Transplantation of the Canine Larynx

CARL E. SILVER, M.D., RICHARD G. ROSEN, M.D., IRVING DARDIK, M.D., HUGH EISEN, M.D., BARRY H. SCHWINNER, M.D., MAX L. SOM, M.D.

From the Head & Neck Service, Surgical Division, Montefiore Hospital and Medical Center, and the Albert Einstein College of Medicine, Bronx, New York

Replacement of the human larynx with an allograft from a cadaver would provide a means of rehabilitation for laryngectomized patients. This problem has been under investigation in our laboratory since 1965. Early studies described a technic for excision and reimplantation of the canine larynx and related the short and long-term results following autotransplantation of the larynx. The present report summarizes experience with laryngeal transplantation in dogs during the past 3½ years. This work includes 1) investigation of the anatomy and blood supply of the canine larynx; 2) comparison of various modifications in transplantation technic with evolution of an optimal procedure; 3) studies on laryngeal autografts indicating the feasibility of transplantation and the anatomic and functional results in surviving animals; 4) studies of laryngeal allografts to determine the nature and timing of rejection and 5) attempts to prolong laryngeal allograft survival with immunosuppressive drugs.

Ogura and co-workers developed a successful method for transplantation of the larynx in dogs and reported results of physiologic studies and comparison of various transplant technics. The findings of Ogura’s group and basic principles of transplantation are similar to ours, and reinforce a conviction that canine experience is applicable to humans.

Anatomy and Blood Supply of the Canine Larynx

The cartilagenous and mucosal relations of the canine larynx are similar to those of humans. The hyoid bone and strap muscles are shaped differently, but have the same basic attachments. The canine thyroid gland consists of two separate lobar structures, each deep to the ribbon like sternothyroid muscles which receive blood supply from a single descending branch of the cranial thyroid artery.

The arterial supply of the larynx arises from the cranial thyroid and laryngeal arteries (Fig. 1). The cranial thyroid, a direct branch of the common carotid, provides a descending branch to the superior pole of the thyroid gland, then enters the substance of the cricothyroid muscle as the major arterial supply to the larynx. The small laryngeal artery is a branch of the external carotid. It penetrates the thyrohyoid membrane as does the superior laryngeal artery in humans, but unlike the human situation, provides only a minor contribution to laryngeal blood supply. The internal carotid artery of the dog is a small vessel which is not of critical importance to the brain. This fact assumes significance in designing procedures for revascularization of the transplanted larynx in dogs.

Venous drainage of the larynx issues through penetrating vessels in the thyrohyoid membrane to a transverse venous sinus called the “laryngeal impar.” The laryngeal impar which is superficial to the
body of the hyoid bone, sends numerous suprahypoid tributaries to the lingual veins, and laterally, empties into two large tributaries which join the external jugular veins. The latter vessels are the major veins of the neck, the canine internal jugulars being insignificantly small.

Transplantation Technic

a) Basic Principles: The key to successful transplantation of any organ consists of the isolation, interruption and reestablishment of blood supply and other essential functional attachments. Because the larynx is supplied by several small vessels and has complex attachments, the technic is more difficult than with many other organs. Reliable anastomosis of small laryngeal and cranial thyroid arteries has not been possible.\(^6\)\(^7\) It is, however, possible to isolate small vessels supplying the larynx in continuity with the major carotid and external jugular vessels. All attachments of these vessels, other than those directly entering or issuing from the larynx can be divided, and vascular interruption and reanastomosis can be performed on major arteries and veins. These vessels are large enough to be anastomosed by direct suture or by stapling methods or prosthetic ring technics.

b) Arterial Revascularization: Three methods of arterial revascularization have been employed (Fig. 2). Type I employs the laryngeal and cranial thyroid vessels bilaterally which provides an excellent blood supply to the larynx, but is time consuming and technically difficult. Type II was in-
vestigated briefly because of reports that a single cranial thyroid artery provides 40% of the laryngeal blood supply, and is sufficient to support the entire larynx. Type III employs the cranial thyroid arteries bilaterally. The relative successes achieved with these procedures are summarized in Table 1. Type III was employed in all recent cases.

In 48 laryngeal transplants in this series the carotid system distal to the laryngeal blood supply was completely ligated, with no attempt to revascularize the internal carotids. In no instance could neurological complications attributable to this technic be detected. Reestablishment of the carotid-cerebral blood supply is unnecessary.

