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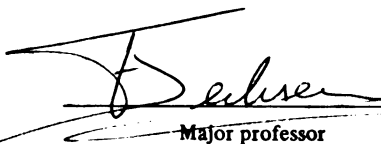
The Effectiveness of a Nerve Muscle
Pedicle Graft for the Treatment of
Equine Left Laryngeal Hemiplegia

presented by

Ian Charles Fulton

has been accepted towards fulfillment
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M.S. degree in Large Animal
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**THE EFFECTIVENESS OF A
NERVE MUSCLE PEDICLE GRAFT
FOR THE TREATMENT OF
EQUINE LEFT LARYNGEAL HEMIPLEGIA**

By

Ian Charles Fulton

A THESIS

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ABSTRACT

THE EFFECTIVENESS OF A NERVE MUSCLE PEDICLE GRAFT FOR THE TREATMENT OF EQUINE LEFT LARYNGEAL HEMIPLEGIA

By

Ian Charles Fulton

The efficacy of a nerve muscle pedicle (NMP) graft for the treatment of induced left laryngeal hemiplegia (LLH) was evaluated in exercising horses by measuring the following variables of upper airway function: Peak inspiratory and expiratory airflow ($\dot{V}_{\text{IMAX}}$ & $\dot{V}_{\text{EMAX}}$), trans-upper airway pressure (P_{UI} & P_{UE}), impedance (Z_{I} & Z_{E}), tidal volume, minute ventilation, heart rate and respiratory frequency. Measurements were recorded before left recurrent laryngeal neurectomy (LRLN) - baseline, 4 weeks following LRLN and 12, 24 and 52 weeks after NMP graft or sham operation.

Following LRLN, horses exercising at 7.0 m/sec had a significantly increased P_{UI} and Z_{I} , while $\dot{V}_{\text{IMAX}}$ decreased compared to baseline values. The sham operation did not improve airway function. Twelve and 24 weeks after NMP graft, Z_{I} was still significantly increased above baseline values but by 52 weeks no significant difference existed. These results prove that the NMP graft can restore upper airway function within 52 weeks in horses with induced LLH.

DEDICATION

To my late father Dr. Len Fulton,
who bestowed upon me the joys of being
a veterinary surgeon.

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I. INTRODUCTION

The inability of horses with left laryngeal hemiplegia (LLH) to exercise at high speeds for a prolonged time, often with inspiratory noise production, is well documented (Flemming,1882; Cook,1970; Haynes,1984:I). This exercise intolerance is a direct result of paralysis of the left cricoarytenoideus dorsalis (CAD) muscle. The left arytenoid cartilage can no longer be abducted, creating a partial obstruction in the air passage and increasing upper airway resistance to airflow (Robinson and Sorrenson,1978; Derksen et al,1986).

In the majority of cases, paralysis of the left CAD muscle is associated with a peripheral neuropathy of the left recurrent laryngeal nerve (Cole,1946; Duncan et al,1974), which has recently been defined as a distal axonopathy (Cahill and Goulden,1986:I,II&III). The cause of this axonopathy is still unknown.

While the search for a cause of LLH has continued, treatment of clinically affected horses has been necessary to allow these horses to pursue their athletic endeavors. Treatments include ventriculectomy (Hobday,1936), tracheostomy (Cook,1970), prosthetic laryngoplasty (Marks et al,1970), and arytenoidectomy (Haynes et al,1984:II). Until recently the success, or failure of surgical techniques for treatment of equine LLH was based on the subjective findings of decreased noise production on inspiration during exercise (Baker,1983:I), or an apparent

improvement in athletic performance (Speirs,1980). Recently at Michigan State University, objective evaluation of upper airway function in exercising horses demonstrated the efficacy of the prosthetic laryngoplasty technique for improving upper airway resistance in horses with LLH (Derksen et al,1986). Ventriculectomy (Shapell et al,1988) and subtotal arytenoidectomy (Belknap et al,1990) have also been quantitatively assessed and proved to be ineffective treatments of LLH. At present, prosthetic laryngoplasty is the only proven efficacious treatment for LLH in horses. A number of complications have been reported with prosthetic laryngoplasty, including nasal discharge of food, chronic coughing, and prosthetic failure (Speirs,1987). These complications have stimulated the investigation of a new treatment for LLH using a nerve muscle pedicle graft (NMP graft) technique.

In dogs (Lyons and Tucker,1974; Greenfield et al,1988) and people, (Tucker,1978:I; Fernandes et al,1987) the NMP graft procedure has been used to treat a variety of neurological diseases including laryngeal paralysis (Tucker,1978:I) and facial nerve paralysis (Anonsen et al,1986). In the horse, histological evidence of reinnervation in the CAD muscle has been reported following a NMP graft created from the second cervical nerve and omohyoideus muscle (Ducharme et al,1989:I), but arytenoid abduction was not observed. However in the study of Ducharme et al, horses were not evaluated during exercise when the recipient CAD muscle would be stimulated to contract (Ducharme et al,1989:I). The purpose of this study was to evaluate at exercise, the efficacy of a NMP graft as a treatment of LLH in horses.

II. STRUCTURE AND FUNCTION OF THE EQUINE LARYNX.

A. Anatomy of the Larynx

The larynx of the horse connects the pharynx with the trachea. In addition to providing a patent airway for respiration, the larynx is involved in coughing, phonation and swallowing. An intricate structure of cartilages, muscles, ligaments, afferent and efferent nerves allows the larynx to participate in this range of physiologic activities.

The skeleton of the equine larynx is composed of a single **cricoid**, **thyroid** and **epiglottic** cartilage and paired **arytenoid**, **corniculate** and **cuneiform** cartilages. The ring like cricoid cartilage encircles the laryngeal lumen posteriorly and has a large dorsal median ridge for muscle attachment. The thyroid cartilage has a "V" shape maintaining the lateral and ventral dimensions of the larynx. The arytenoid cartilages sit within the thyroid laminae, cranial to the cricoid cartilage with which they articulate. The arytenoid cartilages have a large, dorsally situated, muscular process, a ventral vocal process, a base that articulates with the cricoid cartilage and an apex that fuses with the corniculate cartilage. The epiglottis extends rostrally from the thyroid cartilage with the small cuneiform cartilages projecting laterally from the base of the epiglottis. The cricoid, thyroid and arytenoid cartilages are composed of hyaline cartilage, whereas the epiglottic, corniculate and cuneiform are elastic cartilage.

The thyroid and arytenoid cartilages have a synovial articulation with the cricoid cartilage. The articulation between the thyroid cartilage and thyrohyoid

bone of the hyoid apparatus is also synovial. Fused cartilaginous joints exist between the arytenoid and corniculate cartilages, and also between the epiglottis and thyroid and cuneiform cartilages (Getty,1975). Of the laryngeal cartilages, the arytenoid cartilages and epiglottis have the greatest range of motion.

The laryngeal cartilages are held together by fibro-elastic ligaments. The circumferential crico-tracheal ligament connects the cricoid with the first tracheal cartilage. The large crico-thyroid ligament lies on the ventral surface of the larynx. Rostral to this is the thyro-epiglottic ligament. The crico-arytenoid ligaments support the cricoarytenoid articulation. The transverse arytenoid ligament connects the arytenoid cartilages across the dorsal aspect of the larynx. The vestibular ligament extends from the cuneiform and lateral epiglottic cartilage to the ventral aspect of the arytenoid cartilage. The vocalis ligament spans the distance between the vocal process of the arytenoid cartilage and caudal border of the thyroid.

Movement of the larynx as a unit is achieved primarily by extrinsic laryngeal muscles; the **thyrohyoideus**, **hyoepiglotticus**, **sternothyrohyoideus** and **omohyoideus** muscles. The efferent (motor) nerve supply of the thyrohyoideus and hyoepiglotticus muscles is the **hypoglossal** nerve (cranial nerve XII), with the cell bodies located in the hypoglossal nucleus of the medulla oblongata. The ventral branches of the **first** and **second cervical** nerves innervate the sternothyrohyoideus and omohyoideus muscles. Cell bodies of these spinal nerves are situated in the ventral gray columns of the spinal cord.

The intrinsic laryngeal musculature moves individual cartilages in relation to each other. The **cricothyroideus** muscle tenses the vocal ligaments by moving the thyroid cartilage caudally. The **thyroarytenoideus** muscle (vocalis and vestibular components), **arytenoideus transversus** and **cricoarytenoideus lateralis** muscles all produce axial displacement (adduction) of the vocal process of the arytenoid cartilages. The **cricoarytenoideus dorsalis** (CAD) muscles displace the arytenoid cartilages dorsally and laterally, producing abduction of the arytenoid cartilage and vocal folds.

The efferent (motor) nerves of the intrinsic laryngeal muscles, except the cricothyroid muscle, are the left and right **recurrent laryngeal nerves** (RLN), which are branches of the vagus nerve (Getty,1975). The cell bodies of the RLN nerves are situated in the nucleus ambiguus of the medulla oblongata, on the ipsilateral side of the brain (Quinlan et al,1982). Nerve fibers that will become the recurrent laryngeal nerves travel from the brain within the vagus nerves. The right RLN branches from the vagus at the level of the first or second rib and returns to the larynx along the ventral surface of the common carotid artery. The left RLN branches from the vagus at the aortic arch, passes over the ligamentum arteriosum and then travels to the medial side of the aorta before ascending to the larynx using a similar pathway as the right RLN. The left RLN is on average 20 cm longer than the right RLN (Cole,1946). The cricothyroid muscle is innervated by an external branch of the **cranial laryngeal nerve**, another branch of the vagus

nerve. The majority of afferent laryngeal nerves travel in the internal branch of the cranial laryngeal nerve but some also travel within the RLN.

The blood supply to the larynx is provided by the caudal laryngeal artery (a branch of the cranial thyroid artery) and the ascending pharyngeal artery. Both vessels are branches of the common carotid artery. The laryngeal veins drain into the external jugular vein. Lymphatic vessels serving the larynx drain via the retropharyngeal and deep cervical lymph nodes into the thoracic duct and common jugular vein.

The lumen of the larynx is lined with a mucous membrane that follows the outlines of the laryngeal cartilages. In addition, the mucous membrane is reflected around the vocal and vestibular ligaments creating bilateral **vocal and vestibular folds**. The vocal folds and medial surfaces of the arytenoid cartilages are the boundaries of the **rima glottis**, the narrowest cross section of the laryngeal airway. Between the vocal and vestibular folds, on both sides of the larynx, a cul-de-sac of mucous membrane forms the **lateral ventricles**. The mucous membrane that covers the epiglottis reflects off the lateral margins to form the **aryepiglottic folds**. The mucomembranous aryepiglottic folds are supported by the cuneiform cartilages and blend with the mucous membrane of the corniculate and arytenoid cartilages. Rostral to the vocal folds, the mucous membrane has a stratified squamous epithelium. Caudally it becomes a pseudostratified, columnar, ciliated epithelium. Serous and mucous glands are present in the lamina propria of the laryngeal mucous membrane. A concentration of lymphoid tissue is found in the submucosa

covering the corniculate and arytenoid cartilages and within the lateral ventricles (Getty,1975).

B. Physiologic Function of the Larynx.

The complex structural anatomy of the larynx has evolved to perform a multitude of physiologic functions. The mammalian larynx actively participates in breathing, swallowing, phonation, coughing, expulsive efforts (straining) and airway protection from entry of foreign materials (Bartlett,1989). Also, when jumping (a postural change) horses momentarily inhibit respiration by closure of the rima glottis (Attenburrow,1983:I).

Coordination of all laryngeal movements during respiration occurs in the central nervous system. The appropriate laryngeal response to match respiratory demands relies on the motor fibers of only 4 nerves: the left and right recurrent laryngeal nerves and the 2 external branches of the cranial laryngeal nerves. For the larynx to participate in respiration, the vocal folds and arytenoid cartilages must be in the correct position. Full laryngeal adduction stops airflow while abduction allows it. Vocal fold position during breathing was thought to be under the control of extrinsic and intrinsic laryngeal muscles (Fink et al,1956), but subsequent studies have shown that only intrinsic muscles determine vocal fold position (Bartlett,1989). Extrinsic muscles however, are involved with the respiratory cycle, controlling the position of the larynx as a unit (Andrew,1955; Armstrong and Smith,1955). During inspiration, vocal fold abduction occurs primarily from contraction of the CAD

muscles in response to activity in the motor fibers of the RLN (Suzuki and Kirchner,1969). This abduction takes place prior to contraction of the diaphragm produced by phrenic nerve activity, ensuring minimal resistance to airflow at the larynx during inspiration. Contraction of the cricothyroid muscles can contribute to vocal fold abduction (Suzuki and Kirchner,1969). A significant decrease in motor activity of the RLN occurs on expiration and subsequently the vocal folds relax into a partially adducted position (Suzuki and Kirchner,1969).

The ventilatory movements of the vocal folds are under the influence of reflexes arising from various receptors of the pulmonary system (Bartlett,1989). Receptors sensitive to changes in the pH of cerebrospinal fluid, stretch receptors in the lung and chemoreceptors in the carotid sinus all supply information to the respiratory center in the brain stem which controls the rhythmic activity of the larynx during respiration. Changes in physiologic states can produce reflex activity in the larynx. Hypercapnia causes prolonged abduction of the vocal folds that can remain even during expiration (Bartlett,1989). Increased resistance to airflow produces greater discharge from motor nerves in the RLN in an attempt to maximally abduct the arytenoid cartilage and vocal folds (Glogowska et al,1974). Stimulation of irritant receptors in the epithelium of the airway causes adduction of the vocal folds and an increase in laryngeal resistance to airflow (Stransky et al,1973).

Receptors in the laryngeal mucosa may also influence laryngeal movement during respiration. Receptors sensitive to narrowing of the laryngeal lumen and

those sensitive to the evaporative cooling effect of inspiration have been described (Bartlett,1989). Also, receptors responding to laryngeal movement may be involved in regulation of laryngeal activity whilst breathing (Bartlett,1989). Chemoreceptors in the laryngeal mucosa act to protect the airway as their stimulation produces vigorous adduction of the larynx. This same response is observed with mucosal irritation, again protecting the airway.

Changes in respiratory pattern and depth of breathing have been observed to accompany reflex activity of the larynx. These findings confirm that afferent laryngeal nerves are involved with the control of ventilation (Bartlett,1989).

III. AIRFLOW IN THE UPPER RESPIRATORY TRACT

The upper airway acts as a conduit for air flow from the atmosphere to the lung. The upper airway begins at the nostrils and includes the nasal cavity, the pharyngeal cavity and finally the larynx. The lower airway consists of the trachea and its multiple branching system of bronchi and bronchioles. The physical characteristics of airflow within these structures will be reviewed.

For air to flow into the lung, the pressure within the alveoli must be less than atmospheric pressure. A negative intraluminal pressure is produced within the pleural cavity and alveoli by contraction of the diaphragm and intercostal muscles which enlarge the pleural space. The pressure difference created between the pleural cavity and the atmosphere (driving pressure) must be sufficient to overcome a number of impeding factors to ensure that airflow will occur. First, the elastic recoil of the lung parenchyma must be overcome. The presence of surfactant in the alveoli greatly reduces the pressure necessary to overcome this elasticity (Murray,1976). Secondly, the inertial forces created by the reversal of direction of airflow add to the magnitude of the driving pressure needed. At rest inertance is of minor significance but with exercise it increases. Finally, the driving pressure has to overcome the resistance to airflow in the airway serving the lung. The resistance to airflow is a combination of the friction between air and the airway walls, resistance created by irregularities of the airway and the shearing forces developed within the flowing gas (Olson et al,1970). The physical characteristics of fluid flow

have been applied to the respiratory tract to explain the flow of air resulting from the driving pressure.

In a straight cylinder at a low rate of flow, flow is laminar and Poiseuille's equation is used to describe the relationship between pressure and flow:

$$\dot{V} = \frac{P \cdot \pi \cdot r^4}{8 \cdot n \cdot l}$$

Where $\dot{V}$ =flow, P=driving pressure, r=tube radius, n=gas viscosity and l=tube length (West,1979). Since resistance (R) to flow is calculated as driving pressure divided by flow, reworking Poiseuille's equation produces:

$$R = \frac{8 \cdot n \cdot l}{\pi \cdot r^4}$$

When this equation is analyzed, the cylinder radius becomes critical because when it is reduced by one half, a 16 fold increase in resistance occurs. This fact becomes clinically applicable as only a slight reduction of the airway lumen will greatly increase the work of breathing.

