inch radius of curvature. The $\delta^{18}O$ and $\delta^{17}O$ measurements were corrected for tailing, valve leak rate, and background (*2*).
7. As an analytical check, a sample set was isotopically analyzed at the laboratory of R. N. Clayton at the University of Chicago. Figure 1 shows that the measurements at Chicago lie along the slope 1 line defined at La Jolla.
8. N. J. Turro and B. Kraeutler, *J. Am. Chem. Soc.* **100**, 7432 (1978); M. H. Thiemens and R. N. Clayton, *Meteoritics* **14**, 595 (1979).
9. R. K. Sander, T. R. Loree, S. D. Lockwood, S. M. Freund, *Appl. Phys. Lett.* **30**, 150 (1977).
10. R. J. Cicerone and J. L. McCrumb, *Geophys. Res. Lett.* **7**, 251 (1980); J. E. Heidenreich III and M. H. Thiemens, *J. Chem. Phys.*, in press; S. Epstein, *Lunar Planet. Sci.* **11**, 259 (1980).
11. G. Herzberg, *Spectra of Diatomic Molecules* (Van Nostrand, New York, 1950).
12. W. D. Langer, *Astrophys. J. Lett.* **212**, L39 (1977).
13. J. Lequeux, *Geochim. Cosmochim. Acta* **46**, 777 (1982).
14. K. J. Zahnle and J. C. G. Walker, *Rev. Geophys. Space Phys.* **20**, 280 (1982).
15. V. M. Canuto, J. S. Levine, T. R. Augustsson, C. L. Imhoff, *Nature (London)* **296**, 816 (1982); C. L. Imhoff and M. S. Giampapa, *Astrophys. J. Lett.* **239**, L115 (1980); G. F. Gahm, K. Kredga, R. Liseau, D. Dravins, *Astron. Astrophys.* **73**, L4 (1979).
16. J. Bally and W. D. Langer, *Astrophys. J.* **255**, 143 (1982).
17. R. N. Clayton and T. K. Mayeda, *Geophys. Res. Lett.* **4**, 295 (1977); G. J. Wasserburg, T. Lee, D. A. Papanastassiou, *ibid.*, p. 299; T. Lee, T. K. Mayeda, R. N. Clayton, *ibid.* **7**, 493 (1980).
18. Y. Kolodny, J. F. Kerridge, I. R. Kaplan, *Earth Planet. Sci. Lett.* **46**, 149 (1980); J. Geiss and H. Reeves, *Astron. Astrophys.* **93**, 189 (1981); F. Robert and S. Epstein, *Geochim. Cosmochim. Acta* **46**, 81 (1982); R. H. Becker and S. Epstein, *ibid.*, p. 97.
19. We are grateful to R. N. Clayton and T. K. Mayeda for mass spectrometric analysis of the samples. We are particularly indebted to G. Lugmair, who offered valuable advice throughout the project and who patiently read and corrected several manuscripts. Thanks to T. Vander Wood and J. H. Reynolds for their scholarly comments. Support for the project was provided to M.H.T. from the Department of Chemistry, University of California, San Diego.

24 September 1982; revised 17 December 1982

Hysteresis in the Force-Calcium Relation in Muscle

Abstract. *Calcium ions activate muscle contraction. The mechanism depends on the calcium sensitivity of the proteins that regulate contraction. Evidence is presented for the reverse phenomenon, where contraction modulates calcium sensitivity. Increasing the force level increased calcium sensitivity in intact fibers, showing that the relation between force and calcium is not unique. A particular calcium concentration can maintain a higher force level than it can create. The results were confirmed in skinned fiber experiments. Transient reduction of the force led to a transient reduction in calcium binding, suggesting a simple mechanism for the hysteresis.*

It is now well established that muscle contraction is activated through the binding of calcium to calcium-sensitive myofibrillar (*1*) or sarcoplasmic proteins (*2*). The details vary among muscle types, but in all cases calcium binding allows actin and myosin to interact and leads eventually to the production of force and shortening through cross-bridge interaction. In this report we examine the question of whether contraction influences calcium sensitivity and calcium binding.

