagus provides the motor supply for this muscle while Sha Ban (pers. comm.) contends that its motor fibres are derived from the maxillary division of the trigeminal nerve.

The palatopharyngeal muscle of man is supplied by motor fibres from both the glossopharyngeal and vagus nerves (Dominech-Ratto, 1977). This appears to apply to the horse also as both Heffron and Baker (1979b) and Sha Ban (pers. comm.) have stated that the pharyngeal branch of the vagus is involved and Sha Ban added that the glosso-pharyngeal nerve also provides motor fibres via the pharyngeal plexus.

In man the palatine or uvular muscle is supplied from the vagus nerve (Dominech-Ratto, 1977) as it is in the horse (Heffron and Baker, 1979b). Sha Ban however has stated that the accessory palatine nerve, a branch of the maxillary division of the trigeminal provides the motor supply for this muscle.

The implications of this information are, that in the horse, those muscles with direct attachments to the soft palate may be provided with motor fibres from the trigeminal, glossopharyngeal and vagal nerves. It also appears that unlike the recurrent laryngeal nerves these cranial nerves and their branches in the horse, should have bilaterally symmetrical anatomical relationships. The characteristics of the motor innervation of the palatal muscles therefore, appear to be considerably different to those of the intrinsic laryngeal muscles where denervation and reinnervation are so common.

When this study was undertaken there was no pathological evidence available to support the concept that laryngo-palatal dislocation was associated with neurogenic disease of the palatal muscles. For this reason it was decided to apply to some of the palatal muscles the same techniques used for investigating the incidence of denervation and reinnervation in the laryngeal muscles.

No previous information was available on palatal muscle weights. Blythe et al. (1980) made pathological and histochemical observations on the terminal fibres of palatine muscles which were obtained by removing a portion of the free border of the soft palate during the staphylectomy operation in horses which had been diagnosed as having "elongated soft palates". They also examined a series of similar samples from control horses. According to these authors the muscle abnormalities they observed were characteristic of a low-grade, degenerative, non-specific,

myopathy rather than a neuropathy. The abnormalities were more frequent in the muscles from horses with elongated soft palates and from control horses greater than eight years of age. Apart from this limited study, no information was available on the histochemical features of equine palatal muscles. In fact there appeared to be very little information on the histochemistry of the palatal muscles of any species although Orvis and Cardinet (1981) examined the canine palatine tensor muscle and found that it was composed of Type 2C myofibres and a variant of the Type I myofibre.

Leeson and Leeson (1969) in a study of the skeletal muscles of the soft palate of the rat found that they had a high mitochondrial population and that the mitochondria had morphological features suggestive of a high degree of metabolic activity. Nakachi and Oota (1978) later provided support for this observation when they found that the succinate dehydrogenase activity of the feline palatine levator, palatine and palatine tensor muscles was relatively high.

Again, apart from Blythe et al's. (1980) observations, no information was available on the histopathology of equine palatal muscles.

6.2 Materials and Methods

Details of the ages and sexes of the horses used in the study are shown in Table 1 and Table 21 shows the number of horses from which each of the palatal muscles were collected.

TABLE 21 THE NUMBER OF HORSES FROM WHICH PALATAL MUSCLES WERE STUDIED

	"Normal" horses		Abnormal horses		
Muscle	three years and under	over three years	three years and under	over three years	Total
palatopharyngeal	23	17	2	4	46
palatine levator	25	21	3	4	53
palatine	20	16	3	4	43
palatine tensor	24	19	3	3	49

The methods used for processing these muscles, and collecting and analysing the data were the same as described in Chapter 2. Only the

palatine levator and palatine tensor muscles were included in the weight study as, of the muscles with direct attachments to the soft palate these were found to be the only two with discrete enough attachments and insertions to ensure consistently accurate dissection.

The sections made from each muscle were used to measure the proportions of the different fibre types present; the size of groups of AL fibres; and the cross sectional area of fibres. The sections were also examined individually and the presence of any other histological signs of denervation and reinnervation were noted. As was the case with the intrinsic laryngeal muscles, the numbers of palatal muscles available from the two age groups of horses with a known history of upper respiratory tract abnormality, were too small to be statistically analysed with confidence.

6.3 Results

6.3.1 Palatal Muscle Weights

The mean muscle weights (grams) of the different age groups of "normal" horses are shown in Table 22.

TABLE 22 THE MEAN WEIGHTS (GRAMS) OF EQUINE PALATINE LEVATOR AND PALATINE TENSOR MUSCLES FROM HORSES WITH NO HISTORY OF UPPER RESPIRATORY TRACT ABNORMALITIES

	palatine levator	palatine tensor
all ages	2.49	5.18
three years and under	1.86	3.59
over three years	3.50	7.48

6.3.1.1 Palatine Levator Muscle

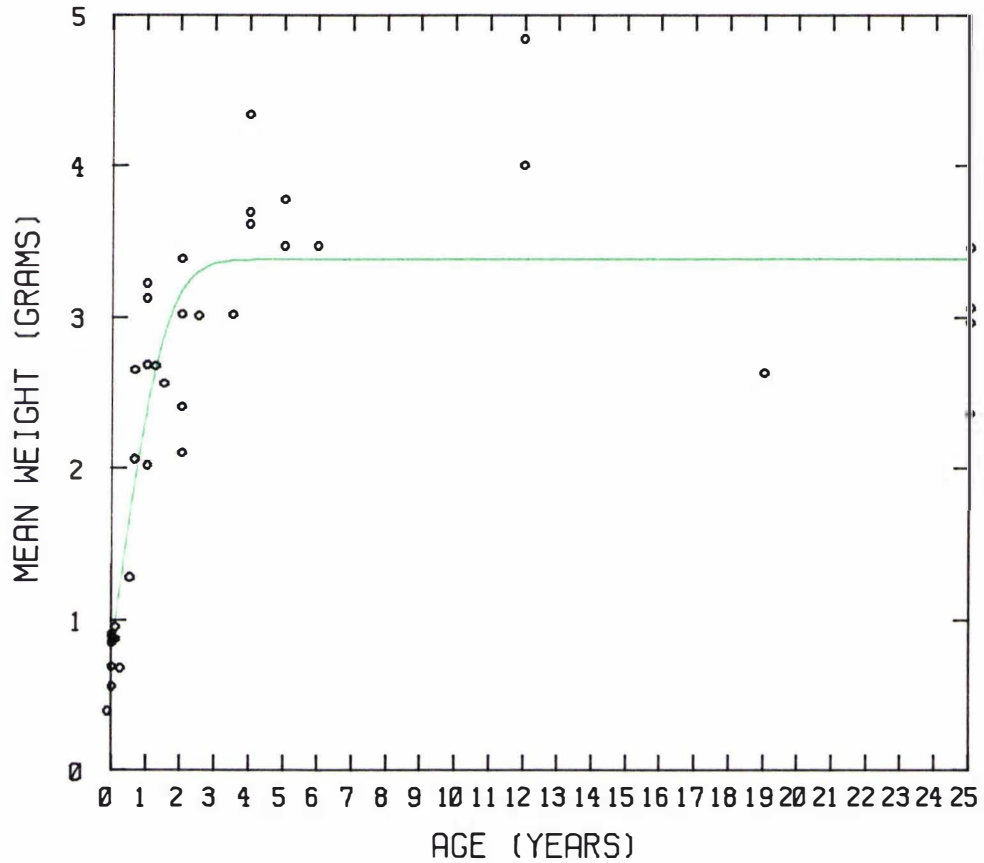
The palatine levator muscle like the other muscles weighed during this study was heavier in the older than the younger horses. This increase in weight with age is illustrated in Fig. 43 and was responsible for the only significant differences noted between

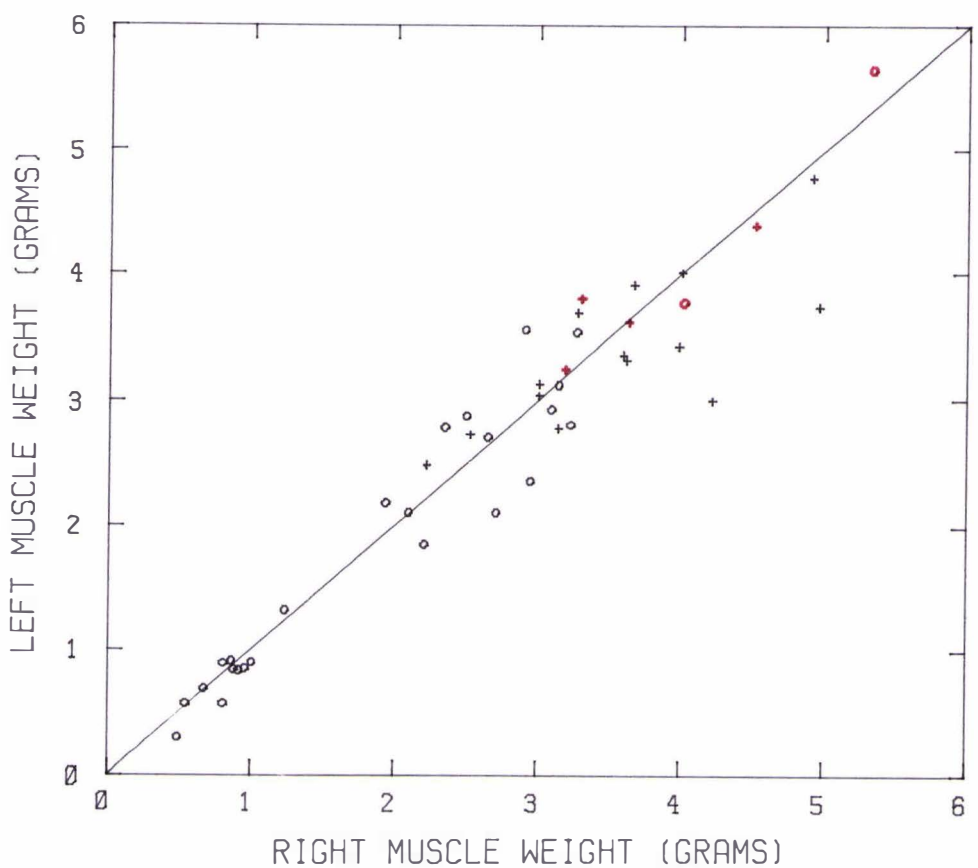
FIGURE 43 The relationship between age (years) and the mean weights of the left and right palatine levator muscles (grams) of the "normal" horses

FIGURE 44 The weights of the left palatine levator muscles (grams) plotted against the weights of the right muscles

o = "normal" horses three years and under
+ = " " over three years

⊙ = abnormal horses three years and under
⊕ = " " over three years


MEAN WEIGHT (GRAMS)
0
1
2
3
4
5
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
AGE (YEARS)


LEFT MUSCLE WEIGHT (GRAMS)
0
1
2
3
4
5
6
0 1 2 3 4 5 6
RIGHT MUSCLE WEIGHT (GRAMS)

the mean muscle weights from the different groups of horses. The sex of a horse, the side of its pharynx from which the muscles came or whether the horse was "normal" or abnormal did not appear to have a significant influence on the weight of its palatine levator muscle. The similarity between the weights of the left and right palatine levator muscles from the "normal" and abnormal horses is illustrated in Fig. 44.

6.3.1.2 Palatine Tensor Muscle

The analysis of palatine tensor muscle weights provided results similar to those already described for the palatine levator muscle. The increase in the weight of the palatine tensor muscle with age is illustrated in Fig. 45. The similarity of the weights of the left and right palatine tensor muscles from "normal" and abnormal horses is illustrated in Fig. 46.

6.3.2 The Predominant Fibre Types Found and Their Proportions

The predominant fibre types and their proportions found in the palatal muscles from the "normal" horses are shown in Table 23.