Whether flow is laminar or turbulent is determined by calculation of the Reynolds number - a dimensionless unit that incorporates pressure and flow characteristics of a gas through a tube. The formula for the Reynolds number is:

$$Re = \frac{2 \cdot r \cdot v \cdot d}{n}$$

Where r=tube radius, v=average velocity, d= gas density and n=gas viscosity. For values less than 2000, flow in a cylinder is laminar and with a Reynolds number

greater than 4000, turbulent flow exists. When the number is between 2000 and 4000, flow is transitional (Olson et al,1970).

Laminar flow in the upper respiratory tract occurs only at very low flow rates. In people, once airflow is greater than 1 L/sec, turbulence is present (Olson et al,1970). In the horse it has been reported that tracheal airflow greater than 1.3 L/sec is turbulent (Attenburrow et al,1983:II). Airflow in the upper respiratory tract of the resting horse is approximately 5 L/sec, thus it is more reasonable to describe upper airway flow mechanics with principles developed for turbulent flow.

Pressure and flow characteristics in a system where turbulent flow exists are described by Rohrer's equation:

$$\Delta P = K_1 \dot{V} + K_2 \dot{V}^2$$

Where P= driving pressure, K_1 = mechanical energy loss due to laminar flow and K_2 = mechanical energy loss due to turbulent flow. Resistance (R) to airflow under conditions of turbulent flow is still defined as the ratio of change in pressure over the change in flow;

$$\text{Resistance} = \frac{\Delta P}{\Delta \dot{V}}$$

(Hamilton,1978), but now resistance is dependent upon the flow rate at which it is measured.

Resistance to airflow can be compartmentalized within the respiratory tract. In the upper respiratory tract of horses at rest, approximately 80% of resistance to airflow is at the nostrils with the larynx contributing the other 20%, but overall the upper respiratory tract is believed to only contribute approximately 30% of total

airway resistance (Robinson and Sorrenson,1975). In contrast, Art et al report that at rest and at exercise the upper respiratory tract, specifically the nostrils, contributes 82% to the total respiratory resistance. The differences in the two studies above may be due in part to the different techniques used to measure pleural pressure, or different positioning of tracheal catheters to measure tracheal pressure. Art et al placed their tracheal catheter at the junction of the intra- and extrathoracic airway. As a result of this placement, the pressure gradient measured between trachea and pleural cavity may have been small and subsequently lower airway resistance could appear less than if the tracheal catheter was placed higher in the trachea as in the study of Robinson et al.

The Rohrer equation described above was derived for constant flow in a cylinder and does not appropriately describe the upper respiratory tract because it does not take into account either inertia resulting from directional changes in airflow or compliance of the airway walls. By utilizing parallels from the field of electrical engineering, it is possible to combine inertance and compliance in formulating the relationship between pressure and flow. In such a system the relationship is called **impedance** (Young and Hall,1989). Impedance represents the total opposition to airflow in the part of the respiratory tract examined. Impedance (Z) is calculated at a specific frequency of respiration as:

$$Z = \frac{\Delta P}{\Delta \dot{V}}$$

Measurement of impedance to airflow in the equine upper respiratory tract using the above equation has been used as a method of evaluating treatments for left

laryngeal hemiplegia - (Shapell et al,1988; Belknap et al,1990). Although inertia and compliance were not measured in these studies, using impedance as the calculated parameter rather than resistance provided a more apt description of the situation in the horse's upper airway.

IV. EQUINE LEFT LARYNGEAL HEMIPLEGIA - A HISTORY.

In the eighteenth and nineteenth centuries much was written about horses that produced a noise associated with their respiratory system during strenuous activity. Of all the terms used to describe this noise, "roaring" appears to have been the most popular. As was discovered then, and is still recognized today, "roaring" is a symptom, not a disease and can occur with any obstruction to airflow in the nasal passages, pharynx, larynx or trachea of the horse (in MacQueen,1896). The symptom of "roaring", usually accompanied by a diminished tolerance to work, has been reported as early as 1664 (in MacQueen,1896).

The degeneration of muscles on the left side of the larynx in horses clinically affected with "roaring" was described by Dupuy in 1807 (in MacQueen,1896). The relationship between left laryngeal paralysis and "roaring" was confirmed when Dupuy (1815), F. Günther (1830) and Field (1837) induced "roaring" in horses by transecting the left recurrent laryngeal nerve (in MacQueen,1896). In 1866, F. Günther reported that 96% of horses with clinical signs of chronic roaring had wasting of the muscles on the left side of the larynx (in Flemming,1882), suggesting that left laryngeal hemiplegia (LLH) was the most common cause of "roaring" in horses. Despite the widely held opinion by men of science that "roaring" was often the result of left laryngeal paralysis, it is interesting to note that in 1882, the law in England still stated that roaring was the result of thick mucus adhered to the walls of the larynx (in MacQueen,1896).

In some cases of LLH, the presence of cervical or thoracic abscesses (often the result of Strangles) explained the damage to the recurrent laryngeal nerve. However, in the majority of horses with LLH, a cause could not be identified. "Roaring" due to LLH was believed by some to have a hereditary origin; especially following an increased incidence of LLH after the introduction of a group of Danish stallions into Normandy in 1764 (in MacQueen,1896). This belief was upheld by the Royal College of Veterinary Surgeons when in 1890, affected Thoroughbred stallions were disqualified from breeding (in MacQueen,1896). The anatomical pathway of the left recurrent laryngeal nerve around the aorta was proposed by some to result in damage to that nerve and be the cause of left laryngeal paralysis. The astute observation that left laryngeal paralysis was not seen in all types of horses did not support this theory (in MacQueen,1896). Breeds most commonly affected with LLH were the large breeds such as saddle horses and draught horses, with the condition usually manifesting itself at three to five years of age (Flemming in McCall,1889).

The "roaring" noise produced by horses with LLH was thought to be the result of a narrowed airway. Flemming stated that during inspiration, the left lateral ventricle fills with air and billows into the airway and upon severe exercise the arytenoid cartilage contributes to this airway narrowing (Flemming, in McCall,1889).

Initially the diagnosis of LLH in horses was purely clinical, relying mainly on the production of the noise during exercise. Threatening a horse with a stick or hitting the animal on the ventral abdomen and the subsequent production of a

grunting noise was said to indicate the presence of LLH (in MacQueen,1896). A detailed description of percutaneous laryngeal palpation in horses with left laryngeal paralysis was an early attempt to distinguish horses with LLH from those with other causes of "roaring" (Williams,1911).

Although it was impossible to visualize the larynx of a "roaring" horse, once it was discovered that many of these animals had left laryngeal hemiplegia, the search for a treatment was undertaken. In 1866, F. Günther and his son K. Günther, attempted a variety of surgical procedures. These included vocal cordectomy, ventriculectomy and partial and total arytenoidectomy (in Williams,1911). K. Günther reported in 1896 that these procedures were not satisfactory as many of the horses died from aspiration pneumonia or overwhelming infection following surgery. Tracheostomy was considered the best and safest method to improve the inspiratory airflow in affected horses (in Williams,1911). Arytenoidectomy was attempted by a number of other surgeons between 1860 and 1900 but with little success at eliminating "roaring" and often with fatalities (in Williams,1911). In North America in 1906, Williams began treating horses with left LLH by ventriculectomy. After a number of modifications to the laryngotomy incision and a reduction of the amount of tissue removed with the lateral ventricle, he reported in 1911 that of 21 horses treated with ventriculectomy, 71% no longer produced a respiratory noise when exercising. Hobday began performing ventriculectomies in England in 1909 and in 1911 he reported on 405 cases. In 1935 Hobday claimed ventriculectomy allowed 85 - 95% of horses with LLH to

return to useful endeavors. However only 20% of these no longer produced noise when exercising.

In summary, veterinary surgeons initially attempted to elucidate the cause of "roaring" in horses. In the majority of cases they found wasting of the muscles on the left side of the larynx, which naturally implied damage or dysfunction of the nerve supplying those muscles - the left recurrent laryngeal nerve. Although a reason for the nerve damage was not often established, surgical treatments to relieve the symptoms of "roaring" were attempted. Between 1866 and 1970 the only treatment reported to improve the horses' exercising capabilities, without severe complications, was ventriculectomy as described by Williams in 1911. Since 1970 other surgical treatments have been developed and evaluated. These will be discussed in a later section.

V. EQUINE LEFT LARYNGEAL HEMIPLEGIA - CURRENT STATUS.

A. Incidence and Type of Horse affected.

Respiratory disorders of horses are second only to lameness as the most common reason for horses not competing in their athletic field of training (Rossdale et al,1985). After infectious respiratory diseases, left laryngeal hemiplegia (LLH) is considered the most commonly diagnosed respiratory disorder (Cook,1970). A number of surveys have attempted to estimate the incidence of LLH in a variety of populations. In North America, an incidence of 2.6% (Pascoe et al,1981), 3.3% (Raphael,1982) and 4.7% (Baker,1983:I) has been recorded. On a single breeding farm in South America, 8% of horses inspected had LLH (Hillidge,1985). A population of Thoroughbreds in England revealed a 2.75% incidence (Lane et al,1987:I) while the same breed in New Zealand had 2% of horses affected (Goulden and Anderson,1981:I). A group of Clydesdale horses, also in New Zealand, had 9% affected with LLH (Goulden et al,1985).

In most surveys, entire or castrated males present with LLH more frequently than females (Cook,1970 Goulden and Anderson,1981:I; Hillidge,1985). However in the Clydesdale study, more females than males were affected. Typically, horses with LLH have been observed to be taller at the withers than unaffected horses within the same population (Marks et al,1970; Rooney and Delaney,1970; Cook,1970; Hillidge,1985; Duncan,1974). In one group, the height difference was shown to be statistically significant (Goulden and Anderson,1981:I).

Horses as old as 22 years have been diagnosed with LLH, however most affected horses are considerably younger. Foals have been observed with LLH (Hillidge,1985) but more commonly the condition is diagnosed between the ages of 1 and 5 years. In the past, diagnosis of LLH at an early age may have been frequently overlooked as endoscopic examination at yearling sales has only taken place on a large scale recently (Lane et al,1987:I). An acceptable summary up to this point would be that LLH is a condition seen most commonly in young, tall male or gelded horses used for athletic purposes and between 2% and 9% of a population may be affected. Classically, the breeds of horses recognized to be commonly affected with LLH are the Thoroughbred, Standardbred, Quarterhorse and heavy draught type horses. This may be a true breed predisposition or because these breeds are used for strenuous exercise, bringing closer scrutiny from the people using them.

Clinical observations by Cook in 1988 suggested that the information cited above on incidence and type of horse affected was not entirely accurate. Cook redefined LLH and termed it **recurrent laryngeal neuropathy**. Four grades of recurrent laryngeal neuropathy were described. Grades I,II and III represented various stages of CAD muscle atrophy and these horses were referred to as hemiparetic. Grade IV horses represented those with LLH. Cook's opinion was that females are as frequently affected as males, size of the horse is not important and up to 80-90% of Thoroughbred and Standardbred horses are affected. These findings were based primarily on subjective percutaneous palpation of the laryngeal

musculature. Interpretation of such subjective assessments should be made with caution.

B. Etiology

In the majority of cases, the search for a cause of LLH has been unrewarding. This has resulted in the term "idiopathic left laryngeal hemiplegia" being used to describe most of the horses with LLH. In one survey, only 8/127 affected horses had an identifiable cause of LLH (Goulden and Anderson,1981:II). There are however sporadic reports describing a definitive cause of laryngeal hemiplegia.

Guttural pouch mycosis and poisoning with the plant **Lathyrus sativus** (Indian vetch) have been associated with laryngeal hemiplegia (Cook,1970). A mid cervical laceration involving the right jugular vein resulted in right sided laryngeal paralysis in one horse (Gilberts,1972). Blunt trauma to the neck has also caused laryngeal hemiplegia (Goulden and Anderson,1981:II). Accidental injection of irritant drugs external to the jugular vein such as phenylbutazone (Helper and Lerner,1980) and barbiturates (Goulden and Anderson,1981:II) have produced LLH in horses. A retropharyngeal abscess caused by Staph. aureus has been associated with LLH (Barber,1981). Organophosphate poisoning has resulted in LLH and bilateral laryngeal paralysis in foals (Rose,1978) and mature horses (Duncan and Brook,1985). With organophosphate poisoning, the laryngeal paralysis is irreversible and may take up to 4 weeks to appear after exposure. Lead poisoning may produce persistent bilateral laryngeal paralysis accompanied by dysfunction of other

peripheral nerves (Burrows,1982). Left and right laryngeal hemiplegia is frequently seen in horses with Australian stringhalt, a disease most likely resulting from a plant toxicity (Huntington et al,1989). Thiamine (vitamin B₁) deficiency was proposed as a possible cause of LLH, as a similar association has been recognized in people and cattle (Loew,1973). A significantly low plasma thiamine level in some horses with LLH has been demonstrated (Cymbaluk et al,1977). However until LLH can be induced by a thiamine deficient diet in normal horses, caution should be used interpreting these findings. Unfortunately these cases of LLH with identifiable causes are in the minority and in most cases of LLH the etiology is unknown. Despite this, several theories have been advanced to explain the occurrence of idiopathic LLH. A brief discussion of these hypotheses follows.

Left laryngeal hemiplegia was thought to occur following respiratory tract infections (Cook,1970; Goulden and Anderson,1981:II). As LLH does not occur in all breeds following respiratory infection and because LLH predominately affects the left side of the larynx, an association between the two conditions seems tenuous.

Anatomical findings have provided the basis for two theories of the cause of LLH. Firstly, a ribbon like flattening of the left RLN as it passes around the aorta has been observed in some horses (Argyle in Mason,1973). Some believed this to be a site of trauma to the nerve and the cause of LLH (Halsam in Mason,1973). The lack of pathologic changes in this area of the nerve and the flattening that has also been observed in the right RLN, do not support this anomaly as a cause of LLH (Mason,1983 Cahill and Goulden,1986:I).

The second theory is based on the observation that the left RLN is approximately 20cm longer than its counterpart on the right (Cole,1946). This, and the position of the nerve in the neck led Rooney (1970) to suggest that tensile forces acting on the left RLN were greater than those on the right. He considered that in the mid-cervical area, the nerve would be stretched periodically by head and neck movements possibly causing ischemia and resulting in nerve damage. However, peripheral nerves can be stretched up to 8% of their length before vascular supply is compromised (Lundborg,1988). Also, nerves suffering from ischemia, not caused by pressure (i.e an embolus) can retain function even 6 hours after loss of their blood supply (Lundborg,1988). These two findings do not support Rooney's theory. Further evidence that adds doubt to this theory is that the microscopic lesions in the left RLN of horses with LLH are not typical of ischemic nerve injury (Duncan,1987).

A hereditary basis to LLH in horses has been suggested for a long time and recently more convincing evidence of this has appeared. A variety of authors in the late 1800's and 1900's described some of the progeny of affected horses to be "roarers" themselves (Mason,1983). Neurogenic muscle atrophy in the left laryngeal muscles of a fetus led to the classification of LLH as congenital, but more fetuses need to be examined to substantiate this finding (Gunn,1973). Cook postulated that a single recessive factor was responsible for LLH (Cook,1981). However the mating of a stallion and mare both affected with LLH has not produced 100% affected foals as would be expected with this mode of inheritance (Hillidge,1985).

The inheritance of phenotype was thought to be the only genetic component to LLH since affected horses tended to be taller and heavier (Marks et al,1970). A dominant gene has recently been hypothesized to be involved with the occurrence of LLH in horses. A French stallion with LLH sired 47 foals, of which 11 had LLH and another 11 were suspected because of abnormal arytenoid cartilage movements (Poncet et al,1989). Although the exact mode of inheritance is unknown, there is substantial evidence that left LLH has a hereditary basis rather than just a congenital one (Cook,1988). The exclusion of stallions with LLH from horse improvement programs in Switzerland (Poncet et al,1989) should stimulate discussion about the inclusion of affected horses in all breeding programs.

C. Pathologic Findings.

The most obvious lesion associated with LLH is atrophy of all the intrinsic muscles on the left side of the larynx except the cricothyroideus (Cahill and Goulden,1986:IV). However, laryngeal muscle atrophy is not limited to horses with clinical evidence of LLH. In one study only 25% of horses with muscle atrophy had clinical signs of LLH (Cole,1946) and others report similar findings (Quinlan et al,1975; Anderson et al,1980). Horses with asynchronous movements of the arytenoid cartilages or asymmetry to their rima glottis, are thought to represent those horse with subclinical pathologic changes in their intrinsic laryngeal muscles (Duncan et al,1977).

