Effects of Arytenoid Adduction on Laryngeal Function Following Ansa Cervicalis Nerve Transfer for Vocal Fold Paralysis in an In Vivo Canine Model

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Laryngeal reinnervation with the ansa cervicalis has been proposed as a treatment for human unilateral vocal fold paralysis (UVFP). This study tested the assumption that results from reinnervation could be improved if combined with medialization surgery. Six canine subjects underwent recurrent laryngeal nerve section and reinnervation with a branch of the ansa cervicalis. After reinnervation, vocal function was assessed before and after arytenoid adduction. Although laryngeal function improved significantly following reinnervation, results were significantly enhanced by the addition of medialization surgery. The implications for the treatment of human unilateral vocal fold paralysis are discussed.

INTRODUCTION

Many treatments are available for unilateral vocal fold paralysis (UVFP). Thyroplasty is preferred by many laryngologists, although implant selection and methods are continuing sources of controversy. Other clinicians recommend reinnervation of the paralyzed recurrent laryngeal nerve (RLN) with a branch of the ansa cervicalis nerve, citing the possibility of a "normal" voice following the procedure.[1–5]

Although most laryngologists agree that medialization is an important component of the treatment of vocal fold paralysis, the optimal procedure is still debated. Both Teflon® injection and arytenoid adduction have proponents. A canine study comparing medialization surgeries suggested that arytenoid adduction produced better results than thyroplasty or Teflon injection.[6] Arytenoid adduction is particularly useful when there is a persistent posterior glottic chink during the closed part of the vocal cycle, and is also helpful in conjunction with type I thyroplasty in cases of marked atrophy or bowing of the membranous portion of the vocal fold.[7]

In this study, an in vivo canine model was used to explore the possibility of combining reinnervation with arytenoid adduction to improve functional results of treatment for UVFP. Experimental evidence suggests that vibration is more symmetric under conditions of symmetric vocal fold tension, thus producing a better voice.[8] By combining medialization with ansa cervicalis reinnervation, we hoped to provide both a midline vocal fold and greater tone to the muscular portion of the fold.

MATERIALS AND METHODS

Experimental Design

Six adult mongrel dogs (approximately 25 kg each) were studied using the in vivo canine model of phonation (vide infra). Animals were assigned to one of two groups. The first group (group A; 4 dogs) received transtracheal nerve stimulation (vide infra). Phonation was evaluated with and without superior laryngeal nerve (SLN) stimulation; RLN stimulation was always present for this group. Measurements were made under four conditions: prior to creation of paralysis (normal condition), after paralysis but prior to reinnervation (paralyzed condition), after reinnervation but prior to arytenoid adduction, and after arytenoid adduction.

A second group (group B; 2 dogs) received direct stimulation to the RLN and SLN. In this group, phonation was evaluated only for the normal, reinnervated, and reinnervation plus adduction conditions. Group B was studied because direct nerve stimulation is a much more precise phonation model, allowing multiple levels of nerve stimulation; transtracheal stimulation does not provide adequate control of nerve stimulation levels. It was not possible to obtain data

Presented at the Meeting of the Western Section of the American Laryngological, Rhinological and Otological Society, Inc., La Jolla, Calif., January 8, 1994. Recipient of the Shirley Barron Award.

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This research was supported by an NIH grant (R01 DC 00855-01) and by Veterans Administration research funds.

This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Los Angeles.

