to LCA1, LCA3, LCB2, and LCB3 that we have obtained from rat. If we align corresponding chains, we observe identity at more than 93 percent of the amino acid positions and conservation of the tissue-specific splicing sites. However, from our analyses of the amino acid sequences we come to different conclusions about secondary structure. We find that the repeat of ten heptad motifs, typical of α-helical coiled coils, constitutes a key element in all classes and types of light chains regardless of the species studied. In contrast, Jackson *et al.* have not noticed the heptad elements. Rather, they propose homology of intermediate filaments and clathrin light chains. They suggest that the similarity is highest between amino acid residues 107 and 236 of the bovine light chains (15 percent). In our opinion, the homology is not extensive enough to allow the interpretation that the proteins derive from a common ancestor gene. We propose here that the central segment containing the heptad motifs mediate the binding of light chains to clathrin heavy chain. In a publication in the same issue of *Nature*, Brodsky *et al.* [figure 3 in (*27*)], using monoclonal antibodies to light chains to block interactions of light chains and heavy chains, conclude that residues 93 to 157 mediate the interaction. This conclusion is precisely consistent with our assignment.

REFERENCES AND NOTES

1. J. L. Goldstein, R. G. W. Anderson, M. S. Brown, *Nature (London)* **279**, 679 (1979); B. M. F. Pearse, *Trends Biochem. Sci.* **5**, 131 (1980); S. C. Harrison and T. Kirchhausen, *Cell* **33**, 650 (1983).
2. B. M. F. Pearse, *J. Mol. Biol.* **97**, 93 (1975); R. A. Crowther, J. T. Finch, B. M. F. Pearse, *ibid.* **103** 785 (1976).
3. T. Kirchhausen and S. C. Harrison, *Cell* **23**, 755 (1981).
4. E. Ungewickell and D. Branton, *Nature (London)* **289**, 420 (1981).
5. T. Kirchhausen *et al.*, in preparation.
6. T. Kirchhausen, S. C. Harrison, P. Parham, F. Brodsky, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2481 (1983).
7. E. Ungewickell, E. Unanue, D. Branton, *Cold Spring Harbor Symp. Quant. Biol.* **57**, 723 (1982).
8. E. Ungewickell, *EMBO J.* **2**, 1401 (1983).
9. S. L. Schmid, W. A. Braell, D. M. Schlossmann, J. E. Rothman, *Nature (London)* **311**, 228 (1984); J. E. Rothman and S. L. Schmid, *Cell* **46**, 5 (1986).
10. B. M. F. Pearse, *J. Mol. Biol.* **126**, 803 (1978); F. M. Brodsky and P. Parham, *ibid.* **167**, 197 (1983).
11. N. Holmes, J. S. Biermann, F. M. Brodsky, D. Bharucha, P. Parham, *EMBO J.* **3**, 1621 (1984).
12. F. K. Winkler and K. K. Stanley, *ibid.* **2**, 1393 (1983).
13. M. J. Geisow and R. D. Burgoyne, *Nature (London)* **301**, 432 (1983); C. E. Creutz and J. R. Harrison, *ibid.* **308**, 208 (1984).
14. F. M. Brodsky, *J. Cell Biol.* **101**, 2047 (1985).
15. M. P. Lisanti *et al.*, *Eur. J. Biochem.* **125**, 463 (1982).
16. T. Kirchhausen and S. C. Harrison, *J. Cell Biol.* **99**, 1725 (1984).
17. M. W. Hunkapiller, E. Lujan, F. Ostrander, L. E. Hood, *Methods Enzymol.* **91**, 227 (1983); A. N. Glazer, R. J. DeLange, D. S. Sigman, *Laboratory Techniques in Biochemistry and Molecular Biology*, T. S. Work and E. Work, Eds. (Elsevier, New York, 1976), vol. 4, p. 78; K. S. Huang *et al.*, *Cell* **46**, 191 (1986).
18. I. Mocchetti, R. Einstein, J. Brosius, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7221 (1986).
19. A. Ullrich, C. H. Berman, T. J. Dull, A. Gray, J. M. Lee, *EMBO J.* **3**, 361 (1984); T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
20. P. Scarmato, J. Brosius, T. Smith, T. Kirchhausen, in preparation.
21. J. Keen, personal communication.
22. D. J. Lipman and W. R. Pearson, *Science* **227**, 1435 (1985).
23. G. Payne and R. Schekman, *ibid.* **230**, 1009 (1985).
24. S. E. Schwarzbaur, J. W. Tamkun, I. R. Lemischka, R. O. Hynes, *Cell* **35**, 421 (1983); T. A. Cooper and C. P. Ordahl, *J. Biol. Chem.* **260**, 11140 (1985); K. E. M. Hastings, E. A. Bucher, C. P. Emerson, Jr., *ibid.*, p. 13699; B. A. Murray *et al.*, *J. Cell Biol.* **103**, 1431 (1986).
25. A. D. McLachlan and J. Karn, *Nature (London)* **299**, 226 (1982).
26. A. P. Jackson, H.-F. Seow, N. Holmes, K. Drickamer, P. Parham, *ibid.* **326**, 154 (1987).
27. F. M. Brodsky *et al.*, *ibid.*, p. 203.
28. R. F. Grantham, *Nucleic Acids Res.* **9**, 1 (1981).
29. L. J. McBride and M. H. Caruthers, *Tetrahedron Lett.* **24**, 245 (1983); R. B. Pepinsky *et al.*, *J. Biol. Chem.* **261**, 4239 (1986).
30. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
31. B. P. Wallner *et al.*, *Nature (London)* **320**, 77 (1986).
32. U. Nudel *et al.*, *Nucleic Acids Res.* **11**, 1759 (1983).