We used aequorin-injected voltage-clamped barnacle single muscle fibers (*3*) to investigate the "quasi-steady state" relations between calcium concentration and force level. In an experiment with a long stimulation pulse (note the slow horizontal sweep rate in Fig. 1A) a constant depolarization leads to a calcium transient that goes through an initial peak and then settles down to a fairly steady level maintained for several seconds. Force behaves similarly. Near the end of the stimulation (marked by arrows) we can measure a quasi-steady-state relation between calcium concentration and force. If the depolarizing pulse is initially stepped briefly to a higher level and then returned to the same level as before, we obtain the result shown in Fig. 1B. Here, despite the initial overshoot, both the membrane depolarization and the calcium transient eventually (see arrow) arrive at levels indistinguishable from those achieved in Fig. 1A, but the force trace does not. It remains at more than twice its previous value. Thus the relation between calcium concentration and force is not unique, but depends on the immediate history of the muscle. This is clear in Fig. 1C, which shows the force-calcium relation after a 10-second depolarization with three different force wave forms produced by a manual control of the stimulation pulse: 1, initial overshoot returning to a steady level (as in Fig. 1B); 2, rapid rise (2 seconds) to a steady level; or 3, slow rise (4 seconds) to a steady level. In each case, the "steady" calcium level produces more force when the fiber previously experienced a higher force (or calcium) level; that is, the steady force for time course $1 > 2 > 3$. An obvious interpretation is that increased force and increased cross-bridge interaction occurring early in the contraction heightened the calcium sensitivity. In other words, in this new condition a much higher force can be maintained with a lower calcium concentration (*4*).

Our interpretation of the experiment illustrated in Fig. 1, A to C, can be criticized in several ways. For example, there might be a long-term change in the intracellular magnesium concentration during the pulse which in turn could change the calcium sensitivity of the myofilaments or the sensitivity of the aequorin. Or there could be adenosine triphosphate (ATP) depletion, or a change in *p*H. To explore these possibilities, we turned to the "skinned" fiber preparation (*5*), where there is complete control over the solutions bathing the myofilaments.

To prepare a barnacle muscle skinned fiber (*6*), we first soak an intact fiber for 1 hour in a relaxing solution (*7*) with a detergent (1 percent Triton X-100) added to disrupt the sarcolemma and the sarcoplasmic reticulum. The fibers are next split longitudinally down to bundles of myofibrils of about 50 to 100 μm in diameter (1 to 1.5 mm long) to reduce the diffusion path lengths. The bundle is then attached to a force transducer (*7*) and immersed (stepped) into solutions (*8*) that can induce contraction or relaxation depending on the calcium concentration. These solutions (*5*, *9*) are well buffered against changes in *p*H, in calcium concentration, and in Mg-ATP concentration, which obviates the criticisms mentioned above. Figure 2A illustrates the results of transferring the fiber consecutively from a relaxing solution, to a submaximal calcium activating solution, to a maximal one, and then back to the same submaximal calcium activating solution before returning to a relaxing solution. It can be seen that the same (submaximal) calcium concentration produces more force when the calcium concentration is stepped down (decreasing Ca) from a maximum activation than when it is stepped up (increasing Ca) from the relaxing solution. This difference in force appears to represent a true hysteresis, since leaving the fiber in the submaximal calcium activating solutions for a longer period during either stepping up or stepping down in calcium does not result in the two forces tending to approach one another. If the solution used between the two identical submaximal calcium activating solutions is not maximal, the hysteresis becomes less as the intervening calcium activation is lower. Also, once the fiber relaxes completely in the relaxing solution, the contraction series shown in Fig. 2A can be repeated with the same results, showing that a few minutes of relaxation wipes out the "memory" of the maximal contraction. Data similar to those in Fig. 2A can be obtained for a variety of submaximal calcium concentrations, so that we can plot the relation between calcium concentration and force for the two condi-

tions, stepping up and stepping down in free calcium. The hysteresis between the two resulting curves is shown in Fig. 2B. A shift in the relation between force and calcium level is seen, with the muscle being about 0.13 *p*Ca unit more sensitive to calcium when stepping down in calcium (relaxing) (*10*). Hence, the skinned fiber experiments (Fig. 2) confirm and extend the results shown in Fig. 1, A to C. They also demonstrate that the force-*p*Ca relation is not unique for a given chemical internal environment (as has usually been tacitly assumed). Instead, the muscle contracts along one *p*Ca curve and relaxes along another.

