Autotransplantation of skeletal muscle into myocardium

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ABSTRACT In a series of 15 studies in dogs, sternocleidomastoid muscle was used to replace deficits created in left ventricular myocardium and sternohyoid muscle was used to replace portions of right myocardial wall. The five right ventricular autotransplants resulted in a 100% surgical success rate, with animals electively killed between 3 and 55 weeks after surgery. In 10 left ventricular studies excision of areas varying from 12 × 46 mm to 30 × 60 mm and incisions of from 40 mm to 70 mm in length were performed. Left ventricular studies resulted in a 60% surgical success rate, with clinically healthy animals being killed for study between 2 weeks and 59 weeks after surgery. Animals surviving the critical surgical recovery period showed no loss of weight or changes in activity. Gross findings at autopsy confirmed the viability of the skeletal muscle transplants. Borders were well healed and the grafted tissue was pliable. Histologic studies suggest that revascularization of skeletal muscle occurred from the myocardial side, and that there were healthy myocardial and skeletal muscle fibers at the junction, with evidence of regeneration.


BEFORE this study there have been no reports of skeletal muscle autotransplants to left ventricular myocardium to provide a model for studying the adaptation of skeletal muscle to repetitive stretch over an extended period of time. We postulated that the inability to produce such a model was more likely attributable to the surgical procedures and techniques used than to characteristics of the muscles themselves.

In 1974, Katz1 noted several biochemical similarities between human slow fibers and myocardial fibers. He compared the molecular weights of myosin, actin, troponin, and tropomyosin in slow skeletal and cardiac muscle and found them to be quite similar. Van Winkle and Entman2 pointed out that the general subcellular organization is similar in cardiac and skeletal muscle, including organelles such as mitochondria, sarcoplasmic reticulum, and transverse tubules. Lutz et al.3 reported that fast and slow myosin can coexist within a single muscle fiber under conditions of long-term electrical stimulation. Other studies have suggested that physiologic and morphologic characteristics of a muscle may be determined by its anatomic position and possibly by its innervation.4–10

Several investigators have attempted to transplant skeletal muscle onto or into the right ventricle, using both paced and unpaced onlay grafts of hemidiaphragm muscle.11 Unpaced grafts resulted in decreased bulk and contractility, whereas paced grafts increased in thickness, indicating a possible myotrophic effect produced by electrical stimulation. Inlay grafts in 16 dogs resulted in the survival of three animals for at least 24 hr; however, the denervated inlay grafts showed motion paradoxical to that of the heart.11 In one animal studied 6 weeks after grafting circulation had been established at the junction of systemic arterial blood supply and coronary arteries. Microscopic studies revealed an increase in collagen but otherwise normal-appearing muscle cells. In recent work by Mocovii et al.12 a diaphragmatic flap was grafted to the right ventricle and stimulated by electrically pacing the phrenic nerve and the result was paradoxical thinning of the grafted muscle during systole. Microscopic studies were not performed by these authors.

The purpose of the series of studies reported here was first, to study the surgical outcome of autotransplantation of skeletal muscle introduced into myocardium under conditions of stretch and second, to document gross and histologic findings related to the

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viability and adaptation of skeletal muscle for an extended period of time after autotransplantation.

Materials and methods

Fifteen adult mongrel dogs of both sexes ranging in weight from 17.2 to 22.3 kg (average weight = 19.3 kg) were used. Group I consisted of five animals in which the sternohyoid (SH) muscle was autotransplanted as an inlay graft into the right ventricle. Group II consisted of 10 animals in which the sternocleidomastoid (SCM) was autotransplanted into the free wall of the left ventricle (table 1). Three different approaches were used in the second group. In three animals no ventricular myocardium was excised. A pedicle of SCM muscle was transplanted into a longitudinal myocardial incision. In six animals a portion of ventricular wall was excised and the medial portion of SCM muscle was grafted as replacement. One animal underwent excision and replacement with both lateral and medial portions of the SCM muscle.