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Skeletal Muscle as the Potential Power Source for a Cardiovascular Pump: Assessment in Vivo

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Skeletal muscle ventricles (SMVs) were constructed from canine latissimus dorsi and connected to a totally implantable mock circulation device. The SMVs, stimulated by an implantable pulse generator, pumped continuously for up to 8 weeks in free-running beagle dogs. Systolic pressures produced by the SMVs, initially of 139 ± 7.2 mmHg and after 1 month of continuous pumping of 107 ± 7 mmHg, were comparable to normal physiologic pressures in the adult beagles (114 ± 21 mmHg). After 2 weeks of continuous pumping, the mean stroke work of the SMVs was 0.4 × 10^6 ergs, a performance that compares favorably with the animal's cardiac ventricles. This study shows that canine skeletal muscle which has not received prior training or electrical conditioning can perform sustained work at the high levels needed for an auxiliary cardiovascular pump. It might be possible eventually to use such muscle pumps in humans to assist the failing circulation and to provide support in children with certain types of congenital heart defects.

SKELETAL MUSCLE IS CAPABLE OF transforming chemical energy into mechanical energy with an efficiency unmatched by man-made machines. It represents a source of autogenous contractile tissue that could in principle be used to augment the function of the failing heart. Skeletal muscle can respond to an increased pattern of use with a series of adaptations that include a greatly enhanced resistance to fatigue. This response is seen at its greatest extent in adult fast skeletal muscle that has been subjected to long-term, low-frequency stimulation (*1*). Such stimulated muscle shows increases in capillary density, mitochondrial volume fraction, and enzymes of oxidation metabolism, and a switch from the synthesis of fast to the synthesis of slow isoforms of myosin, changes that result in increased resistance to fatigue (*1–3*).

In previous studies in dogs, we used electrical stimulation to render skeletal muscle fatigue-resistant and then used this muscle to construct skeletal muscle pumping chambers. When these chambers were connected to the animal's own circulation for short periods, they maintained for several hours flows that were equivalent to 20% of the canine cardiac output (*4*). In another study, pumping chambers constructed from electrically preconditioned muscle were tested with a mock circulation device for several days (*5*). In the present experiments we assessed the skeletal muscle pumping chambers for several weeks without subjecting the muscles to any preconditioning electrical stimulation; instead the muscles were al-

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lowed a period of vascular recovery and were then called upon immediately to perform a typically cardiac pattern of work. We found that skeletal muscle can perform continuous work while undergoing adaptive transformation, and our results suggest that muscle chambers similar to those described here might be useful in patients with end-stage heart failure or with congenital heart defects.