During earlier experiments, all arterial and venous anastomoses were performed by direct suture. This method, although time-consuming, was adequate. Later, the "American Vascular Stapler" was employed. The stapler saved time, and resulting patency was at least as good as with direct suture. Some dogs died during the first postoperative week from blowouts or complete disruptions of staple carotid arterial anastomoses. This occurred only in dogs with marsupialized larynxes but not with direct reanastomoses to the trachea. The completely reconstructed laryngotracheal complex served as a splint, and protected the vascular anastomoses from distracting forces of motion of the head and neck. Routine reinforcement of the stapled anastomoses with three or four interrupted fine silk sutures eliminated anastomotic disruptions.

c) Venous Reconstitution: The method of reestablishment of venous drainage is diagrammed in Figure 1. This has proven satisfactory and has not been altered.

d) Pharyngeal Reconstruction: Pharyngeal mucosa was anastomosed with a continuous circumferential suture of 000 chromic catgut by a simple over-and-over technic, with no attempt to invert mucosa. The closure was reinforced by approximation of the suprahyoid and inferior constrictor muscles of the transplant with those of the recipient. Fewer pharyngeal leaks have been encountered with this technic than with inversion of the mucosal layer and reinforcing sutures. Pharyngeal leaks were tolerated by the dogs and closed spontaneously if the larynx was viable.

e) Laryngotracheal Reconstruction: Figure 3 shows the two types of laryngotracheal

---

* Codman & Shurtleff, Randolph, Mass.

---

**TABLE 1. Vascular Reconstruction—Incidence of Ischemic Necrosis According to Type of Revascularization**

<table>
<thead>
<tr>
<th>Dogs Survived</th>
<th>Ischemic Necrosis</th>
<th>% Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 or more days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Type II</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Type III</td>
<td>21</td>
<td>6*</td>
</tr>
</tbody>
</table>

* In 2 instances, could not distinguish ischemic necrosis from rejection.
cheal reconstruction employed. Type A, direct anastomosis of the laryngeal transplant to the stump of the recipient trachea, with a distal temporary tracheostomy, was employed in the first 30 operations. Analysis of results indicated that only 60% of the animals survived the immediate postoperative period. Of surviving animals, occlusion of the airway or pneumonia due to aspiration accounted for 61% of the mortalities (Table 2).

Type B reconstruction was employed in the next 18 operations. This technique consisted of marsupialization of the subglottic region of the transplant into the upper part of the midline incision by circumferential mucocutaneous suture and creation of a permanent type tracheostomy with the recipient tracheal stump. In this manner, aspiration of food and saliva into the distal tracheobronchial tree was prevented, and a wide open airway was created which was less subject to obstruction from plugging or dislodgement of the tracheotomy tube. As indicated in Table 2, immediate and long-term survival were considerably improved, and pneumonia and tracheal occlusion were eliminated as causes of death in the Type B series.

f) *Performance of the Transplant:* One or two teams operated depending on whether an auto or allograft was performed. Through a midline incision the donor larynx and blood supply were completely mobilized, while the recipient larynx was excised and the vessels prepared. The donor larynx was placed in cold heparinized saline as soon as it was excised. Perfusion of the arteries with cold heparinized saline during the period of ischemia was found to impede the procedure and was not of demonstrable benefit. The pharyngeal mucosa was anastomosed first, in the manner described. Revascularization was accomplished by anastomosing first one vein, then one artery, followed by the second vein and artery. Approximation of the muscles, creation of the laryngostomy and tracheostomy and skin closure completed the procedure. A #12 laryngectomy tube was inserted in the tracheostomy.

Total ischemic time averaged about 20 minutes. At least 30 minutes of ischemia appears to be tolerated by the canine larynx. Most instances of ischemic necrosis occurred when at least 45 minutes of ischemia time had elapsed. Completion of the pharyngeal and venous anastomoses before the first arterial anastomosis greatly facilitated the technical procedure. The time elapsed did not significantly prolong the period of ischemia. The procedure considered optimal for canine laryngeal transplantation (Type III-B) is diagrammed in Figure 1.