TABLE 23 THE PERCENTAGES OF FIBRE TYPES PRESENT IN THE PALATAL MUSCLES FROM HORSES WITH NO HISTORY OF UPPER RESPIRATORY TRACT ABNORMALITIES

Muscle	Fibre type %		
	AH:SH:PH	AL:SH:PH	AL:SH:PL
palatopharyngeal	92	7	1
palatine levator	86	10.6	3.4
palatine	91	8	1
palatine tensor	57	35	8

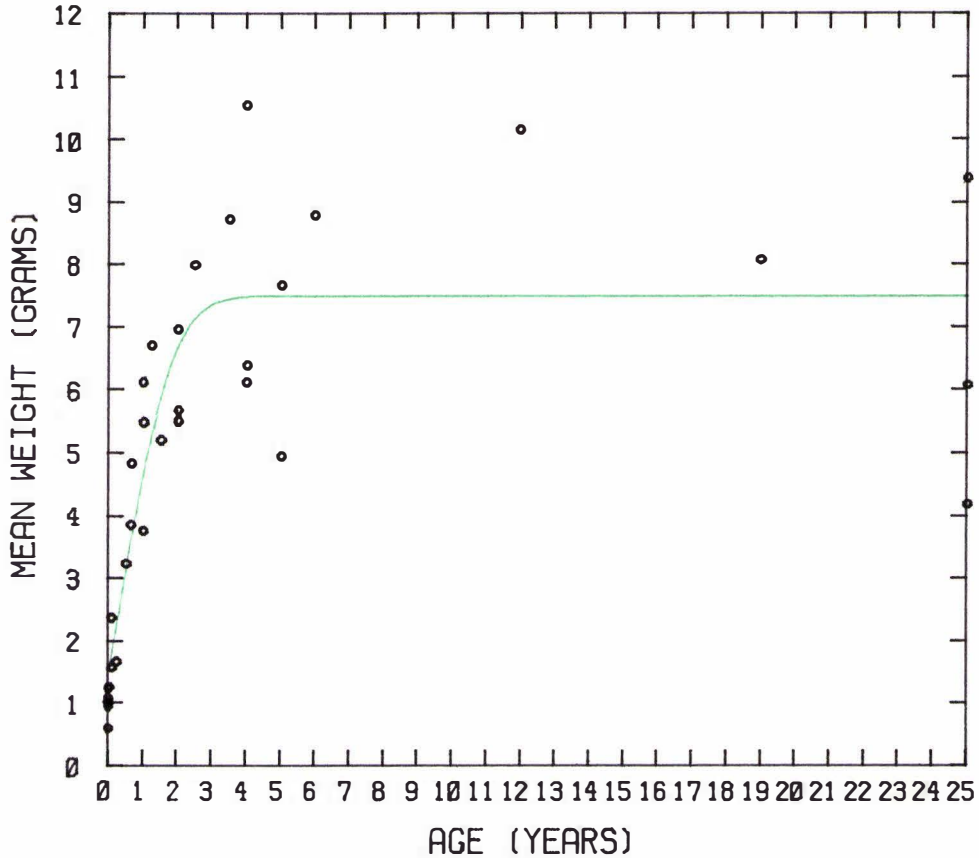
The majority of the fibres in the palatal muscles studied were highly reactive for ATPase, SDase and GPase (Figs. 47a,b and c). Only a small proportion of the fibres, except in the case of the palatine

FIGURE 45 The relationship between age (years) and the mean weights of the left and right palatine tensor muscles (grams) of the "normal" horses

FIGURE 46 The weights of the left palatine tensor muscles (grams) plotted against the weights of the right muscles

o = "normal" horses three years and under
\+ = " " over three years

o = abnormal horses three years and under
+ = " " over three years


MEAN WEIGHT (GRAMS)
AGE (YEARS)
0 1 2 3 4 5 6 7 8 9 10 11 12
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

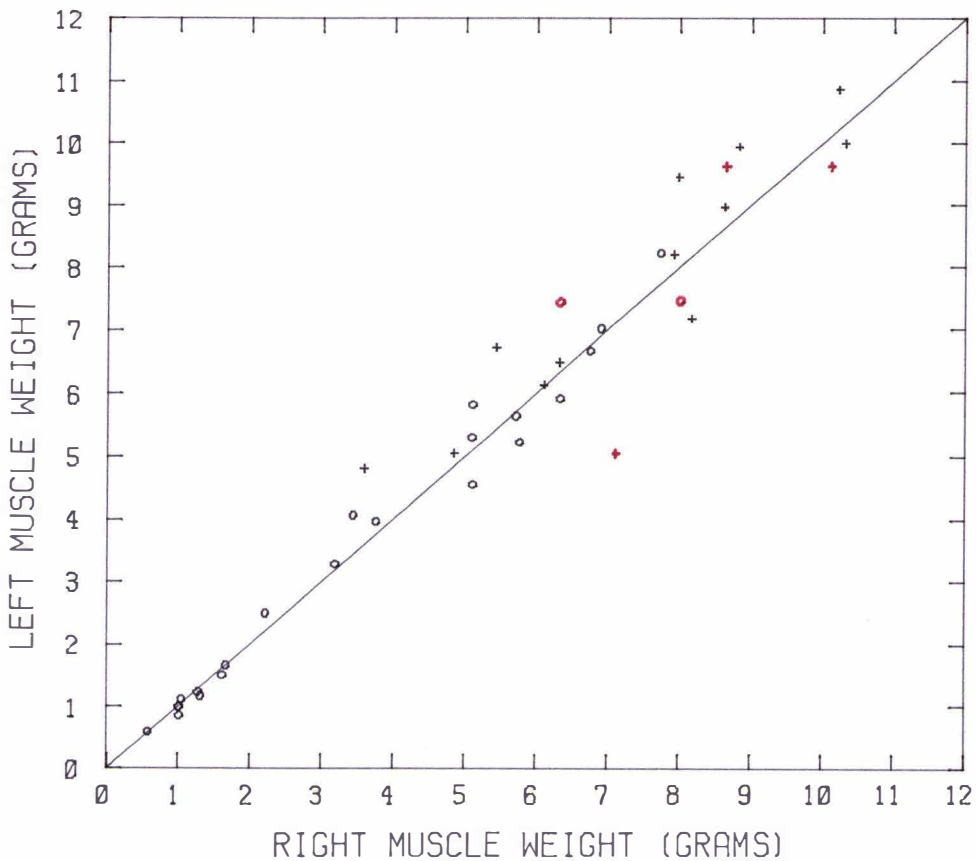

LEFT MUSCLE WEIGHT (GRAMS)
RIGHT MUSCLE WEIGHT (GRAMS)
0 1 2 3 4 5 6 7 8 9 10 11 12

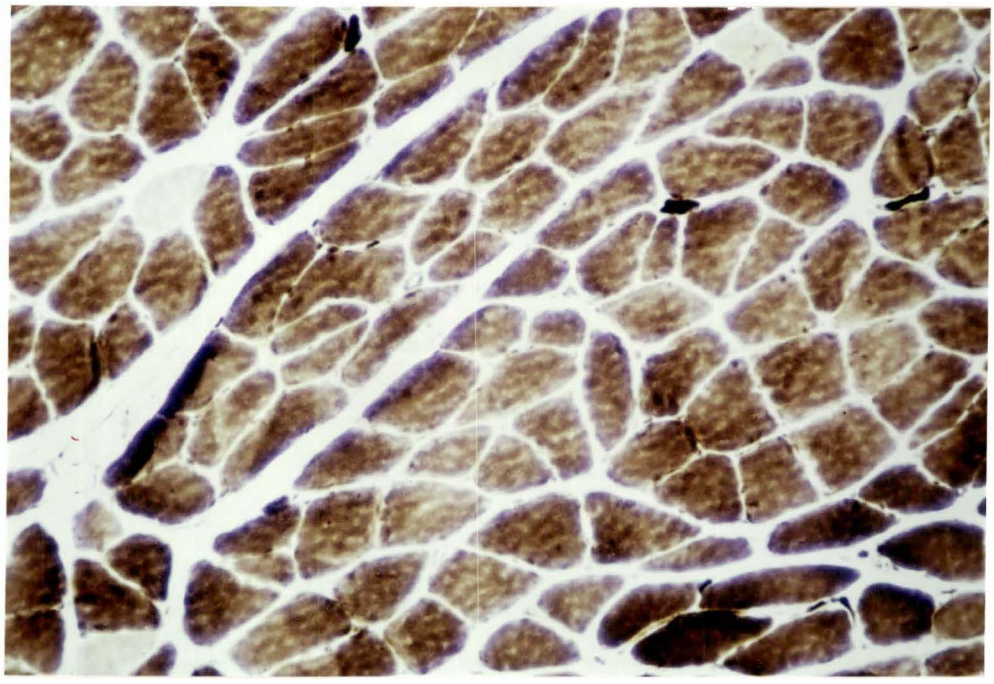
FIGURE 47 Transverse serial sections of the palatine muscle of a "normal" horse (x260). Sections stained to demonstrate the activity of:-

a. Myosin ATPase

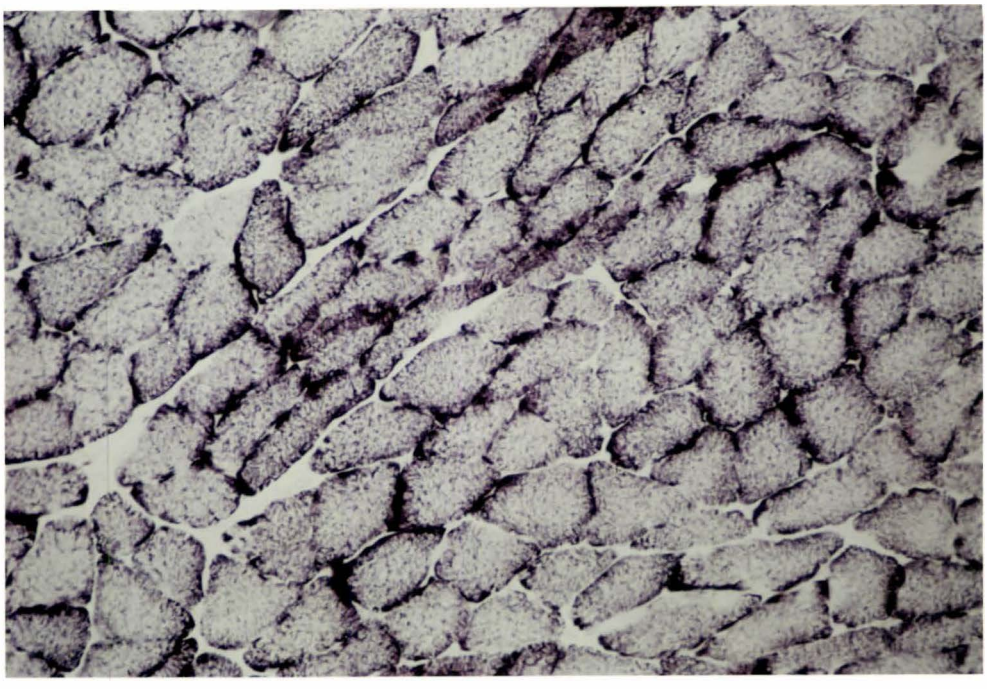
b. SDase

c. GPase

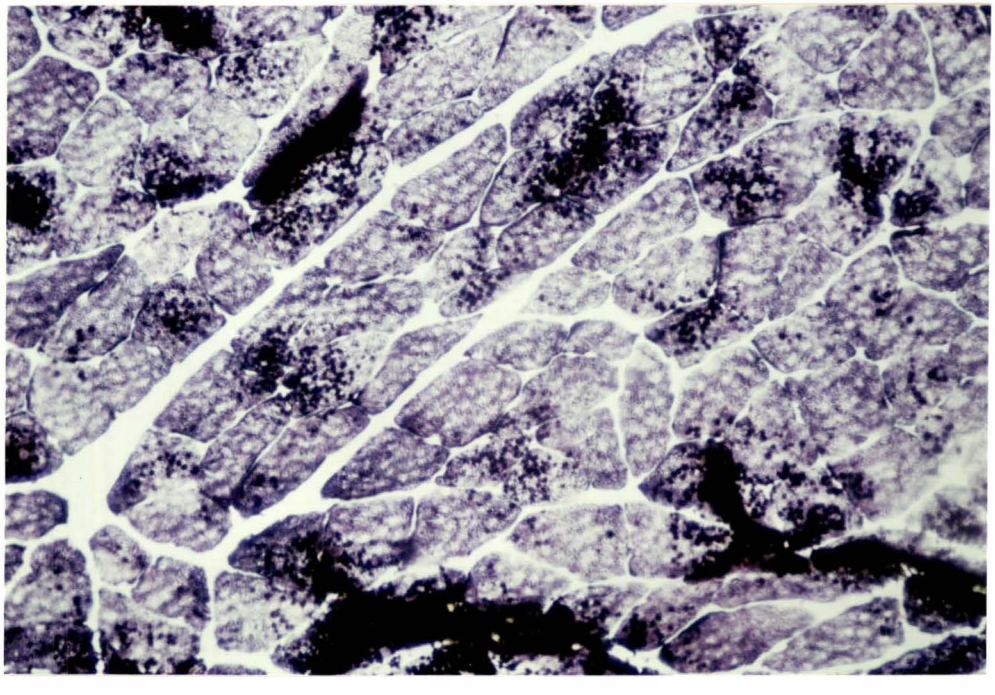
a



b



c



tensor muscle (Fig. 48a) were classed as AL and as was the case with the intrinsic laryngeal muscles only a relatively small proportion of these fibres were PL.