Skeletal muscle ventricles (SMVs) were constructed in each of six beagle dogs by freeing the latissimus dorsi muscle and wrapping it around a cone-shaped Teflon mandrel of 17-ml volume (*6, 7*). A rest period of 4 to 6 weeks allowed the unstimulated muscle to reestablish blood supply lost by division of collaterals (*8*). A second operation was performed to remove the Teflon mandrel from the SMV and to implant a mock circulation device. This enabled us to measure on a long-term basis the pressures and flows generated by the SMV, and to exercise independent control over the preload (ventricular filling pressure) and afterload conditions. The mock circulation device was totally implantable. No tubes or wires crossed the skin barrier except when measurements were actually in progress. The device was located on the left lateral chest wall under the skin and subcutaneous tissue. The heart was not interfered with. Animals moved about freely without apparent discomfort or locomotor deficit.

The mock circulation device (Fig. 1) consisted of two polyurethane bladders fixed to each end of an acrylic conduit. Each bladder was 80 mm in length with a volume of 20 ml. One bladder was inserted into the SMV cavity and the second, similar polyurethane bladder was fixed within a hermetically sealed acrylic cannister. The two main compartments of the device were fitted with pressure ports similar to those used for long-term intravenous therapy. These Vascular Access Ports (Norfolk Medical Projects) were secured under the skin for percutaneous access. The pressure port communicating with the SMV bladder permitted direct adjustment of filling volumes and measurement of pressure produced by the SMV. The pressure port connected to the cannister allowed direct adjustment and measurement of the pressure within the cannister.

At implantation, the bladders and conduit were filled with saline. Air was then injected into the cannister so that the bladder within the cannister collapsed completely. This isolated that pressure in the cannister (P_1) from the pressure in the SMV bladder when the muscle was relaxed. The filling pressure applied to the SMV (representing the preload) and the cannister air pressure (representing the afterload) could thus be adjusted

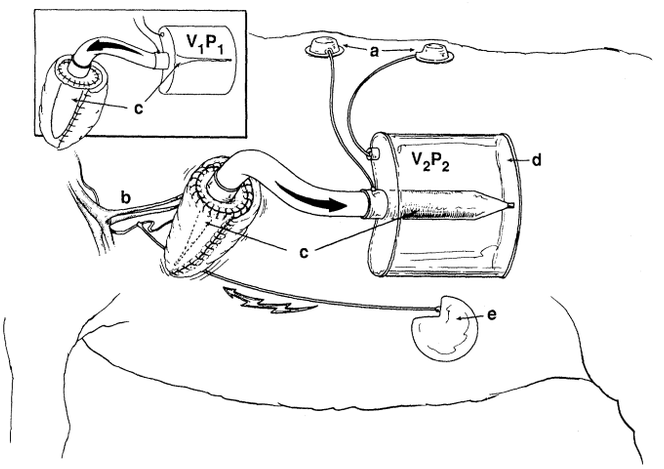

Fig. 1. Diagram showing the SMV mock circulation system. The inset depicts the SMV during diastole; the main figure depicts the SMV during systole. V_1P_1 and V_2P_2 are explained in the text (*9*); a, vascular access ports; b, neurovascular bundle; c, bladders; d, pressurized cannister, e, electrical stimulator.

independently. When the SMV was stimulated, the fluid in its bladder was not ejected until the pressure exceeded the cannister resting pressure (P_1); the fluid then entered the cannister bladder. As the volume of air within the cannister decreased, the air pressure within the cannister increased according to Boyle's law. The actual volume change was thus easily computed (*9*). When the SMV relaxed, the cannister bladder then collapsed under the higher cannister pressure (P_2) and returned fluid to the SMV bladder. In this way the air in the cannister regained its initial volume (V_1) and pressure (P_1) in preparation for the next cycle (Fig. 1).

Baseline settings were established so that the SMV would contract 54 times a minute against an afterload of 80 mmHg with a filling pressure of 40 mmHg (*10*). Pressures and flows were measured daily in the conscious animals, both at the baseline settings and over a range of preloads, afterloads, and burst frequencies. A typical pressure recording, obtained after 3 weeks of continuous pumping, is shown in Fig. 2.

