tial functions of Hagler *et al.* is +7 kcal (*21*). Ab initio molecular orbital calculations (*21, 22*) also give differences close to this value and a calculation with the use of flexible geometry gives a difference of +4.5 kcal (*21*). The dipeptide-water energy difference is thus of the same order as the energy difference in vacuo but of the opposite sign. This component of the water-peptide interaction energy would tend to stabilize the α-helical conformation relative to the C_7 in aqueous solution (*23*). This stabilization of the α_R conformation by water might be expected from the larger dipole moment of the α_R conformation due to the alignment of the two amide dipoles, 7.12 *D*, as compared to the C_7 whose dipole moment is 2.71 *D* and has an internal hydrogen bond. The results of the continuum reaction field method, which represents the interaction of the induced (macroscopic) dipole of the solvent with the solute, gives a difference between the α_R and C_7 of greater than −4 kcal (*7*). The results presented here were obtained with the Rowlinson potential. Similar results have been obtained with the ST2 potential (*23*).

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References and Notes

1. A. T. Hagler and J. Moult, *Nature (London)* **272**, 222 (1978).
2. I. D. Kuntz and W. Kauzmann, *Adv. Protein Chem.* **28**, 239 (1968); C. Tanford, *ibid.* **23**, 722 (1968).
3. P. J. Flory, *Statistical Mechanics of Chain Molecules* [Interscience (Wiley), New York, 1969]; G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.* **23**, 283 (1968); G. Nemethy and H. A. Scheraga, *Q. Rev. Biophys.* **10**, 239 (1977); A. Pullman and B. Pullman, *ibid.* **7**, 505 (1975).
4. K. D. Gibson and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 420 (1967); A. J. Hopfinger, *Macromolecules* **4**, 731 (1971).
5. B. P. Pullman and H. Berthod, *Theor. Chim. Acta* **36**, 317 (1975).
6. S. Krimm and C. M. Venkatachalam, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2468 (1971).
7. R. Rein, V. Renugopalakrishnan, S. Nir, T. J. Swissler, *Int. J. Quantum Chem. Symp.* **2**, 99 (1975); V. Renugopalakrishnan, S. Nir, R. Rein, in *Environmental Effects on Molecular Structure and Properties* (Reidel, Dordrecht, 1976), p. 109.
8. The Monte Carlo method used here has also been applied to compute the thermodynamic properties of pure water (*9*), ions in water (*10*), and methane in water (*11*). The water structure in the triclinic lysozyme crystal was simulated by these techniques (*1*), and the validity of these methods has been tested by comparing the calculated water structure with that observed by x-ray in a cyclic hexapeptide crystal (*12*). More recently the dynamics of the alanine dipeptide in aqueous solution was studied by an alternative, equally potent computer simulation method (*13*).
9. D. L. Beveridge and S. Swaminathan, *J. Am. Chem. Soc.* **99**, 8392 (1977); J. C. Owicki and H. A. Scheraga, *ibid.*, p. 7403.
10. J. Fromm, E. Clementi, R. O. Watts, *J. Chem. Phys.* **62**, 1388 (1975).
11. J. C. Owicki and H. A. Scheraga, *J. Am. Chem. Soc.* **99**, 7413 (1977); V. G. Dashevsky and G. N. Sarkisov, *Mol. Phys.* **27**, 1271 (1974).
12. A. T. Hagler, J. Moult, D. J. Osguthorpe, *Biopolymers* **19**, 395 (1980).
13. M. Karplus and P. J. Rossky, *J. Am. Chem. Soc.* **101**, 1913 (1979); P. J. Rossky, M. Karplus, A. Rahman, *Biopolymers* **18**, 825 (1979).
14. L. Boje and A. Hvidt, *J. Chem. Thermodyn.* **3**, 663 (1971).
15. W. W. Wood, in *Physics of Simple Liquids*, H. N. V. Temperely, J. S. Rowlinson, G. S. Rushbrooke, Eds. (North-Holland, Amsterdam, 1968), p. 117.
16. J. S. Rowlinson, *Trans. Faraday Soc.* **47**, 120 (1951).
17. F. H. Stillinger and A. Rahman, *J. Chem. Phys.* **60**, 1545 (1974).
18. A. T. Hagler, E. Huler, S. Lifson, *J. Am. Chem. Soc.* **96**, 5319 (1974); A. T. Hagler and S. Lifson, *ibid.*, p. 5327; ———, P. Dauber, *ibid.* **101**, 5122 (1979).
19. N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. Teller, E. Teller, *J. Chem. Phys.* **21**, 1087 (1953).
20. J. E. Worsham, Jr., H. A. Levy, S. W. Peterson, *Acta Crystallogr.* **10**, 319 (1975).
21. B. Robson and A. T. Hagler, in preparation.
22. I. H. Hillier and B. Robson, *J. Theor. Biol.* **76**, 83 (1979); B. Robson, I. H. Hillier, M. Guest, *Trans. Faraday Soc. Ser. 2* **74**, 1311 (1978).
23. The total stabilization energy also includes the difference in the water-water interaction energy in the presence of the two conformers. This component takes significantly longer to converge than other averages (A. T. Hagler, D. J. Osguthorpe, B. Robson, in preparation).
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Calcification Inside Artificial Hearts: Inhibition by Warfarin-Sodium