The common histologic appearance of these muscles is referred to as "neurogenic atrophy". This is represented by the presence of atrophic and hypertrophic fibers, centrally placed nuclei, increased perifasicular fat and connective tissue, loss of myelinated fibers from intramuscular nerves and "fiber type grouping". This last phenomenon is a characteristic of denervation that occurs when a surviving nerve fiber innervates a denervated muscle group using collateral axon sprouts. The denervated muscle fiber will assume the metabolic muscle type as determined by the new nerve (Cahill and Goulden,1986:IV). Similar histologic findings as those described above have been reported in the right laryngeal intrinsic muscles, but with much less frequency and severity. In all of the studies referred to, neurogenic atrophy in the cricothyroideus muscles was absent. The cricothyroideus is the only intrinsic muscle not innervated by the recurrent laryngeal nerve. The pathologic findings in horses with LLH are one of the few aspects of LLH that finds uniform agreement among authors (Cole,1946; Gunn,1973; Duncan et al,1974; Cahill and Goulden,1986:IV).

Laryngeal cartilages of horses with LLH have not been studied in detail. In people with long standing laryngeal paralysis, few cartilage lesions are described (Elies and Pusalkar,1983). Elies in 1983, also reports on an equine study where bony ankylosis of the cricoarytenoid joint occurred in 10/30 horses following transection of the recurrent laryngeal nerve. Similar findings have not been recorded elsewhere.

The histologic appearance of the RLN of horses with LLH has been extensively reported. Two groups of researchers, one originally from Scotland led by Duncan and the other from New Zealand led by Cahill, have both described similar light and electron-microscope findings in the RLN. These include lesions referred to as "onion bulbs" - concentric layers of Schwann cell processes resulting from repeated injury to the nerve fiber that causes demyelination and remyelination. The occurrence of thin myelinated internodes and decreased internode length also indicates remyelination. Split myelin sheaths, the accumulation of axonal organelles and proliferating Schwann cells known as "bands of Bungner" have also been observed. These changes to nerve microanatomy were only found in the distal RLN which led to the conclusion that a distal axonopathy occurs in horses with LLH. The left RLN had loss of many myelinated fibers with an abundance of the lesions mentioned above, whereas the right RLN had only a few pathologic changes. A low number of similar lesions were reported in the tibial and peroneal nerves of horses with LLH (Cahill and Goulden,1986:I,II&III), but the significance of this is uncertain.

Segmental demyelination of nerves as seen in horses with LLH is thought to result from axonal degeneration suggesting the primary lesion occurs within the axon (Cahill and Goulden,1986:III). Duncan (1987) provided evidence for abnormal axonal transport of cytoplasm supporting the theory that the axon is the primary site of pathologic change. Since axonal metabolism is under the influence of the cell body, the nucleus ambiguus has been examined (Cahill and Goulden,1986:V).

No changes indicative of axon damage were seen in the cell bodies studied. However, examination of the lateral cuneate nucleus, which contains cell bodies of long nerves of the central nervous system (CNS), revealed axonal "spheroids" - areas of degenerating and regenerating axons (Cahill and Goulden,1986:V). Spheroids are present in normal horses and people but an increased prevalence of spheroids is found in patients with axonopathies. Significantly more spheroids were observed in horses with LLH compared to normal horses (Cahill and Goulden,1989). The actual significance of this is uncertain and the statement that a component of LLH has its origins in the CNS should be made with caution. The fact that the cricothyroideus muscle does not undergo neurogenic atrophy does not support a CNS origin for LLH as the cell bodies for this nerve also originate in the nucleus ambiguus.

In summary, idiopathic LLH in horses is a distal axonopathy, the left recurrent laryngeal nerve being more severely affected than the right. Many horses have subclinical levels of laryngeal muscle atrophy and the term coined by Cook (1988) of "recurrent laryngeal neuropathy" is probably applicable to these horses. A causative agent such as a virus, bacteria or toxin has not been identified for idiopathic LLH but this may not be unexpected since considerable evidence does exist to classify LLH as a hereditary condition.

D. Diagnosis

The use of the flexible fiberoptic endoscope for examination of the upper respiratory tract of horses revolutionized the accuracy with which LLH could be diagnosed (Cook,1974). This technique however, is a single diagnostic test and should not be relied on entirely as some horses with LLH have a normal appearance to their larynx at rest.

A thorough history and physical examination of a horse suspected of having LLH is necessary as many conditions mimic the clinical signs of LLH. The owner of the horse should be questioned explicitly as to onset and duration of the clinical signs the horse has exhibited. Information about the horses' fitness, the type of respiratory noise being made and the degree of exercise necessary to produce the noise is essential to help formulate an accurate diagnosis. Increased turbulence of airflow in the respiratory tract causes the inspiratory noise associated with exercise (Attenburrow et al,1983:II), but this is not diagnostic for LLH. Whether a change in phonation has occurred and whether a decrease in performance is associated with the noise must be ascertained. Training and race times are useful for establishing this information (Baker,1983:I).

The physical examination of the respiratory system must be complete to rule out other diseases, or to rule in conditions occurring concurrently with LLH. Auscultation of lung fields and trachea, assessment of airflow from both nostrils, presence or absence of nasal bone symmetry and nasal discharge must all be noted. Structures to be palpated include the trachea, submandibular lymph nodes and

especially both jugular grooves to detect evidence of previous trauma or inflammation associated with perivascular injection. The larynx should then be palpated percutaneously. Fibrous scars in the skin from previous laryngeal surgery should be noted; clipping of the hair may be necessary to observe these. The muscular process of the arytenoid cartilage in horses with LLH is prominent and with experience easily palpated (Cook,1988:I). While palpating the muscular process, the arytenoid depression test can be performed. This is reported to create axial displacement of the left arytenoid cartilage and produce an inspiratory noise at rest (Marks et al,1970). This test can give false positive results (Cook,1988).

Even if a presumptive diagnosis of LLH has been made, confirmation rests with endoscopic examination of the larynx. Rigid rhinolaryngoscopes were originally used to visualize the larynx (Cook,1965; Mackay-Smith and Marks,1968. Marks,1970) but because of risk to horse and operator and the limited field of vision, the use of this instrument has fallen into disfavor, especially following the introduction of the flexible fiberoptic endoscope (Cook,1974). Structures to be examined during endoscopy include the nasal turbinates, the soft palate, guttural pouch openings, pharyngeal walls, the larynx and trachea (Lane,1987:II). Endoscopically the normal larynx appears symmetrical. A diagnosis of LLH can be aided by the following endoscopic observations:

1. Asymmetry of the rima glottis due to axial displacement of the left arytenoid and corniculate cartilage. A ballooning lateral ventricle may contribute to this asymmetry.

2. Absence of abduction of the left arytenoid and corniculate cartilages after swallowing (Goulden and Anderson,1981:II), or following occlusion of both nostrils to increase inspiratory efforts.
3. Absence of adduction of the vocal folds during swallowing or during the "slap test" (Greet,1983) which tests the integrity of the cervical spinal cord and recurrent laryngeal nerves. A positive slap test produces adduction of the contralateral vocal fold.

Electromyographic studies can detect paralysis of the CAD muscle (Moore et al,1988). However, this diagnostic technique appears impractical because of its invasive nature, cost and the operator skill required.

Several other conditions may cause the larynx to appear asymmetrical. Changes in rima glottis symmetry can occur with arytenoid chondritis (Haynes et al,1980:II) and ossification of laryngeal cartilages (Shapiro et al,1979). Radiographs may be helpful in distinguishing between these conditions. The eccentric position of the end of the endoscope may result in the appearance of laryngeal asymmetry and if any doubt exists, examination via the opposite nostril is advised (Lane,1987:II). Observation of asynchronous movement of the arytenoid cartilages is considered a variation of normal in the horse due to imbalance of abductor and adductor muscle activity (Baker,1983:II). Only 40% of horses examined in Baker's study demonstrated rhythmic arytenoid movement and over a 5 year period none of the horses with asynchrony progressed to develop LLH. Endoscopic examination of horses exercising on a treadmill did not find asynchronous movement at rest a

reliable indicator of LLH (Rakeshaw et al,1990). Cook (1988:II) maintains asynchrony is a subclinical manifestation of laryngeal hemiplegia. However present, evidence suggests that laryngeal asynchrony should not preclude a horse from passing a pre-purchase examination if other parts of the examination are normal.

The use of tranquilizers during endoscopic examination is controversial. Cook recommends the use of tranquilizers for examination of the larynx (1988:II). Robinson and Sorrenson reported that the tranquilizer xylazine alters upper airway function by increasing resistance to airflow in the resting horse (Robinson and Sorrenson,1975). Since respiratory movements of the larynx are under influence of pulmonary function via respiratory system receptors, tranquilization may not be an ideal aid to endoscopy. However no evidence has been found that misdiagnosis occurs because of their use (Lane,1987:II).

In some horses the larynx is normal at rest but following exercise, signs of LLH may be present. Thus, exercise may be helpful in the diagnosis of LLH. Exercise should be sufficiently strenuous to demonstrate the respiratory noise and exercise intolerance as described by the owner. Endoscopic examination should follow exercise as soon as possible. Failure to maintain abduction of the left arytenoid and corniculate cartilage during expiration as well as inspiration is indicative of LLH (Lane,1987:II). If exercise is not possible intravenous administration of doxapram will increase respiratory efforts and may help support the diagnosis (Lane,1987:II). Recently, videoendoscopy during exercise on a treadmill has proven effective in demonstrating the obstruction to airflow in horses

with LLH (Derksen,1988 Morris and Seeherman,1988) and also in a horse with a failed prosthetic laryngoplasty (Stick and Derksen,1989). This may be of valuable assistance in diagnosing horses believed to have subclinical LLH but which have a normal appearance to their larynx at rest.

Several other conditions of the upper airway can cause exercise intolerance and noise production. These include epiglottic entrapment, dorsal displacement of the soft palate, arytenoid chondritis and subepiglottic cysts. Conditions that have been reported in conjunction with LLH include dorsal displacement of the soft palate, palatopharyngeal arch displacement and inflammation of the lower airway (Goulden and Anderson,1981:II). If observed, interpretation of the significance of respiratory disorders occurring with LLH is essential prior to recommending surgical management for LLH.

VI. TREATMENTS OF LEFT LARYNGEAL HEMIPLEGIA.

Prior to discussing the surgical treatments recommended for laryngeal hemiplegia it is pertinent to remember that idiopathic LLH is not a life threatening condition. With today's high cost of surgery, retirement from performance sports may be a viable option for some horses. However, return of a horse with LLH to previous athletic ability requires surgery. Before surgery it is imperative that the owner of the horse be informed of the advantages and disadvantages of laryngeal surgery. Also accurate assessment of the horse is essential as a pre-existing musculoskeletal disorder or concomitant respiratory disease could render the horse incapable of performing despite successful laryngeal surgery. Frequent and accurate client communication is paramount to avoid unrealistic expectations of owners who often do not appreciate the intricate details of laryngeal surgery in the horse.

The basic principle of all the techniques attempted for treatment of LLH is to relieve obstruction to airflow, by either removal or stabilization of the lateral ventricle and arytenoid and corniculate cartilages. The section on the history of equine laryngeal hemiplegia commented on early treatment attempts and left us in 1935 with ventriculectomy as the only technique in common use (Hobday,1935).

A. Ventriculectomy

Ventriculectomy involves the removal of the lateral ventricle by resecting the mucosal lining, creating a fibrous adhesion between the vocal fold, thyroid and arytenoid cartilages. This adhesion was believed to prevent collapse of the

arytenoid cartilage into the airway during inspiration. Ventriculectomy can be performed in the standing, tranquilized horse but general anesthesia with the horse in dorsal recumbency is recommended (Haynes,1984:I).

The lateral ventricle is exposed through a laryngotomy created by a midline incision through the skin, subcutaneous tissues and entire length of the cricothyroid ligament and membrane. Lateral retraction of the thyroid cartilage provides access to the laryngeal lumen. A variety of techniques for removal of the ventricle have been described but the generally accepted method is to place a toothed burr in the ventricle, engage the mucosa and exteriorize the lining. A clamp beneath the burr then provides traction to ensure that an adequate amount of mucosa is removed. Suturing the incised edges of the ventricle (Pouret,1966), or removal of the vocal fold in addition (Baker,1983:I), are no longer considered necessary for ventriculectomy (Haynes,1984:I). The surgery is relatively fast and usually performed after removing the endotracheal tube once a satisfactory level of anesthesia is achieved. The cricothyroid membrane, subcutaneous tissues and skin are routinely left open to heal by second intention. Recently ablation of the lateral ventricle by a laser beam under endoscopic vision was attempted with reasonable success in removing the lateral ventricle (Shires et al,1990). Food is withheld for 12-24 hours post operatively.

Healing of the laryngotomy takes 21-30 days and cleaning twice daily is necessary due the continuous flow of mucus from the trachea. Horses are rested for 30 days and usually do not begin training until 45 days post operatively,

provided endoscopic appearance of the surgical site is satisfactory. Complications following ventriculectomy are few. Excessive removal of mucosa lining the larynx can produce granulomas that may require surgical removal.

The success of ventriculectomy has been evaluated using the subjective variables of noise reduction when exercising or improved tolerance to exercise. Not all authors use both criteria, leading to a wide range of reported success rates from 99% (Pouret,1966) and 75% (Barber,1984), to 28% (Baker,1983:I). In 1988, a quantitative study demonstrated the inability of unilateral ventriculectomy to improve resistance to airflow in exercising horses with induced LLH (Shappell et al,1988). Ventriculectomy has been classified as a moderately useful technique for horses with "partial hemiplegia" or for those that do not merit the cost of a prosthetic laryngoplasty (Haynes,1980:I). However ventriculectomy is also deemed an unjustified procedure on its own in horses with LLH (Speirs,1987; Cook,1988:II).

B. Prosthetic Laryngoplasty

The unreliable success rates associated with ventriculectomy led to development of the prosthetic laryngoplasty technique. Use of this procedure began in the late 1960's and was first reported in detail in 1970 (Marks et al,1970). Placement of a prosthetic suture to mimic the abductor function of the CAD muscle is the basis of this surgery.

Under general anesthesia, the horse is placed in lateral recumbency with the affected side of the larynx up. A curvi-linear incision is made along the ventral

margin of the linguo-facial vein. A plane of dissection between this vein and the omohyoideus muscle is established to reveal the crico and thyropharyngeus muscles overlying the lateral wall of the larynx. The cricoid cartilage and muscular process of the arytenoid cartilage are then identified. A non absorbable suture (braided lycra - Marks et al,1970:II) is passed under the posterior border of the cricoid lamina for 1-1.5 cm, keeping the needle submucosally before penetrating through the dorsal lamina as close as possible to the median ridge of the cricoid cartilage. The suture is then passed under the cricopharyngeus muscle emerging at the septum between the cricopharyngeus and thyropharyngeus muscles. Using a 16 gauge needle a hole is drilled through the muscular process of the arytenoid cartilage and the cranial end of the prothesis pulled through with a #10 crochet hook. The suture is firmly tied to create permanent abduction of the arytenoid cartilage. The tissue planes and skin are closed in a routine manner. Subsequently a ventriculectomy may be performed as previously described. The results of Shappell's work questions the value of the ventriculectomy. Following laryngoplasty, food is withheld for 12-24 hours. Resumption of exercise can occur 45-90 days after surgery depending on the endoscopic appearance of the larynx.

In the original study, which combined the prosthetic laryngoplasty with ventriculectomy, few complications were described and 87% of horses treated no longer made a respiratory noise associated with exercise (Marks et al,1970). Since this original report many variations of the surgery have been described including an approach dorsal to the linguo-facial vein (Merriam,1973). Elastic and non-elastic

prostheses were evaluated and the latter recommended because of greater long term strength and less inflammation associated with its use (Merriam,1973). The use of two prostheses was considered better than a single one (Speirs,1983). Further variations on the original technique include placing a second suture through the arytenoid and thyroid cartilage as well as the prosthesis (Goulden and Anderson,1982), eliminating the ventriculectomy (Speirs,1972), and using a trocar pointed surgical needle to pass the prosthesis through the muscular process rather than a crochet hook (Shappell et al,1988). Accurate placement of the suture through the muscular process of the arytenoid cartilage is the most important step in performing a laryngoplasty. In vitro studies indicate that the muscular process is the most common place for the prosthesis to pull through the cartilage when tension is applied (Dean et al,1990).