There was a decline in both pressure and flow generated after 30 minutes, and a further decline during the first 24 hours of pumping (Fig. 3a), presumably reflecting the lack of conditioning, particularly of the fatigue-susceptible, type II fiber component. Thereafter, skeletal muscle ventricular function stabilized. Figure 3 shows the systolic pressures and flows generated by the SMV over 1 month of continuous pumping, measured at baseline settings. Systolic pressure was initially 139 ± 7 mmHg (mean ± SD, $n = 6$), and was 107 ± 7 mmHg after 1 month ($n = 4$). Flow was initially 518 ± 105 ml/min, and declined to 224 ± 85 ml/min at 1 month (Fig. 3b). Systolic aortic blood pressure for the adult beagle is 114 ± 21 mmHg. Cardiac outputs in beagles (8 to 12 kg) range from 900 to 2700 ml/min under anesthesia (*11*).

We originally intended to follow the six dogs with SMVs pumping continuously for 1 month. One dog was killed at 1 week after a leak appeared in the polyurethane ventricular bladder. Another was killed at 3 weeks after an ulcer developed in the skin overlying the mock circulation cannister. Of the remaining four dogs, two were killed at 1 month as planned and the remaining two were studied for 2 months. After 2 months of continuous pumping one SMV was capable of producing systolic pressures of 160 mmHg and flows of 422 ml/min; the other had declined to 100 mmHg and flows of 85 ml/min.

Variation in afterload, preload, and burst frequency during the measurement procedure provided some indication of the capabilities of the SMV. The highest pressures were recorded with a burst frequency of 85 Hz, an afterload of 200 mmHg, and a preload of 60 mmHg (Fig. 3a). These were not necessarily the maximum performance characteristics of this model, but rather were the highest pressures and flows recorded with the protocol we used. The highest flows were recorded with a burst frequency of 85 Hz, an afterload of 60 mmHg, and a preload of 50 mmHg (Fig. 3b). Such figures

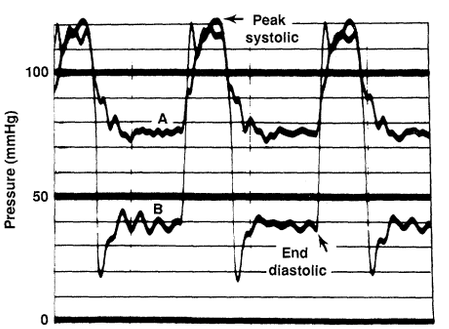

Fig. 2. Mock circulation pressure trace obtained on day 21 of continuous pumping. Trace B shows the pressure generated by the SMV. At end diastole, the filling pressure is 40 mmHg. At peak systole, the pressure is 120 mmHg. Trace A shows the pressure within the cannister (afterload) against which the SMV is pumping. The afterload at rest is 80 mmHg. The change in pressure A represents flow (*9*).