One simple hypothesis to account for the changed calcium sensitivity is that a change in force leads to a change in calcium binding. We believe that the mechanism for changing calcium binding may involve a cross-bridge–induced change in the calcium affinity of calcium binding sites on the myofilaments. Although we could not test the binding hypothesis directly, we made an indirect test with the aequorin-injected voltage-clamped single muscle fibers from the barnacle; the results of this test are shown in Fig. 1D. One of the easiest methods for transiently perturbing the force is to stimulate a muscle to contract, wait until force has developed, then suddenly allow the muscle to shorten (by releasing it) for a distance sufficient to break many of the cross-bridges. If activation remains after the shortening step, some force may redevelop as new cross-bridges form (see Fig. 1D). A striking result of the length change is a transient burst of "extra" calcium in the sarcoplasm, which appears as a shoulder on the falling phase of the calcium transient (trace c in Fig. 1D). Subtracting the control calcium transient yields the time course and magnitude of the "extra" calcium, and this is shown in trace e of Fig. 1D. Thus, quickly releasing the muscle transiently reduces force and transiently increases free calcium (*11*), presumably by releasing it from binding sites on the myofilaments (*12*). Increased calcium binding as a result of cross-bridge interaction was previously shown by Bremel and Weber (*13*) and by Fuchs (*14*) on the basis of measurements on isolated proteins and myofibrils under rigor conditions and was suggested by Adelstein and Eisenberg (*15*), Taylor (*16*), and Shiner and Solaro (*17*) on theoretical grounds. We have demonstrated this phenomenon under physiological conditions.

On the basis of results from intact (Fig. 1, A to C) and skinned (Fig. 2) fibers, we conclude that contraction modulates calcium sensitivity. The results of Fig. 1D strongly suggest that the mechanism responsible for changes in calcium sensitivity depends on changes in calcium binding and calcium affinity. Therefore the force-*p*Ca relation of the muscle is not unique, but depends on the contraction history. The hysteresis is quite large compared to the working *p*Ca range (Fig. 2B) so that the phenomenon is important.

The feedback from force to calcium sensitivity can help explain several previously puzzling phenomena in muscle, including (i) the relatively high force-to-