Hypothermia and surgical approach

Anesthesia. Atropine sulfate (0.03 mg/kg) and penicillin plus streptomycin (1 million U) were given before surgery. Anesthesia was induced by 18 mg/kg thiamylal sodium and maintained with the halothane-diethyl ether azeotrope in a mixture of 95% O₂/5% CO₂. Anesthesia was administered through a Fluotec Mark III vaporizer at flow rates of 8 to 10 liters/min. All animals were intubated with a cuffed endotracheal tube and ventilated with an open (nonbreathing) system adjusted to provide a tidal volume of 20 cc/kg at a rate of 20 breaths/min. Arterial and central venous pressures were determined with use of transfemoral polyethylene catheters inserted to the midabdominal level of the aorta and inferior vena cava. Rectal and esophageal temperatures were measured with digital thermometers.

Electrocardiograms. Electrocardiographic analyses were taken in leads I, II, III, aVR, aVL, and aVF. Electrocardiograms were recorded before and after surgery and at the time the animals were killed.

Hypothermia. Surface-induced deep hypothermia was used in preference to cardiopulmonary bypass because of its relative ease and low complication rate. Hypothermia was induced by water bath (1°C) immersion until rectal temperature reached 24°C. Temperature continued to fall during simultaneous exposure of the heart and neck muscle. After the animal was raised from the hypothermia tank it was aseptically prepared for an intercostal thoracotomy. Rectal temperature averaged 20°C by the time the pericardectomy had been completed and circulatory arrest was induced.

Surgical technique. In group I, the SH muscle was transected near its point of insertion, i.e., at the level of the hyoid bone. In group II the SCM was divided near its point of insertion at the mastoid bone. Nerves were divided in both SH and SCM muscles, but blood supply was left intact to the lower portion of the muscle. Mediastinal access was created by blunt transthoracic dissection of the anterior chest wall and the inverted pedicle was carefully delivered into the thoracic cavity parallel to the anterior surface of the trachea. The intent of the technique was to supply a well-vascularized pedicle. After pericardectomy, total circulatory arrest was achieved by inflow and outflow occlusion of the superior vena cava, inferior vena cava, ascending aorta, and pulmonary artery. Cardioplegia was induced with a 30 ml bolus of cold Young’s solution* that was injected directly into the aortic root proximal to the aortic cross-clamp. Ventriculotomy was conducted on a bloodless, flaccid, nonbeating heart.

Each animal in group I underwent excision of a portion of the right ventricle. Full-thickness incisions were made parallel to the ventricular septum, taking care to avoid papillary muscles and the larger vessels of the coronary vasculature. The amount of myocardium excised was determined on a case-by-case basis, and ranged from 10 × 30 mm to 30 × 80 mm. In studies of the left ventricle (group II) both full-thickness incisions and excisions were performed. The longitudinal incisions ranged from 40 to 70 mm in length. Areas of excisions varied from 12 × 46 mm to 30 × 60 mm.

The distal pedicle was stretched longitudinally and laterally as it was sutured into the myocardium so that when it replaced excised muscle a minimum of normal tonus was present at systole, increasing during diastole (figure 1). Continuous full-thickness sutures of 3-0 Prolene were used with several superficial interrupted stitches at the cranial suture line to avoid vascular compromise distal to the superior point of pedicle attachment. Superficial interrupted stitches were also placed circumferentially at the site of graft/myocardium juxtaposition. Upon closure of the myocardium, air was evacuated and replaced with a preventricular lumen of normal saline.

Rewarming to normothermia was initiated by water bath (41°C) flotation. Occluding ties and clamps were removed and resuscitation was accomplished with manual massage and intravenous administration of calcium chloride, epinephrine, lidocaine hydrochloride, and sodium bicarbonate. Ventricular fibrillation was converted by electrical countershock. Routine chest and neck closures were performed when the animal’s temperature had reached between 28° and 30°C during rewarming. Surgical autotransplantation from time of arrest to beginning of resuscitation averaged 27 min. Suturing of the chest wall at the site where the skeletal muscle pedicle entered the thoracic cavity was not necessary because of adjacent tissue pressure.