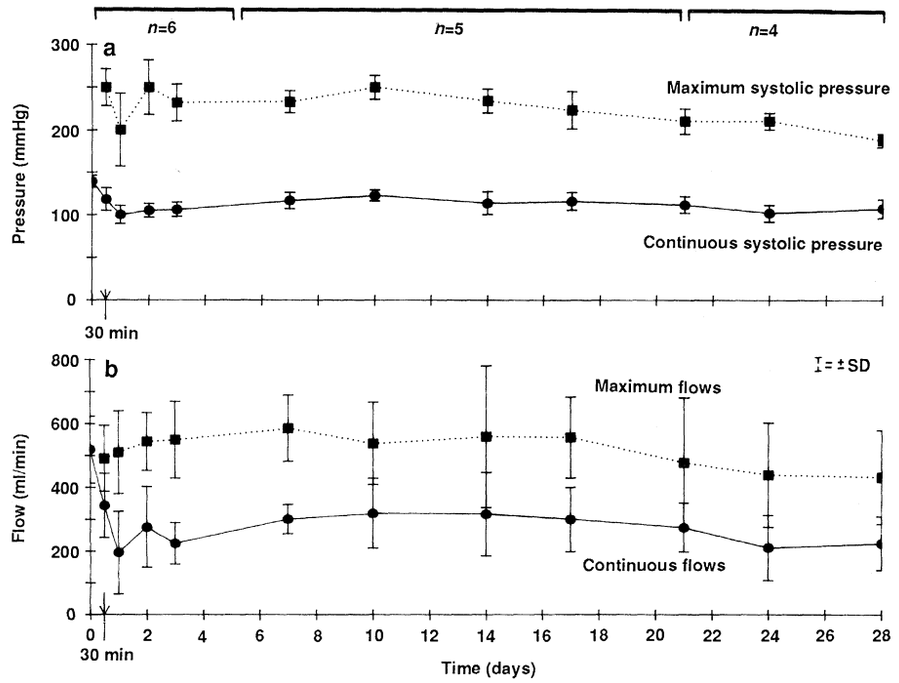

Fig. 3. (**a**) Continuous and maximum systolic pressures produced by the SMVs over 4 weeks. (**b**) Continuous and maximum flows during the same period. Continuous refers to the systolic pressures and flows that were generated at the baseline settings of 25 Hz, a preload of 40 mmHg, and an afterload of 80 mmHg.

are of more than theoretical interest: the SMV of one dog pumped continuously for 2 months at a burst frequency of 85 Hz.

In an SMV, unlike the heart, a single electrical stimulus elicits a muscle twitch, which would not normally be sufficient to augment cardiac function. Tetanic, or burst stimulation of the SMV is necessary to produce adequate summation of force, and up to a certain point more work can be elicited from the muscle by increasing the burst frequency.

In previous experiments we found the stroke work (pressure × volume) of the canine left and right cardiac ventricles to be 1.83×10^6 ergs and 0.22×10^6 ergs, respectively (*4*). After 2 weeks of continuous pumping into the mock circulation, the five SMVs in the present experiment generated stroke work of $0.40 \pm 0.14 \times 10^6$ ergs (mean ± SD) at the baseline settings (25 Hz, preload 40 mmHg; afterload 80 mmHg). When the burst frequency was increased first to 43 Hz and then to 85 Hz at the same preload and afterload, the corresponding stroke work increased to $0.54 \pm 0.2 \times 10^6$ ergs and $0.78 \pm 0.4 \times 10^6$ ergs, respectively (excluding one dog which generated stroke work of 2.05×10^6 ergs at 85 Hz). Although the SMVs in this experiment pumped continuously at preloads of 40 mmHg, significant stroke work was produced at physiologic preloads of 10 to 20 mmHg (Fig. 4). It is encouraging that even at this stage of development, SMVs could be made that were capable of generating a substantial fraction of the normal left ventricular stroke work after pumping unceasingly for weeks.