The greater success of the laryngoplasty compared to the ventriculectomy in improving exercise ability is widely reported. Success rates reported range from 44% (Goulden and Anderson,1982) through 60% (Baker,1983:I) to 80-90% (Haynes,1980:I). The racing performance of horses treated using a prosthetic laryngoplasty was compared to that of normal horses of similar ability and no significant differences in their racing performances were found (Speirs,1980). Although no significant improvement in success was found by combining ventriculectomy with laryngoplasty (Speirs et al,1983), it is still often recommended that ventriculectomy be performed. At Michigan State University this is not routinely done following the findings of Shappell et al (1988) mentioned earlier.

The original reported success of the prosthetic laryngoplasty was based on subjective findings. In 1986 Derksen et al demonstrated that laryngoplasty significantly reduces the resistance to inspiratory airflow in horses with induced LLH. This was substantiated at faster speeds in a later study (Shappell et al,1988). Although the speed of exercise used in these studies was less than those attained by horses during racing, these studies were the first to quantitatively demonstrate improvement in upper airway function following laryngoplasty. Laryngoplasty has also been shown to reduce the level of hypoxemia recorded in horses with LLH (Bayly et al,1984; Tate et al,1990).

Unfortunately, a long list of complications associated with prosthetic laryngoplasty have been described over the past 20 years. These are listed below.

1. Nasal discharge of feed and water (Mackay-Smith et al,1973; Haynes,1980:I; Goulden and Anderson,1982).
2. Need for emergency tracheostomy (Mackay-Smith et al,1973).
3. Pneumonia (Mackay-Smith et al,1973; Haynes,1980:I).
4. Esophageal obstruction (Mackay-Smith et al,1973)
5. Laryngeal granuloma following penetration of the mucosa under the cricoid cartilage (Haynes,1980:I)
6. Intra operative hemorrhage (Haynes,1980:I).
7. Broken needles embedded in the cricoid lamina (Haynes 1980:I).
8. Chronic coughing (Haynes,1984:I; Speirs,1987)

9. Aspiration of feed and water while eating (Greet et al,1979).
10. Death from asphyxiation (Merriam,1973)
11. Chondritis of the cricoid cartilage (Goulden and Anderson,1982)
12. Seroma formation at the surgical site (Merriam, 1973).
13. Fistulous tracts associated with a contaminated prosthesis (Marks et al,1970).
14. Failure of the prosthesis to maintain arytenoid abduction (Speirs,1987).
15. Cartilage ossification (Speirs,1987)
16. Wound infection and dehiscence (Speirs,1987).

Fortunately all of these complications do not occur frequently. Acute and chronic coughing and nasal discharge of food and water appear to be the most common problem. Nasal discharge of food and water may be a result of hyperabduction of the left arytenoid cartilage caused by excessive tension created when tying the prosthesis. Since preventing dynamic collapse of the arytenoid cartilage during exercise is the reason for success of the laryngoplasty technique, full abduction may not be necessary (Derksen et al,1986). Dysfunction of pharyngeal muscles leading to coughing and dysphagia may be caused by trauma to the cranial laryngeal nerve at surgery (Greet et al,1979). Many of the post operative complications can be avoided by employing meticulous surgical technique. Some horses with chronic coughing may require the prosthesis to be cut or

removed to reduce the problem. As a result of some of the above complications, a third surgical procedure has been reintroduced and evaluated as another potential treatment for LLH.

C. Arytenoidectomy

The modern day use of arytenoidectomy was reported in 1980 (White and Blackwell,1980). Unilateral arytenoidectomy of two horses, both with a failed laryngoplasty, returned one to performance without the major complications of aspiration pneumonia and dysphagia previously reported (Williams,1911). Following the report of White et al, a resurgence of the arytenoidectomy procedure has been seen, especially for the treatment of arytenoid chondropathy (Haynes et al,1980:II; Tulleners et al,1988:I).

Three types of arytenoidectomy are described (Speirs,1987). **Total** arytenoidectomy removes the entire cartilage with the attached corniculate cartilage. **Partial** arytenoidectomy leaves behind the muscular process of the arytenoid while **subtotal** arytenoidectomy leaves both muscular and corniculate processes. All three procedures require general anesthesia with the horse placed in dorsal recumbency. Anesthesia is maintained via an endotracheal tube placed through a tracheotomy which can be performed before or after induction of general anesthesia (Haynes et al,1984:II). A ventral laryngotomy as described for ventriculectomy provides access to the affected arytenoid cartilage. The lateral ventricle on the same side is usually excised first, followed by dissection and removal of the arytenoid

cartilage. Suturing the mucosal edges was recommended but recently this has been demonstrated not to be essential as the mucosal deficit can heal satisfactorily by second intention (Tulleners et al,1988:I). The laryngotomy is routinely left to heal by second intention. A tracheostomy tube is often required for up to 7 days post operatively with appropriate nursing care.

Complications associated with arytenoidectomies include hemorrhage, cartilage mineralization (Tulleners et al,1988:I), coughing, dysphagia (Haynes,1984:II) and nasal discharge of food and water (Speirs,1986). The latter three of these complications are minimized by the use of the subtotal technique (Haynes et al,1984:II). The partial arytenoidectomy is reported to have a higher number of post operative complications (Speirs,1986). In a study where the left corniculate cartilage was removed using a neodymium:yttrium aluminum garnet laser, post operative complications were not reported (Tate et al,1989). However post operative endoscopic examination of horses in Tate's study suggested that the procedure would not provide horses with LLH with an adequate airway for maximal exercise. (Tate et al,1989). Although the complications of arytenoidectomy sound devastating, treated horses have successfully competed (Haynes et al,1984:II). Both partial (Speirs,1986) and subtotal arytenoidectomy (Haynes et al,1984:II) have been reported to improve exercise capacity in a small number (10) of horses with LLH.

A quantitative evaluation of subtotal arytenoidectomy for treatment of LLH unequivocally demonstrated the failure of this technique to improve upper airway

flow mechanics in exercising horses (Belknap et al,1990). This is in contrast to the report by Haynes et al, (1984:II). A difference in the amount of corniculate cartilage removed may explain these different findings. Improvement in upper airway function has been reported after total arytenoidectomy in one horse suggesting that removing the entire corniculate process may be of benefit (Stick and Derksen,1989).

In summary, ventriculectomy, laryngoplasty and arytenoidectomy are treatments for LLH in the horse which have varying degrees of success because of either ineffectiveness or post operative complications. Therefore, investigation of another treatment for LLH is reasonable. Treatment of laryngeal paralysis in people and dogs by laryngeal reinnervation using a nerve muscle pedicle graft has been described (Lyons and Tucker,1974; Tucker,1976). The use of this technique in dogs, people and horses will be reviewed in the following chapter.

VII. LARYNGEAL REINNERVATION

A. Reinnervation of Skeletal Muscle

The need for a functional repair of a paralyzed muscle in people has stimulated investigation into skeletal muscle reinnervation. In cases of bilateral laryngeal paralysis and facial nerve paralysis, reinnervation has allowed return of function to denervated muscles.

The nerve used to reinnervate a muscle should allow normal function. For example, abductors of the larynx must be innervated with a nerve that is activated on inspiration. A number of different techniques for reinnervating a muscle have been reported. These include anastomosis of a transected nerve (Badke,1989), anastomosis of dissimilar nerves (Rice,1982), implantation of a cut nerve end into a muscle (Meikle et al,1987) and the nerve muscle pedicle graft technique (Hall et al,1988). The two reinnervation procedures that have few complications and good success rates are the **nerve muscle pedicle (NMP) graft** and nerve implantation techniques. The NMP graft may be the better technique as reinnervation is faster and muscle contraction stronger than with the nerve implantation technique (Hall et al,1988). Activity within reinnervated muscles, as determined by electromyography (EMG), is usually evident 8 weeks following the NMP graft surgery (Hall et al,1988). The process by which a nerve reinnervates a muscle is controversial and warrants discussion.

One theory put forward to explain the rapid return of muscle activity with the NMP technique is the transplantation of **motor end plates** (the synapse of a nerve

and muscle fiber) in the muscle pedicle. Once fibers of the recipient muscle and NMP heal, propagation of depolarization from the pedicle to the denervated muscle is possible producing a contraction (Johnson and Tucker,1977). An alternative explanation is that the NMP contains a number of transected axons that each serve as a nerve implant. When a nerve fiber is transected the most distal end undergoes Wallerian degeneration of the myelin sheath and axon within it. Retrograde degeneration of the axon toward the cell body usually stops at the first collateral axon reached (Kelly,1987). Two to three days after distal axon transection changes occur within the cell body and if severe enough, the cell may die. If the cell does not die, there is synthesis of new proteins for regrowth of the axon. Following implantation of a severed nerve end into the denervated muscle, reinnervation occurs by axons sprouting and forming junctions with the recipient muscle fibers. Rapid regeneration is possible as only a short distance has to be traversed by the regenerating axon sprouts (Hall et al,1988).

Finally, it has been suggested that reinnervation following a NMP graft is not the result of the pedicle itself but because of "muscular neurotization". In this process, axons sprout from an adjacent innervated muscle into the denervated muscle. Thus, surgical manipulation of muscle overlying the denervated muscle may allow this process to proceed (Neal et al,1981). However, horse-radish peroxidase injected into reinnervated muscle is only taken up by the nerve used to reinnervate and not into the nerves of adjacent muscles, lending support to the first two mechanisms of reinnervation and refuting muscular neurotization (Meikle et

al,1987). With the NMP graft or the nerve implantation technique, minimal fibrosis at the surgical site is essential as electrical impulses and axon sprouts cannot successfully traverse a fibrous scar.

In people and animals, reinnervated muscle groups have a distinct histologic appearance. In normal muscle, all muscle fibers of a **motor unit** (1 axon and its muscle fibers) stain the same histochemically. The presence of 2 - 3 different fiber types in normal muscle (due to differences in myofibril adenosine triphosphate activity) produces a mosaic pattern of muscle fibers when viewed under a microscope. After reinnervation "fiber type grouping" is observed. This grouping is the result of collateral sprouting of axons and is the hallmark of a reinnervated muscle (Eisele et al,1988). This phenomenon allows histologic confirmation of successful muscle reinnervation (Eisele et al,1988).

The majority of research concerning muscle reinnervation has taken place in animals and successful adaptation of the techniques in people has followed (Tucker,1976 Anonsen et al,1986). In the following section this research will be reviewed.

B. Discovery of the Nerve Muscle Pedicle Graft

Early unsuccessful investigations on laryngeal reinnervation occurred with the objective to develop techniques useful in the treatment of bilateral vocal cord paralysis in people (McCall,1946). Later, a resurgence of interest in reinnervating the laryngeal muscles came with research into transplantation of the larynx. With

the popularity of organ transplants in the late 1960's, it was thought laryngeal transplantation might be a viable treatment for people with extensive laryngeal cancer.

Early work on laryngeal transplantation took place in dogs and concentrated on the blood supply to the larynx and methods of small vessel anastomosis (Yagi et al,1966; Ogura et al,1966). Successful revascularization was found to be possible. Function of the transplanted larynx was then evaluated. Initially anastomosis of the recurrent laryngeal nerve was attempted. Although axons did regenerate, neuroma formation, misdirection of regenerating fibers and loss of motor units gave less than satisfactory results, often with uncoordinated muscle contraction (Harvey et al,1970). Further studies led to the development of a technique where a small piece of muscle was transplanted with its nerve to the denervated muscle; this was termed the neuromuscular, or **nerve muscle pedicle** (Tucker et al,1970). Studies were also performed on a variety of immunosuppressive drugs as the rejection phenomena of transplanted organs had to be overcome (Ogura et al,1970). Despite all this work it was still not considered justifiable to transplant the human larynx as immunosuppression of patients often led to proliferation of cancer, the primary reason for laryngeal transplantation (Tucker,1974).

Successful transplantation of the larynx has occurred in dogs, however human laryngeal transplantation seems unlikely (Tucker,1978:I). Thus although the development of the NMP graft has not led to laryngeal transplantation, the

technique has proved useful in the treatment of peripheral nerve paralysis, especially of the larynx.

C. Experimental Studies in Laryngeal Reinnervation

Bilateral paralysis of the vocal cords occurs in some people after thyroidectomy often necessitating tracheotomy or arytenoidectomy (Evoy,1961; Tucker,1980). Although usually effective in providing an airway, the loss or decreased ability for speech with these procedures, provided impetus to investigate the possibility of returning laryngeal function by reinnervation.

In 1946 anastomosis of the vagus and recurrent laryngeal nerves was performed in dogs to reinnervate the laryngeal muscles but with little success (McCall,1946). Other attempts at laryngeal reinnervation have included direct implantation of the cut end of the recurrent laryngeal nerve (RLN) into a laryngeal muscle (Doyle et al,1967), section and anastomosis of the RLN (Boles and Fritzell,1969; Murikami and Kirchner,1971) and implantation of the phrenic nerve into a laryngeal muscle (Fex,1970. Taggart,1971). The aim of all these procedures was to reinnervate the posterior cricoarytenoid muscle (equivalent to the cricoarytenoideus dorsalis muscle of horses), to allow abduction of one arytenoid cartilage providing an airway for breathing, thereby removing the need for tracheotomy or arytenoidectomy. The only procedure that met with some success was implantation of the phrenic nerve. Unfortunately function of the reinnervated muscle took 9-12 months to return. Many of the nerve anastomoses produced

muscle contractions but not synchronous with respiration. This was thought to be due to aberrant reinnervation where adductor fibers would reinnervate abductor muscles and vice versa (Boles and Fritzell,1969). A random pattern of abductor and adductor nerve fibers does exist within the RLN explaining this aberrant innervation despite meticulous surgical technique (Crumley et al,1980).

Further studies on the use of the phrenic nerve both as an implant into laryngeal muscles (Morledge et al,1973) and in anastomosis to the RLN (Iwamura,1974) drew criticism because function on one side of the diaphragm was sacrificed. This complication has been overcome by splitting the phrenic nerve (Crumley et al,1980) or using one of its branches (Baldissera et al,1986) allowing preservation of diaphragm function. Although reinnervation has been reported with anastomosis of the phrenic nerve to the nerves innervating the larynx, a minimum of 6 months was required for return of function.

In 1970, Tucker in his work with laryngeal transplantation, attempted to reinnervate the posterior cricoarytenoid (PCA) muscle in dogs. An island of PCA muscle with the RLN attached was removed and immediately sutured back to the parent muscle. Within 2 weeks normal arytenoid movement and vocal fold function had returned. Histologic examination of the nerve and muscle confirmed reinnervation (Tucker and Ogura,1971). Since the RLN would not always be available for reinnervation in cases of bilateral cord paralysis, other potential donors of a "nerve muscle pedicle" (NMP) were investigated. Using a NMP graft created from the **ansa hypoglossi** nerve and **sternohyoid** muscle, return of function

to the posterior cricoarytenoid muscle was seen between 6 and 12 weeks post operatively (Hengerer and Tucker,1973). Later studies indicated that even after 6 months of denervation the posterior cricoarytenoid muscle could be reinnervated with this NMP technique (Lyons and Tucker,1974). Use of a NMP from the ansa hypoglossi nerve and **sternothyroid** muscle also produced reinnervation (Sato and Ogura,1978) but in one study complete return of function was not evident until 36-44 weeks (Greenfield et al,1988). Recent work with nerve muscle pedicles and nerve implantation to other skeletal muscles indicate that both transfer of motor endplates and axonal sprouting of transplanted nerves leads to early return of function (Anonsen et al,1986).

The NMP does appear to be a viable technique for reinnervation of muscle although some disagreement exists as to the mechanism by which it works. Technically it is less difficult to perform than nerve anastomosis and successful use of the NMP in the human field has substantiated the validity of this procedure.