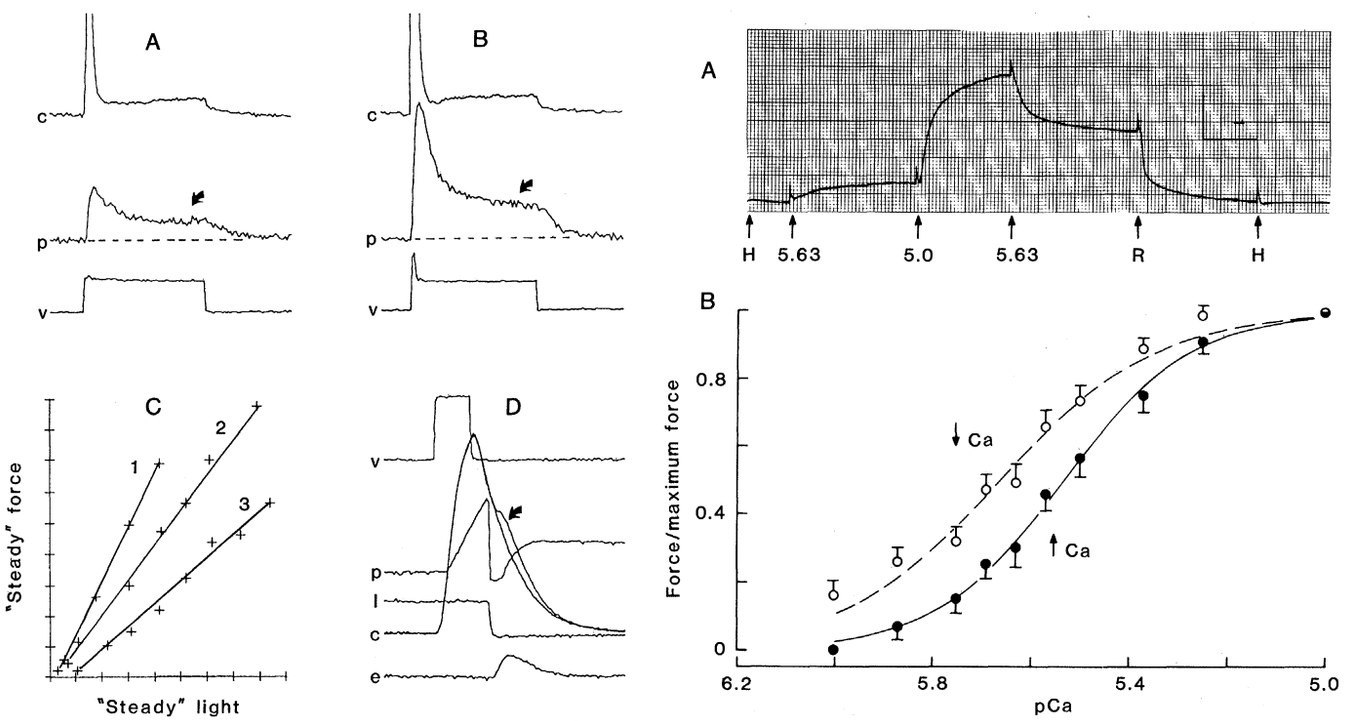

Fig. 1 (left). (A to C) Quasi-steady-state hysteresis force-calcium relation. (A) Long stimulus pulse at constant amplitude. (B) Long stimulus with brief initial extra depolarization: trace v, membrane potential at 40 mV/cal; trace c, aequorin light signal (calcium transient) at 1.0 μA/cal; and trace p, isometric force at 46 mN/cal. Horizontal sweep, 4 sec/cal; temperature, 7°C; fiber length, 23 mm. (C) Quasi-steady-state force-*p*Ca relation with the "steady" force and "steady" light at the end of a 10-second depolarization whose amplitude was controlled manually to give force wave forms 1 to 3 (see text). (D) Response to a transient change in force and length produced by a quick release (shortening) (at point shown by arrow): trace v, membrane potential at 40 mV/cal; trace p, isometric force at 66 mN/cal; trace l, fiber length at 2.5 mm/cal; trace c, aequorin light signal (calcium transient) at 1.0 μA/cal superimposed on control light signal without the length change; and trace e, the extra light—the difference between the experimental and control calcium transients. Temperature, 9°C; fiber length, 23 mm. Fig. 2 (right). Hysteresis in the force-*p*Ca relation in skinned fibers. (A) At points shown by the arrows the fiber is transferred consecutively to a relaxing solution with EGTA as the calcium buffer (*R*) (not shown), a relaxing solution with HDTA as the calcium buffer (*H*, shown on the left), solutions with *p*Ca values as indicated (5.63, 5.0, and 5.63, again with EGTA as the calcium buffer), a relaxing solution with EGTA (*R*), and finally HDTA solution (*H*) to test force return to baseline. Horizontal and vertical bars represent 1 minute and 100 N, respectively. Fiber diameter, 50 m; temperature, 22°C. (B) Force as fraction of maximum plotted against *p*Ca for two conditions: (●) stepping up and (○) stepping down calcium concentration. Standard error bars are shown (N = ten fibers). Curves were fitted by the Hill equation: fractional force = $[Ca]^N/([Ca]^N+K^N)$, with N and *p*K = 3.3 and 5.53, respectively, for increasing calcium and 2.7 and 5.66 for decreasing calcium.

calcium ratio observed early in relaxation (*3*); (ii) shifts in the force-*p*Ca relation with changes in sarcomere length (*18, 19*); (iii) the apparent positive "cooperativity" in calcium activation that steepens the force-*p*Ca curve (*19–21*); and (iv) an effect on calcium sensitivity of factors that may affect actomyosin interaction and influence cross-bridge turnover (for instance, Mg-ATP, Mg, *p*H, and fiber type) (*20, 21*).