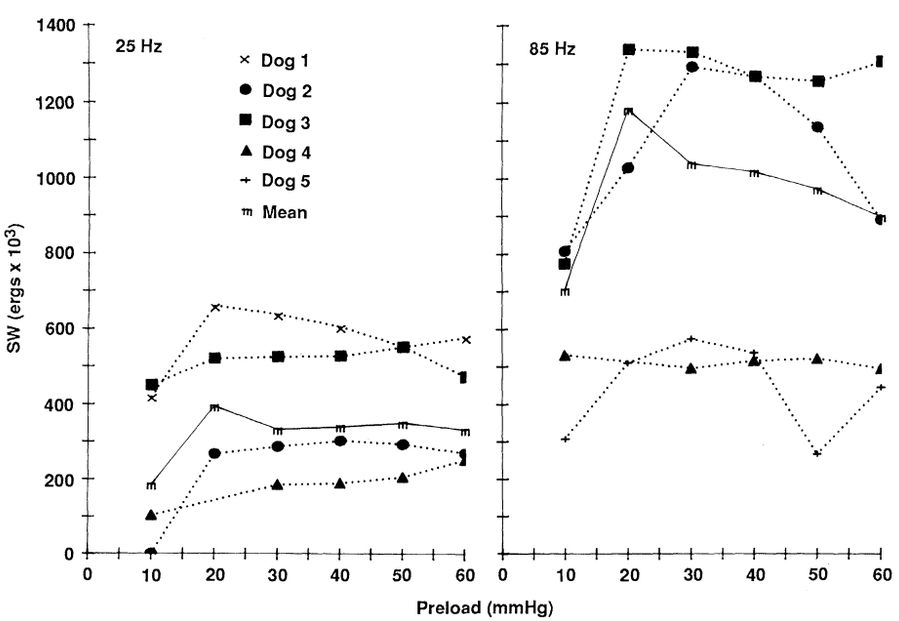

Fig. 4. Stroke work (SW) plotted against preload (filling pressure) for SMVs of five dogs after 2 weeks. Curves were obtained at 25 Hz and 85 Hz with afterload of 80 mmHg. Measurements were made in dog 1 only at 25 Hz and in dog 5 only at 85 Hz. The plotted mean values therefore represent only the three dogs (Nos. 2, 3, and 4) that were measured at both 25 Hz and 85 Hz. Note significant stroke work at preloads of 10 to 20 mmHg.

In a second group of five beagles, the right latissimus dorsi muscles were stimulated to contract in situ, instead of being raised as SMVs. The stimulation pattern was identical to that used in the first group for muscles formed into SMVs. The left muscle served as an unstimulated control. As in the SMV group, the dogs tolerated the stimulation well and showed no evidence of discomfort. After 2 months of repetitive burst stimulation, the dogs were anesthetized and a fatigue test (*2*) was performed in which isometric tension was measured with the same stimulation pattern as had been imposed on the muscle of the right side. Over an 11-minute period the percentage fatigue was 34 ± 12% for the unconditioned left side and 11 ± 11% for the conditioned right side.

Histological and histochemical examination of muscle samples from the SMVs (*12*) revealed a substantial increase in the proportion of (fatigue-resistant) type I fibers, compared to the 45% in the undisturbed contralateral latissimus dorsi muscles that had not received stimulation. SMVs that had been pumping for 2 months showed a dense capillary blood supply. There was some thickening of perimysial septa, which were continuous with the newly formed connective tissue lining of the SMV cavity. There was occasional vacuolation and splitting of fibers.

Some samples (Fig. 5, a and b) contained fascicles in which the fibers were small, showed internal nuclei, and stained darkly for myofibrillar adenosinetriphosphatase after incubation with acid and alkali, signs consistent with regeneration after damage. Other fibers showed core-like structures. These abnormalities were not seen in the second group of dogs, in which the muscles had been subjected to the same pattern of

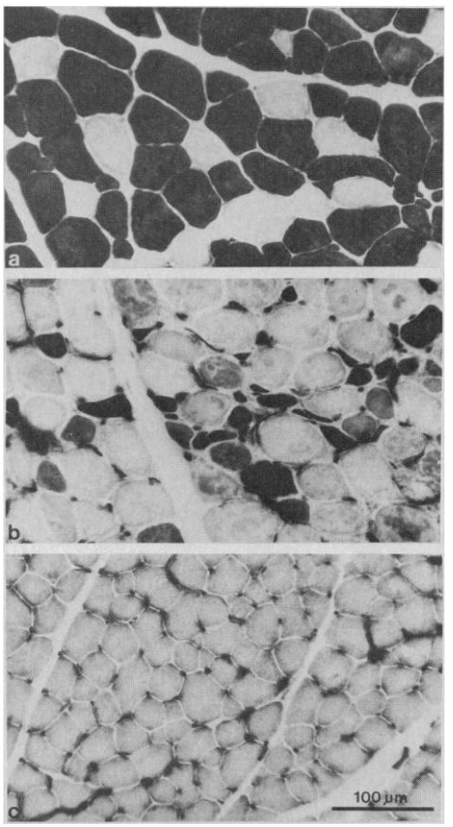

Fig. 5. Myosin adenosinetriphosphatase staining of (**a**) control unstimulated latissimus dorsi, (**b**) SMV, and (**c**) latissimus dorsi stimulated in situ. The dark-staining fibers (alkaline preincubation) are type II fast fibers and the light-staining fibers are type I slow fibers. Note that in (c), 100% transformation has occurred in the fibers of the latissimus dorsi stimulated in situ. The muscle from the SMV (b) is in the process of undergoing transformation.

stimulation but had not been surgically disturbed (Fig. 5c). Some damage may have been sustained as a result of vascular or mechanical disruption during the formation of the SMV. We suspect, however, that much of the damage resulted from the high filling pressure used in this experiment. The preload pressure of 40 mmHg probably overstretched the muscle fibers and forced the SMV to function on a less than optimal part of the length-tension curve (Fig. 4). This situation would be analogous to eccentric exercise of limb muscles, the effects of which are known to be damaging (*13*).