In the myosin ATPase sections made from the palatine tensor muscles the mosaic pattern of AH and AL fibres characteristic of the other muscles, was not always present. Sometimes the whole section was composed of fibres with a low reactivity for myosin ATPase. It was found that sections made from the medial side of the muscle tended to show the characteristic mosaic pattern (Fig. 48a) while those from the lateral side tended to be predominantly AL.

There were few changes in the proportions of fibre types present in each of the palatal muscles from the different groups of horses. Where significant differences did occur they were infrequent and isolated cases and unifying trends did not appear to be present. The only possible exception to this was the tendency in some groups for the proportion of PH fibres to be higher in the muscles from older horses and from those which were in training.

6.3.3 Other Fibre Types

As was the case with the laryngeal muscles the only other fibre type which occurred with any frequency in the palatal muscles was the AH:SH:PL type. Again, this fibre type appeared more frequently in the muscles from the younger horses. It was present in at least one palatal muscle from all the horses less than one year of age but only in the muscles of approximately half the horses over one year of age. This fibre type appeared with approximately equal frequency (30-40% of the muscles examined) in the palatopharyngeal, palatine levator and palatine tensor muscles but less frequently (16% of the muscles examined) in the palatine muscle. In each of the muscles where it occurred this fibre type accounted for approximately 2-3% of the fibres counted except in the palatine tensor muscle where a mean of 6.6% of the fibres counted were of this type.

6.3.4 Other Staining Characteristics and Changes in Fibre Architecture

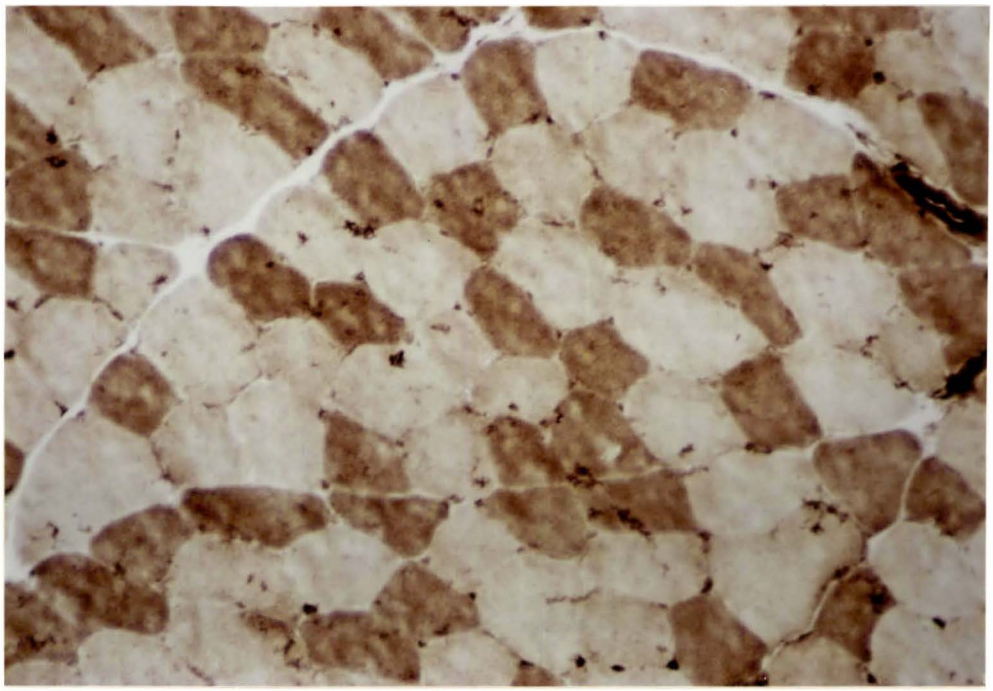
All the fibres of the palatal muscles studied took up the

FIGURE 48 Transverse serial sections of the palatine tensor muscle of a "normal" horse (x260). Sections stained to demonstrate the activity of:-

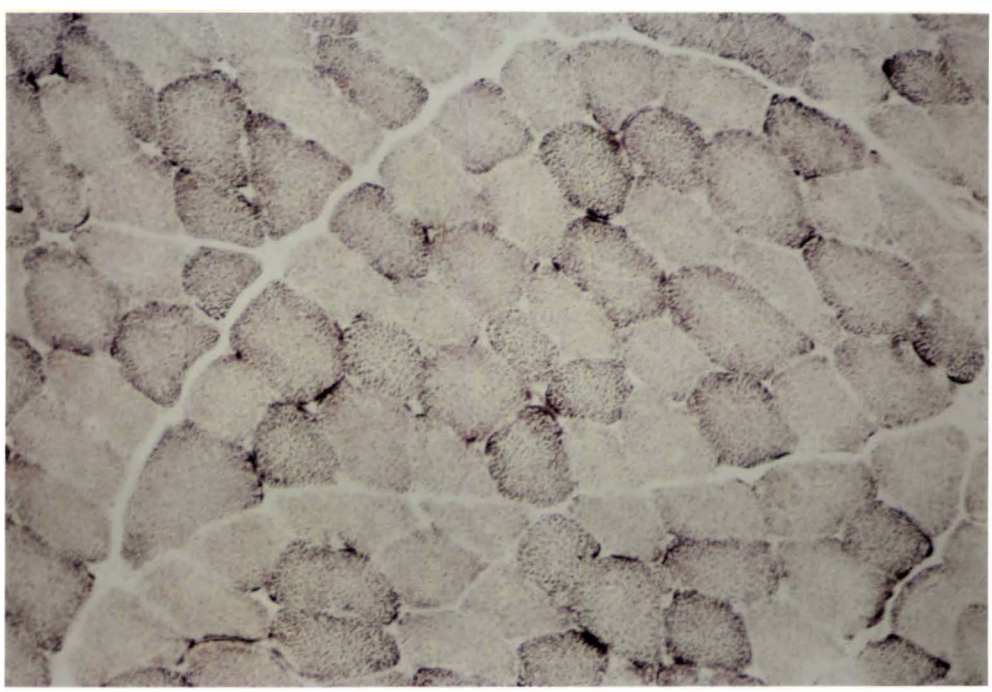
a. Myosin ATPase

b. SDase

a



b



blue diformazan deposited during the SDase reaction to a greater or lesser extent. Where there was variation in staining intensity between fibres within a muscle this was usually not distinct enough to allow for reliable separation of fibre types using this reaction. This variation between fibres was most evident in the palatine tensor muscle, where relatively large numbers of fibres stained with a lower intensity of blue diformazan were present (Fig. 48b).

In some of the older horses, pale or mottled fibres appeared occasionally, and one tangetoid fibre was noted in the left palatine levator muscle of a 20 year old stallion which had suffered from idiopathic laryngeal hemiplegia.

In most cases the glycogen phosphorylase reaction stained palatal muscle fibres with the dark blue colour which is consistent with a high content of this enzyme (Fig. 47c). There were usually a few PL fibres present and these were most numerous in the palatine tensor muscle. This pattern of glycogen phosphorylase staining was consistent in the palatal muscles studied from most of the horses. In some of the older horses large diameter fibres which were PL were noted in the palatine levator and tensor muscles.

6.3.5 Size of Groups of AL Fibres

The mean numbers of AL fibres per group observed in the palatal muscles from "normal" horses are shown in Table 24.

TABLE 24 THE MEAN NUMBER OF AL FIBRES PER GROUP IN THE PALATAL MUSCLES FROM HORSES WITH NO HISTORY OF UPPER RESPIRATORY TRACT ABNORMALITIES

Muscle	Mean number of AL fibres per group
palatopharyngeal	1.02
palatine levator	1.60
palatine	1.17
palatine tensor	4.55

The differences between the mean number of AL fibres per group in the muscles from the different categories of horses were in most cases not significant. Where significant differences did occur they were isolated cases and unifying trends did not appear to be present.

6.3.6 Fibre Cross Sectional Area

The mean cross sectional areas of the AH and AL fibres measured in the palatal muscles from the "normal" horses are shown in Table 25.

TABLE 25 THE MEAN CROSS SECTIONAL AREAS (μm^2) OF THE AH AND AL FIBRES IN THE PALATAL MUSCLES OF HORSES WITH NO HISTORY OF UPPER RESPIRATORY TRACT ABNORMALITIES

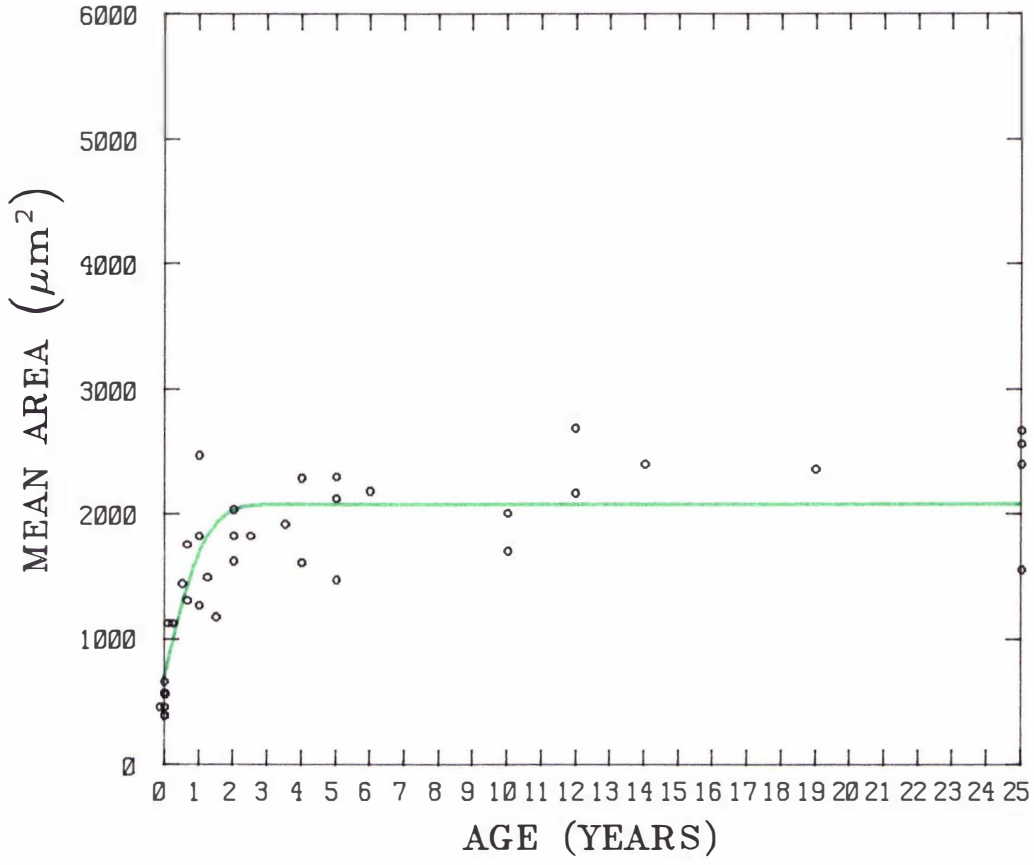
Muscle	Fibre Type	Mean cross sectional area
palatopharyngeal	AH	1796.52
	AL	1018.66
palatine levator	AH	1980.40
	AL	2125.42
palatine	AH	1140.95
	AL	1138.31
palatine tensor	AH	1401.09
	AL	1309.95

In each of the palatal muscles the mean cross sectional areas of both fibre types were larger in the older groups of "normal" and abnormal horses than in the younger groups. These differences were statistically significant in most cases. This increase in fibre cross sectional area with age is shown in Figs. 49-52.