g) *Postoperative Care:* The dogs were given large doses of parenteral antibiotic drugs until complete healing. Fluids were given daily by clysis as necessary. Most Type B dogs ate and swallowed well by the fifth postoperative day. A great deal of personal attention was given to tracheostomy cannulae and airways. Suction was rarely necessary, nor was artificial humidification employed. Immunosuppressive agents were given parenterally or orally, depending on the animal's ability to swallow. Biopsies of laryngeal mucosa were obtained readily through the laryngostomy in Type B dogs.
Autologous Transplants

Eighteen laryngeal autotransplants were performed. Ten dogs survived 4 days or longer. Four survived more than one week. The longest survivor lived 55 days. All autografts had Type A laryngotracheal reconstructions. Tracheal occlusions accounted for five deaths, and pneumonia for one. Necrosis of the larynx in four dogs, was manifest by the fourth or fifth postoperative day. Two of these were Type I arterial reconstructions, with intraoperative ischemic intervals lasting over 30 minutes in both. The other two were Type II procedures.

a) Gross Anatomical Findings: The reimplanted larynx developed erythema and edema during the first few days, which gradually subsided. The glottic region was usually the most persistently edematous, requiring 2 to 3 weeks before resolution. By the fourth week, the larynx was normal in appearance (Fig. 4).

b) Functional Changes: No attempt was made to restore innervation to the transplants. The vocal cords in all long-term survivors became fixed in an abducted position, thus providing a good airway when glottic edema subsided (Fig. 5). Feeding, in Type A dogs, was associated with minimal aspiration and occasional regurgitation, but swallowing function was satisfactory. The dogs maintained their weight by oral feedings and only one, the 55-day survivor, had moderate bronchopneumonia at autopsy. Cine-esophograms usually demonstrated a minimal degree of aspiration.

c) Evaluation of Results: The autologous transplantation experiments indicated the feasibility of laryngeal transplantation from the viewpoint of reestablishment of vascular supply, tissue survival, healing and preservation of normal anatomy. The airway was adequate, functionally.

The main difficulty was swallowing in animals with intact laryngotracheal bronchial systems. This may not be resolved by animal experimentation alone, for dogs cannot be taught how to swallow. Some animals are capable of swallowing without aspiration with denervated larynges. Despite aspiration, pneumonia occurred in only one dog, the longest survivor.

Most of the animals which did not have immune mechanisms suppressed by drugs, tolerated the aspiration which occurred. Procedures such as teflon injection into the vocal cords and cricopharyngeus myotomy have not been evaluated. No nerve anastomoses or other reinnervation procedures have been attempted. Nor has long-term function been evaluated in Type B experiments with reanastomosis of the airway.

Allografts without Immunosuppression

Eleven allografts were performed without administration of immunosuppressive agents during the postoperative period. This experiment was to study the nature and timing of rejection of the transplanted canine larynx. Six animals survived 8 days
or more, sufficient time to show signs of rejection. All operations were the III-B type. The laryngeal mucosa was observed through the laryngostomy daily, and biopsies were taken. Animals were sacrificed when necrosis was obvious, usually on the fourteenth day.

a) Rejection vs. Ischemic Necrosis: It is possible to distinguish necrosis of the larynx resulting from ischemia, from that due to rejection. Ischemic necrosis was always manifested by the fifth postoperative day by disruption of the wound and profuse salivary discharge, followed by exposure of the sloughing, foul smelling larynx. In allografted dogs, necrosis was considered due to rejection if the mucosa necrosed on the eighth postoperative day or later, and if, at autopsy at least one artery and vein were patent (Fig. 6). In these animals, wound disruption, fistula and slough did not occur. The necrotic larynx was incorporated into surrounding tissues without fistula, indicating that healing occurred before necrosis supervened (Fig. 7). In two animals that received immunosuppressive agents, the time of necrosis could not be determined and vessels could not be identified at autopsy (Table 1).

b) The Rejection Reaction: No instance of ischemic necrosis occurred in the allograft-without-immunosuppression animals. The laryngostomy revealed pink mucosa until the seventh or eighth day when increased erythema was followed by edema, friability and a grey-yellow exudate within a few days. By the twelfth to fourteenth day, the mucosa was necrotic with exposure of unhealthy-looking cartilage.