The concept of using skeletal muscle in this way is not new, but previous attempts failed because muscle function deteriorated within minutes to hours (*14*). We incorporated a delay of 4 to 6 weeks between the ligation of muscle collateral blood vessels and the onset of electrical stimulation. In previous experiments, disruption of collateral circulation in unconditioned latissimus dorsi muscle drastically reduced the blood flow available to the exercising muscle; however, 3 weeks later, there was a substantial recovery of the exercise-induced increase in muscle blood flow (*8*). Vascular delay thus reduces the likelihood of ischemia arising from inadequate perfusion of the stimulated graft.

The conclusion that such SMVs might have clinical application needs to be extrapolated with caution, since canine muscles have an unusually oxidative character (*15*). It should be possible, however, to achieve a similar result in less inherently fatigue-resistant muscles, such as those of humans, either by a moderate degree of preconditioning or by escalating more gradually the functional demands placed on the mobilized muscle. SMVs are probably not suitable for patients in cardiogenic shock from acute myocardial infarction because a few weeks are necessary for recovery of collateral muscle blood supply before the SMV can be activated. However, many people have chronic heart failure caused by diseases of the heart muscle, including previous heart attacks. Their hearts can only pump a half to a third of the blood of a normal heart and thus their physical activity is limited. In such patients, an SMV might be used to assist the failing heart by pumping a few extra liters of blood per minute, skeletal muscle pumps might also be used in children with certain types of congenital defects, such as hypoplastic right and left ventricles where the cardiac pumping chambers are underdeveloped.

Several issues must be resolved, however, before it can be determined whether muscle pumps will prove clinically feasible. Muscle fibrosis was detected to some extent in the SMVs of this study, and we do not know whether the fibrosis is progressive. In separate studies, skeletal muscle stimulated continuously in situ at rates of 120 to 240 per minute for 1 year underwent adaptive transformation without evidence of muscle damage (*16*). High preloads may cause muscle stretch and could result in muscle hypertrophy or hyperplasia (*17*), and these effects, as well as such problems as the potential for thrombus formation, require further study.