In most of the muscles examined the mean cross sectional area of AL fibres tended to be less than that of the AH fibres. This difference

FIGURE 49 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the palatopharyngeal muscles from the "normal" horses

FIGURE 50 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the palatine levator muscles from the "normal" horses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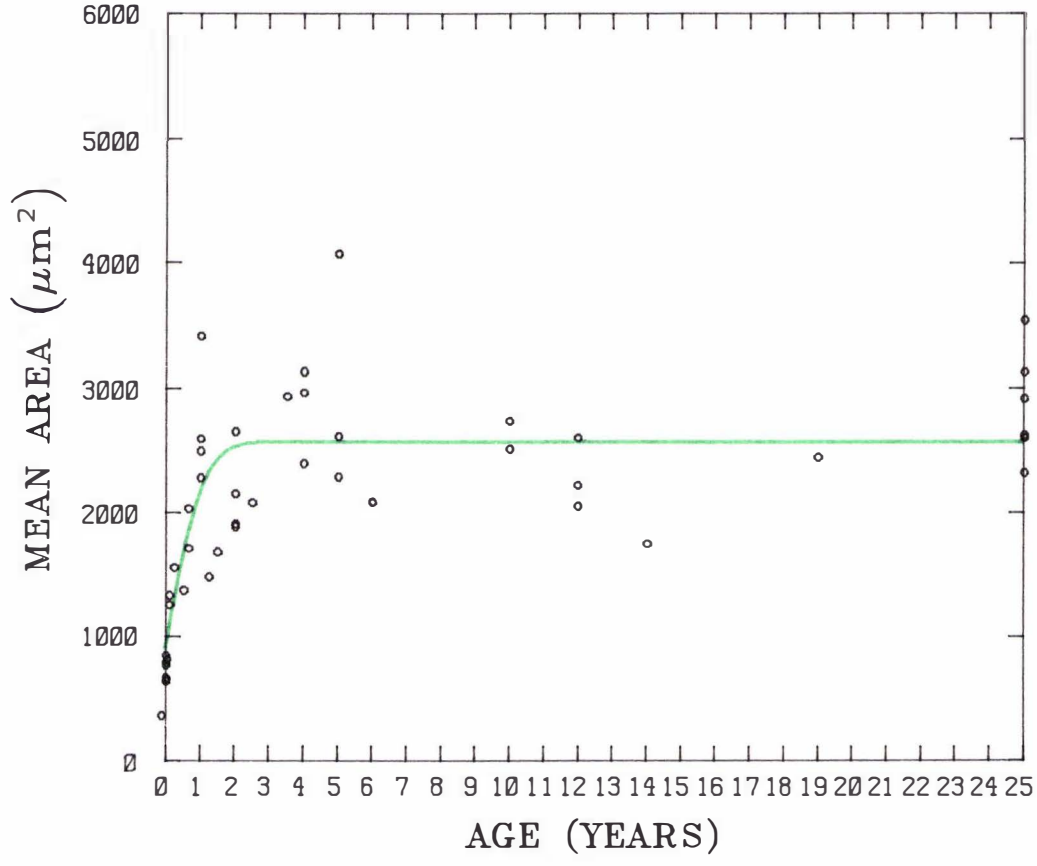
MEAN AREA (μm^2)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
AGE (YEARS)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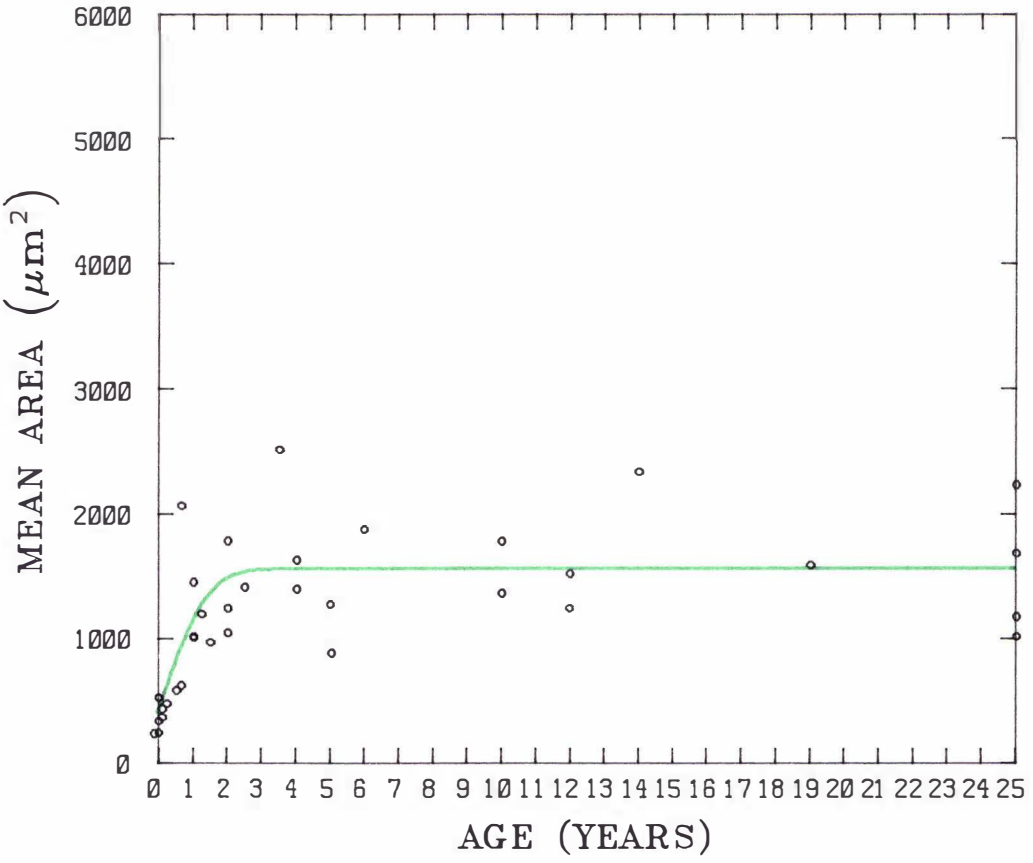
MEAN AREA (μm^2)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
AGE (YEARS)

FIGURE 51 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the palatine muscles from the "normal" horses

FIGURE 52 The relationship between age (years) and the mean cross sectional areas of the AH and AL fibres (μm^2) in the palatine tensor muscles from the "normal" horses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MEAN AREA (μm^2)
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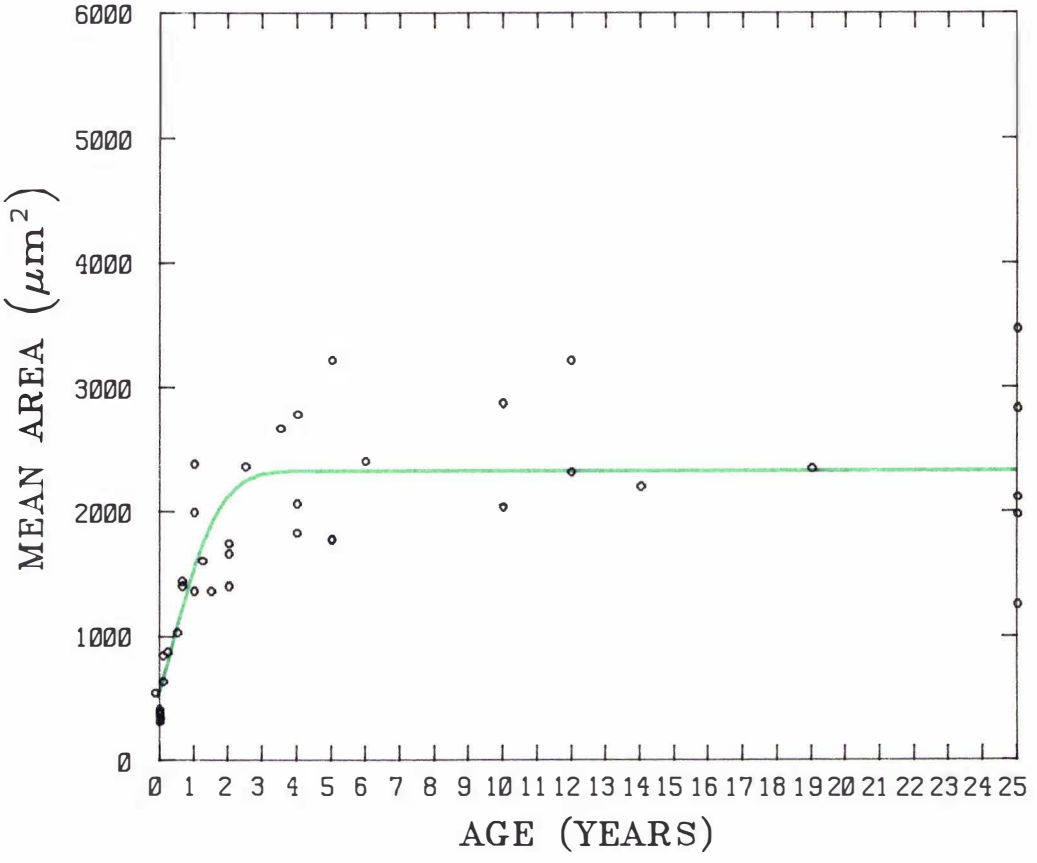
AGE (YEARS)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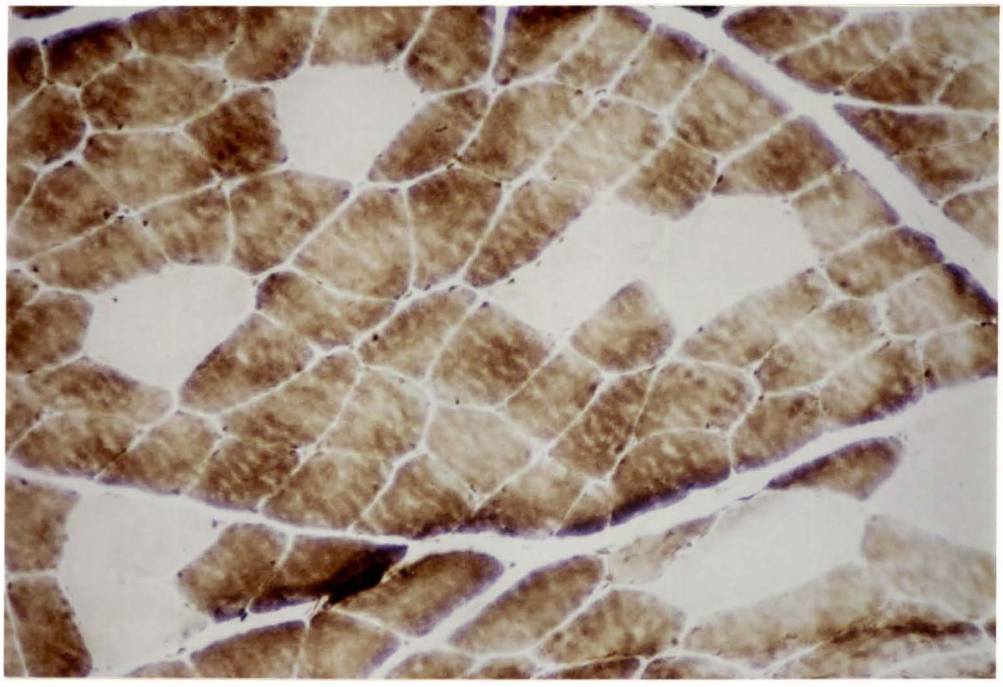
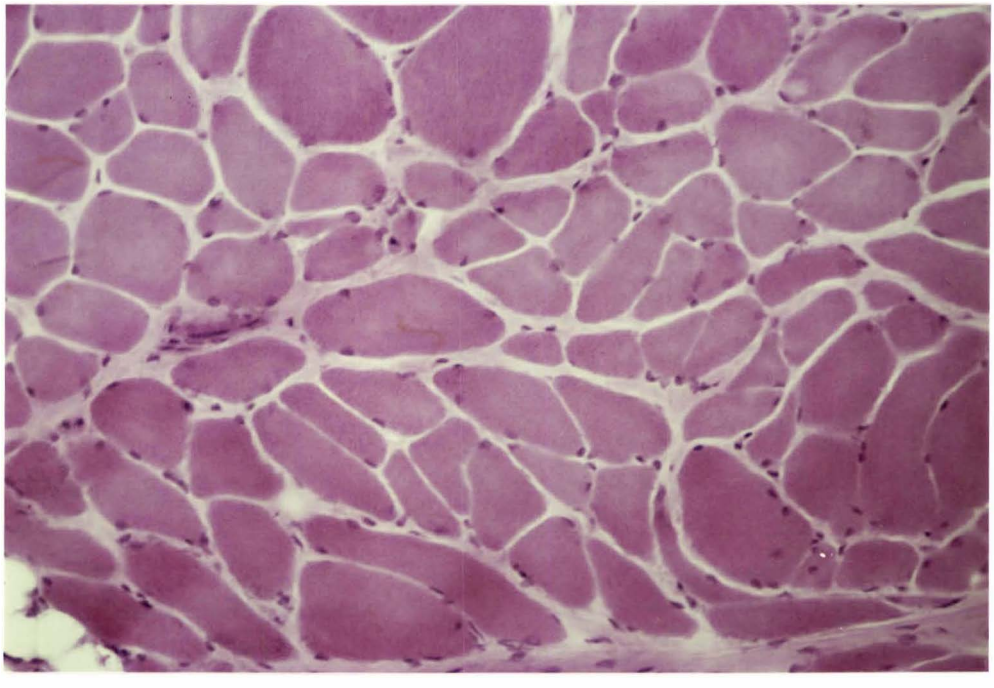
MEAN AREA (μm^2)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
AGE (YEARS)

FIGURE 53 A transverse section of the palatine levator muscle from a "normal" horse (x260). Section stained to demonstrate the activity of myosin ATPase

FIGURE 54 A transverse section of the palatine muscle from a "normal" horse (x260). Section stained with haematoxylin and eosin





however was usually not significant.