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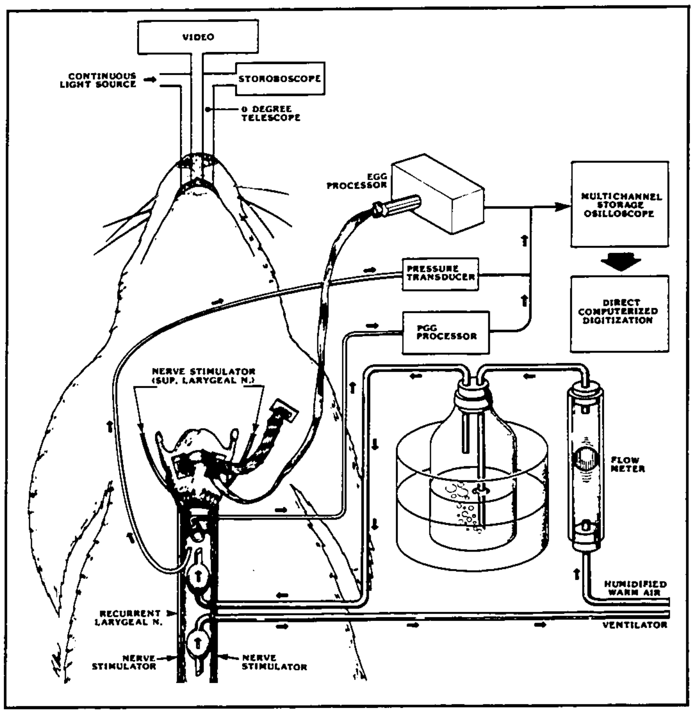

Fig. 1. The in vivo canine preparation with computer, videostroboscopic, glottographic, and aerodynamic equipment. (Reprinted with permission from Sloan, S.H., *et al.*: Effect of Asymmetric Vocal Fold Stiffness on Traveling Wave Velocity in the Canine Larynx. *Otolaryngol Head Neck Surg*, 107:516–526, 1992.)

for the paralyzed state for group B without excessive dissection and subsequent scarring of perineural tissues.

In Vivo Canine Model of Phonation

All dogs were premedicated with acepromazine maleate intramuscularly. Intravenous pentobarbital sodium was given to the level of corneal anesthesia, and additional doses were used as needed to maintain this level of anesthesia throughout the experiment.

The animal was placed supine on the operating table. A midline incision was made to expose the trachea from the sternal notch to the hyoid bone (Fig. 1). A 1-cm button was used to suspend the epiglottis from a fixed point to allow direct visualization of the larynx. In the normal state, both RLNs were identified and preserved. After reinnervation, the RLN on one side and the ansa cervicalis-RLN anastomosis on the opposite side were identified and preserved. The superior laryngeal nerves were identified and preserved on both sides in both states.

A low tracheostomy was performed at the level of the suprasternal notch, through which an endotracheal tube was passed to allow ventilator-assisted respiration. A second tracheostomy was performed in a higher location, through which an endotracheal tube was passed cephalad with the tip positioned approximately 10 cm below the level of the vocal folds. The cuff was inflated to just seal the trachea and humidified heated air was passed through this tube rostrally. Flow was monitored with a Gilmont flowmeter, and remained constant at 318 mL/s. The air was humidified and heated by being bubbled through 5 cm of heated water so that its temperature was 37°C when measured at the glottic outlet.

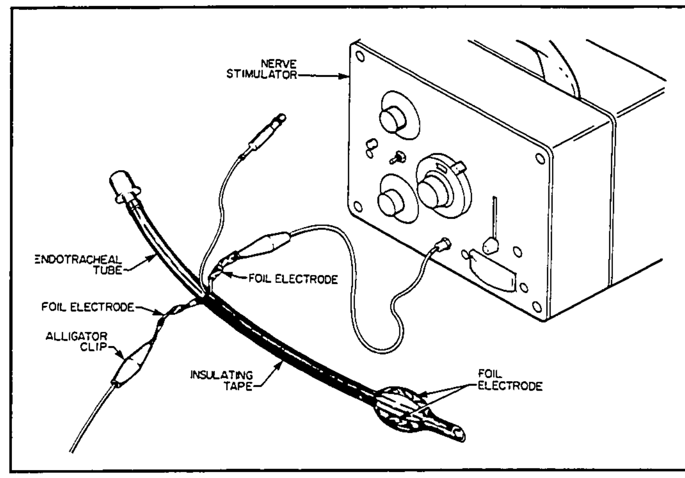

Fig. 2. Modified endotracheal tube and nerve stimulator used for transtracheal stimulation in group A canines.