Results

All animals in group I survived (table 2). Four dogs in group II died subsequent to surgery; one failed to resuscitate and another died from bleeding that resulted from suture line dehiscence. Two of the three dogs that underwent longitudinal incision without myocardial excision died between 12 and 16 days after surgery. Autopsy revealed an aneurysmal dilation of the graft in both of these animals (table 3). Most tolerated the procedure well and were ambulatory with good appetites on the first postoperative day. Some developed “flu-like” upper respiratory symptoms that persisted about 48 hr and then subsided without additional treatment. Hematomas of the neck occurred in several animals. These were successfully treated by aspiration. The six surviving dogs in group II and the five from group I were electively killed at between 3 weeks and 14 months after surgery (table 4).

Gross findings. Paradoxical motion of grafted skel-

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*0.81 g potassium citrate, 2.46 g magnesium sulfate, 0.001 g neostigmine methyl sulfate, H₂O qs to 100 cc, and pH adjusted to 7.4 with NaHCO₃.

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et al muscle was not present in any of the survivors when they were killed, an observation that was not consistent with results reported by others.\textsuperscript{11,12} A clearly defined scar had formed between the walls of cardiac and skeletal muscle in each animal (figure 2, A).

Pedicles composed of SH muscle (group I) underwent moderate narrowing and loss of elasticity in contrast to a widening of skeletal muscle in the graft. Although considerable tension was present the heart remained in a relatively normal position. All SH transplants to the right ventricle showed diminution in size. Aneurysms were not seen in survivors but examination of cross sections revealed an indentation of the endocardial surface at the site of implantation. All animals in group I were free of thrombi.

In group II, the portion of lateral and medial SCM muscle that was available for the pedicle stalk had shortened by day 14 to the extent that the heart was lifted from its bed. Pedicle division was performed under normothermic conditions in two dogs at 11 and 20 weeks, respectively. The pedicle was divided 2 to 4 cm proximal to the superior point of myocardial attachment. Pedicles of SCM underwent minimal narrowing. The stalk developed a gristy quality and when it was transected retrograde blood flow was observed. Revascularization from the endothelial surface of the graft extended upward into the pedicle. The closer the proximity to the graft and the longer the period of transplantation, the greater the amount of retrograde bleeding. Two animals that died at 14 and 16 days had large newly organized thrombi at the endocardial surface of the graft. One dog killed at 133 days also had a thrombus fused with the underlying scar tissue. In contrast to right ventricular transplants, the area of skeletal tissue grafted into the left ventricle was longer than the original implants in animals killed at 14 and 36 days. At 84 days, grafts had shrunk to about two-thirds of their original size. One graft examined at 133 days showed only a small difference from the area measured during transplantation.

**Histologic findings.** Animals were killed at between 2 weeks and 14 months after surgery. Hematoxylin and trichrome stains were made of samples taken from pedicles, skeletal muscle, cardiac muscle, and at the interface of skeletal/myocardial tissue. In a few cases tissues were prepared for electronmicroscopic studies. The general response of SH and SCM muscles followed the same pattern. There was initial denervation atrophy with myolysis of the distal part of the pedicle graft followed by a gradual return to a near-normal appearance of the skeletal muscle. Relatively few changes occurred in cardiac muscle and those that did were in close proximity to scar tissue.

**Group I (right ventricle)**

Fascicles. At 49 days after transplantation, fascicles were about two-thirds normal size. The overall width of pedicle muscle had similarly diminished but fascicles were well filled with considerable variation in diameter. At 202 days there was a further decrease in

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**TABLE 3**

| Group II (SCM to left ventricular transplant) — nonsurvivors |
|---|---|---|---|
| Dog No. | Day of death | Procedure | Cause |
| 7 | 14 | excision | Suture line deficiency |
| 9 | 1 | excision | Not resuscitated |
| 10 | 12 | incision | Aneurysmal dilation/rupture |
| 12 | 16 | incision | Aneurysmal dilation/rupture |

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**TABLE 4**

| Group II (SCM to left ventricular transplant) — survivors |
|---|---|
| Dog No. | Day killed |
| 6 | 435 |
| 8 (pedicle cut 141 days) | 395 |
| 11 | 84 |
| 13 (pedicle cut 77 days) | 133 |
| 14 | 36 |
| 15 | 14 |
graft size. By contrast, fascicles in the animal that lived to day 397 appeared almost normal.