The palatal muscles from the horses suffering from idiopathic laryngeal hemiplegia had larger AH and AL fibres than those from the normal horses. However, when the fibres of laryngeal hemiplegic horses were compared with those from normal horses of a similar age the differences in their cross sectional areas were not significant except in the case of the palatopharyngeal muscle. The mean cross sectional area of AH fibres from these muscles of the laryngeal hemiplegic horses greater than three years of age was 3025.50 μm^2 while that from the same group of normal horses was 2433.52 μm^2 ($P < 0.01$).

6.3.7 Histochemical and Histological Features of Palatal Muscles

The general histological features of the palatal muscles studied were similar in most respects to those of the juvenile and mature laryngeal muscles already described (Chapter 5). The most notable difference was the absence of the fibre type grouping which was so commonly demonstrated in many of the laryngeal muscles by the myosin ATPase stain.

In the palatopharyngeal and palatine muscles the AL fibres were present either singly or in small groups of two or three (Fig. 47a). Groups of 10-16 AL fibres were sometimes seen in the left or right palatine levator muscle although most groups of AL fibres in this muscle were smaller than this (Fig. 53). It was only in the palatine tensor muscle that larger groups of AL fibres were present and they were observed in both the left and right muscles of many of the horses (Fig. 48a).

In the haematoxylin and eosin stained sections of the palatal muscles studied it was not uncommon to observe certain of the features which were considered to be associated with denervation and reinnervation when they occurred in laryngeal muscles. Marked variation in fibre size and well developed endomysial connective tissue appeared to be a feature of all the palatine muscles (Fig. 54).

In most of the palatine levator muscles and not infrequently in the other muscles, there were areas of the sections which were very

nuclear and had occasional fibres with central nuclei (Fig. 55). These areas often exhibited variation in fibre size and prominant endomysial connective tissue. Except in the case of the palatine tensor muscle these histological features were not accompanied by grouping of AL fibres in the corresponding section stained to demonstrate the activity of myosin ATPase. These histological features were just as likely to be present in left or right muscles and were seen in the muscles from horses of all ages.

6.3.8 Sarcosporidiosis

As was the case with the intrinsic laryngeal muscles, sarcocysts occurred in the palatal muscles studied. They were not observed in horses under two years or over 15 years but were seen in the muscles of horses between these ages. The palatine tensor was the muscle most often involved and the cysts were observed in the left or right or both muscles. The cysts were less frequently observed in one or both sides of the palatine levator, palatopharyngeal and palatine muscles.

6.4 Discussion

6.4.1 Palatal Muscle Weights

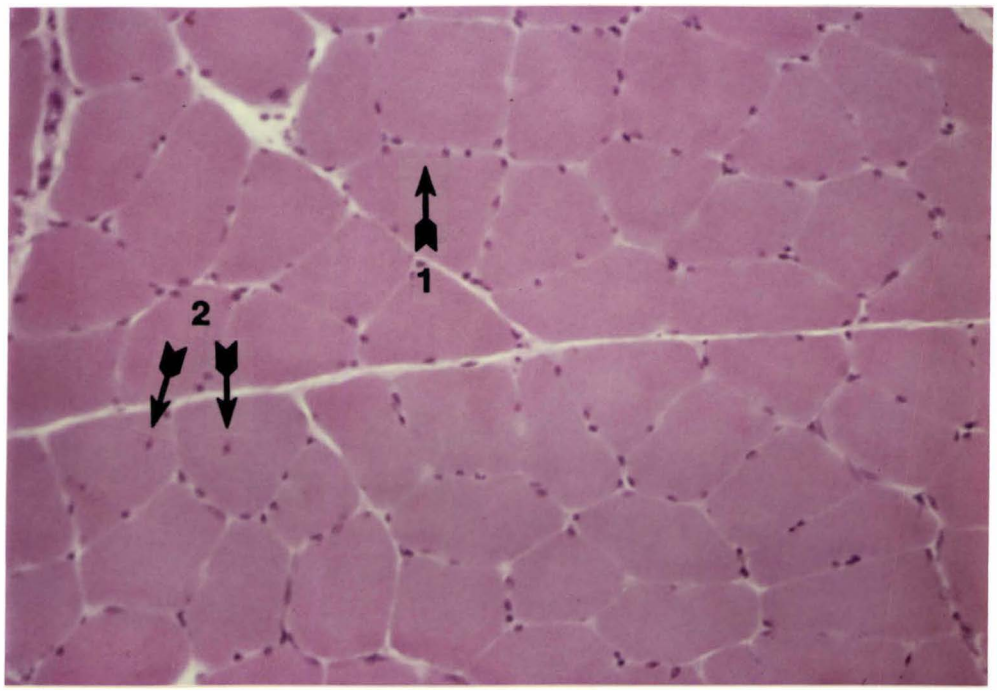
The increase in weight with age of the palatine levator and palatine tensor muscles, follows a similar growth curve to those plotted for the intrinsic laryngeal muscles, and the weight of these palatal muscles peaks when a horse is approximately three years of age. Idiopathic laryngeal hemiplegia had no apparent influence on palatal muscle weights and a difference between the weights of the left and right muscles was not present in the palatal muscles studied. Thus the unilateral atrophy which was so common in some of the intrinsic laryngeal muscles was not present in either of the palatal muscles studied.

6.4.2 The Fibre Types Observed in Palatal Muscles

The fibre types observed were those which would be expected in terms of what is known of the function of these muscles. The palatopharyngeal muscle which had the highest proportion of AH fibres would be expected to be a very rapidly contracting muscle as it is

FIGURE 55 Transverse sections of the left palatine levator muscles from a "normal" horse (x260). Sections stained with haematoxylin and eosin and illustrate:-

1. Fibres with numerous sarcolemmal nuclei
2. Internal nuclei
3. Prominant endomysium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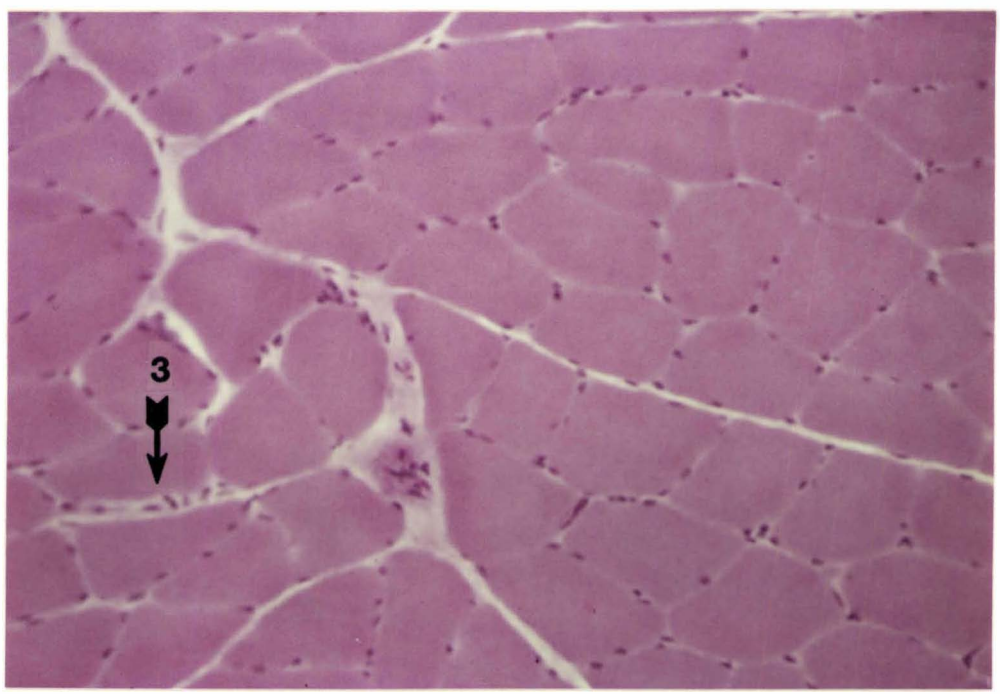
3

important in closing the nasopharyngeal sphincter and perhaps opening the ostia of the auditory tube during swallowing (Heffron and Baker, 1979a). The high proportion of AH fibres observed in the palatine muscle suggest that it is also rapidly contracting. Its action in shortening the soft palate is probably involved in the rapid movements made by the soft palate during swallowing. The palatine levator muscle also had a relatively high proportion of AH fibres and this is consistent with its function in closing the nasopharyngeal sphincter during swallowing (Heffron and Baker, 1979c; Cook, 1981). Only the palatine tensor muscle had approximately equal proportions of AH and AL fibres and their distribution as noted during this study is interesting in terms of what is known of this muscle in other species. Doyle and Rood (1980) have shown that in man and the Rhesus monkey the palatine tensor muscle can be divided into two fibre bundles. The lateral bundle proceeds as a tendon around the hamulus of the pterygoid bone and onto the palatine bone and aponeurosis. The medial bundle is attached to the posterior third of the lateral membranous wall of the eustachian tube and is responsible for its active dilation (Honjo et al., 1980). In the horse the muscle does not appear grossly to have two separate divisions but the attachments of its various aspects are similar to those described in other species. The lateral aspect of the muscle probably contains the fibres which activate the tendon around the hamulus of the pterygoid bone and so tense and depress the rostral third of the soft palate. The medial fibres which attach to the lateral cartilagenous lamina of the eustachian tube are probably the fibres which are involved in tubal dilation during swallowing. It would be expected that the lateral fibres would be capable of sustained tonic activity and as demonstrated by this study they were in fact predominantly AL. The medial fibres would be expected to be faster contracting and it was in the medial aspect of the muscle that the characteristic mosaic of AH and AL fibres was noted.

It was not possible to compare the fibre types found in the equine palatine tensor muscle during this study with those observed in the dog by Orvis and Cardinet (1981) as acid preincubation of ATPase sections was not performed for the reasons outlined in Chapter 4.

The SDase and GPase reactions of the fibres of the muscles studied in this survey indicated that the palatal muscles of the horse are capable of both aerobic and anaerobic metabolism. It was only in the muscles of some of the older horses that occasional pale mottled fibres were demonstrated by the SDase reaction and occasional large pale fibres by the GPase reaction. These fibres are probably similar to those exhibiting the ageing changes previously noted in some of the laryngeal muscles. This study did not provide evidence that the changes in fibre proportion and architecture which appeared to result from neurogenic disease in some of the laryngeal muscles, occurred in the palatal muscles.

6.4.3 Fibre Type Grouping in Palatal Muscles

It is possible that fibre type grouping as a result of denervation and reinnervation may not be particularly obvious in muscles such as the palatopharyngeal and palatine which have predominantly AH fibres, or the palatine tensor where large areas of AL fibres normally occur. If an AH muscle fibre was denervated in the first two muscles it would most likely be reinnervated by one of the axons supplying another AH fibre, the most numerous type. A change of fibre type would not occur unless the reinnervating axon came from one of the AL fibres which make up less than 10% of the total fibre population.

The proportion of AH:AL fibres observed in the palatine levator muscle was closer to that of the intrinsic laryngeal muscles where fibre type grouping of AL fibres did occur. Large groups of AL fibres however were not observed in the equine palatine levator muscles examined.