In group A, the rostral tube was modified by the placement of foil electrodes on its cuff (Fig. 2).[9–11] The cuff was rotated in the trachea so that the electrodes were situated adjacent to the tracheoesophageal groove. The ground electrode was secured to the anterior tracheal wall. Electrodes were placed directly in the cricothyroid muscles to represent superior laryngeal nerve stimulation. Phonation was evaluated both with and without SLN stimulation; RLN stimulation was always present for these dogs. Stimulation frequency was 80 Hz, with intensity ranging from 3.0 to 6.0 mA. A pulse duration of 1.5 msec was used for each trial. Stimulation levels were chosen to provide optimal quality and intensity of phonation. Three trials were performed for each animal at each combination of nerve stimulation levels. Each trial contained a 2-second period of phonation. A 500-msec segment of this phonation was recorded on the personal computer, and 200 msec were analyzed.

In group B, a 1-cm segment of each superior laryngeal nerve was isolated, and Harvard miniature electrodes were applied bilaterally. In the normal condition (prior to creation of paralysis), electrodes were applied to both RLNs; after reinnervation, electrodes were applied to one RLN and one ansa cervicalis (anastomosed to RLN) on the opposite side. A Grass stimulator (model 54H; Quincy, Mass.) provided stimulation to both SLNs, with current set at 0.5 to 1.2 mA and a pulse duration of 1.5 msec. A constant current stimulator (WR Medical Electronics RLN Stimulator, Model S2LH, St. Paul, Minn.) provided electrical current to the RLNS and the ansa cervicalis. Stimulation frequency was 80 Hz, with intensity ranging from 0.1 to 2 mA and pulse duration of 1.5 msec.

Three trials were performed for each animal at each combination of nerve stimulation levels. The levels of nerve stimulation were selected by determining the range of stimulation which would produce phonation, then selecting three representative levels within this range. Each trial contained a 2-second period of phonation; a 500-msec segment was recorded on the personal computer, and 200 msec were analyzed.

Surgical Technique

The RLN and ansa cervicalis nerves were identified

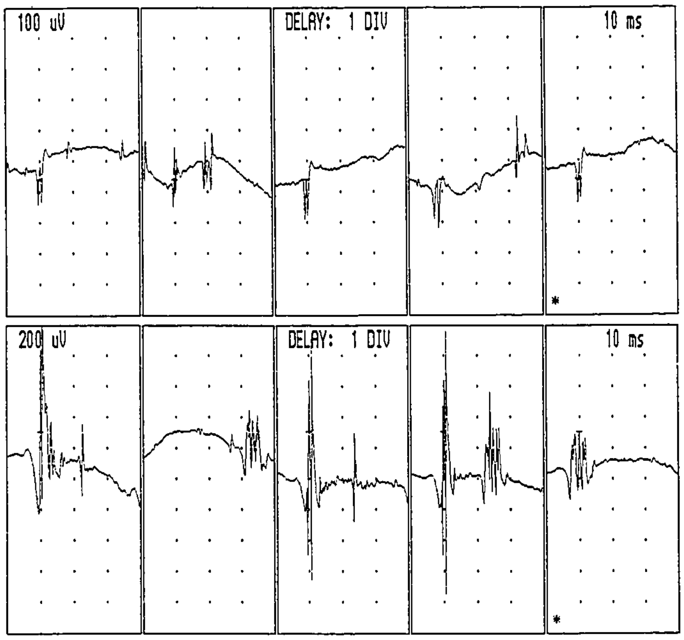

Fig. 3. Representative electromyographic (EMG) tracings following ansa cervicalis reinnervation from posterior cricoarytenoid muscle demonstrating small motor units **(top)** and polyphasic motor units **(bottom).**