Abstract: *Intracavitary calcium phosphate deposits were observed in smooth, elastomeric blood pump sacs implanted in male calves for periods of 115 to 166 days. These deposits occurred predominantly on the flexing surface of the sacs. In contrast, similar pump sacs remained generally free of mineral deposits for up to 150 days in calves treated with the anticoagulant warfarin-sodium. These results implicate a vitamin K-dependent process in calcium phosphate deposition on elastomeric sacs.*

Twenty years have elapsed since the first attempts were made to use implantable blood pumps to support the circulation for prolonged periods (*1*). Although the ultimate goal of implantable blood pump programs is the development of an artificial heart, a highly desirable intermediate goal is the development of an implantable, long-term, left ventricular assist pump. Initial problems of thromboembolism and device breakage have been reduced to tolerable levels. Gradual improvements in pump design, pump fabrication, and operative techniques have enabled investigators to provide continuous left ventricular support in calves for periods exceeding 3 months (*2–4*). Moreover, three groups found that calves could survive more than 5 months after heart replacement with two pneumatically powered implanted blood pumps (*5*).

As longer-term animal studies have become possible, dystrophic calcification has been observed on the blood-contacting surfaces of the pumps removed from animals at autopsy. Calcification has been observed on pump linings fabricated of segmented polyether-type polyurethane, segmented polyurethane-polydimethyl siloxane copolymer, Dacron flock-lined polyurethane, and glutaraldehyde-treated, gelatin-coated synthetic rubber (*2, 6*). This calcification has caused stiffening, flexion failure, and perforation of the pump linings. Although this degree of calcification may be unique to the growing calf, it is now limiting the duration of studies in these animals.

In 1976, our group began to use an implantable, pneumatically powered blood pump consisting of a segmented polyurethane sac contained within a rigid plastic case (*4, 7*). The pump has been used as a left ventricular assist device or, with two such pumps, as an artificial heart, in a series of male Holstein-Friesian calves weighing 90 to 110 kg. To minimize the incidence of thromboembolism, we administered the anticoagulant warfarin-sodium and the platelet protective agents aspirin (20 mg/kg-day) and

Table 1. Sac calcification in calves not treated with warfarin-sodium.

Calf No.*	Period of continuous pumping (days)	Portion of flexing side of sac covered by gross calcification (%)†
176R	115	68.6
176L	115	59.3
108R	159	45.4
108L	159	57.1
141	166	100

*R and L refer to right or left ventricle of an artificial heart. Calf No. 141 had an implanted left ventricular assist pump. †As determined by planimetry.