D. Reinnervation of the Human Larynx

Bilateral laryngeal paralysis is most commonly observed in people following external neck trauma as can occur in car accidents or from neck surgery for tumor removal. Tracheostomy is essential to provide an adequate airway in these patients. To allow removal of the tracheotomy tube, unilateral arytenoidectomy was developed (Woodman,1944). Unfortunately this results in marked loss of voice quality. The need to provide a method for vocal fold abduction and still allow

adduction for speech, stimulated the attempt at PCA muscle reinnervation with a NMP graft. In 1976 five patients were reported to have been successfully reinnervated, three of which no longer required a tracheostomy tube (Tucker,1976). The ansa hypoglossi nerve and omohyoideus muscle were used to create the NMP. Only 6-8 weeks were necessary to see improvement in these patients, one of which had suffered paralysis for 22 years.

The success of the procedure stimulated the use of a NMP to reinnervate laryngeal adductor muscles in patients with unilateral paralysis who requested improvement in their voice quality. Good to excellent results by 12 weeks post operatively were observed in 60% of patients (Tucker,1977 Tucker and Rusnov,1981). Over almost a 10 year period, using the nerve muscle pedicle technique, Tucker reported a 90% success with treatment of unilateral paralysis and 80% with bilateral paralysis (Tucker,1978:II Tucker,1983). Success was not always a 100% return to function but deemed a significant improvement in either voice quality or ability to perform everyday activities without the tracheostomy tube. Other surgeons also reported rapid return of function using the NMP graft for both uni- and bilaterally paralysed vocal folds (Applebaum et al,1979; May et al,1980; Fernandes et al,1987).

Unfortunately these high success rates were not reported by all surgeons. Subsequently Tucker reported on the possible reasons for failure (Tucker,1982). Thorough patient evaluation prior to surgery is essential. An unmovable arytenoid cartilage or stenotic area elsewhere in the upper respiratory tract could result in

failure of the NMP to improve respiration or phonation. Meticulous surgical technique in creating the pedicle and identifying the correct muscle of the larynx into which to implant the pedicle were stressed. Also it was recommended to study patients at exercise post operatively to demonstrate abduction of the vocal cord.

Despite the apparent success of the NMP graft technique there have been investigations into laryngeal pacemakers as a potential treatment for laryngeal paralysis (Bergmann et al,1984). Other investigators have successfully performed studies using laryngeal pacemakers to improve function of muscles reinnervated with a nerve muscle pedicle (Broniatowski et al,1985). In this latter study a linear strain gauge that spans 3-4 tracheal cartilages is placed on the outside of the trachea. The strain gauge relays tracheal movement synchronous with respiration to an amplifier. The amplifier is connected to an electrode placed around the nerve of a NMP used to reinnervate the paralyzed muscle. Once miniaturization of the amplifier is achieved, the authors believe this technique will be applicable in people. Since reinnervation with the NMP graft technique has not been uniformly successful for all surgeons, research into such laryngeal pacemakers would appear justified.

E. Reinnervation of the Equine Larynx

In 1989 Ducharme and co-workers described three different techniques of laryngeal reinnervation as potential treatments for LLH in horses. In horses with

LLH, the cricoarytenoid dorsalis (CAD) muscle, the major abductor of the larynx (Martin et al,1986), is the only muscle that requires reinnervation.

The first technique described by Ducharme et al (1989:I) involved the use of a NMP graft from the second cervical nerve and omohyoideus muscle. EMG studies had identified the omohyoideus as an accessory muscle of respiration that is active during inspiration. After 30 weeks, 3 out of 4 ponies showed histologic evidence of reinnervation but no obvious evidence of arytenoid abduction at rest. In an attempt to minimize the fibrosis at the insertion of the NMP graft in the CAD muscle, a second study was performed using a nerve implantation technique (Ducharme et al,1989:II). Again, histologic evidence of reinnervation was apparent but even after 6 months the minimal and inconsistent movement of the arytenoid cartilage was considered inadequate to allow a horse to return to peak exercise.

In the third study, the first cervical nerve was anastomosed to the abductor branch of the RLN (Ducharme et al,1989:III). This technique although technically more difficult, provided better endoscopic evidence of reinnervation of the CAD muscle than the NMP or nerve implantation. Clonic movement of the arytenoid cartilage synchronous with inspiration was observed in some ponies treated with the nerve anastomosis. The authors concluded that the reinnervation achieved using the nerve anastomosis would be inadequate for horses to return to maximal exercise even though evaluations were performed in resting ponies.

In the above studies the NMP graft and nerve implantation procedures were performed immediately after denervation of the CAD muscle. In contrast, horses

with LLH have an atrophied CAD muscle, therefore the significance of these studies may be questioned.

The feasibility of a motor nerve anastomosis to the distal RLN in horses with idiopathic LLH is questionable. The distal recurrent laryngeal nerve in horses with LLH has a number of pathologic changes as described earlier and axonal regeneration may not be possible along this conduit because of these lesions. For this reason, I chose to evaluate the NMP graft procedure as a potential treatment of equine LLH. Since LLH in horses primarily manifests as exercise intolerance, the horses in this study were evaluated at exercise using pulmonary function tests. The following is a description of that project.

VIII. MATERIALS AND METHODS

A. Horses

Seven adult Standardbred horses (4.4 ± 0.64 years old, weighing 380 ± 8.3 kg) were used in this study. The horses were vaccinated against tetanus, equine influenza, and rhinopneumonitis. The endoscopic appearance of their upper airway, including the cervical trachea, was normal prior to any surgical procedures. The horses were trained to exercise on a treadmill^a (6.38° incline) while wearing a fiberglass face mask that covered the nostrils and mouth. Horses were kept at pasture between surgical procedures and measurement protocols.

B. Measurement Techniques

Measurement of upper airway function has been previously described (Derksen et al,1986; Shappell et al,1988; Belknap et al,1990). Briefly, a fiberglass face mask with attached pneumotachograph^b is placed over the nostrils and mouth. The mask allows complete dilation of the nostrils and is sealed against the face using a rubber shroud and adhesive tape. A wire mesh^c is present between the nostrils and pneumotachograph. The resistance of the pneumotachograph is 0.04 cm H₂O/L/sec up to an airflow rate of 90 L/sec. Pressure changes across the

^a Jetline, Desales Inc., Sand Lake, MI.

^b Merriam Instruments, Grand Rapids, MI.

^c Mesh SS Screen, McMaster Carr, Chicago, IL.

pneumotachograph are measured using a differential pressure transducer,^d which produces a signal proportional to airflow. The flow signal is integrated to give tidal volume (V_T). The pneumotachograph is calibrated before each measurement protocol using a rotameter flowmeter^e capable of measuring rates up to 90 L/sec. Although flow is measured continuously, only peak flows are reported in this study.

Transupper airway pressure (P_U) was defined as the pressure difference between a lateral tracheal catheter (Derksen et al,1986) and lateral mask catheter positioned just cranial to the nostrils. The P_U was measured using a differential pressure transducer^d calibrated using a water manometer prior to each protocol. All signals were recorded on a physiograph.^f Inspiratory and expiratory impedance (Z_I and Z_E) were calculated as the ratio of peak P_U and peak airflow over 10 breaths. Pressure and flow catheter systems were evaluated for phase differences as previously described (Derksen et al,1980). Phase differences were not detected up to a frequency of 10 Hz.

Heart rate (HR) was recorded on a heart rate computer.^g Respiratory rate (f) was calculated from the physiograph recording and minute ventilation ($\dot{V}_E$) was calculated as the product of f and inspiratory V_T .

^d Model DP45-22, Validyne Sales, Northridge, CA.

^e Model FP-2-37-P-10/77, Fischer and Porter Co. Warminster, PA.

^f Model 8188, Gould, Inc., Madison Heights, MI.

^g Equistat, Biomechanics and Exercise, Unionville, PA.

C. Experimental Design

Measurements of upper airway function were made while the horses were resting and during exercise on a 6.38° inclined treadmill at 4.2 m/sec for 2 minutes and 7.0 m/sec for 4 minutes. A two-minute rest was allowed between exercise periods. Baseline measurements were made before left recurrent laryngeal neurectomy (LRLN), 28 days after LRLN to ensure atrophy of the CAD muscle, and then at 12, 24, and 52 weeks after NMP graft. Endoscopic appearance of the larynx was noted at rest prior to each measurement protocol.

D. Surgical Procedures

For all surgical procedures anesthesia was induced with glycerol guaiacolate^h and thiamylal sodium,ⁱ then maintained with halothane^j in oxygen via an endotracheal tube and semi-closed anesthetic system. Left recurrent laryngeal neurectomy was performed in the mid-cervical area. After a 3 cm long segment of the recurrent laryngeal nerve was removed, the proximal and distal ends of the nerve were folded over and tied with 3-0 polydioxanone suture.^k Subcutaneous tissues and skin were closed in a routine manner.

^h Guaiacol Glycerol Ether, USP, Aceto Chemical Company, Flushing, NY.

ⁱ Biotal, Boehringer Ingelheim Animal Health, Inc., St. Joseph, MO.

^j Fluorothane, Ayerst Laboratories, Inc., New York, NY.

^k Ethicon Inc., Sommerville, NJ.

The NMP graft was performed using a modification of the technique described by Ducharme (Ducharme et al,1989:I). A 12-cm linear skin incision was made along the ventral border of the linguofacial vein. Blunt dissection between the omohyoideus muscle and linguofacial vein was used to identify the first cervical nerve as it travelled caudal to the left cricopharyngeal muscle to its insertion in the omohyoideus muscle. Cranial retraction of the cricopharyngeus muscle allowed visualization of the pale, atrophied CAD muscle in which a 1-cm opening was created between and parallel to atrophied muscle fibers. The NMP was created at the point of entry of one branch of the first cervical nerve into the omohyoideus muscle. A pedicle of muscle approximately 5 x 5 mm was removed with the nerve intact and was transposed to the previously created opening in the CAD muscle. Often other branches of the first cervical nerve were transected to allow the NMP to be positioned without tension. Two simple interrupted sutures of 4-0 polydioxanone suture^k were used to secure the cranial and caudal aspect of the pedicle. The omohyoideus muscle and linguofacial vein were apposed with 3-0 polydioxanone suture^k and the skin closed with stainless steel staples. The two control horses had the same surgical procedure performed, but in addition, a 5-cm section of the first cervical nerve was removed after the NMP had been sutured into the CAD muscle. All horses were treated with procaine penicillin (25,000 iu/kg) two hours prior to surgery and twice post operatively at 12 hour intervals.

E. Statistical Analysis

A three factorial analysis of variance was used according to the model $Y_{ijk} = \mu + A_i + B_j + AB_{ij} + C_k + AC_{ik} + BC_{jk} + \text{error}$ (Gill, 1978); where A_i was the fixed effect of exercise (3 levels), B_j was the fixed effect of surgical procedure (5 levels), and C_k the random effect of the 5 horses. Where F values were significant at $p < 0.05$, treatment means were compared using the Tukey test.

IX. RESULTS

In the 5 principal horses prior to LRLN, increasing exercise speed from rest to 4.2 m/s and then to 7.0 m/s significantly increased the following variables: HR, f , maximal inspiratory and expiratory flow ($\dot{V}_{\text{IMAX}}$ and $\dot{V}_{\text{EMAX}}$), peak inspiratory and expiratory pressure (P_{UI} and P_{UE}), tidal volume (V_{T}), and minute ventilation ($\dot{V}_{\text{E}}$). The inspiratory and expiratory impedance (Z_{I} and Z_{E}) did not change significantly (Table 1).

In the five principal horses exercising at 7.0 m/s, LRLN caused a significant decrease in the $\dot{V}_{\text{IMAX}}$ and a significant increase in P_{UI} and Z_{I} when compared to baseline values (Figure 1). Similar changes were recorded in the control horses (Table 2). Left recurrent laryngeal neurectomy did not significantly affect any of the other variables measured at any level of exercise (Figures 2, 3, 4).

Following the sham procedure Z_{I} and P_{UI} remained increased and $\dot{V}_{\text{IMAX}}$ decreased at the 12, 24, and 52 week measurement periods. The other variables recorded were not altered by the sham procedure compared to LRLN values (Table 2).

Twelve weeks after the NMP graft, with the principal horses exercising at 7.0 m/sec, Z_{I} was significantly decreased and the $\dot{V}_{\text{IMAX}}$ significantly increased when compared to values obtained after LRLN. However, Z_{I} was still significantly elevated above baseline values (Figure 1). The P_{UI} remained significantly elevated over baseline values, similar to the LRLN value (Figure 1).

Table 1. The effect of exercise on the measured variables in the 5 principal horses before left recurrent laryngeal neurectomy (baseline).

Variable	Exercise level		
	Rest	4.2 m/s	7.0 m/s
HR (beats/min)	39.6 $\pm$ 2.24	176.4 $\pm$ 2.29*	201.6 $\pm$ 2.25†*
<i>f</i> (breaths/min)	17.4 $\pm$ 1.33	69.3 $\pm$ 3.67*	86 $\pm$ 2.92†*
V_T (l)	7.87 $\pm$ 0.97	13.94 $\pm$ 1.45*	17.76 $\pm$ 1.44†*
$\dot{V}_E$ (l/min)	133.2 $\pm$ 10.18	968 $\pm$ 128*	1518 $\pm$ 148†*
P_{UI} (cm of H ₂ O)	2.22 $\pm$.193	20.66 $\pm$ 2.31*	34.42 $\pm$ 3.35†*
P_{UE} (cm of H ₂ O)	1.29 $\pm$.315	10.71 $\pm$ 2.20*	13.03 $\pm$ 2.49*
$\dot{V}_{I_{max}}$ (L/sec)	5.78 $\pm$ 0.79	47.59 $\pm$ 6.40*	75.46 $\pm$ 6.21†*
$\dot{V}_{E_{max}}$ (L/sec)	6.52 $\pm$ 0.91	46.09 $\pm$ 3.67*	66.94 $\pm$ 5.87†*
Z_I (cm H ₂ O/L/sec)	0.399 $\pm$ 0.04	0.454 $\pm$ 0.069	0.459 $\pm$ 0.037
Z_E (cm H ₂ O/L/sec)	0.198 $\pm$ 0.03	0.289 $\pm$ 0.094	0.211 $\pm$ 0.058

HR = heart rate, *f* = respiratory frequency, V_T = tidal volume, $\dot{V}_E$ = minute ventilation, P_{UI} = inspiratory pressure, P_{UE} = expiratory pressure, $\dot{V}_{I_{max}}$ = peak inspiratory flow, $\dot{V}_{E_{max}}$ = peak expiratory flow, Z_I = inspiratory impedance, Z_E = expiratory impedance.

* Data significantly different ($p < 0.05$) from the same measurement at rest.

† Data significantly different ($p < 0.05$) from the same measurement at 4.2 m/s.

Table 2. Mean data from the two sham operated horses. Variables at all exercise levels before and after all surgical procedures.