6.4.4 Fibre Cross Sectional Area in Palatal Muscles

The increase in mean cross sectional area of palatal muscle fibres with age, noted during this study is consistent with the changes which occur in muscle as an animal grows. Similar changes were noted in the intrinsic laryngeal muscles. The only significant interaction which was probably not due to this age effect was noted in the palatopharyngeal muscle. The mean cross sectional area of the fibres from the muscles of the laryngeal hemiplegic horses over three years of age (mean age 11.25 years) was greater than that of the fibres from similar aged normal horses (mean age 12 years). This trend was

present but not significant in the other palatal muscles from the laryngeal hemiplegic horses. While the laryngeal hemiplegic horses were not older than the normal horses they may have been larger as a relationship between large body size and laryngeal hemiplegia has been demonstrated by Goulden and Anderson (Appendix 9). The possibility also exists that the larger cross sectional area of the fibres of this muscle may have been a response to laryngeal hemiplegia.

6.4.5 Histological Features of Palatal Muscles

The histological features described in the palatal muscles would, if they were accompanied by fibre type grouping, have been classed as subtle pathology using the grading system devised to describe the changes produced by denervation and reinnervation in the laryngeal muscles. They were however extremely common in the palatal muscles from both the left and right sides of young and old horses. They were not apparently accompanied by fibre type grouping, at least in the muscle where groups of AL fibres would be expected to be seen if they occurred. The signs of advanced neurogenic atrophy, for example, marked fibre atrophy and hypertrophy, fascicular atrophy and fibrous replacement of muscle fibres, were not observed in any of the palatal muscles studied.

Some of the histological features observed during the present study may have been similar to the minor myopathic changes such as randomly placed nuclei, described by Blythe et al. (1989). The more severe histopathology which they described, such as myonecrosis and phagocytosis was not observed in the palatal muscles examined. Blythe et al. (1980) commented that the myopathy they observed was more severe in horses with "elongated soft palates" and control horses greater than eight years of age. While only one of the horses included in the present study could perhaps be considered similar to those horses with "elongated soft palates", a considerable number of horses were over eight years of age.

6.4.6 Sarcosporidiosis

As was the case with the laryngeal muscles the occurrence of sarcocysts in palatal muscles did not appear to be related to histological abnormalities and was probably an incidental finding.

6.4.7 Concluding Comments

The results of this study of equine palatal muscles suggest that if neurogenic atrophy of these muscles does occur it is not as common as, or necessarily associated with, the neurogenic atrophy of some of the intrinsic laryngeal muscles which results in idiopathic laryngeal hemiplegia. If laryngo-palatal dislocation and idiopathic laryngeal hemiplegia have a common aetiology it does not manifest itself nearly as frequently in the motor nerves of the palatal muscles as it does in the left recurrent laryngeal nerve.

One of the major problems in attempting to understand the pathology of the soft palate and pharynx of the horse is a lack of real knowledge about normal pharyngeal function. Electromyography has been a significant aid in understanding the biomechanics of the equine larynx and it is used extensively to investigate palatal problems in man. For example, the palatine tensor muscle fires during inspiration in man (Hairston and Sauerland, 1981) and this inspiratory firing has been shown to cease just prior to pharyngeal obstruction in a case of obstructive sleep apnoea (Anch et al. 1981), a syndrome during which snoring and sterterous respiration occur. If the difficulties associated with electromyography of equine palatal muscles could be overcome this approach may be very rewarding. Access to these muscles for the placement of needle electrodes could be possible via the guttural pouches. It would also be necessary to record electromyograms from these muscles in conscious horses as some are probably most active during swallowing. As clinical laryngo-palatal dislocation only occurs in exercising horses it may also be necessary to consider the use of telemetry.

The possibility remains that laryngo-palatal dislocation is not directly associated with the palatal muscles at all. Cook's (1981) suggestion that dislocation of the soft palate may result from overactivity of the sternothyrohyoid muscle, still requires further investigation. The different histochemical features of the hyoepiglottic muscle of the horse with laryngo-palatal dislocation (Chapter 4) when compared with the other horses in this series, also warrants further study. In addition it is conceivable that the common pathology of the laryngeal

muscles may indirectly contribute to laryngo-palatal dislocation. If the arytenoid cartilage is not fully abducted during inspiration, due to partial denervation of the dorsal cricoarytenoid muscle it is likely that less than maximal tension may be exerted on the cuneiform process of the epiglottic cartilage via the ventricular ligament. This could lessen the forces depressing the epiglottis and perhaps facilitate laryngo-palatal dislocation. Whatever the cause of this dislocation it will probably not be clarified until normal pharyngeal and palatine function in the horse is more completely understood.

7. CONCLUSIONS

7.1 The intrinsic laryngeal muscles of the New Zealand Thoroughbred reached their mature weight when the horses were between three and four years of age.

7.2 Atrophy of some of the intrinsic laryngeal muscles supplied by the left recurrent laryngeal nerve was very common in "normal" horses and more severe in laryngeal hemiplegic horses.

7.3 At least one of the adductor muscles was more severely affected by neurogenic disease than the abductor muscles studied.

7.4 The fibres of most of the intrinsic laryngeal muscles were predominantly fast twich and capable of aerobic and anaerobic metabolism.

7.5 Neurogenic disease appeared to have an influence on the proportion of AH fibres in affected muscles and on the level of one of their enzymes of aerobic and anaerobic metabolism.

7.6 Fibre type grouping of AL fibres was very common in some of the left intrinsic laryngeal muscles from "normal" horses. It tended to be more marked in some of the muscles of the older horses but it appeared as early as six weeks of age.

7.7 The mean cross sectional area of the fibres of equine intrinsic laryngeal muscles increased till the horse was approximately three years of age.

7.8 There was no apparent difference between the areas of AH and AL fibres in the intrinsic laryngeal muscles studied.

7.9 Neurogenic disease appeared to alter the mean cross sectional area of fibres both directly in affected muscles and indirectly in muscles related to them.

7.10 The histological signs of neurogenic disease demonstrated by haematoxylin and eosin preparations appeared in some of the intrinsic laryngeal muscles almost as frequently as the fibre type grouping demonstrated by enzyme histochemistry. They were not present in the muscles of horses less than one year of age and were more severe in some of the muscles of the laryngeal hemiplegic horses.

7.11 Evidence of neurogenic disease may be present in one or more

of the intrinsic laryngeal muscles of the majority of New Zealand Thoroughbred horses. No evidence of neurogenic disease however, was observed in any of the intrinsic laryngeal muscles of the three ponies studied.

7.12 There was no evidence of unilateral atrophy of the palatal muscles studied. Fibre type grouping as a result of denervation and reinnervation was not observed in these muscles, nor were histological signs of severe neurogenic disease.

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APPENDIX 1

Davies' and Gunn's modification of the calcium-cobalt method of Padykula and Herman for demonstrating the activity of the enzyme myosin ATPase in transverse frozen sections of muscle.

Reagents

1. Substrate:

1.0M tris (hydroxymethyl) aminomethane (M.W. = 121.14)		12ml
0.18M calcium chloride hexahydrate (2g/100ml)		6ml
adenosine triphosphate disodium salt		90mg
distilled water		45ml
adjust pH to 9.5 with 0.1N hydrochloric acid		
distilled water	to	60ml

2. Cacodylate buffered formaldehyde (pH 7.)

2.14g sodium cacodylate $3H_2O$ in 50ml distilled water		
0.2M hydrochloric acid		6.3ml
40% formaldehyde		20ml
distilled water	to	200ml

3. 2% cobalt chloride

4. 1% ammonium sulphide

Method

1. Fresh frozen sections, cut at 10 µm mounted on clean microscope slides
2. Place in solution (2) for two minutes
3. Wash in two changes of distilled water
4. Incubate in solution (1) for 20 minutes at 37°C
5. Wash in two changes of distilled water
6. Solution (3) for three minutes
7. Wash in two changes of distilled water
8. Solution (4) for 30 seconds
9. Wash and mount in glycerine jelly.

APPENDIX 2

Nachlas et al.'s method for demonstrating the activity of the enzyme SDase.

Reagents

1. Substrate

0.1M phosphate buffer pH 7.6	11ml
0.2M sodium succinate	11ml
nitroblue tetrazolium (1mg/ml)	22.5ml

2. Phosphate buffer (Culling, 1974)

potassium dihydrogen orthophosphate 0.9g in 100ml distilled water	10ml
disodium hydrogen orthophosphate 0.94g in 100ml distilled water	90ml

3. 10% formol.

Method

1. Fresh frozen sections cut at 10 µm and mounted on clean microscopic slides
2. Incubate at 37°C in solution (1) for 20 minutes
3. Wash in two changes of distilled water
4. Fix in solution (3) for 10 minutes
5. Wash and mount in glycerine jelly.

APPENDIX 3

Takeuchi's (1956) modification of the method of Takeuchi and Kuriaki (1955) to demonstrate the activity of the enzyme GPase.

Reagents

1. Substrate

glucose-1-phosphoric acid	150mg
adenosine-5-monophosphoric acid	30mg
distilled water	45ml
0.1M acetate buffer pH 5.9	30ml
protamine zinc insulin	2 I.U.
absolute ethanol	15ml

2. acetate buffer pH 5.9

0.1M sodium acetate	190ml
0.1M acetic acid	10ml

3. absolute ethanol

4. Lugol's iodine

iodine		1g
potassium iodide		2g
distilled water	to	100ml

Method

1. Fresh frozen sections mounted on clean microscope slides
2. Incubate in solution (1) for three hours at 37^{o}C
3. Wash in distilled water
4. Dry in an oven at 37^{o}C
5. Solution (3) for two minutes
6. Dry in air
7. Solution (4) for three minutes
8. Mount in glycerine jelly.

The blue colouration induced by iodine staining of the polyglucose chain tended to fade with time. Consequently it was necessary to repeat the iodine staining immediately before subsequent use of the section.

APPENDIX 4.1 EXAMPLE OF THE DATA COLLECTED FROM THE CRICOTHYROID MUSCLE OF HORSE 12, ILLUSTRATING ITS ARRANGEMENT FOR ANALYSIS

Horse Number	Health 1 = normal 2 = abnormal	Sex 1 = entire ♂ 2 = female 3 = gelding	Age in Years	Weight of left muscle in grams	Weight of right muscle in grams
12	1	2	0.25	1.6	1.27

	Total number of fibres counted	Number of AH fibres	Number of AL fibres	Number of AHSH^{P}H fibres	Number of ALSH^{P}H fibres	Number of ALSHPL fibres	Mean size of groups of AL fibres	Standard deviation of size, of groups of AL fibres	Number of groups of AL fibres with more than 10 fibres per group	Mean area of AH fibres μm^2	Mean area of AL fibres μm^2
Bundle A left muscle	114	69	45	69	34	11	3.21	4.02	1	865	763
Bundle B left muscle	129	79	50	79	29	21	3.33	2.89	0	1077	897
Bundle A right muscle	107	69	38	69	22	16	2.38	2.09	0	829	631
Bundle B right muscle	143	109	34	109	19	15	2.13	1.4	0	592	538

AH = myosin ATPase high; AL = myosin ATPase low; SH = succinate dehydrogenase high; ^{P}H = glycogen phosphorylase high; PL = glycogen phosphorylase low.