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References and Notes

1. S. Ebashi, A. Kodama, F. Ebashi, *J. Biochem.* **64**, 465 (1968); S. Ebashi and M. Endo, *Prog. Biophys. Mol. Biol.* **18**, 123 (1968); A. G. Szent-Györgyi, *Biophys. J.* **15**, 707 (1975); W. Lehman, *Int. Rev. Cytol.* **44**, 55 (1976).
2. A. Sobieszek and J. V. Small, *J. Mol. Biol.* **102**, 75 (1976); R. Dabrowska, D. Aromatorio, J. M. F. Sherry, D. Hartshorne, *J. Biochem.* **17**, 253 (1978).
3. E. B. Ridgway and C. C. Ashley, *Biochem. Biophys. Res. Commun.* **29**, 229 (1967); C. C. Ashley and E. B. Ridgway, *J. Physiol. (London)* **209**, 105 (1970).
4. The data in Fig. 1C imply that the increased calcium sensitivity is not simply a function of the previous force level but is also dependent on the time spent at that force.
5. R. Natori, *Jikeikai Med. J.* **1**, 119 (1954).
6. C. C. Ashley and D. G. Moisescu, *J. Physiol. (London)* **270**, 627 (1977).
7. The relaxing solution is identical to that described in (*8*) with no added calcium and with EGTA as the chelator for calcium.
8. D. C. Hellam and R. J. Podolsky, *J. Physiol. (London)* **200**, 807 (1969).
9. The solutions contained 170 m*M* K propionate, 30 m*M* Na propionate, 4.5 m*M* Mg-ATP, 1.5 m*M* Mg^{2+}, 30 m*M* EGTA, 60 m*M* Tes (*p*H 7.1), 20 m*M* creatine phosphate, and 10 U/ml creatine phosphokinase. Calcium EGTA was added to achieve the desired *p*Ca ($-\log_{10}$[Ca]). The relaxing solution contained no added calcium. Solutions were mixed as described in (*5*). Before each contraction, the fiber was transferred from an EGTA relaxing solution to one with HDTA (hexamethylenediamine-*N,N,N',N'*-tetraacetic acid, a weak Ca^{2+} buffer) replacing the EGTA, in which the fiber remained relaxed but from which force developed more rapidly when the fiber was transferred into a Ca^{2+} activating solution. Fibers immersed in an EGTA-buffered subthreshold Ca^{2+} solution showed the same effect and also the force-*p*Ca hysteresis. All solutions were stirred to increase the speed of the force response to a solution change.
10. In addition, the slope of the relaxation curve is slightly less steep. We also observed this hysteresis in skinned frog and rat skeletal muscle.
11. We examined the effects of changing force in both directions. Stretch causes free calcium to disappear; release causes free calcium to appear (Fig. 1D) (E. B. Ridgway and A. M. Gordon, in preparation).
12. This is supported by the facts that the magnitude of extra free calcium (i) is increased under conditions when more activating calcium is present (increased or paired stimulation), (ii) is correlated with the force redeveloped after the length change (like the classical "active state" experiments), and (iii) has a time course intermediate between the calcium transient and force, as would be required for calcium bound to an activating site. Alternative sources of extra calcium—the sarcoplasmic reticulum (SR) or the surface membrane—can be ruled out on the basis of control experiments. See A. M. Gordon and E. B. Ridgway [*Eur. J. Cardiol.* **7**, 27 (1978)] and E. B. Ridgway and A. M. Gordon [*Biophys. J.* **33**, 30a (1981)] for the SR; and A. M. Gordon and E. B. Ridgway [*J. Gen. Physiol.* **68**, 653 (1976)] for the surface membrane, where it is shown that a shortening step increases the outward current and not the inward current under voltage clamp.
13. R. Bremel and A. Weber, *Nature (London)* **238**, 97 (1972).
14. F. Fuchs, *Biophys. J.* **17**, 161 (1977).
15. R. S. Adelstein and E. Eisenberg, *Annu. Rev. Biochem.* **49**, 921 (1980).
16. E. W. Taylor, *CRC Crit. Rev. Biochem.* **6**, 103 (1979).
17. J. S. Shiner and R. J. Solaro, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
18. M. Endo, *Nature (London)* **237**, 211 (1972); M. G. Hibberd and B. R. Jewell, *J. Physiol. (London)* **290**, 30P (1979).
19. A. Fabiato and F. Fabiato, *J. Gen. Physiol.* **72**, 667 (1978).
20. F. J. Julian, *J. Physiol. (London)* **218**, 117 (1972).
21. P. W. Brandt, R. N. Cox, M. Kawai, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4717 (1980).
22. We thank A. O. D. Willows and the staff at Friday Harbor Laboratories for their hospitality. We thank O. Shimomura for the native aequorin. We thank S. Price, A. Fabiato, A. Snow, B. Hille, and W. Almers for useful comments on earlier drafts. This work was supported by grants NS 10919 and NS 08384 from the National Institutes of Health and by the Muscular Dystrophy Association.