	BASELINE			LRLN			SHAM12			SHAM24			SHAM52		
	Rest	4.2	7.0	Rest	4.2	7.0	Rest	4.2	7.0	Rest	4.2	7.0	Rest	4.2	7.0
HR	39	166	207	38.5	169	220	39.5	161	195	34.5	170	216	38	186	226
<i>f</i>	28	87	88	40.5	72.5	73	36.5	77.5	75	29	75	70	36	70	60
V_T	5.7	12.3	14.7	3.8	14.8	19.6	7.3	16.7	21	6.6	18.3	24.4	5.8	19.1	26.2
$\dot{V}_E$	159	1101	1369	166	1076	1421	260	1298	1576	192	1393	1677	207	1335	1570
P_{UI}	3.4	19.8	30.8	2.9	21.8	45.9	3.7	27.5	43.5	2.3	25.6	48.6	2.8	26.3	51.4
P_{UE}	2.8	10.4	14.2	2.2	11.0	14.1	1.8	12.4	16.1	2.4	14.6	17.0	3.1	14.8	21.3
$\dot{V}_{IMAX}$	7.5	54	75	8.0	52.8	54.3	11.6	51.4	55.1	8.6	45.1	50.1	8.7	50.8	52.11
$\dot{V}_{EMAX}$	8.9	47.9	58.5	8.6	43.9	64.2	11.3	47	61.9	7.2	46.7	50.3	10.3	50.2	42.7
Z_I	.53	.37	.41	.48	.47	.86	.32	.53	.79	.28	.56	.97	.32	.52	.99
Z_E	.37	.23	.27	.25	.21	.23	.16	.29	.31	.17	.33	.34	.30	.29	.49

HR = heart rate (beats/min), *f* = respiratory frequency (breaths/min), V_T = tidal volume (l), $\dot{V}_E$ = minute ventilation (l/min), P_{UI} = inspiratory pressure (cm of H₂O), P_{UE} = expiratory pressure (cm of H₂O), $\dot{V}_{IMAX}$ = peak inspiratory flow (L/s), $\dot{V}_{EMAX}$ = peak expiratory flow (L/s), Z_I = inspiratory impedance (cmH₂O/L/s), Z_E = expiratory impedance (cmH₂O/L/s)

BASELINE = Prior to surgical procedures

LRLN = Left recurrent laryngeal neurectomy

SHAM12 = 12 weeks after sham nerve muscle pedicle (NMP) graft procedure

SHAM24 = 24 weeks after sham NMP graft procedure

SHAM52 = 52 weeks after sham NMP graft procedure (n=1)

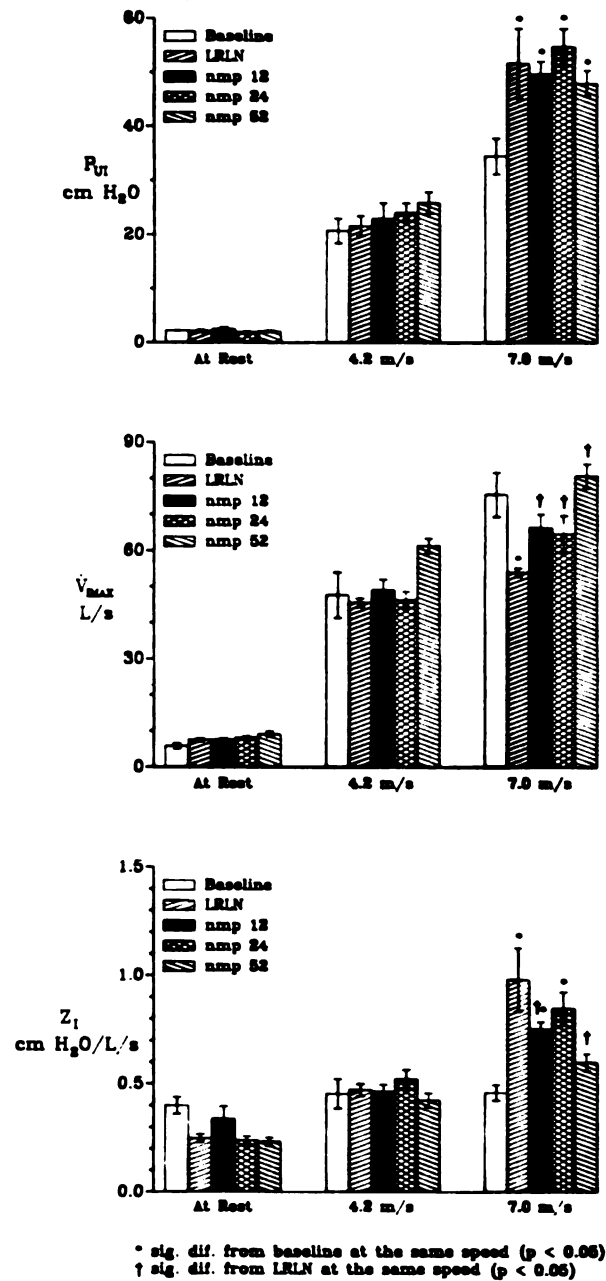


Figure 1. Peak inspiratory pressure (P_{Ul}), maximal inspiratory flow ($\dot{V}_{MAX}$) and inspiratory impedance (Z_I) before and after surgical procedures at rest and during exercise. Baseline = prior to surgical procedures; LRLN = left recurrent laryngeal neurectomy; NMP12 = 12 weeks after NMP graft procedure; NMP24 = 24 weeks after NMP graft procedure; NMP52 = 52 weeks after NMP graft procedure.

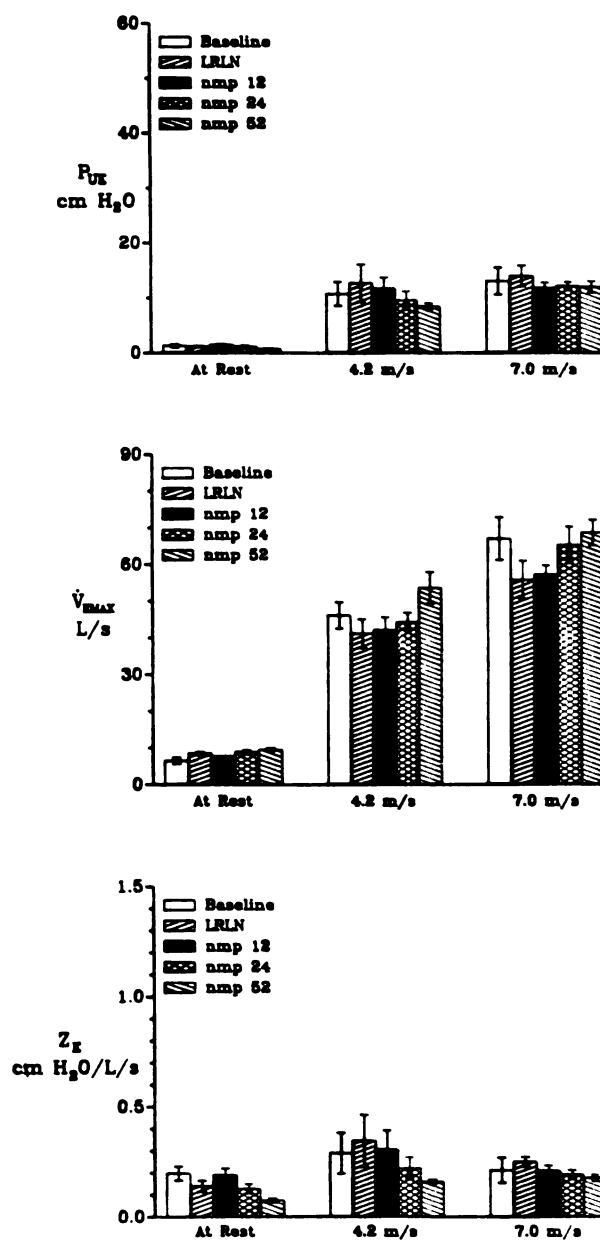


Figure 2. Peak expiratory pressure (P_{UE}), maximal expiratory flow ($\dot{V}_{EMAX}$) and expiratory impedance (Z_E) before and after surgical procedures at rest and during exercise. Baseline = prior to surgical procedures; LRLN = left recurrent laryngeal neurectomy; NMP12 = 12 weeks after NMP graft procedure; NMP24 = 24 weeks after NMP graft procedure; NMP52 = 52 weeks after NMP graft procedure.

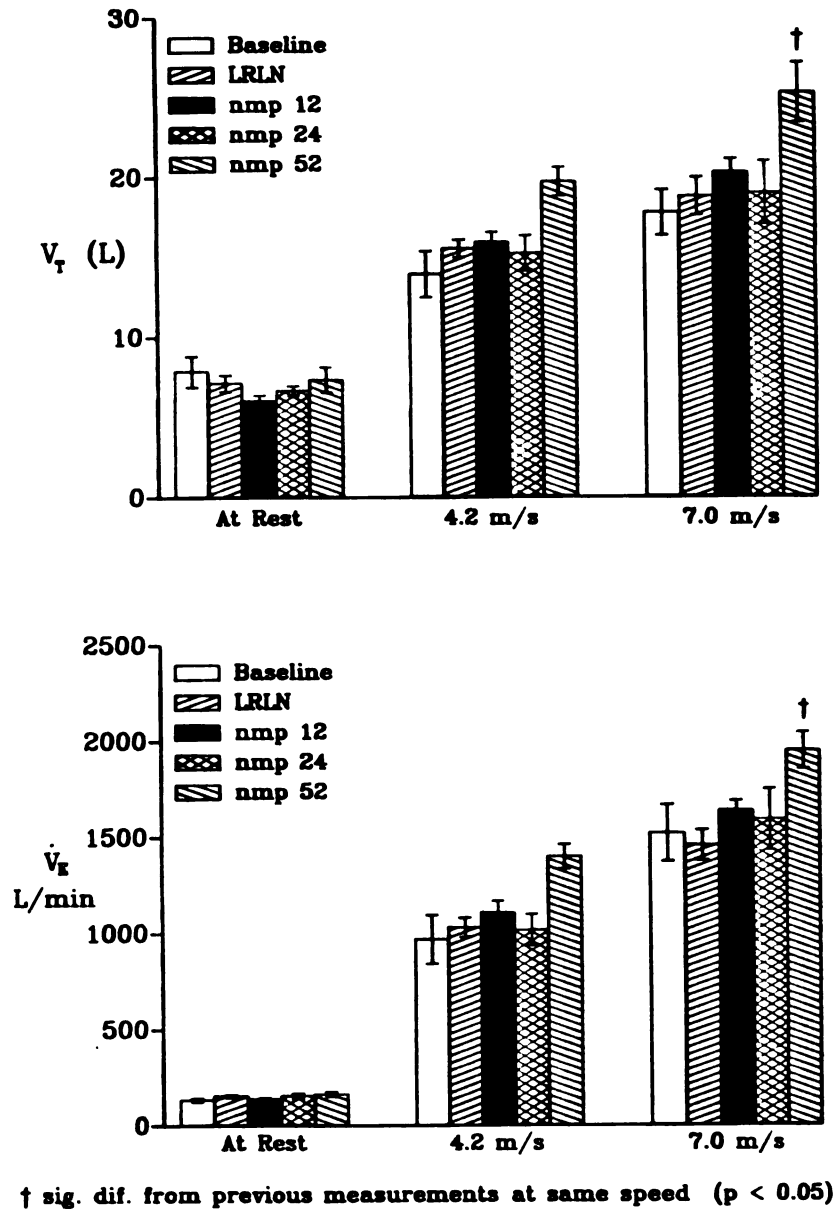


Figure 3. Tidal volume (V_T) and minute ventilation ($\dot{V}_E$) before and after surgical procedures at rest and during exercise. Baseline = prior to surgical procedures; LRLN = left recurrent laryngeal neurectomy; NMP12 = 12 weeks after NMP graft procedure; NMP24 = 24 weeks after NMP graft procedure; NMP52 = 52 weeks after NMP graft procedure.

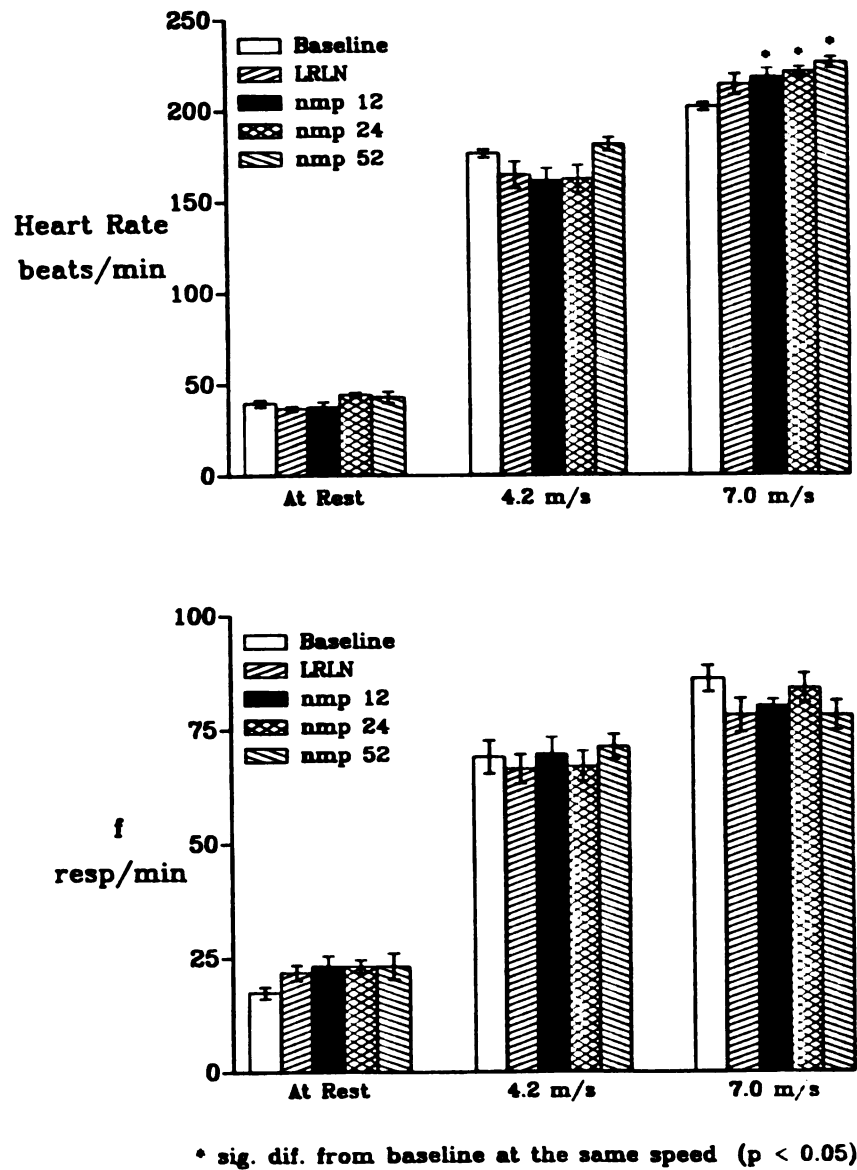


Figure 4. Heart rate (HR) and respiratory frequency (f) before and after surgical procedures at rest and during exercise. Baseline = prior to surgical procedures; LRLN = left recurrent laryngeal neurectomy; NMP12 = 12 weeks after NMP graft procedure; NMP24 = 24 weeks after NMP graft procedure; NMP52 = 52 weeks after NMP graft procedure (n=1).

Twenty-four weeks after the NMP graft, with horses exercising at 7.0 m/sec, Z_I was no longer significantly decreased when compared to the LRLN value, primarily because of an increased Z_I in one horse. The $\dot{V}_{\text{IMAX}}$ remained significantly greater than the LRLN value and the P_{UI} was still significantly elevated compared to baseline (Figure 1).

Fifty-two weeks after the NMP graft with horses exercising at 7.0 m/sec, Z_I and $\dot{V}_{\text{IMAX}}$ were not significantly different from baseline values (Figure 1). Although the P_{UI} remained significantly greater than the baseline value (Figure 1), the Z_I had improved in all the principal horses compared to the 24-week measurement period.

Other variables that altered significantly after the NMP graft with horses exercising at 7.0 m/sec included the HR, which at the 12, 24, and 52 week measurements was greater than baseline values (Figure 4). Also the V_T and $\dot{V}_E$ at 52 weeks post operatively were greater than values obtained at all previous measurement periods (Figure 3). The NMP graft did not affect any other variables at any level of exercise (Figure 2, 3, 4).

Prior to obtaining baseline measurements, endoscopic examination of the upper airway with horses standing at rest, failed to reveal any abnormalities. Following LRLN, in principal and control horses, there was axial displacement of the left arytenoid and corniculate cartilage and vocal fold indicative of LLH (Lane,1987:II). Twelve, 24, and 52 weeks after NMP graft or sham procedure, the endoscopic appearance of the larynx was similar to that following LRLN. In 2 horses there

was a mild post operative swelling at the surgical site for 2 days following NMP graft, but this resolved without further treatment.

X. DISCUSSION AND CONCLUSIONS

Before surgical intervention, increasing levels of exercise caused an elevation in HR, f , V_T , $\dot{V}_E$, peak P_{UI} , $\dot{V}_{IMAX}$ and $\dot{V}_{EMAX}$, while Z_I and Z_E remained unchanged. Similar changes with exercise have been previously reported (Shappell et al,1988; Belknap et al,1990). Inspiratory flow rates were slightly higher than a previous report (Derksen et al,1986), but comparable to others (Belknap et al,1990; Hornicke et al,1983). The higher exercise speeds in our study could account for the greater $\dot{V}_{IMAX}$ recorded compared to the study of Derksen et al (1986).

Following LRLN, Z_I almost doubled when horses were exercising at 7.0 m/sec. The peak P_{UI} dramatically increased, whereas peak $\dot{V}_{IMAX}$ decreased (Figure 1). These changes occur because relative to atmospheric pressure, a negative pressure is generated in the airway during inspiration, causing unsupported soft tissue structures to collapse into the airway lumen and obstruct airflow (Robinson and Sorrenson,1978). These changes are most apparent at exercise when pressure changes in the airway during breathing are greatest. In horses with LLH, the paralyzed CAD muscle cannot support the arytenoid cartilage, resulting in dynamic collapse of this structure during inhalation at maximal exercise (Derksen,1988). For the horse to maintain adequate air flow during exercise through an obstructed airway, P_{UI} is increased. The further decrease in intraluminal pressure that results exacerbates the dynamic collapse limiting airflow and increasing Z_I (Derksen,1988). This dynamic collapse of the arytenoid cartilage in exercising horses with LLH has

been documented by videoendoscopy during exercise (Derksen,1988 Morris and Seeherman,1988).