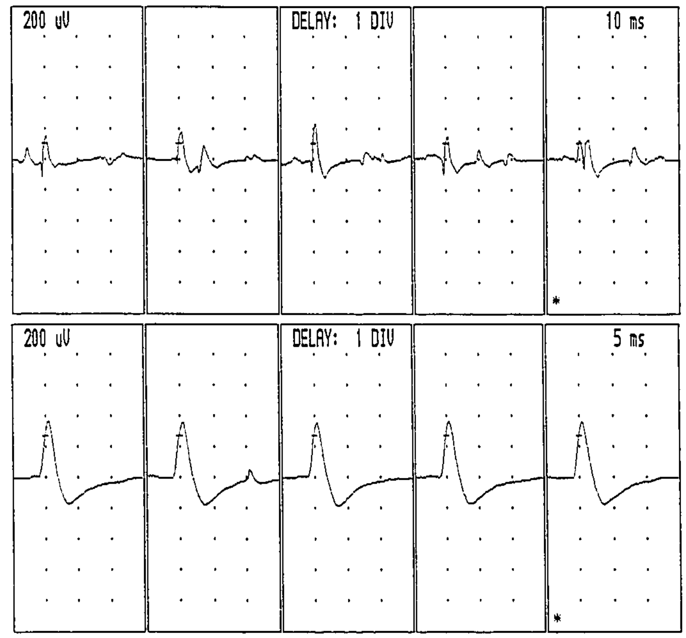

Fig. 4. Representative EMG tracings following ansa cervicalis reinnervation from thyroarytenoid **(top)** and lateral cricoarytenoid **(bottom)**, both showing motor unit activity.

bilaterally. On the basis of proximity of the two, one side was chosen for sectioning. At approximately 5 cm from the cricothyroid joint, the RLN was sharply sectioned. The proximal end was tied off with a 2-0 silk suture. The distal end was tagged with a 2-0 Prolene® suture. The wound was closed, and the canines recovered so that the effects of chronic paralysis could be measured. Three months later, the distal end of the sectioned recurrent laryngeal nerve was identified, freshened, and anastomosed end-to-end to the ansa cervicalis with a 10-0 nylon. The wound was closed in 3 layers with 3-0 Vicryl®.

Five to 6 months later, phonatory characteristics were evaluated with the in vivo canine model of phonation, as described previously. Immediately after this evaluation, arytenoid adduction was performed as described by Isshiki, *et al.*[12] The thyroid cartilage at the side of the anastomosis was exposed to the posterior margin. The constrictor muscles were sectioned. The mucosa of the pyriform sinus was lifted to identify the muscular process of the arytenoid. A 3-0 nylon suture was placed around the muscular process with the use of a 16-gauge angiocatheter. The tension on the stitch was adjusted to maximum acoustic intensity.

Measurements of Vocal Function

Voice signals were recorded with a 1-inch Bruel & Kjaer condenser microphone placed 30 cm from and level with the glottic outlet. The microphone was directed 90 degrees from the direction of the sound source. The sound level was measured in decibels with a Bruel & Kjaer C-scale sound meter (type 2209). Background noise was 35 dB lower than signal levels. The acoustic signal was low-pass filtered at 3 kHz, digitized at 20 kHz, and stored on a hard disk of a personal computer. Digitized acoustic signals were analyzed with a signal processing package (C-Speech). Percent jitter, shimmer, and signal-to-noise ratio were calculated for each trial.

Subglottic pressure was measured with a Millar Micro-tip catheter pressure transducer (Model SPC-330, size 3F, Millar Instruments, Inc., Houston, Tex.) passed rostrally through the superior tracheotomy and placed 5 cm below the glottis. This signal was low-pass filtered at 3 kHz, digitized at 20 kHz, and stored in a personal computer. Because of variations in subglottic pressure during a cycle, peak pressure was used as the dependent variable.[13] The peak was identified with a commercially available signal processing package (C-Speech).

Vocal efficiency was calculated as the ratio of the acoustic power of the voice to the subglottic power. The total acoustic power was calculated using the formula[14]:

$$\text{Total sound power} = 2r^2P_e^2/P_0c$$

This formula applies to the sound power radiating with no directivity into a hemisphere of area $2r^2$, where r is the distance away from the source. P_0c is the specific acoustic impedance, and equals 41.1 dynes × s/cm³ in air at 20°C. P_e is the root mean square sound pressure in dynes per cubic centimeter at a distance r from the sound source. The subglottic power is the product of the flow rate and the subglottic pressure.

Videostroboscopy and Videolaryngoscopy

Videostroboscopy was performed before and after arytenoid adduction using a Storz laryngostrobe unit (model 8000). The stroboscope was connected to a Storz 0-degree telescope via a fluid-filled light cable. Images were recorded with a Jedmed CCD (charge-coupled device) video camera (model 70-5110) and a Sony U-matic videocassette recorder (VO-5850). The video images were analyzed frame by frame.