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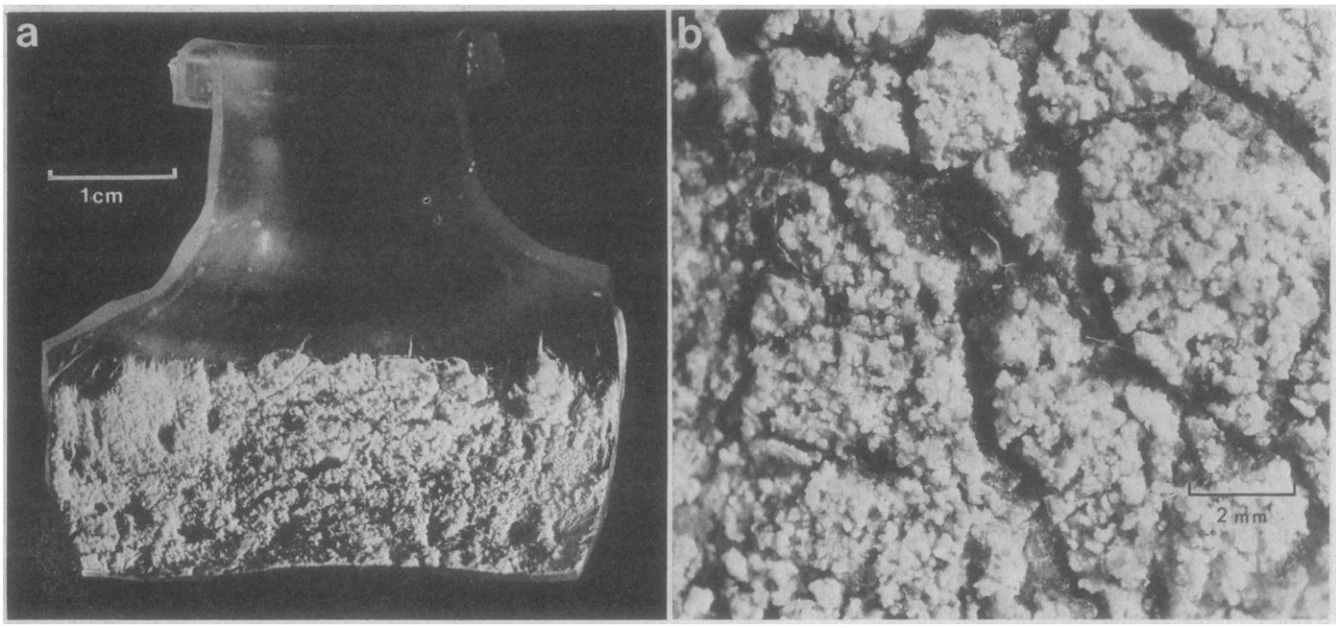

Fig. 1. Representative photographs of the blood pump sac in a calf not treated with warfarin-sodium (calf No. 176R). (a) A section of the outlet neck. A dense calcium phosphate deposit was present on the moving portion of the sac, whereas the stationary portion was free of grossly visible deposits. A rather well-delineated edge, which marks the boundary between the moving and stationary sac walls, separates the two zones. (b) A magnified view of the calcific deposit. The calcium phosphate crystals were firmly adherent to a proteinaceous coating on the underlying segmented polyurethane.

dipyridamole (3 mg/kg-day). The dose of warfarin-sodium (5 to 10 mg/day) was adjusted to maintain a prothrombin time of 25 to 30 seconds (*8*). Six calves (in which were implanted a total of seven blood pumps, that is, five calves having assist pumps and one calf having an artificial heart) lived over 90 days with one of them surviving 150 days. From the mean survival time of the calves we calculated that the blood pumps worked effectively for 115 ± 22 days (mean ± standard deviation, $N = 7$) (*9*). When we examined the animals at autopsy, we found that the pump sac surfaces were substantially free of cells and gross deposits of either mineral or organic matter. However, the pump sacs from calf No. 433 (survival time 100 days) did show thin calcium phosphate deposits along the line of maximum flexion, and these deposits covered < 1 percent of the area of the flexing side of the sac.

Having proved that use of this type of pump resulted in relatively few thromboembolic complications, we implanted pumps in another series of calves without administering warfarin-sodium (in order to eliminate risks of bleeding associated with anticoagulation); but we continued to use the platelet protective drugs. We used three calves (in which were implanted five blood pumps, that is, one calf having an assist pump and two calves having artificial hearts). From the mean survival time of the calves we calculated that the blood pumps worked effectively for 142 ± 25 days (mean ± standard deviation, $N = 5$). The period of continuous pumping was not significantly different from that of the warfarin-sodium treated calves ($P < .20$, by a nonparametric Student's t-test). However, the appearance of the sacs at autopsy was considerably different from what had been observed previously. White microcrystalline deposits generally limited to the intracavitary portion of the flexing side were found in all five pump sacs, with secondary entrapment of some red blood cells in the most encrusted sacs (Fig. 1). The firmly adherent deposit was thickest at the periphery of the moving side of the sac (line of maximum flexion). The flexing zone deposits varied from thicknesses exceeding 3.0 mm to nonmeasurable amounts (Table 1). Subsequent scanning electron microscopic and energy-dispersive x-ray examinations of the interior sac surfaces showed abundant, calcium phosphate-rich deposits on the entire blood-contacting surface, the largest amounts being located along the line of maximum flexion. Data obtained by x-ray diffraction indicate that microcrystalline hydroxyapatite or chlorapatite is the predominant mineral form.