Muscle fibers and nuclei. Cross sections of the pedicle revealed muscle fibers that varied widely in diameter and there were several hypertrophic cells in most fascicles. Some fascicles showed advanced atrophy of muscle fibers. At 49 days the grafts had a predominance of rounded fibers scattered with small fibers. Striations were not seen in longitudinal sections; nuclei were well preserved and showed only mild proliferation. Few skeletal fibers were present in sections taken at 202 days; however, those present were rounded and had an increased number of small nuclei with many small muscle cells present. Cardiac fibers were arranged in a parallel pattern in one area, i.e., a “streaming” pattern. At 379 days after surgery (figure 2, A) the majority of cardiac and skeletal fibers were normal in size and a moderate number of hypertrophied skeletal fibers were present. Striations were absent and considerable nuclear proliferation was present. Several of the nuclei were enlarged and vacuolated.

Connective tissue and inflammatory reaction. At 49 days, scar tissue was approximately 1.5 cm in width. Analysis revealed the presence of fiber atrophy, fibrosis, collagen, and, at the cardiac border, some fat cells. There was minimal phagocytosis throughout the scar and few apatite deposits (calcium crystals) were present. An increase in the amount of interfascicular or perimysial collagen tissue was present in the skeletal muscle. Minimal increase in connective tissue had occurred in the pedicle. At 202 days, scar tissue was large, calcified, and contained a moderate number of fat cells. At this time small skeletal muscle fibers were entrapped in the scar tissue. Few histocytes were present.

Cross sections obtained 379 days after surgery showed marked diminution in scar area. A band of connective tissue approximately 1 mm thick enveloped the skeletal muscle, with the inferior portion forming a smooth endothelial surface (figure 2, A). A small amount of calcification was present beneath the endothelial surface and there were fat cells in the lateral aspect of the scar adjacent to cardiac muscle. Minimal perimysial connective tissue was seen in the graft and pedicle. No inflammatory response was seen at this time.

Blood vessels. Samples of scar and connective tissue taken at 49 days after transplantation showed dilated spaces with little or no epithelial lining. By contrast, old arterial walls appeared considerably thicker than normal. The pedicle contained a fairly uniform scattering of small sinusoidal vessels. At 202 days, one of the sinusoids in cardiac muscle adjacent to scar tissue measured 1 mm in diameter. Additional small sinusoids were scattered throughout the scar. At 379 days, sinusoidal vessels were present in scar, skeletal (including pedicle), and cardiac fibers.

Group II (left ventricle)

Fascicles. Group II animals underwent SCM trans-
plantation to the left ventricle. Most of the changes that occurred were found at the edge of skeletal and cardiac muscle where a subendothelial or central scar separated the cardiac muscle from skeletal muscle grafted into the deficit. Fascicles examined on the fourteenth day after grafting showed mild diminution and some apoptosis near the scar. The 36 day graft showed mild diminution with organization changes of the fascicles and fibers. By 84 days the fascicle was 50% of its original size and had retracted from interfascicular connective tissue. This appearance was also noted at 133 days (figure 2, B).

Muscle fibers and nuclei. The transplanted skeletal muscle fibers at 14 days showed focal infarction with hyalinization, loss of striations, and absence of nuclei. Many fibers appeared rounded. Degeneration of muscle fibers were more pronounced at 36 days, with a moderate decrease in diameter and advanced atrophy adjacent to and mixed with scar tissue. Striations varied from minimal to normal away from the scar tissue. In contrast to earlier findings, a moderate proliferation of muscle nuclei could be seen. Cardiac fibers had developed a parallel alignment and appeared to be “streaming” toward scar tissue. Necrosis of skeletal fibers was found at the periphery of the scar and degenerating fibers were incorporated within the scar tissue. Muscle fibers examined 84 days after surgery showed moderate variability in the diameter of fibers with smaller fibers at the periphery of fascicles and areas of necrosis and degeneration near the edge of the graft. Marked proliferation of nuclei were present in the pedicle but not in the graft. Again, streaming of both cardiac and skeletal fibers toward the scar was seen. Few striations were present. Denervation atrophy, central nuclei, and proliferation of histiocytes were more prevalent in the pedicle stalk than in the graft. At 133 days, the number of normal-appearing cells had increased at the periphery of the sections. Striations were present in some areas including the pedicle. There was marked atrophy of skeletal muscle fibers near the scar and streaming of cardiac fibers predominantly oriented toward the scar. The latter fibers were striated with vacuolization of the nuclei. The pedicle revealed mild denervation atrophy, proliferation of nuclei, and occasional hypertrophic cells.