Appendix 4.2

The data from the dorsal cricoarytenoid, lateral cricoarytenoid, transverse arytenoid, ventricular, cricothyroid, hyoepiglottic, palatopharyngeal, palatine levator, palatine and palatine tensor muscles from 56 horses, arranged as in Appendix 4.1

Dorsal cricoarytenoid muscle.

```
1 1 2 -.16 .69 .84
   162 147 15 142 15 0 1.07 .27 0 326 181
   288 253 35 243 35 0 1.46 .72 0 286 269
   297 271 26 271 24 2 1.04 .2 0 200 160
   155 142 13 142 13 0 1.44 1.01 0 175 161
2 1 1 .001 1.39 1.47
   116 86 30 80 21 9 2.31 2.1 0 776 693
   186 144 42 133 23 19 1.68 1.03 0 550 492
   125 80 45 63 31 14 3 2.42 0 503 532
   165 117 48 116 14 34 1.66 1.01 0 827 538
3 1 1 .002 .6 .58
   176 146 30 146 30 0 1.3 .56 0 270 206
   111 75 36 75 36 0 1.8 1.06 0 372 337
   164 124 40 107 29 11 2.35 1.5 0 482 573
   106 81 25 80 19 6 2.08 1.78 0 584 540
4 1 1 .003 1.56 1.55
   137 110 27 56 6 21 1.23 .43 0 712 562
   147 117 30 71 4 26 1.36 .58 0 596 523
   351 244 107 206 101 6 2.06 1.65 0 421 366
   264 190 74 148 18 56 1.41 .71 0 696 506
5 1 1 .003 1.33 1.3
   367 300 67 298 30 37 1.6 1.35 0 312 325
   339 282 57 282 25 32 1.43 .64 0 384 381
   184 154 30 153 10 20 1.43 .68 0 472 429
   263 219 44 219 20 24 1.42 .67 0 303 356
6 1 2 .003 * *
   251 185 66 184 29 37 2.44 3.92 1 382 320
   235 174 61 172 3 58 2.35 2.59 1 793 751
   227 171 156 169 8 48 1.7 1.1 0 498 501
   185 146 39 145 8 31 1.56 1.08 0 377 344
7 1 2 .005 1.4 1.3
   418 350 68 330 68 0 1.51 .89 0 272 204
   276 211 65 186 65 0 2.03 1.45 0 257 232
   223 181 42 148 42 0 1.4 .72 0 240 223
   233 201 32 158 32 0 1.23 .59 0 274 250
8 1 2 .005 1.75 1.81
   190 129 61 102 43 18 3.21 2.78 1 641 651
   189 144 45 124 40 5 1.88 1.48 0 638 605
   191 131 59 109 51 8 2.45 2.64 1 645 588
   137 100 37 81 29 8 2.64 1.15 0 479 504
9 1 1 .01 1.75 1.75
   197 148 49 146 16 33 1.88 1.34 0 487 425
   262 212 50 212 37 13 1.43 .81 0 347 317
   167 135 32 127 28 4 1.39 .66 0 601 569
   125 105 21 95 18 3 1.31 .48 0 637 594
10 1 * .08 1.78 1.64
   164 107 57 107 57 0 4.07 3.89 2 590 607
   110 69 41 69 41 0 3.73 3.41 1 721 783
   165 107 58 107 58 0 5.27 8.36 1 698 653
   115 75 40 75 40 0 3.08 3.28 0 880 880
11 1 1 .1 2.19 2.15
   149 107 42 106 20 22 2.63 1.96 0 887 791
   178 130 48 130 31 17 2 1.69 0 854 639
   * * * * * * * * * * *
   * * * * * * * * * * *
12 1 2 .25 2.13 2
   107 74 33 74 19 14 2.2 1.93 0 1255 1129
   181 122 59 122 41 18 2.46 1.67 0 1040 871
   167 120 47 118 25 22 2.24 2.51 1 753 743

13 1 2 .5 4.12 4
   79 48 31 48 31 0 4.43 3.95 0 2329 2400
   134 83 51 83 51 0 4.25 3.59 1 1840 1849
   133 105 28 105 28 0 1.65 1.11 0 1345 1201
   118 93 25 93 25 0 1.79 1.25 0 1662 1162
14 1 2 .5 3.72 3.28
   156 111 45 111 39 6 1.96 1.4 0 1073 682
   206 142 64 142 24 40 2 2.06 1 1397 1246
   186 103 83 100 77 6 4.88 5.68 3 1057 849
   180 97 83 88 70 13 5.18 8.38 2 922 829
15 1 2 .66 5.24 4.98
   145 86 59 86 57 2 2.68 2.83 1 2596 1546
   186 122 64 123 57 6 2.37 2 0 1693 1334
   167 99 68 99 68 0 3.23 2.96 1 1872 1525
   99 45 54 45 54 0 9 10.14 2 1992 1928
16 1 2 .66 4.45 4.8
   128 71 57 71 27 30 3.35 3.79 1 1702 1293
   134 75 59 75 10 49 3.69 3.98 1 1638 1270
   118 79 39 79 28 11 3.27 1.95 0 1668 1446
   127 90 37 90 15 22 2.06 1.47 0 1254 1140
17 1 1 1 9.17 8.57
   83 55 28 55 28 0 5.6 7.54 1 1992 1799
   44 24 20 24 21 0 3.3 4.32 1 1856 2066
   76 54 22 54 22 0 1.69 .95 0 3394 2463
   128 79 49 79 48 1 2.22 2.18 0 2735 1549
18 1 2 1 7.79 6.67
   121 76 45 73 37 4 4.5 5.89 1 2685 3101
   150 96 54 96 54 0 6 5.1 1 1986 2252
   121 84 37 84 37 0 2.85 2.73 0 1621 1916
   79 64 15 64 15 0 1.67 .87 0 1692 1830
19 1 2 1 7.14 7.06
   133 80 53 76 29 24 2.79 1.96 0 2789 2527
   65 37 28 37 9 19 4 2.94 0 1908 1615
   78 49 29 48 12 17 2.42 1.24 0 2388 1685
   105 56 49 55 22 27 4.45 3.36 1 1665 1393
20 1 2 1 8.97 8.84
   69 51 18 51 18 0 1.8 1.23 0 3911 4326
   90 66 24 66 24 0 1.85 1.14 0 3296 3414
   79 52 27 52 27 0 1.93 1.94 0 2396 2811
   79 55 24 55 24 0 1.41 .87 0 4460 4742
21 1 1 1.25 8.36 7.65
   96 70 26 70 17 9 2.17 1.59 0 2224 2097
   105 78 27 78 27 0 2.25 2.05 0 2276 2035
   67 43 24 43 22 2 2.4 2.37 0 2146 1595
   109 80 29 79 29 0 1.81 1.22 0 2440 1670
22 1 1 1.5 3.17 5.84
   180 116 64 116 64 0 4 3.21 1 429 905
   102 59 43 59 43 0 6.14 5.87 2 390 652
   86 51 35 51 35 0 3.89 4.89 1 1849 1438
   118 85 33 85 31 2 1.65 .88 0 1504 1116
23 1 1 2 10.16 9.8
   75 41 34 41 34 0 8.5 12.48 1 2675 3833
   88 68 20 68 20 0 2.86 2.12 0 2481 2675
   68 20 48 20 48 0 24 32.53 1 2694 2192
   75 44 31 44 31 0 3.1 2.81 0 1788 1225
24 1 1 2 7.84 7.26
   88 67 21 67 7 14 2.1 1.2 0 1759 2071
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Lateral cricoarytenoid muscle.

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Tranverse arytenoid muscle.

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   79 72 7 72 7 0 1 0 0 1439 505
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Ventricular muscle.

```
6 1 2 .005 * *
   * * * * * * * * * * * *
   * * * * * * * * * * * *
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   79 52 27 52 27 0 1.93 1.94 0  2362 1488
   79 55 24 55 24 0 1.41 .87 0   2169 1310
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   72 64 8 64 8 0 1.86 .9 0 1298 723
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29 2 1 3 * *
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34 1 2 4 2.93 2.79
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   103 97 6 97 6 0 1 0 0 830 485
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   * * * * * * * * * * * *
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   86 84 2 84 2 0 1 0 0 1723 1021
56 1 3 10 1.6 1.69
   55 55 0 55 0 0 0 0 0 2793 0
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   91 88 3 88 3 0 1.5 .71 0 1749 1526
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Cricothyroid muscle.

```
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2 1 1 .001 .93 1
   112 97 15 95 13 2 1.15 .38 0 694 500
   154 129 25 127 20 5 1.47 .8 0 784 532
   117 92 25 85 14 11 2.08 1.16 0 801 807
   137 123 14 116 11 3 1.56 .88 0 925 693
3 1 1 .002 .54 .53
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   84 81 3 81 3 0 1 0 0 241 221
   175 162 13 160 10 3 1.08 .29 0 365 249
   186 176 10 174 9 1 1.11 .33 0 300 211
4 1 1 .003 1.22 1.25
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   141 122 19 78 9 10 1.12 .33 0 432 381
   125 70 55 61 12 43 4.23 5.9 1 1075 733
   158 102 56 80 18 38 4.3 4.07 1 940 829
5 1 1 .003 1 1
   * * * * * * * * * * *
   * * * * * * * * * * *
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   157 136 21 136 11 10 1.33 1.22 0 386 355
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   163 128 34 95 34 0 1.48 1.16 0 462 419
   173 124 49 112 49 0 2.04 1.49 0 477 393
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   202 183 19 180 19 0 1.27 .46 0 596 452
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   143 109 34 109 19 15 2.13 1.4 0 592 583
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   212 112 100 112 82 18 5.56 6.59 4 1267 840
   173 122 51 120 25 26 2.13 1.39 0 776 631
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   96 52 44 52 22 22 4.4 4.9 2 1197 1621
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```

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Hyoepiglottic muscle.

```
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7 1 2 .005
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20 1 2 1
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22 1 1 1.5
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24 1 1 2
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   74 34 40 30 15 25 10 13.04 1 2103 2620
26 1 2 2
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27 2 2 2
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29 2 1 3
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   151 97 54 97 49 5 2.45 1.74 0 2124 2779
30 2 2 3
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34 1 2 4
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35 1 3 4
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40 2 3 6
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   98 52 46 52 44 2 5.11 4.76 2 1888 604
43 1 2 12
   63 27 36 27 14 22 12 16.53 1 3935 1767
   73 30 43 30 29 19 7.17 12.75 1 2523 3164
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54 1 2 25
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Palatopharyngeal muscle.

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1 1 2 -.16 .41 .90
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2 1 1 .001 * *
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   134 118 16 117 11 5 1.23 .44 0 560 515
3 1 1 .002 .42 .53
   136 126 10 119 10 0 1.43 .79 0 420 492
   132 121 11 121 4 2 1.38 .74 0 406 399
   168 154 14 154 14 0 1.08 .28 0 338 292
   139 123 16 120 13 0 1.78 1.09 0 352 333
4 1 1 .003 .87 .89
   134 95 39 72 36 3 2.44 1.5 0 484 468
   163 128 35 113 22 13 2.33 2.12 0 626 597
   217 150 67 126 53 14 2.91 2.63 1 569 547
   144 95 49 71 43 6 3.77 4.83 1 606 603
5 1 1 .003 * *
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   141 137 4 137 4 0 1 0 0 332 380
   106 105 1 105 1 0 1 0 0 324 805
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   127 127 0 127 0 0 0 0 0 474 0
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   180 160 20 160 20 0 1.05 .23 0 417 342
8 1 2 .005 * *
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   78 71 7 71 5 2 1.17 .41 0 724 551
   60 57 3 57 3 0 1.5 .71 0 763 529
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   110 94 16 94 6 10 1.33 .49 0 1451 796
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   93 78 15 78 15 0 1.15 .38 0 1688 1495
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16 1 2 .66 2.28 1.93
   77 71 6 71 2 3 1 0 0 1657 889
   113 102 11 102 11 0 1.1 .32 0 226 1420
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   * * * * * * * * * * *
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   88 69 19 69 19 0 1.73 1.01 0 826 791
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   67 59 8 59 8 0 1 0 0 2323 1440
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Palatine levator muscle.

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```

Palatine muscle.

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   126 126 0 110 0 0 0 0 0 510 0
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   132 110 22 110 16 6 1.83 .94 0 246 260
5 1 1 .003
   167 160 7 160 7 0 1.17 .41 0 696 425
   131 128 3 128 3 0 1 0 0 459 512
8 1 2 .005
   150 136 14 136 14 0 1.27 .65 0 377 304
   * * * * * * * * * * *
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   160 122 38 122 38 0 2.92 2.72 0 365 242
   90 71 19 71 19 0 1.46 .78 0 520 361
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   96 96 0 96 0 0 0 0 0 425 0
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   * * * * * * * * * * * *
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   110 98 12 98 12 0 1.5 1.07 0 912 899
56 1 3 10 *
   74 71 3 71 3 0 1 0 0 1897 1397
   49 47 2 47 2 0 1 0 0 1987 1793
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Palatine tensor muscle.