Stabilization of the arytenoid cartilage in horses with LLH using a non-absorbable prosthetic suture has been demonstrated to decrease Z_1 and P_{UI} and return $\dot{V}_{MAX}$ to normal levels during exercise (Derksen et al,1986; Shappell et al,1988). However, a number of post operative complications have been reported. These include failure of the prosthetic suture to maintain abduction, wound dehiscence, infection of the tissue around the suture, and chronic suture sinus tract associated with the prosthesis (Haynes,1984:I). Coughing (acute and chronic), aspiration of feed into the trachea (Greet et al,1979), pneumonia, chondritis, cartilage ossification, and laryngeal granulomas are also potential problems following prosthetic laryngoplasty (Speirs,1987). Functional stabilization of the arytenoid cartilage, by reinnervating the CAD muscle, could potentially achieve improvement of upper airway function similar to laryngoplasty without the associated complications as there is no prosthesis or permanent abduction of the arytenoid cartilage.

A NMP graft for the treatment of laryngeal muscle paralysis, has been performed extensively in dogs (Hengerer and Tucker,1973; Lyons and Tucker,1974; Greenfield et al,1988) and people (Tucker,1978 Applebaum et al,1979; May et al,1980; Fernandes et al,1987). Excellent success and minimal complications have been reported. Attempts to restore arytenoid abduction by reinnervating the left CAD muscle using three different techniques in the horse have been reported.

One technique involved implanting the cut-end of the second cervical nerve directly into the CAD muscle. Although partial arytenoid abduction was present in four out of six ponies six months post operatively, convincing histologic evidence of reinnervation in the CAD muscle was not demonstrated (Ducharme et al,1989:II). A second study, where a branch of the first cervical nerve was anastomosed to the left recurrent laryngeal nerve, was reported to be successful in restoring some arytenoid abduction, but not sufficient for high-speed exercise (Ducharme et al,1989:III). The third study utilized a NMP graft from the second cervical nerve and omohyoideus muscle. Histologic evidence of reinnervation (muscle fiber hypertrophy and fine regenerating axons) was observed, but abduction of the arytenoid cartilage was not observed in the resting horse after 30 weeks (Ducharme et al,1989:I). The lack of arytenoid abduction at rest, as determined by endoscope examination of the larynx, is not unexpected. The NMP graft is created from an accessory muscle of respiration, the omohyoideus muscle (Ducharme et al,1989:I). The omohyoideus muscle is quiescent at rest and activated during inspiration only under exercise conditions. Thus in treated horses, arytenoid cartilage abduction is only expected during exercise. A similar lack of arytenoid abduction at rest has been observed in 50% of patients following laryngeal reinnervation (Tucker,1978).

In the present study we chose to evaluate the NMP technique by measuring variables of respiratory function. Twelve weeks following the NMP graft, with horses exercising at 7.0 m/sec, the Z_1 in 3 out of 5 horses had decreased when

compared to values obtained after LRLN. This decrease in Z_1 resulted in a significant rise in $\dot{V}_{\text{IMAX}}$. The Z_1 of one horse had improved dramatically at this point. Return of function to reinnervated laryngeal muscles is seen at 6-12 weeks in people (Fernandes et al,1987) and in dogs neurogenic activity was reported to be present 6-9 weeks following NMP graft (Hengerer and Tucker,1973). The rapid reinnervation of laryngeal muscles is thought to be due to transplantation of motor endplates present in the muscle pedicle (Tucker,1977), allowing propagation of incoming impulses to the muscle fibers once the pedicle has healed to the recipient muscle. It is reasonable to assume that motor endplates were transplanted in one horse due to the rapid reduction in Z_1 that then persisted throughout the remainder of the study.

In two horses, 12 weeks after NMP graft, Z_1 had improved but not as dramatically as the previously mentioned horse. By 52 weeks both horses had shown further improvement in Z_1 at 7.0 m/sec. A study in dogs has reported a similar trend following NMP graft into the CAD muscle with partial improvement at 19 weeks and complete recovery by 36-44 weeks (Greenfield et al,1988). Partial and slow return of function is thought to be due to transplantation of only a few motor endplates along with transected nerve fibers in the pedicle increasing the time necessary for complete reinnervation to occur (Tucker,1982).

As a group, a 52 week post-operative period was necessary before the Z_1 at 7.0 m/sec was no longer significantly different from the baseline value. If motor endplates were not transplanted in some of the horses and simply the transected

end of the first cervical nerve, this finding is compatible with other studies. Implantation of the phrenic nerve, also a cervical nerve, into the CAD muscle is reported to take 6-12 months to achieve reinnervation (Fex,1970 Taggart,1971).

The lower heart rate at baseline compared to subsequent measurement periods with the horses exercising at 7.0 m/sec may be explained by the 2-3 week training period necessary to acquaint horses with the treadmill. This training period was not required before the other measurement periods. The V_T and $\dot{V}_E$ 52 weeks post operatively (Figure 3) were greater than the previous measurement periods. This is most likely because the horses had spent 6 months at pasture prior to the measurement period and their lack of conditioning necessitated greater respiratory effort for the same workload.

The absence of serious post operative complications make the NMP graft an inviting surgical technique. Rapid reinnervation (12 weeks post operatively) is possible, but was not consistently achieved as 52 weeks was necessary to observe improvement of respiratory function in all principal horses. At present the NMP graft cannot be recommended as a routine treatment for LLH in athletic horses as the convalescent time before racing can be resumed is greater than with prosthetic laryngoplasty. However, with improvement of the surgical technique, the nerve muscle pedicle may indeed be a suitable treatment for LLH.

XI. RECOMMENDATIONS

The success of this project is a significant step towards an uncomplicated surgical treatment of LLH in horses. Consultation with Dr. Harvey Tucker of the Cleveland Clinic, Ohio; father of human laryngeal reinnervation, has provided ideas for improving the success of the NMP graft procedure in the horse.

Firstly, the CAD muscle in the horse is large enough to have two or three NMP grafts implanted. Improved surgical exposure of the CAD muscle will be necessary to allow multiple NMP grafts to be inserted, but using a variety of retractors this should be achievable. Two or three NMP grafts may decrease the time necessary for improvement to be seen and ultimately provide a better return of function to the CAD muscle.

Secondly, the use of local anesthetic on the first cervical nerve and omohyoideus muscle prior to creating the pedicle will allow more accurate dissection to occur. With these modifications I believe it will be possible to improve on the already successful technique of laryngeal reinnervation using a NMP graft.

LIST OF REFERENCES

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Anderson LJ, Goulden BE, Munford RE. A study of the weights of some intrinsic laryngeal and palantine muscles in the Thoroughbred horse. N.Zealand Vet J 1988;28:222-225.

Andrew BL. The respiratory displacement of the larynx: A study of the innervation of accessory respiratory muscles. J Physiol 1955;130:474-487.

Anonsen CK, Duckert LG, Cummings CW. Preliminary observations after facial rehabilitation with the ansa hypoglossi pedicle transfer. Otolaryngol-Head neck surgery 1986;94:302-304.

Applebaum EL, Allen GW, Sisson GA. Human laryngeal reinnervation: The Northwestern experience. Laryngoscope 1979;89:1784-1787.

Armstrong BW, Smith DJ. Function of certain neck muscles during the respiratory cycle. Am J Physiol 1955;143:599-600.

Art T, Serteyn D, Lekeux P. Effect of exercise of the partitioning of equine respiratory resistance. Eq Vet J 1988;20:268-273.

Attenburrow DP. Respiration and locomotion. In: Snow DH, Persson SGB, Rose RJ, eds. Equine exercise physiology. Cambridge, England: Granta Editions, 1983:I;17-22.

Attenburrow DP, Flack FC, Hornicke H, Meixner R, Pollmann U. Respiratory airflow and sound intensity. In: Snow DH, Persson SGB, Rose RJ, eds. Equine exercise physiology. Cambridge, England: Granta Editions, 1983:II;23-26.

Badke A, Irintchev AP, Wernig A. Maturation of transmission in reinnervated mouse soleus muscle. Muscle and Nerve 1989;12:580-586.

Baker GJ. Laryngeal hemiplegia in the horse. Comp Cont Ed Pract Vet 1983:I;5:561-567.

Baker GJ. Laryngeal asynchrony in the horse: Definition and significance. In: Snow DH, Persson SGB, Rose RJ, eds. Equine exercise physiology. Cambridge, England: Granta Editions, 1983;II:46-50.

Baldissera F, Cantarella G, Marini G, Ottaviani F. Restoring abduction of paralyzed vocal cords in the cat using selective laryngeal reinnervation by phrenic motoneurons. Laryngoscope 1986;96:1399-1404.

Barber SM. Paralaryngeal abscess with laryngeal hemiplegia and fistulation in a horse. Can Vet J 1981;22:389-392.

Barber SM, Fretz PB, Bailey JV, McKenzie NT. Analysis of surgical treatments for selected upper respiratory tract conditions in horses. Vet Med 1984;678-682.

Barlett D. Respiratory functions of the larynx. Physiol Rev 1989;69:33-57.

Bayly WM, Grant BD, Modransky PD. Arterial blood gas tensions during exercise in a horse with laryngeal hemiplegia, before and after corrective surgery. Res Vet Sci 1984;36:256-258.

Belknap JK, Derksen FJ, Nickels FA, Stick JA, Robinson NE. Subtotal arytenoidectomy fails to improve upper airway flow mechanics in exercising horses with induced laryngeal hemiplegia. Am J Vet Res (in press,1990).

Bergmann K, Warzel H, Eckhardt HU, Gerhardt HJ. Respiratory rhythmically regulated electrical stimulation of paralysed laryngeal muscles. Laryngoscope 1984;94:1376-1380.

Boles R, Fritzell B. Injury and repair of the recurrent laryngeal nerve in dogs. Laryngoscope 1969;79:1405-1418.

Broniatowski M, Kaneko S, Jacobs G, Nose Y, Tucker HM. Laryngeal Pacemaker II. Electronic pacing of reinnervated posterior cricoarytenoid muscles in the canine. Laryngoscope 1985;95:1194-1198.

Burrows GE. Lead poisoning in the horse. Eq Pract 1982;4:30-36.

Cahill JI, Goulden BE. Equine laryngeal hemiplegia: Part I. A light microscopic study of peripheral nerves. N.Zealand Vet J 1986;I;34:161-169.

Cahill JI, Goulden BE. Equine laryngeal hemiplegia: Part II. An electron microscope study of peripheral nerve. N.Zealand Vet J 1986;II;34:170-175.

Cahill JI, Goulden BE. Equine laryngeal hemiplegia: Part III. A teased fibre study of peripheral nerves. N.Zealand Vet J 1986;III;34:181-185.

Cahill JI, Goulden BE. Equine laryngeal hemiplegia: Part IV. Muscle Pathology. N.Zealand Vet J 1986;IV;34:186-190.

Cahill JI, Goulden BE. Equine laryngeal hemiplegia: Part V. Central nervous system pathology. N.Zealand Vet J 1986;V;34:191-193.

Cahill JI, Goulden BE. Nervous system component in equine laryngeal hemiplegia. N.Zealand Vet J 1989;37:89-90.

Cole CR. Changes in the equine larynx associated with laryngeal hemiplegia. Am J Vet Res 1946;7:69-77.

Cook WR. The diagnosis of respiratory unsoundness in the horse. Vet Rec 1965;77:516-528.

Cook WR. A comparison of idiopathic laryngeal paralysis in man and horse. J of Laryngo and Otol 1970;84:819-835.

Cook WR. Some observations on diseases of the ear, nose and throat in the horse, and endoscopy using a flexible fibreoptic endoscope. Vet Rec 1974;94:533-539.

Cook WR. Some observations on form and function of the equine upper airway in health and disease. Proc 27th AAEP 1981;393-451.

Cook WR. Diagnosis and grading of hereditary recurrent laryngeal neuropathy in the horse. J Eq Vet Sci 1988;I;8:432-455.

Cook WR. Recent observations on recurrent laryngeal neuropathy in the horse: Applications to practice. Proc 34th AAEP 1988;II:427-478.

Crumley RL, Horn K, Clendenning D. Laryngeal reinnervation using the split phrenic nerve graft procedure. Otolaryngol: Head Neck Surg 1980;88:159-164.

Crumley RL, Izdebski K. Voice quality following laryngeal reinnervation by ansa hypoglossi transfer. Laryngoscope 1986;96:611-616.

Cymbaluk NF, Fretz PBA, Loew FM. Thiamin measurements in horses with laryngeal hemiplegia. Vet Rec 1977;101:97-98.

Dean PW, Nelson JK, Schumacher J. Effects of age and prosthesis material on in vitro cartilage retention of laryngoplasty prosthesis in horses. Am J Vet Res 1990;51:114-117.

Derksen FJ, Robinson NE. Esophageal and intrapleural pressures in the healthy conscious pony. Am J Vet Res 1980;41:1756-1761.

Derksen FJ, Stick JA, Scott EA, Robinson NE, Slocombe RF. Effect of laryngeal hemiplegia and laryngoplasty on airway flow mechanics in exercising horses. Am J Vet Res 1986;47:16-20.

Derksen FJ. Physiology of airflow in the athletic horse. Proc 34th AAEP 1988:149-158.

Doyle PJ, Brummett RE, Everts EC. Results of surgical section and repair of the recurrent laryngeal nerve. Laryngoscope 1967;77:1245-1254.

Ducharme NG, Horney FD, Partlow GD, Hulland TJ. Attempts to restore abduction of the paralysed equine arytenoid cartilage I. Nerve-muscle pedicle transplants. Can J Vet Res 1989;I;53:202-209.

Ducharme NG, Horney FD, Hulland TJ, Partlow GD, Schnurr D, Zutrauen K. Attempts to restore abduction of the paralysed equine arytenoid cartilage II. Nerve implantation (Pilot Study) Can J Vet Res 1989;II;53:210-215.

Ducharme NG, Viel L, Partlow GD, Hulland TJ, Horney FD. Attempts to restore abduction of the paralyzed equine arytenoid cartilage III. Nerve anastomosis. Can J Vet Res 1989;III;53:216-223.

Duncan ID, Griffiths IR, McQueen A, Baker GJ. The pathology of equine laryngeal hemiplegia. Acta Neuropath (Berl) 1974;27:337-348.

Duncan ID, Baker GJ, Heffron CJ, Griffiths IR. A correlation of endoscopic and pathological changes in subclinical pathology of the horses' larynx. Eq Vet J 1977;9:220-225.

Duncan ID, Brook D. Bilateral laryngeal paralysis in the horse. Eq Vet J 1985;17:228-233.

Duncan ID. Ultrastructural observations of organelle accumulation in the equine recurrent laryngeal nerve. J Neurocytol 1987;16:269-280.

Eisele DW, Hillel AD, Trachy RE, Little JW. Reinnervation of the trapezius muscle. Otolaryngol-Head Neck Surg 1988;98:34-43.

Elies W, Pusalkar A. Histological findings in arytenoid cartilages after long-lasting recurrent nerve paralysis. J Laryngol and Otol 1983;97:949-957.

Evoy MH. Paralysis of the vocal folds after thyroidectomy. Am J Surg 1961;102:73-78.

Fernandes CMC, Samuel J, Steinberg JL. Nerve/muscle pedicle graft for bilateral vocal cord palsy. J Laryngol: Otol 1987;101:471-474.

Fex S. Functioning remobilization of vocal cords with permanent recurrent laryngeal paresis. Acta Otolaryngol 1970;69:294-301.

Fink BR, Basek, Epanchin V. The mechanism of opening of the human larynx. Laryngoscope 1956;66:410-425.

Fleming G. Laryngismus paralytica ("roaring"). Vet J of Comp Path 1882;14:1-12.

Getty R, ed. Sisson and Grossman's The anatomy of the domestic animals. 5th ed. WB Saunders Co. Philadelphia, 1975;376-454, 498-524, 650-670.

Gilbert GH. Laryngeal hemiplegia following jugular injury. JAVMA 1972;171:1686-1687.