A composite of the microscopic process of rejection is shown in serial biopsies and autopsy specimens (Fig. 8). Initially, the mucosa was intact with a mixed inflammatory infiltrate in the submucosa. Submucosal inflammation varied in degree from subject to subject, but usually tended to regress by the fifth postoperative day. By the sixth day, in some specimens, the inflammatory reaction increased with proliferation of endothelium and early necrosis. On the seventh and eighth days, the epithelium sloughed, edema became predominant, and the inflammatory infiltrate became chronic, with lymphocytes and plasma cells predominating. At this time, skeletal muscles showed myolysis, and major donor vessels, although patent, were necrotic. By the tenth day all specimens were completely necrotic, with varying degrees of secondary infection. In all specimens, cartilage was intact histologically, even on the fourteenth day.

Allografts with Immunosuppression

Immunosuppressive agents were given postoperatively to 17 allografted dogs in an attempt to prolong survival of the transplant. Only five dogs survived 8 or more days, or long enough to show signs of re-

Fig. 5. Glottic view of laryngeal autograft—55 days after transplantation. Note abducted vocal cords with good airway.
jection. Of these, one larynx was viable and histologically unremarkable at time of autopsy on the eighth day. The other surviving dogs rejected transplants by the ninth or tenth day.

a) Management of Immunosuppression:
The first 12 operations were of the III-A type. Gastrotomies had been performed 2 weeks prior to transplantation, and azathioprine, 4–6 mg./Kg. was administered orally during the 3 days preceding operation. After transplantation, azathioprine was administered intravenously, in doses adjusted to maintain the leukocyte count at approximately 5,000/mm³. Hydrocortisone was given intramuscularly in daily doses of 50 mg. Only two of 12 animals lived 8 days or longer. Four did not recover from anesthesia, two others died of tracheal occlusion, two of pneumonia, and two died without recognizable lesions at autopsy, but were believed to have generalized sepsis related to leukopenia.

This discouraging experience with transplants treated with immunosuppression prompted revision of the regimen in hope
Fig. 8. Serial biopsies demonstrating progress of rejection of the laryngeal allograft shown in Figures 6 and 7. A. (top, left) Third post-transplant day. B. (top, right) At seven days. C. (bottom, left) Eight days. D. (bottom, right) Tenth day.
of reducing the physiological insult which the animals could not tolerate. Type B laryngotracheal reconstruction was developed and tested in allografted dogs without immunosuppression. Preliminary gastrostomy and pretreatment with azathioprine were eliminated. Five allografts with immunosuppression were performed, Type III-B, starting with azathioprine doses of 2 mg./Kg./day in the first dog with increments to 4 mg./Kg./day in the fifth. Hydrocortisone, 60–100 mg./day was given intramuscularly. Three of these dogs survived 8 days or longer. All rejected the transplants.

b) Evaluation of Results: We believe that larger doses of immunosuppressive agents will be required to prolong allograft survival. Earlier experiments with immunosuppression indicated that Type A animals, weakened by operation and preoperative leukopenia, could not tolerate doses of azathioprine as high as 6 mg./Kg./day. Modifications of the procedure and improvements in animal care may permit use of azathioprine in more effective levels.

Conclusions

Forty-eight canine laryngeal transplants have been performed. The animals were divided into three experimental groups: autografts, allografts without immunosuppression, and allografts with immunosuppression. In addition to alterations in the supportive regimen, modifications of the technical procedures have been tested to develop the simplest and most reliable operation. Information has been obtained on anatomical and functional changes following transplantation, the nature and timing of the rejection reaction, and development of a plan of management aimed at prolonged allograft survival.

We observed physiologic adaptations of recipients, and survival of recipient and transplant up to 55 days. The most satisfactory transplant operation employed both cranial thyroid arteries in continuity with the common carotids (Type III), the lateral tributaries of the laryngeal impar in continuity with the external jugular veins, and marsupialization of the subglottic region with creation of a permanent tracheostomy below (Type B). Unilateral revascularization did not prove satisfactory. It was not necessary to reestablish cerebral blood flow via the carotid system in any animal; interruption of the carotid system did not lead to neurologic deficit.

Ischemic necrosis of the larynx is usually associated with either a prolonged (greater than 30 minutes) ischemic interval during operation or with inadequate revascularization. Rejection of laryngeal allografts occurs uniformly when immunosuppression is not employed. Evidence of rejection can be seen in microscopic sections by the sixth to seventh day, and is grossly apparent on the seventh to eighth day after transplantation. Efforts to obtain prolonged allograft survival through the use of larger doses of agents have been hampered by inability of recipient animals to tolerate large doses of the drugs. Recent improvements in technic and in animal care may enhance the possibility of future success.

References