15 June 1982; revised 10 September 1982

Dwarf Males in the Teredinidae (Bivalvia, Pholadacea)

Abstract. *Extreme sexual dimorphism in the Bivalvia is rare. The occurrence of dwarf males in* Zachsia *appears to be the first case in the Teredinidae and the first outside the Leptonacea. Female* Zachsia *release straight-hinge larvae that develop in the plankton and settle on living rhizomes of* Phyllospadix. *Larvae entering mantle pouches of females become males. Evolution of this life history pattern is tied to problems of living in a fragile, patchy habitat—that is, the rhizomes of* Phyllospadix.

Studies of the life history of *Zachsia zenkewitschi* Bulatoff and Rjabtschikoff 1933 (*1*) conducted at the Vostok Field Station in the Soviet Union (*2*) revealed a case of marked sexual dimorphism—that is, dwarf males (*3*) in the Teredinidae. We investigated the "tailed larvae" of this little known species. These were described as occurring in lateral mantle pouches just anterior to the siphons (*1*). It was these so-called larvae that proved to be dwarf males.

The occurrence of dwarf males among the Bivalvia is rare and otherwise known only in the Veneroida, superfamily Leptonacea (*4*). Species with dwarf males are small, parasitic on or commensal with a marine invertebrate (except *Z. zenkewitschi*), and so far as known, brood their larvae.

Like other species with dwarf males, *Z. zenkewitschi* lives in a restricted, patchy habitat, but differs in that it inhabits living rhizomes of the sea grass *Phyllospadix iwatensis* Makino rather than associating with a marine invertebrate. Specimens studied were dissected from *Phyllospadix* collected at six localities in Vostok Bay. Additional specimens were collected by Y.Y. at Putjatin Island, about 25 km west of Vostok, and at Vladivostok, the type locality of *Z. zenkewitschi*. The following observations were based on the study of over 100 specimens collected from these various localities.

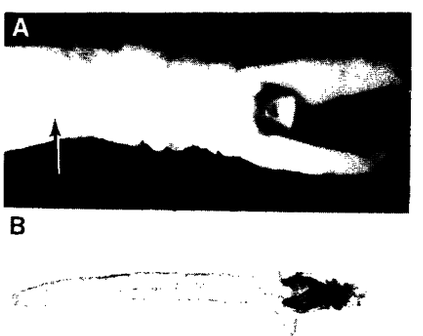

Fig. 1. (A) Lateral view of posterior end of a female *Zachsia zenkewitschi* showing siphons, left pallet, mantle collar, and left mantle pouch (arrow) containing dwarf males (preserved specimen, ×9). (B) Mature dwarf male *Zachsia zenkewitschi* removed from the pouch of the female (preserved specimen, ×30).

Superficially adult female *Z. zenkewitschi* look like other short-term larviparous shipworms. The larvae are brooded until the straight-hinge stage and released en masse when 80 μm long and 70 μm high. They reach the pediveliger stage after feeding in the plankton for 2 to 3 weeks. In laboratory cultures most larvae swim near the bottom of the culture dish, suggesting that in the field they probably swim in the sea grass beds rather than the turbulent surface waters and that wide-ranging dispersal is probably by adults in floating sea grass.

Experimentally we have not succeeded in getting pediveliger larvae to metamorphose, but field and laboratory observations indicate that larvae which settle on *Phyllospadix* that is not inhabited by *Zachsia* will penetrate and metamorphose into females. Pediveligers settling on rhizomes already inhabited by a female will crawl into one of the mantle pouches, which open into the cavity of the mantle collar at the base of the siphons of the female, and metamorphose into males (*5*). The pouch contain-