Gill JL. Design and analysis of experiments in the animal and medical sciences. Vol 1. Ames, Iowa: Iowa State University Press, 1978.

Glogowska M, Stransky A, Widdecombe JG. Reflex control of discharge in motor fibres to the larynx. J Physiol 1974;239:365-379.

Goulden BE, Anderson LJ. Equine laryngeal hemiplegia Part I: Physical characteristics of affected animals. N. Zealand Vet J 1981;I;29:150-154.

Goulden BE, Anderson LJ. Equine laryngeal hemiplegia Part II: Some clinical observations. N. Zealand Vet J 1981;II;29:194-198.

Goulden BE, Anderson LG. Equine laryngeal hemiplegia Part III: Treatment by laryngoplasty. N. Zealand Vet J 1982;30:1-5.

Goulden BE, Anderson LJ, Cahill JI. Roaring in Clydesdales. N. Zealand Vet J 1985;33:73-76.

Greenfield CL, Walshaw R, Kumar K, Lowrie CT, Derksen FJ. Neuromuscular pedicle graft for restoration of arytenoid abductor function in dogs with experimentally induced laryngeal hemiplegia. Am J Vet Res 1988;49:1360-1366.

Greet TRC, Baker GJ, Lee R. The effect of laryngoplasty on pharyngeal function in the horse. Eq Vet J 1979;11:153-158.

Greet TRC. The slap test for laryngeal adductory function in horses with suspected cervical spinal cord damage. Eq Vet J 1983;12:127-131.

Gunn HM. Further observations on laryngeal skeletal muscle in the horse. Eq Vet J 1973;5:77-80.

Hall SJ, Trachy RE, Cummings CW. Facial muscle reinnervation: A comparison of neuromuscular pedicle with direct nerve implant. Ann Otol Rhinol Laryngol 1988;97:229-233.

Hamilton LH. Nasal airway resistance: Its measurement and regulation. Physiologist 1978;22:43-49.

Harvey JE, Ohyama M, Ueda N, Mogi G, Ogura JH. The effect of 1,1,3-Tricyano-2-Amino-1-Propene(U-9189) on reinnervation of the canine larynx "Electrophysiological observations" Laryngoscope 1970;80:1646-1664.

Haynes PF. Surgical failures in upper respiratory surgery. Proc 26th Ann Gen Meet AAEP 1980:I:223-249.

Haynes PF, Snider TG, McClure JR, McClure JJ. Chronic chondritis of the equine arytenoid cartilage. JAVMA 1980;II:177:1135-1142.

Haynes PF. Surgery of the equine respiratory tract. In: Jennings PB, ed. The practice of large animal surgery Vol I. Philadelphia: WB Saunders and Co.1984:I;435-445.

Haynes PF, McClure JR, Watters JW. Subtotal arytenoidectomy in the horse: An update. Proc 30th Ann Gen Meet AAEP 1984;II:21-33.

Helper LC, Lerner D. Unilateral retinopathy and blindness in a horse following intracarotid injection of phenylbutazone. Eq Pract 1980;2:33-35.

Hengerer AS, Tucker HM. Restoration of abduction in the paralyzed canine vocal fold. Arch Otolaryngol 1973;97:247-250.

Hilledge CJ. Prevalence of laryngeal hemiplegia in a thoroughbred horse farm. Eq Vet Sci 1985;5:252-254.

Hobday F. The merits of Williams' operation for roaring. Proc 48th Ann Conv of AVMA 1911;291-298.

Hobday F. The surgical treatment of roaring in horses. Vet Rec 1935;15:1535-1539.

Hornicke H, Meixner R, Pollmann U. Respiration in exercising horses. In: Snow DH, Persson SGB, Rose RJD, eds. Equine exercise physiology. Cambridge, England: Granta Editions, 1983;7-16.

Huntington PJ, Jeffcott LB, Friend SCE, Luff AR, Finkelstein DI, Flynn RJ. Australian stringhalt - epidemiological, clinical and neurological investigations. Eq Vet J 1989;21:266-273.

Iwamura S. Functioning remobilization of the paralyzed vocal cord in dogs. Arch Otolaryngol 1974;100:122-126.

Johnson J, Tucker Hm. Selective experimental reinnervation of paralyzed facial muscles. Arch Otolaryngol 1987;103:22-25.

Kelly JP. Reactions of neurons to injury. In: Kandel ER, Schwartz JH, eds. Principles of Neural Science 2nd ed. New York, Elsevier, 1987;188-195.

Lane JG, Ellis DR, Greet TRC. Observations on the examination of thoroughbred yearlings for idiopathic laryngeal hemiplegia. Eq Vet J 1987(I);19:531-536.

Lane JG. Fiberoptic endoscopy of the equine upper respiratory tract: A commentary on progress. Eq Vet J 1987(II);19:495-499.

Loew FM. Thiamin and equine laryngeal hemiplegia. Vet Rec 1973;97:372-373.

Lundborg G. Intraneural microcirculation. Orthopedic Clinics of Nth Am 1988;19:1-12.

Lyons RM, Tucker HM. Delayed restoration of abduction in the paralyzed canine larynx. Arch Otolaryngol 1974;100:176-179.

Mackay-Smith MP, Marks D. Television clinical diagnosis of laryngeal hemiplegia in the horse. Proc 14th AAEP 1968:227-235.

Mackay-Smith MP, Johnson JH, Baker RH. Laryngoplasty - A progress report. 19th AAEP 1973:133-146.

MacQueen J. Roaring, whistling and grunting. J Comp Path and Therapy 1896;9:112-119.

Marks D, Mackay-Smith MP, Cushing LS, Leslie JA. Etiology and diagnosis of laryngeal hemiplegia in horses. JAVMA 1970;I;157:429-436.

Marks D, Mackay-Smith MP, Cushing LS, Leslie JA. Use of a prosthetic device for surgical correction of laryngeal hemiplegia in horses. JAVMA 1970;II;157:157-163.

Martin GS, Beadie RE, Haynes PF, Watters JM. Cross-sectional area of the aditus laryngus and rima glottidis before and after transection of the left recurrent laryngeal nerve in the horse. Am J Vet Res 1986;47:422-425.

Mason JE. Laryngeal hemiplegia: A further look at Haslam's anomaly of the left recurrent laryngeal nerve. Eq Vet J 1973;5:150-155.

May M, Lavorato AS, Bleyart AL. Rehabilitation of the crippled larynx: Application of the Tucker Technique for muscle nerve reinnervation. Laryngoscope 1980;90:1-18.

McCall J. A review of: Roaring in horses (laryngismus paralyticus). Its history, nature, causes, prevention and treatment. London: Balliere, Tindall and Lex, 1889. The Vet J 1889;28:265-271.

McCall JW, Hoerr NL. Reinnervation of a paralyzed vocal cord. Laryngoscope 1946;56:527-535.

Meikle D, Trachy RE, Cummings CW. Reinnervation of skeletal muscle: A comparison of nerve implantation with neuromuscular pedicle transfer in an animal model. Ann Otol Rhinol Laryngol 1987;96:152-157.

Merriam JG. Laryngoplasty- an evaluation of three abductor muscle prosthetics. Proc 19th AAEP 1973:123-131.

Miglets AW. Functional laryngeal abduction following reimplantation of the recurrent laryngeal nerves. Laryngoscope 1974;84:1996-2005.

Moore MP, Andrews F, Reed SM, Grant BD. Electromyographic evaluation of horses with laryngeal hemiplegia. J Eq Vet Sci 1988;8:424-427.

Morledge DR, Lauvstad WA, Calcaterra TC. Delayed reinnervation of the paralyzed larynx. Arch Otolaryngol 1973;97:291-293.

Morris EA, Seeherman HJ. The dynamic evaluation of upper respiratory function in the exercising horse. Proc 34th AAEP 1988:159-165.

Murakami Y, Kirchner J A. Vocal cord abduction by regenerated recurrent laryngeal nerve. Arch Otolaryngol 1971;94:64-68.

Murray JF. The Normal Lung. The basis for diagnosis and treatment of pulmonary disease. Philadelphia, Saunders WB and Company, 1978;77-107.

Neal GD, Cummings CW, Sutton D. Delayed reinnervation of unilateral vocal cord paralysis in dogs. Otolaryngol Head Neck Surg 1981;89:608-612.

Ogura JH, Kawasaki M, Takenouchi S, Yagi M. Replantation and transplantation of the canine larynx. Ann Otol Rhinol Laryngol 1966;75:295-312.

Ogura JH, Harvey Je, Mogi G, Ueda N, Ohyama M, Tucker HM. Further experimental observations of transplantation of canine larynx. Laryngoscope 1970;80:1231-1243.

Olson DE, Dart GA, Filley GF. Pressure drop and fluid flow regime of air inspired into the human lung. J Ap Physiol 1970;28:482-294.

Pascoe JR, Ferraro GL, Cannon JH. Exercise-induced pulmonary hemorrhage in racin Thoroughbreds: A preliminary study. Am J Vet Res 1981;42:703-707.

Pascoe JR. Recurrent laryngeal neuropathy. A discussion of Dr. Cook's Paper. Proc 34th AAEP 1988:479-486.

Pavletic MM, Nafe LA, Confer AW. Mucocutaneous subdermal plexus flap from the lower lip for lower eyelid restoration in the dog. JAVMA 1982;180:921-926.

Poncet PA, Montavon S, Gaillard C, Barrelet F, Straub R, Gerber H. A preliminary report on the possible genetic basis of laryngeal hemiplegia. Eq Vet J 1989;21:137-138.

Pouret E. Laryngeal ventriculectomy with stitching of the laryngeal saccules. Proc 12th AAEP 1966:207-213.

Quinlan T, Goulden BE, Davies AS. Bilateral asymmetry of equine laryngeal muscles. N.Zealand Vet J 1975;23:145-147.

Quinlan TJ, Goulden BE, Barnes GRG, Anderson LJ, Cahill JI. Innervation of the equine laryngeal muscles. N.Zealand Vet J 1982;30:43-45.

Rakestraw PC, Hackett RP, Ducharme NG, Nielan GJ, Erb NH. A comparison of laryngeal function in resting and exercising in horses with varying degrees of laryngeal dysfunction. Vet Surg 1990;18:73.

Raphael CF. Endoscopic findings in the upper respiratory tract of 479 horses. JAVMA 1982;181-470-473.

Rice DH. Laryngeal reinnervation. Laryngoscope 1982;92:1049-1059.

Robinson NE, Sorrenson PR, Goble DO. Patterns of airflow in normal horses and horses with respiratory disease. Proc 21st AAEP 1975:11-21.

Robinson NE, Sorrenson PR. Pathophysiology of airway obstruction in horses: A review. JAVMA 1978;172:299-303.

Rooney JR, Delaney FM. A hypothesis on the causation of laryngeal hemiplegia in the horse. Eq Vet J 1970;2:35-37.

Rose RJ. Acute laryngeal paralysis in arabian foals. Aust Vet J 1978;54:154.

Rossdale PD, Hopes R, Digby W, Offord K. Epidemiological study of wastage among racehorses 1982 and 1983. Vet Rec 1985;116:66-69.

Sato F, Ogura JH. Reconstruction of laryngeal function for recurrent laryngeal paralysis: Historical view, advancement of latest investigations and a preliminary experiment. Laryngoscope 1978;88:689-696.

Shapell KK, Derksen FJ, Stick JA, Robinson NE. Effect of ventriculectomy, prosthetic laryngoplasty and exercise on upper airway function in horses with induced left laryngeal hemiplegia. Am J Vet Res 1988;49:1760-1765.

Shapiro J, White NA, Schlafer DH, Rowland GN. Hypertrophic ossification of the laryngeal cartilages of a horse. J Eq Med and Surg 1979;3:370-374.

Shires GMH, Adair HS, Paton CS. The use of the ND:YAG Laser for the removal of the laryngeal ventricles in the equine. Abstract in Vet Surg 1990;18:75.

Siribodhi C, Sundmaker W, Atkins JP, Bonner FJ. Electromyographic studies of laryngeal paralysis and regeneration of laryngeal motor nerves in dogs. Laryngoscope 1962;72:164.

Speirs VC. Abductor muscle prosthesis in the treatment of laryngeal hemiplegia in the horse. Aust Vet J 1972;48:251-254.

Speirs VC. A retrospective survey of the racing performance of Australian horses after surgery for carpal injuries and laryngeal hemiplegia. Proc 26th AAEP 1980:335-345.

Speirs VC, Bourke JM, Anderson GA. Assessment of the efficacy of an abductor muscle prosthesis for treatment of laryngeal hemiplegia in horses. Aust Vet J 1983;60:294-299.

Speirs VC. Partial arytenoidectomy in the horse. Vet Surg 1986;15:316-320.

Speirs VC. Laryngeal surgery- 150 years on. Eq Vet J. 1987;19:377-383.

Stick JA, Derksen FJ. Use of videoendoscopy during exercise for determination of appropriate surgical treatment of laryngeal hemiplegia in a colt. JAVMA 1989;195:619-622.

Stransky A, Szereda M, Widdicombe JG. The effects of lung reflexes on laryngeal resistance and motoneuron discharge. J Physiol 1973;231:417-438.

Suzuki M, Kirchner JA. The posterior cricoarytenoid as an inspiratory muscle. Laryngoscope 1969;79:849-864.

Taggart JP. Laryngeal reinnervation by phrenic nerve implantation in dogs. Laryngoscope 1971;81:1330-1336.

Tate LP, Sweeney CL, Cullen JM, Corbett WT, Newman HC, Brown TC, Ketner MT. Am J Vet Res 1989;50:786-791.

Tate LP, Corbett WP, Bishop B. Investigation of physiological effect of laryngeal hemiplegia followed by laryngoplasty in the exercising thoroughbred. Abstract Vet Surg 1990;18:78.

Tucker HM, Harvey J, Ogura JH. Vocal cord remobilization in the canine larynx. Arch Otolaryng 1970;92:530-533.

Tucker HM, Ogura JH. Vocal cord remobilization in the canine larynx: A histologic evaluation. Laryngoscope 1971;81:1602-1606.

Tucker HM. Laryngeal transplantation: current status 1974. Laryngoscope 1974;85:787-796.

Tucker HM. Human laryngeal reinnervation. Laryngoscope 1976;86:769-779.

Tucker HM. Reinnervation of the unilaterally paralyzed larynx. Ann Otolaryngol 1977;86:789-794.

Tucker HM. Selective reinnervation of paralyzed musculature in the head and neck: Functioning autotransplantation of the canine larynx. Laryngoscope 1978;I;88:162-171.

Tucker HM. Human laryngeal reinnervation: Long term experience with the nerve muscle pedicle technique. Laryngoscope 1978;II;88:598-604.

Tucker HM. Vocal cord paralysis-1979. Etiology and management. Laryngoscope 1980;90:585-590.

Tucker HM, Rusnov M. Laryngeal reinnervation for unilateral vocal cord paralysis: Long term results. Ann Otolaryngol 1981;90:457-459.

Tucker HM. Nerve-muscle pedicle reinnervation of the larynx: Avoiding pitfalls and complications. Ann Otol Rhinol Laryngol 1982;92:440-444.

Tucker HM. Complications after surgical management of the paralyzed larynx. Laryngoscope 1983;93:295-298.

Tulleners EP, Harrison IW, Raker CW. Management of arytenoid chondropathy and failed laryngoplasty in horses. 75 cases (1979). JAVMA 1988;I;192:670-675.

Tulleners EP, Harrison IW, Mann P, Raker CW. Partial arytenoidectomy in the horse with and without mucosal closure. Vet Surg 1988;II;17:252-257.

West JB, Respiratory Physiology - The essentials. 2nd ed. Baltimore,Md: The Williams and Wilkins Co, 1979;86-114.

White NA, Blackwell RB. Partial arytenoidectomy in the horse. Vet Surg 1980;9:5-12.

Williams WL. The Surgical relief of roaring. 48th Ann Conf AVMA 1911:299-317.

Woodman DG. A modification of the extralaryngeal approach to arytenoidectomy for bilateral abductor paralysis. Arch Otolaryngol 1944;36:63-65.

Yagi M, Ogura JH, Kawasaki M, Takenouchi S. Physiological studies of the replanted canine larynx. Ann Otol Rhinol Laryngol 1966;75:849-863.

Young SS, Hall WL. A rapid, non-invasive method for measuring total respiratory impedance in the horse. Eq Vet J 1989;21:99-105.

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