The calcification on the segmented polyurethane sac is the result of an interaction between the prosthetic pump and the biological system. Factors affecting the pump may include flexing diaphragm stresses, local temperature increases, and absorption of blood constituents into the polymer. Our study suggests that alteration in the biological environment can effect the calcification process. There is increasing evidence implicating vitamin K in the biological mineralization process. Warfarin-sodium administration during pregnancy has a definite effect on skeletal calcification in the fetus (fetal warfarin syndrome), although the mechanism is not clearly understood (*10*). Recently, a vitamin K-dependent, γ-carboxyglutamic acid (Gla)-containing protein was identified that has an affinity for insoluble calcium salts and participates in the regulation of calcium salt deposition in mineralized tissue (*11*). Gla-containing proteins have been shown to be present in natural bone and in pathological mineralization as seen in calcific atherosclerosis, renal calculi, and calcific aortic valves (*12*). The apparent inhibitory effects of warfarin-sodium on sac calcification reported herein suggests that such a vitamin K-dependent, protein carboxylation process may be implicated in the calcium phosphate deposition on elastomeric blood pump sacs.

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References and Notes

1. T. Akutsu and W. J. Kolff, *Trans. Am. Soc. Artif. Intern. Organs* **4**, 230 (1958).
2. W. F. Bernhard, C. G. LaFarge, R. H. Liss, M. Szycher, R. L. Berger, V. Poirier, *Ann. Thorac. Surg.* **26**, 427 (1978).
3. T. Washizu *et al.*, *Trans. Am. Soc. Artif. Intern. Organs* **23**, 301 (1977).
4. W. S. Pierce, J. H. Donachy, D. L. Landis, J. A. Brighton, G. Rosenberg, J. J. Migliore, G. A. Prophet, W. J. White, J. A. Waldhausen, *Circulation* **58** (suppl. I), 133 (1978).
5. Calf survival of 210 days following artificial heart implantation has been achieved at the University of Utah, Salt Lake City (W. J. Kolff, personal communication), 196 days at the Free University of Berlin, West Germany (E. S. Bücherl, personal communication), and 157 days at Pennsylvania State University by our group.
6. D. L. Coleman, J. D. Andrade, W. J. Kolff, *Off. Publ. Am. Soc. Artif. Intern. Organs* **8**, 23 (abstr.) (1979); Y. Nosé, R. Kiraly, H. Harasaki, in *Devices and Technology Branch Contractors Conference Proceedings 1977*, K. Gerber, Ed. (Publ. No. NIH 78-1601, Department of Health, Education, and Welfare, Washington, D.C., 1978), p. 63.
7. J. W. Boretos and W. S. Pierce, *Science* **158**, 1481 (1967). The blood-contacting sacs were fabricated of Biomer segmented polyurethane. Microcrystalline wax forms were coated with high-gloss silicone rubber dispersion under a laminar flow hood. The surface was allowed to dry. The forms were dipped repeatedly in a 20 percent solution of segmented polyurethane in *N,N*-dimethylacetamide until the walls were approximately 0.38 mm in thickness, with 20-minute periods of solvent evaporation at 60°C being allowed between successive dips. The sacs were allowed to cure for 24 hours at 60°C, and then the hollow form was crushed and removed. The silicone rubber lining was peeled out. The sac was trimmed and heat-set to fit within the pump housing. A final cure was achieved by autoclaving. Pump components were cleaned ultrasonically in a trisodium phosphate detergent with multiple rinses in distilled water. The completely assembled pumps were sterilized with ethylene oxide and allowed to degas for at least 7 days before being implanted.
8. The normal prothrombin time for calves in our laboratory is 16.5 ± 3.3 seconds (mean ± standard deviation, $N = 57$).
9. For details of these studies and the causes of death, see Pierce *et al.* (*4*); W. S. Pierce, J. A. Brighton, J. H. Donachy, D. L. Landis, G. Rosenberg, G. A. Prophet, W. J. White, J. A. Waldhausen, *Arch. Surg.* **112**, 1430 (1977).
10. W. L. Shaul and J. G. Hall, *Am. J. Obstet. Gynecol.* **127**, 191 (1977); J. M. Pettofor and R. Benson, *J. Pediatr.* **86**, 459 (1975).
11. P. V. Hauschka, J. B. Lian, P. M. Gallop, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3925 (1975); P. A. Price, A. S. Otsuka, J. A. Poser, J. Kristaponis, N. Raman, *ibid.* **73**, 1447 (1976).
12. P. V. Hauschka, J. B. Lian, P. M. Gallop, *Trends Biochem. Sci.* **3**, 75 (1978); R. J. Levy and J. B. Lian, *Circulation* **58** (suppl. II), 54 (1978).