Evoked Electromyography

Concentric electromyography (EMG) needle electrodes were placed transorally into the thyroarytenoid, inter-

TABLE I.
Summary of Stroboscopic Analysis.

Laryngeal State	Glottic Closure	Mucosal Motion	Cord Motion
Normal	Complete	Two-mass	Symmetric
Reinnervation	Incomplete; posterior glottic leak	Two-mass; delay present	Asymmetric
Reinnervation and adduction	Complete	Two-mass; delay absent	Symmetric

arytenoid, posterior cricoarytenoid, and lateral cricothyroid muscles. The results were recorded using a Nicolet Viking II EMG instrument. The signal was high-pass filtered at 20 Hz, low-pass filtered at 10 kHz, digitized at 20 kHz, and recorded into the hard drive of a personal computer.

Spontaneous EMG recordings were made during light anesthesia to detect the response of the thyroarytenoid, interarytenoid, lateral cricoarytenoid, and posterior cricoarytenoid muscles of the reinnervated side. These were compared to the spontaneous EMG from the equivalent intrinsic laryngeal muscles of the intact side.

RESULTS

Electromyography and Stroboscopy

Spontaneous EMG of the posterior cricoarytenoid, lateral cricoarytenoid, thyroarytenoid, and interarytenoid muscles revealed reinnervation potentials in all the muscles. Reinnervation was demonstrated by the presence of motor unit activity. Polyphasic potentials were also noted, indicating incomplete reinnervation (Figs. 3 and 4).

Table I summarizes the stroboscopic data. Phonation in the normal larynx showed complete closure and bilaterally symmetrical mucosal wave motion. In the paralyzed state, vocal fold closure was incomplete and two-mass vibration did not occur. Mucosal wave motion was absent on the paralyzed side. In the reinnervated state, the posterior glottis did not close completely. Arytenoid adduction resulted in complete closure of the glottis, including the posterior glottic chink. Two-mass motion (upper and lower margin) of the mucosa on both cords was absent in the paralyzed state, but was observed in all other conditions. However, there was a slight phase delay between the bilateral mucosal waves in the reinnervated animals. This was corrected by arytenoid adduction.

Acoustic and Aerodynamic Data

Mean values and standard deviations for jitter, shimmer, signal-to-noise ratio, subglottic pressure, and vocal efficiency for the different surgical conditions are shown in Figures 5 through 8. Recall that phonation was evaluated in four settings in group A, and three settings in group B. Because experimental conditions were not directly comparable across the two groups of dogs, separate analyses were undertaken for each group. These are described in turn (vide infra).

Transtracheal stimulation study (group A). All dependent variables (jitter, shimmer, signal-to-noise, vocal efficiency, and subglottic pressure) showed consistent patterns of significant correlation across the four experimental conditions (normal, paralyzed, reinnervated, reinnervated plus adduction). However, multivariate analyses could not be used to control for these intercorrelations, due to the limited number of measurements that could be made for each dog in each condition. For this reason, separate three-way repeated measures analyses of variance (ANOVAs; dog by SLN stimulation level by surgical condition, with repeated measures on surgical condition) were undertaken for each dependent measure. The intercorrelation among dependent measures should be borne in mind in interpreting these analyses.

Analyses showed significant effects of surgical conditions on all dependent variables. Planned comparisons were used to examine the effects of surgical treatments on phonation. Significant differences were found between the paralyzed and reinnervated conditions, between the reinnervated and the reinnervated plus arytenoid adduction condition, and between the reinnervated plus adducted and the normal conditions (Table II). Reinnervation did improve phonation, but improvements were enhanced by the addition of arytenoid adduction. However, phonation after the combined treatment still differed significantly from normal phonation.

Direct stimulation study (group B). As in the transtracheal stimulation study, dependent variables were significantly correlated across experimental conditions, but the limited number of observations again precluded the use of multivariate statistics. Therefore, separate four-way repeated measures ANOVAs (dog by SLN stimulation condition by RLN stimulation condition by surgical condition, with repeated measures on surgical condition) were conducted for each dependent measure.