Connective tissue and inflammatory reaction. The scar tissue, initially highly disorganized, showed progressive cicatrization and collagen formation. At day 14, formation of granulation tissue was seen and the small number of histiocytes indicated minimal chronic inflammation. Plasma cells and polymorphonuclear cells were present and occasionally a giant cell was noted. Fat cells were estimated at 3+ and apatites were present. Fascicles had been completely replaced by fibrous tissue in which histiocytes could be seen in several areas. Samples taken at 36 days showed proliferation of connective tissue between the fascicles and interfascicular fat cells beneath the endocardial surface. Distinct areas of apatite as well as pigmented macrophages and foreign body giant cells were present. By 84 days, there was proliferation of connective tissue in the endomysium and perimysium. The scar was scattered with areas of calcification, fat cells, and small apatites. There was less evidence of chronic inflammation with only occasional giant cells. A fine network of capillaries containing red blood cells had developed in a large area of the subendothelial portion of the scar. Also present were perineural and periartrial fat cells, 2+ calcium, and focal areas of necrotic skeletal muscle. A moderately large thrombus undergoing organization with multiple sinusoids was also present. At 133 days the central scar appeared comparatively smaller than in earlier specimens. There was increased collagen content and, except for a few pigmented cells, almost complete absence of inflammatory cells.

Blood vessels. Sections taken from the wide, thick scar of the graft at 14 days showed several small oval spaces that were subsequently identified as newly forming vessels. These extremely thin-walled areas or sinusoids were seen as dilated spaces beneath a poorly organized endothelial layer at 36 days after surgery. They were distinctly different from arterial walls that had undergone hypertrophy. At 84 days a moderate number of both large and small sinusoids were present in the central scar. By 133 days sinusoids were observed in scar tissue and skeletal and cardiac muscle, but less frequently than before. In one specimen, a sinusoid was seen to extend through the junction of scar and cardiac tissue (figure 3, A).

Electronmicroscopy. The sample taken from scar tissue adjacent to the interface of myocardium revealed excellent ultrastructural preservation (figure 3, B). Individual muscle fibers were separated by considerable quantities of extracellular collagen. The most striking alteration consisted of a decrease in the quantity of myofilaments present in individual muscle cells, with some disruption of the normal Z band pattern. The remaining cell organelles were generally unremarkable. Glycogen content was variable.

In tissue from the mid-scar multiple coarse bundles of type I collagen, fibroblasts, and portions of a large vascular structure were observed.

Tissue taken from the scar near the skeletal muscle graft also revealed excellent ultrastructural preserva-
tion (figure 3, C). Changes in skeletal muscle cells were similar to those seen in cardiac muscle, although to a lesser degree. Occasional cells suggestive of satellite cells were noted. One cell in particular appeared to be multinucleated. Nuclei with a dispersed nuclear chromatin and occasional prominent nucleoli were also observed. Scattered pools of glycogen were present. Compared with cardiac muscle, there appeared to be less connective tissue separating muscle fibers.

The most striking alteration apparent in both areas was a decrease of myofilaments in cells that were otherwise relatively well preserved. This suggests muscle cell regeneration.

Electrocardiography. Electrocardiograms were recorded before and after surgery and just before the animals were killed. The majority of the readings were interpreted as indicative of myocardial infarction, but there was poor correlation between these findings and the postoperative clinical disposition of the survivors.

Discussion

Successful long-term replacement of right or left ventricular myocardium with skeletal muscle has not been previously documented. The emphasis of the work reported here was to develop a surgical procedure that would allow introduction of skeletal muscle into myocardium, and to study the effect of continuous stretch on skeletal muscle after autotransplantation. Surgical success was defined as survival of the animal and restoration to a clinically healthy appearance and normal activity.