13. At the Milton S. Hershey Medical Center of the Pennsylvania State University, we thank O. Ferrari and G. A. Prophet for technical assistance. The Pennsylvania study was supported by NIH grants R01 HL 20356, R01 HL 13426, and the H. G. Barsumian, M.D. Memorial Trust Fund. At Calspan Corporation, we thank C. K. Akers and V. A. DePalma for electron microscopic and x-ray analyses, and R. Zontek and A. Meyer for spectroscopic data. The Calspan study was supported by NIH contract N01 HV 3 2953.

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Alteration in Connections Between Muscle and Anterior Horn Motoneurons After Peripheral Nerve Repair

Abstract. *The connections between the spinal cord and lower leg muscles of the rat are significantly altered by repair of the intervening sciatic nerve. Muscles supplied by the peroneal branch of the sciatic are innervated by fewer motoneurons after sciatic repair. Many of these neurons originally innervated the peroneal muscles, and others formerly served the antagonistic tibial muscles. Perikarya in the size range of alpha motoneurons regained peripheral connections with greater frequency than those in the gamma range. There are thus postoperative defects in the extent and specificity of alpha reinnervation as well as in the degree of gamma control.*

The results of peripheral nerve repair in humans are often disappointing. Fine coordination is impaired, and individual muscles no longer act independently of one another. Reinnervation of muscle by inappropriate motoneurons may be a cause of poor postoperative function (*1*), but has not been clearly demonstrated. We have shown that after repair of the rat sciatic nerve, the peroneal muscles are reinnervated by appropriate motoneurons as well as by many that previously served their antagonists. We have also found that few motoneurons in the gamma size class regain peripheral connections. There are thus anatomical defects in both the specificity of muscle reinnervation and the extent of gamma control after peripheral nerve repair; these defects may result in the deterioration of function commonly experienced.

Experiments were performed on five 250-g female albino rats. In two normal animals horseradish peroxidase (HRP) was injected into the peroneal or the tibial muscle compartments to determine the relative locations of their motoneuron pools. In three additional rats the right sciatic nerve was severed in midthigh, and epineurial repair was performed with 10-0 nylon sutures under magnification (×3 to ×8). After 3 months, HRP was injected into both peroneal compartments of these animals. Each muscle group was injected with 20 μl of 20 percent HRP (Sigma VI) in 5-μl portions under anesthesia (Chloropent, 3 ml per kilogram of body weight). Nerves supplying adjacent muscles were severed through a more proximal incision to limit the central transport of HRP to the chosen pathway (*2*). After 48 hours the animals were reanesthetized and perfused with fixative according to procedure II of Rosene and Mesulam (*3*). The lumbosacral cords were dissected out, cut in 40-μm cross sections, and reacted with H_2O_2 and tetramethyl benzidine (*4*). The sections were serially mounted, counterstained with neutral red, and examined to determine the location, number, and size of labeled cells present in each section. Cell profiles that appeared in two adjacent sections were counted only once. Neuronal diameters were estimated by the method of Burke *et al.* (*2*).

In normal rats, injecting the peroneal or tibial muscle compartments of the lower leg resulted in the labeling of discrete pools of motoneurons in the anterior horn of the spinal cord. Others have demonstrated similar compartmentalization (*2*, *5*). The location of the peroneal motoneuron pool was defined in the coronal plane at different cord levels. Neurons labeled by peroneal muscle injection after nerve repair were scored as "in" or "out" of the normal peroneal pool location by comparing their position with that of the normal pool on the opposite, control side of the same animal.

The six control peroneal pools contained an average of 395 cells (range, 368 to 434) (*6*). In one animal, bilateral peroneal compartment injection labeled 368 cells on the right and 424 on the left. There was thus a variation in pool size of 13 percent from side to side and 15 percent overall. In normal peroneal pools, most cells were concentrated in the fourth lumbar (L_4) segment, with an abrupt proximal termination and gradual attenuation throughout L_5 (Fig. 1). In one animal, the normal tibial pool contained 866 cells extending from L_4 to L_6 and was most prominent in its caudal extreme.

The three postoperative peroneal pools contained an average of 273 cells (range, 245 to 291). The mean postoperative pool was thus 69 percent the size of its normal counterpart, a variation far greater than the 15 percent variability in normal pool size. The anatomical distribution of motoneurons innervating the peroneal muscles was also changed postoperatively. The peak concentration of labeled cells, which normally occurred at the L_4 level, shifted to the L_5 and even L_6 levels (Fig. 1). In addition, 29 to 47 percent of the cells labeled by peroneal muscle injection were within the area normally occupied on coronal section by the tibial pool. Similar overlap was very unusual (0.5 to 1 percent of labeled cells) in the unoperated animals. Furthermore, the size distribu-

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