Results confirm findings of the transtracheal stimulation study. Significant effects of surgical conditions were found for all dependent measures. Planned comparisons again examined differences between surgical conditions. The addition of arytenoid adduction consistently enhanced the effects of reinnervation on phonation. Phonation after the combined surgeries differed significantly from normal phonation for all variables except subglottic pressure (Table III).

DISCUSSION

Results of this study support the conclusion that arytenoid adduction improves phonation in canines with RLN paralyses that have been previously treated with ansa cervicalis reinnervation. Intercorrelations among dependent measures of vocal function preclude

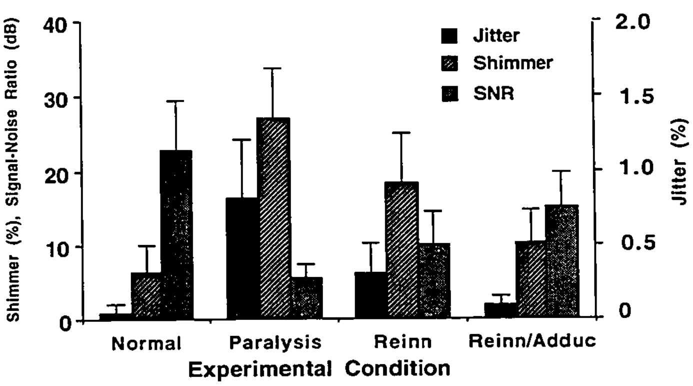

Fig. 5. Acoustic data for group A for the four experimental conditions. The values are means ± standard deviations. SNR = signal-to-noise ratio.

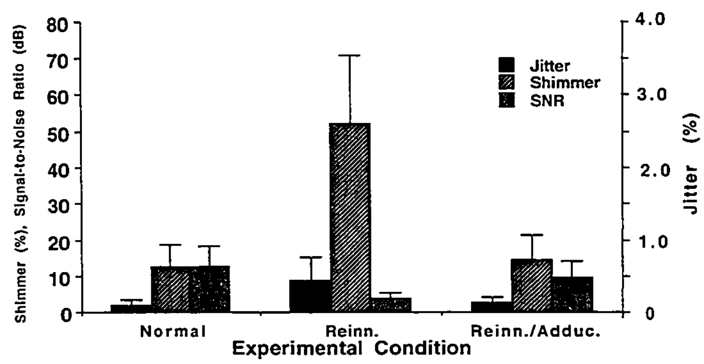

Fig. 7. Acoustic data for group B for the three experimental conditions. The values are means ± standard deviations. SNR = signal-to-noise ratio.

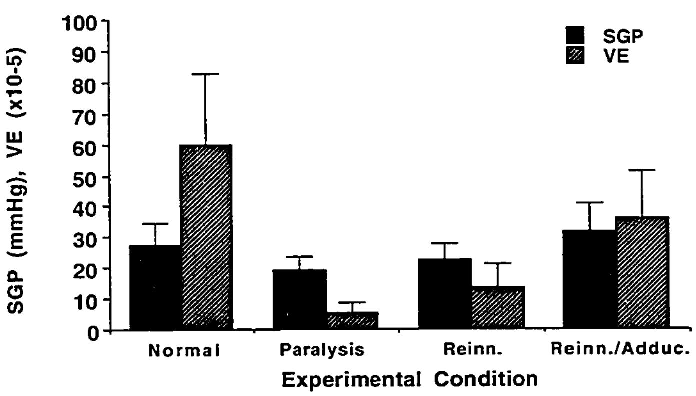

Fig. 6. Aerodynamic data for group A. The values are means ± standard deviations. SGP = subglottic pressure; VE = vocal efficiency.