The premise that denervated skeletal muscle requires considerable stretch for successful introduction into myocardium appears to be substantiated. The procedure used in this series resulted in 100% survival with right ventricular autotransplantation and 60% surgical success in left ventricular studies. Survivors recovered well within a few days without significant loss of weight or activity.

The SH and SCM muscles were selected because of their proximity to the recipient site and their relative similarity in thickness to the right and left ventricular walls. Both muscles proved adaptable as denervated pedicle grafts, although it was found that only the medial head of the SCM was necessary. Gross findings at time animals were killed revealed excellent color and appearance of the skeletal muscle, although a layer of scar tissue could be observed at the borders of the grafts. In many cases adhesions had formed between the grafted muscle and surrounding tissues. The texture of the transplanted muscle was firm but pliable, suggesting that it would be responsive to pacing from an external source. Cardiac function studies were not completed for the series of subjects reported in this study. Early findings in a current series in which a slightly modified surgical procedure is being used to introduce latissimus dorsi muscle into the left ventricle show no significant differences between preoperative function and function at 1 month or more after surgery (table 5).

The amount of stretch placed on both cardiac and

FIGURE 3. A. SCM graft to left ventricle at 133 days. Original magnification of 50 × of midportion of figure 2, B shows sinusoid in connective tissue at junction of myocardium and graft. B, Excellent ultrastructural preservation adjacent to myocardial tissue. C, Elements in scar tissue that were interpreted as early forms of regenerating skeletal fibers.
skeletal muscle may be a factor in the development of scar tissue, which may also be further aggravated by shortening of the pedicle. It is interesting to note that the amount of scar tissue markedly decreased in right ventricular transplants studied at 1 year after surgery. The streaming of cardiac fibers that was observed is occasionally seen in association with heart disease, but it is more likely that the phenomenon was caused by traction on the myocardium resulting from contraction of scar tissue.

One intent of the surgical plan was to provide a well-vascularized pedicle graft. However, acute angulation at the origin of the SH and SCM muscles caused considerable outflow obstruction of the arterial supply. The appearance of sinusoids, forming at the endothelial surface and extending upwards to establish a retrograde flow, was unexpected. These sinusoids were present in the earliest histologic specimens, primarily in scar tissue. Specimens taken after 33 days showed sinusoidal development in both skeletal and cardiac muscle. These findings are consistent with those of Hansen-Smith et al., who observed that the first blood vessel formed in autotransplanted muscle appeared as a large sinusoidal endothelial tube. Mallory et al., studying the morphogenesis of myocardial circulation after acute infarction, reported that newly endothelialized vessels formed as early as 4 days after infarction.

The success of this surgical procedure has provided a model for the study of the effect of continuous repetitive stretch on denervated skeletal muscle. Neck muscles, which ordinarily are not subjected to frequent or rapid motion, were autotransplanted under conditions of stretch and subjected to additional stretch with each heart beat (more than 100,000 times each 24 hr). We are currently investigating the use of latissimus dorsi, which may be preferable to the neck muscle because of its thickness, location, and neurovascular supply. The current studies should also provide additional data regarding scar formation, and efforts will be made to prevent formation of excessive connective tissue. Additional studies are planned to assess the hemodynamic and electrophysiologic responses to autotransplantation and to determine the potential for grafted skeletal muscle to participate in cardiac function.

In summary, several conclusions can be drawn from the current series of denervated pedicle grafts of stretched skeletal muscle. (1) Autotransplantation into either the right or left ventricle is feasible and reproducible. (2) Animals enjoyed good health and normal activity until electively killed, with the longest time period of study being 435 days. (3) Surface-induced deep hypothermia with total circulatory arrest is a reliable adjunct that allows ample time for autotransplantation without the complications of cardiopulmonary bypass. (4) Suturing the pedicle into the graft site under considerable stretch avoids paradoxical motion during normal heart rhythm. (5) Histologic studies showed healthy viable muscle fibers in right ventricular transplants up to 435 days. (6) Revascularization of connective tissue and cardiac and skeletal fibers was observed in the form of sinusoids in early histologic samples. (7) Parallel formation or “streaming” occurred in both cardiac and skeletal fibers.

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