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1 1 2 -.16 * *
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   140 140 0 140 0 0 0 0 0 905 0
   244 244 0 244 0 0 0 0 0 401 0
   117 117 0 117 0 0 0 0 0 480 0
2 1 1 .001 .86 1
   189 139 50 136 32 18 1.79 1.03 0 321 329
   289 215 74 208 50 24 1.9 1.54 0 260 272
   289 208 81 185 59 22 3.12 2.39 0 307 362
   247 144 103 139 77 26 4.16 3.86 2 285 327
3 1 1 .002 .59 .56
   206 164 42 163 27 15 2.47 2.67 0 234 270
   190 160 30 159 23 7 1.58 1.17 0 239 300
   201 159 42 157 27 15 2.47 1.74 0 285 362
   237 171 66 169 49 17 3.3 2.58 1 319 412
4 1 1 .003 1.11 1.04
   220 167 53 165 27 26 1.58 .86 0 381 344
   231 171 60 165 31 29 1.94 1.26 0 302 291
   111 81 30 77 23 7 3 2.58 0 419 643
   143 89 54 82 40 14 3.38 3.26 1 428 242
5 1 1 .003 1 1
   258 250 8 250 8 0 1.33 .52 0 290 350
   176 171 5 171 5 0 1.25 .5 0 261 438
   50 150 0 150 0 0 0 0 0 398 0
   218 218 0 218 0 0 0 0 0 396 0
6 1 2 .003 * *
   128 106 22 106 10 12 1.57 .85 0 471 534
   151 122 29 122 26 3 1.53 .84 0 344 338
   153 118 35 118 17 18 1.46 .93 0 287 328
   152 132 20 132 20 0 1.33 .62 0 258 301
7 1 2 .005 1.16 1.3
   182 136 46 125 146 0 2.42 2.14 0 308 336
   159 120 39 114 39 0 1.63 1.13 0 270 336
   145 110 35 116 35 0 2.19 1.6 0 388 418
   130 108 22 108 22 0 1.22 .55 0 344 332
8 1 2 .005 1 1
   161 136 25 132 22 3 2.72 1.35 0 335 382
   159 101 58 100 42 16 4.14 5.33 3 456 436
   139 87 52 85 47 5 2.89 2.93 1 371 392
   191 123 67 122 59 5 2.48 1.28 0 402 428
9 1 1 .01 1.22 1.27
   209 171 38 153 30 8 02.53 1.68 0 332 361
   136 106 30 102 28 2 1.88 1.36 0 324 367
   212 165 47 159 32 15 3.92 2.43 0 288 340
   247 215 32 211 29 3 2.5 2.2 0 284 341
10 1 * .08 1.5 1.61
   89 44 45 44 45 0 5 5.72 2 1006 744
   153 96 59 94 59 0 4.54 8.94 1 624 514
   123 35 88 35 33 0 29.33 38.1 2 944 804
   103 68 35 68 35 0 5 5.92 1 1046 1015
11 1 1 .1 2.5 2.2
   134 134 0 134 0 0 0 0 0 640 0
   100 100 0 100 0 0 0 0 0 597 0
   168 168 0 168 0 0 0 0 0 604 0
   119 119 0 119 0 0 0 0 0 634 0
12 1 2 .25 1.65 1.65
   108 108 0 108 0 0 0 0 0 798 0
   92 92 0 92 0 0 0 0 0 794 0
   115 73 42 73 42 0 3.82 4.7 2 1049 907
   69 39 30 39 30 0 5 3.52 0 916 908
14 1 2 .5 3.28 3.17
   199 95 104 88 88 16 13 11.65 4 1295 848
   206 95 111 92 95 16 10.09 12.48 3 1309 813
   102 45 57 45 56 1 8.14 13.04 1 1208 806
   92 31 61 31 61 0 12.2 20.6 1 1051 861
15 1 2 .66 3.95 3.74
   93 23 70 23 70 0 1 0 1 1613 1377
   77 2 75 2 75 0 1 0 1 1220 1657
   101 27 74 27 74 0 37 50.91 1 1345 1243
   53 0 53 0 53 0 1 0 1 0 1496
16 1 2 .66 4.55 5.1
   101 74 27 47 0 0 2.7 2.36 0 1512 1337
   73 55 18 37 0 0 2 1.58 0 1450 1073
   90 67 23 44 0 0 1.92 1.38 0 1821 752
   96 70 26 44 0 0 2.17 1.47 0 1825 1346
17 1 1 1 5.91 6.32
   121 57 64 3 64 0 15.75 29.5 1 1788 1922
   97 45 52 1 50 0 7.29 7.87 2 1938 2002
   98 49 49 2 43 0 12.25 20.55 1 1918 2205
   65 26 39 4 37 0 1 0 1 2168 2013
18 1 2 1 5.81 5.1
   115 4 111 1 110 1 55.5 74.3 1 2063 1129
   105 4 101 4 101 0 1 0 1 1300 1247
   122 39 83 39 83 0 41.5 57.3 1 1275 1165
   67 0 67 0 67 0 1 0 1 0 1317
19 1 2 1 4.06 3.43
   66 62 4 58 0 0 1.33 .58 0 2884 3226
   91 91 0 0 0 0 0 0 0 2439 0
   68 68 0 68 0 0 0 0 0 2158 0
   91 91 0 91 0 0 0 0 0 1881 0
21 1 1 1.25 6.65 6.74
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   * * * * * * * * * * * *
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   68 0 68 0 60 8 1 0 1 0 1798
22 1 1 1.5 5.3 5.08
   126 126 0 126 0 0 0 0 0 1217 0
   70 70 0 70 0 0 0 0 0 1489 0
   * * * * * * * * * * * *
   * * * * * * * * * * * *
23 1 1 2 7 6.9
   69 46 23 46 23 0 1.92 1.24 0 1613 1360
   62 37 25 37 25 0 3.57 2.94 0 1786 1699
   80 49 31 49 31 0 6.2 8.98 1 1072 1089
   83 50 33 50 33 0 3.3 4.11 1 1288 1252
24 1 1 2 5.63 5.7
   76 29 47 29 37 10 9.4 14.72 1 1724 1451
   84 28 56 26 51 5 28 21.2 2 1723 1718
   69 58 11 47 0 0 2.2 .84 0 1956 1682
   94 83 11 72 0 0 1.57 .98 0 2066 1540
25 1 3 2 5.23 5.75
   102 72 30 42 0 0 2 1.6 0 1679 1254
   97 69 28 41 0 0 2.55 2.66 0 1583 1109
   79 79 0 79 0 0 0 0 0 2041 0
   98 98 0 98 0 0 0 0 0 1789 0
27 2 2 2 7.44 6.31
   99 0 99 0 99 0 1 0 1 0 1900
   115 0 115 0 115 0 1 0 1 0 1763
   120 0 120 0 120 0 1 0 1 0 1837
   74 0 74 0 74 0 1 0 1 0 1897
```

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   87 45 42 45 42 0 4.2 4.13 1 2881 1725
   87 41 46 41 46 0 5.11 6.27 1 3169 2233
   88 39 49 39 49 0 8.17 15.6 1 2996 1776
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   118 0 118 0 118 0 1 0 1 0 2171
   42 0 42 0 42 0 1 0 1 0 2491
30 2 2 3 7.46 7.99
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   57 0 57 0 57 0 1 0 1 0 2289
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   43 0 43 0 43 0 1 0 1 0 2308
31 1 3 3.5 9.44 7.98
   73 43 30 43 25 5 6 5.57 2 2595 2588
   107 64 43 64 43 0 3.31 2.9 0 2491 2131
   61 45 16 44 10 6 1.6 1.07 0 3474 2500
   57 47 10 46 5 5 1.43 .79 0 3447 2061
32 1 3 4 10.85 10.22
   * * * * * * * * * * *
   * * * * * * * * * * *
   84 20 64 20 64 0 21.33 31 1 2153 1842
   77 1 76 1 76 0 1 0 1 2173 2046
34 1 2 4 6.13 6.09
   90 0 90 0 0 90 0 0 1 0 2011
   114 0 114 0 0 114 0  0 1 0 1680
   72 0 72 0 0 72 1 0 1 0 1937
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   70 0 70 0 70 0 1 0 1 0 3756
   85 0 85 0 85 0 1 0 1 0 3436
   93 0 93 0 93 0 1 0 1 0 2022
   75 0 75 0 75 0 1 0 1 0 1915
36 1 3 5 * *
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   79 22 57 22 59 0 28.5 38.9 1 3685 2959
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   86 32 54 32 54 0 13.5 22.37 1 2371 1490
   90 36 54 36 54 0 18 26 1 2517 1589
   90 47 43 47 43 0 3.07 2.89 1 1892 1266
   83 49 34 49 34 0 3.4 5.58 1 1640 1354
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   89 54 35 54 35 0 3.18 3.22 1 2126 1215
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   121 54 67 54 67 0 6.09 6.16 3 2197 1231
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   55 0 55 0 55 0 1 0 1 0 1600
   96 0 96 0 96 0 1 0 1 0 1896
   68 0 68 0 65 3 1 0 1 0 2213
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   76 15 61 15 61 0 1 1 2524 2431
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   70 26 44 26 44 0 14.67 1 3650 2427
   43 0 43 0 43 0 1 1 * 2546
43 1 2 12 * *
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   77 37 40 37 17 23 5.71 6.75 2 3805 4010
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   77 77 0 77 0 0 0 0 0 2322 0
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   57 42 15 27 0 0 2.14 1.21 0 2163 1919
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   63 24 39 24 39 0 13 20.78 1 2802 2289
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   65 35 30 35 30 0 6 4.9 1 2151 1864
   60 30 30 30 30 0 6 5.39 1 2529 2424
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   93 80 13 67 13 0 1.3 .67 0 2123 1571
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   45 36 9 36 0 1.28 .49 0 2439 1525
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   49 41 8 41 8 0 2 .82 0 2779 1558
   51 44 7 44 7 0 2.33 1.53 0 2541 1469
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APPENDIX 5 EXAMPLE OF THE GENSTAT ANALYSIS USED DURING THIS STUDY, IN THIS CASE THE ANALYSIS IS OF THE AREA OF AH FIBRES FROM THE LATERAL CRICOARYTENOID MUSCLE OF "NORMAL" HORSES OVER THREE YEARS OF AGE

Analysis of Variance

VARIATE: AREAHX

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
HORSE STRATUM						
HLTH_SX	1	2751	0.00	2751	0.001	- N.S.
RESIDUAL	17	50290840	45.83	2958285	4.390	
TOTAL	18	50293584	45.83	2794088	4.146	
HORSE SIDE STRATUM						
SIDE	1	10773924	9.82	10773924	7.999	Significant $p < 0.025$
SIDE.HLTH_SX	1	162938	0.15	162938	0.121	
RESIDUAL	17	22898516	20.87	1346972	1.999	
TOTAL	19	33835376	30.83	1780809	2.642	
HORSE.SIDE.SAMPLE STRATUM	38	25609124	23.34	673924		
GRAND TOTAL	75	109739080	100.00			

GRAND MEAN 2824.04

TOTAL NUMBER OF OBSERVATIONS 76

Tables of Means

VARIATE: AREAHX

GRAND MEAN 2824.04

HLTH_SX	ENTIRE_NM	FEM_NF	GELD_NG
REP	NO NORMAL MALES OVER 3 YEARS OF AGE	2831.09	2818.91
		32	44

NOT SIGNIFICANTLY DIFFERENT

SIDE	LEFT	RIGHT
	3200.55	2447.53
	SIGNIFICANTLY DIFFERENT	P < 0.025

HLTH_SX	ENTIRE_NM	FEM_NF	GELD_NG
SIDE			
LEFT		3153.31	3234.92 ($\bar{x}_1$)
LEFT REP		16	22
RIGHT		2508.88	2402.91 ($\bar{x}_2$)
RIGHT REP		16	22
		NOT SIGNIFICANTLY DIFFERENT	SIGNIFICANTLY DIFFERENT P < 0.05

$t = \bar{x}_1 - \bar{x}_2$ divided by the standard error of the difference of the means with the same level of health and sex and maximum replications i.e. (6) in the standard error table. The degrees of freedom are the residual degrees of freedom in the horse side stratum of the anova table above.

Standard Errors of Differences of Means

TABLE	HLTH_SX	SIDE	SIDE HLTH_SX	
REP	UNEQUAL	38	UNEQUAL	
SED			518.728 MIN REP	(1)
	399.600	266.258	482.064 MAX-MIN	(2)
			442.372 MAX REP	(3)
EXCEPT WHEN COMPARING MEANS WITH SAME LEVEL(S) OF:	HLTH_SX		410.331 MIN REP	(4)
			381.329 MAX-MIN	(5)
			349.931 MAX REP	(6)

To test the significance of the difference between the grand means of different age groups and health groups (i.e. normal or abnormal) the following formula was used:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{SS_1^2}{n_1} + \frac{SS_2^2}{n_2}}}$$

Where $\bar{x}_1 + \bar{s}_2$ are the two grand means SS_1^2 & SS_2^2 are the residual mean squares from the horse strata of the two anova tables and n_1 and n_2 are the two total numbers of observations.

Degress of freedom = $n_1 - 1 + n_2 - 1$.