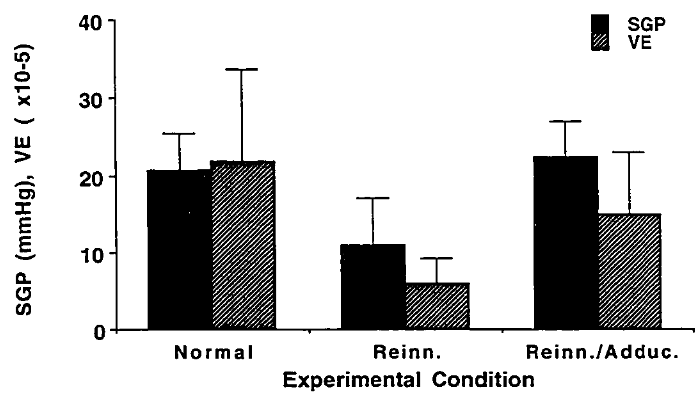

Fig. 8. Aerodynamic data for group B. The values are means ± standard deviations. SGP = subglottic pressure; VE = vocal efficiency.

specification of the precise nature of this improvement. However, global improvement in vocal function following medialization may be related to overall improvement in the symmetry of vibration, improved glottal closure, greater subglottic pressure, and improved entrainment of the vocal folds. Recall that, although the two mass mucosal waves were present on the vocal folds of the reinnervated animals, the innervated side appeared to lag slightly compared to the normal side. This slight phase delay disappeared following arytenoid adduction.

Crumley, *et al.*[3,4] described nerve transfer from the ansa cervicalis to the recurrent laryngeal nerve as a treatment for UVFP, and compared results with Teflon® injection. They reported that the reinnervation procedure was superior to Teflon injection, and claimed that normal voice was produced following the procedure. In a subsequent report,[5] 11 of 12 patients had results "far superior" to Teflon injection or Isshiki thyroplasty. Crumley[4] enumerates several advantages of reinnervation, including restoration of tone to intrinsic laryngeal muscles, more normal compliance of the folds, stabilization of the vocal process and arytenoid, and more symmetrical mucosal wave motion. The increased stiffness of the vocal fold and stabilizing effect of the reinnervated muscle on the arytenoid apparently improve the ability of the larynx to compensate for the lack of volitional vocal fold movement.[3–5] All these factors tend to normalize the position of the folds and provide additional muscular adduction of the arytenoid cartilage. Although this adduction does not occur synchronously with the other side, it does position the vocal folds appropriately for good phonatory quality, at least in most human studies.[3–5] It must be noted, however, that although nerve transfer may provide tone to the vocal fold, the fold remains immobile. This lack of movement is the most significant disadvantage of reinnervation when used alone.

Although the literature contains reports of small numbers of patients with excellent qualitative results from nerve transfer,[15] the canine model was used here because it permits objective comparison of the results of surgical treatments for UVFP. The canine has been used extensively to study laryngeal nerve reinnervation.[16,17] Our results indicate that, although phonation is improved following ansa cervicalis reinnervation, further improvement is possible with a medialization procedure. Reinnervation improves the tone of the laryngeal muscles, resulting in a more symmetric mucosal wave during phonation. However, impaired closure of the glottis may persist due to the position of the fold. By pexy of the vocal fold in the

TABLE II.
Results of Planned Comparisons for Transtracheal Stimulation Group.

Comparison/ Dependent Variable	*F*	*df*	*P*
Paralysis vs. Reinn			
Jitter	76.82	8,10	<.01
Shimmer	48.85	8,10	<.01
SNR	207.50	8,10	<.01
SGP	21.02	8,10	<.01
VE	18.29	8,10	<.01
Reinn vs. Reinn/Adduc			
Jitter	53.73	8,10	<.01
Shimmer	21.87	8,10	<.01
SNR	40.03	8,10	<.01
SGP	21.33	8,10	<.01
VE	79.59	8,10	<.01
Normal vs. Reinn/Adduc			
Jitter	14.76	8,10	<.01
Shimmer	28.38	8,10	<.01
SNR	57.81	8,10	<.01
SGP	5.54	8,10	<.01
VE	54.00	8,10	<.01

Reinn = reinnervation; Adduc = arytenoid adduction; SNR = signal-to-noise ratio; SGP = subglottic pressure; VE = vocal efficiency.

TABLE III.
Results of Planned Comparisons for Direct Stimulation Group.