REFERENCES AND NOTES

1. S. Salmons and G. Vrbova, *J. Physiol. (London)* **210**, 535 (1969); S. Salmons and J. Henriksson, *Musde Nerve* **4**, 94 (1981); D. Pette and G. Vrbova, *ibid.* **8**, 676 (1985).
2. J. D. Mannion *et al.*, *Circ. Res.* **58**, 298 (1986).
3. B. R. Eisenberg and S. Salmons, *Cell Tissue Res.* **220**, 449 (1981); W. E. Brown, S. Salmons, R. G. Whalen, *J. Biol. Chem.* **258**, 14686 (1983); J. Henriksson *et al.*, *Am. J. Physiol.*, in press; M. M.-Y Chi *et al.*, *ibid.*, in press; D. Pette, M. E. Smith, H. W. Staudte, G. Vrbova, *Pfluegers Arch.* **338**, 257 (1973); W. Reichman, H. Hoppeler, O. Mathieu-Costello, F. von Bergen, D. Pette, *ibid.* **404**, 1 (1985); D. Pette, *Med. Sci. Sports Exercise* **16**, 517 (1984).
4. J. D. Mannion, M. A. Acker, R. L. Hammond, L. W. Stephenson, *Surg. Forum* **37**, 211 (1986); J. D. Mannion *et al.*, *Trans. Am. Soc. Artif. Intern. Organs* **15**, 14 (1986).
5. M. A. Acker *et al.*, *J. Thorac. Cardiovasc. Surg.* **92**, 733 (1986).
6. J. D. Mannion, R. Hammond, L. W. Stephenson, *ibid.* **91**, 534 (1986).
7. Six male beagles (9 to 12 kg) were used in this study. They were anesthetized with sodium pentobarbital, and a left flank incision was made. The collateral blood vessels to the latissimus dorsi muscle were divided, and the muscle was freed at its origin and insertion. The neurovascular bundle, consisting of the thoracodorsal artery, vein, and nerve, was left intact. The muscle was wrapped around a cone-shaped Teflon mandrel 80 mm long and 19 mm at its greatest diameter. Two to two-and-a-half spiral wraps of muscle were made with the proximal blood vessels on the external surface. A collar of Teflon felt was sutured to the muscle wraps at the top of the mandrel. A modified Medtronic electrode was placed around the thoracodorsal nerve and connected to a totally implantable unipolar electrical stimulator (Medtronic, Inc., Minneapolis, MN, Model 7421) which was not, however, activated at this stage. The wound was closed and the dog was left to recover for 4 to 6 weeks without further intervention.
8. J. D. Mannion *et al.*, *Abstracts of the 2nd Vienna Muscle Symposium* (1985), p. 28.
9. The cannister had a fixed volume (V_1; between 155 and 175 ml) determined for each device at the time of construction. At completion of the contraction of the SMV, the air within the cannister had a new, smaller volume (V_2) and a new higher pressure (P_2). Since V_1 was known, and both P_1 and P_2 could be measured directly, and V_2 could be determined according to Boyle's law ($P_1V_1 = P_2V_2$ at constant temperature). The ambient atmospheric pressure was added to both P_1 and P_2 so that:
$$V_2 = V_1(P_1 + P_{atm}) \cdot (P_2 + P_{atm})^{-1}$$
The stroke volume of the SMV was simply $(V_1 - V_2)$.
10. Air was added to the cannister via the corresponding port so that the resting cannister pressure (afterload) was 80 mmHg. The amount of saline was adjusted so that the pressure in the relaxed SMV (preload) was 40 mmHg. With the dog still anesthetized, voltages for threshold and supramaximal stimulation were determined. The implantable stimulator was activated at the following settings: frequency within bursts, 25 Hz; duty cycle, 312 msec ON, 812 msec OFF; pulse width, 220 μsec; amplitude, 1 to 3 V, adjusted for supramaximal stimulation. These settings produced 54 contractions per minute.
11. D. K. Detweiler, in *The Beagle as an Experimental Dog*, A. C. Andersen and L. S. Good, Eds. (Iowa State Univ. Press, Ames, 1970), p. 232.
12. For routine histological examination, cryostat sections (10 μm) transverse to the long axis of the fibers were stained routinely by hematoxylin and eosin. Serial sections were stained histochemically for the demonstration of myofibrillar adenosinetriphosphatase. Sections were viewed with a Leitz Diaplan microscope equipped for photomicrography. T. Barka and P. J. Anderson, Eds., in *Histochemistry, Theory, Practice and Bibliography* (Hoeber, New York, 1963), p. 312; L. Guth and F. J. Samaha, *Exp. Neurol.* **28**, 365 (1970).
13. R. B. Armstrong, R. W. Ogilvie, J. A. Schwane, *J. Appl. Physiol.* **54**, 80 (1983); J. Friden, M. Sjostrom, B. Ekblom, *Experientia* **37**, 506 (1981); D. J. Newham, G. McPhail, K. R. Mills, R. H. T. Edwards, *J. Neurol. Sci.* **61**, 109 (1983).
14. M. L. Dewar, D. C. Drinkwater, C. Wittnich, R. C. Chiu, *J. Thorac. Cardiovasc. Surg.* **87**, 325 (1984); E. Kusaba *et al.*, *Trans. Am. Soc. Artif. Intern. Organs* **19**, 251 (1973); H. M. Spotnitz, L. Merker, J. R. Malm, *ibid.* **20**, 747 (1974); B. R. Vachon, H. Kunov, W. Zingg, *Med. Biol. Eng. Comput.* **13**, 252 (1975); A. Kantrowitz and W. M. P. McKinnon, *Surg. Forum* **9**, 266 (1959).
15. D. H. Snow *et al.*, *Histochemistry* **75**, 53 (1982).
16. M. A. Acker *et al.*, *J. Appl. Physiol.* **62**, 1264 (1987).
17. O. M. Sola, in *Biomechanical Cardiac Assist: Cardiomyoplasty and Muscle-Powered Devices*, R. Chiu, Ed. (Futura, Mount Kisco, NY, 1986), p. 32.

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