Comparison/ Dependent Variable	*F*	*df*	*P*
Reinn vs. Reinn/Adduc			
Jitter	118.19	8,8	<.01
Shimmer	190.55	8,8	<.01
SNR	178.16	8,8	<.01
SGP	295.29	8,8	<.01
VE	17.14	8,8	<.01
Normal vs. Reinn			
Jitter	10.99	8,8	<.01
Shimmer	10.35	8,8	<.01
SNR	25.21	8,8	<.01
SGP	3.29	8,8	<.01
VE	79.52	8,8	<.01

Reinn = reinnervation; Adduc = arytenoid adduction; SNR = signal-to-noise ratio; SGP = subglottic pressure; VE = vocal efficiency.

midline, arytenoid adduction corrects this problem.

In a paralyzed vocal fold, incomplete closure may result in a lack of entrainment of the folds and asymmetries in the mucosal traveling wave. Incomplete glottal closure may prevent the development of adequate subglottic pressure, limiting vocal efficiency. In the present study, average peak subglottic pressure in the paralyzed condition was smaller than that in normal phonation. This was only partially corrected by reinnervation. Higher subglottic pressures were generated following arytenoid adduction. Increasing subglottic pressure toward normal values may optimize the synchronization of the vocal folds, resulting in improved entrainment and acoustic measures. It is important to note that posterior glottal gaps are often present in normal human speakers, particularly in women. Therefore, in humans, a posterior glottal gap may not represent an abnormality, nor a deficiency of reinnervation procedure.

Results of this study suggest that, with medialization and improved closure, the phonatory results of ansa cervicalis reinnervation can be improved. After arytenoid adduction, there was marked improvement in the symmetry of stroboscopic waves during phonation. Isshiki[8] first reported that, in asymmetric vocal fold vibration, the degree of closure profoundly affected the symmetry of vibration. In all 6 canines in this study, a persistent posterior glottal chink after reinnervation was closed with arytenoid adduction. This approach has theoretical advantages over the combination of nerve-muscle pedicle and medialization thyroplasty suggested by Tucker.[18] Because thyroplasty does not adduct the vocal processes, it is less useful than arytenoid adduction when combined with reinnervation.

A point of concern with arytenoid adduction following reinnervation in humans is that it could endanger the distal portion of the recurrent laryngeal nerve where it enters the larynx. The muscular process of the arytenoid (through which a suture is passed in arytenoid adduction) is within 2 mm of the distal passage of the recurrent laryngeal nerve, and is close to the exit of the abductor branch of this nerve.

The present data suggest that patients who have undergone nerve transfer might improve further if a medialization procedure such as arytenoid adduction were added to the surgical therapy. These procedures appear to complement each other in treatment of unilateral vocal fold paralysis. The data do not explain the normal voice achieved in a number of patients in the study of Crumley, *et al.*[2] One possibility is that humans with UVFP are better able to compensate for the paralysis than is the canine model of phonation. Further, the posterior glottic chink is larger in canines than in humans. For this reason, the canine data in this study must await verification in human patients.

CONCLUSION

The combination of ansa cervicalis to recurrent laryngeal nerve anastomosis and arytenoid adduction appears to be a desirable option for treatment of the UVFP. In the in vivo canine model, phonation improved significantly compared to reinnervation alone. This combination technique shows promise as a treatment for UVFP. Only prospective trials, however, are capable of determining the best of many available treatments for human UVFP.

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For more information, contact Dr. N. Ravindranathan, Maxillo-Facial Unit, Ripas Hospital, Bandar Seri Begawan, Brunei Darussalam, at fax number 673-2-447583; or the AAOMFS Congress Secretariat at fax number 082-429754/428969.

Congress of Otorhinolaryngologic Societies Set in Pakistan

The Eighth Asia-Oceania Congress of Otorhinolaryngologic Societies will be December 10–14, 1995, in Karachi, Pakistan.

Information regarding registration and submission of abstracts can be obtained via fax contact with Prof. M. Jalisi, Department of ENT, Dow Medical College, Civil Hospital, Karachi, Pakistan. The fax number is 92-21-5689860.