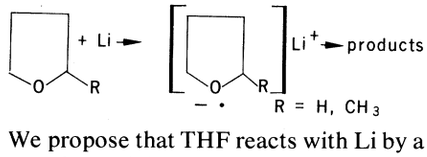
percent as current density is decreased from 5.0 to 0.9 mA/cm².

The cycling behavior of the Li electrode is generally better on Li than on Ni or other substrates. One possible reason for this is that the deposit is nucleated much more easily and is therefore much more uniform with a Li substrate. Lithium may also be less active as an electrode for the (catalyzed) electrochemical reduction of the solvent than is, say, Ni.

Another test of a solvent's suitability as a Li battery medium is its reactivity with plated Li on open-circuit storage. This assesses the intrinsic reactivity of Li with solvent, and the achievement of low reaction rates is a necessary condition for a satisfactory shelf life. The experiment consists of plating 1.1 C/cm² onto a Ni substrate, and then switching the cell to open circuit. After a predetermined time, the Li plate is electrostripped and the efficiency determined.

Figure 2 compares data obtained from $LiAsF_6$-based THF and 2-Me-THF electrolytes. After 96 hours on open circuit, almost 70 percent of Li plated from the 2-Me-THF electrolyte is electroaccessible. With THF, however, all of the plated Li is isolated after 48 hours. These data translate into average isolation rates of 1.1 μA/cm² (2-Me-THF, 96 hours) and 8.3 μA/cm² (THF, 24 hours). At 24 hours, the isolation rate for 2-Me-THF is 1.3 μA/cm², almost an order of magnitude lower than that calculated for THF. Data points at 16 and 24 hours appear to be anomalously high and require comment. In all of our work with cyclic ether electrolytes containing $LiAsF_6$, we have observed a recontacting phenomenon whereby some Li lost to encapsulation reactions may be recovered by storage on open circuit (*9*). We believe that this occurs in these experiments.

The superiority of 2-Me-THF over THF with regard to inertness toward reduction by Li may be rationalized by the following scheme.



We propose that THF reacts with Li by a rate-determining one-electron transfer from Li to the lowest unfilled molecular orbital (LUMO) centered on the electronegative oxygen atom. The ease of this transfer is largely determined by the energy of that vacant orbital (*13*). Thus, by raising the energy of the LUMO, it becomes more difficult to transfer an electron into it.

The energy of the LUMO about the oxygen atom of THF can be perturbed upward by locating an electron-donating group in the 2-position adjacent to the oxygen. The influence of additional electron density on the oxygen atom raises the activation energy required to form the anion radical. Since a methyl group is known to release electron density through covalent bonds (*14*), the 2-Me-THF anion radical becomes more difficult to form. In consonance with this model, preliminary static and dynamic experiments indicate that 3-Me-THF is as reactive as THF, whereas 2,5-di-Me-THF is at least as inert as 2-Me-THF.

Tetrahydrofuran is known to undergo reduction via alpha proton abstraction by a strong base (*15*). Thus, a methyl group in the 2-position could conceivably shield one side of the ring from attack. This effectively halves the number of available hydrogens, which in turn would retard the reaction rate. We do not believe, however, that the large disparity between THF and 2-Me-THF reactivity may be accounted for by the statistical factor alone. Therefore, the LUMO concept of Li–cyclic ether reactivity better accounts for the results at hand and indicates how suitably functionalized THF molecules may be rendered less reactive toward Li.

On the basis of the static tests at 71°C, the dynamic Li-on-Li cycling experiments at 25°C, and isolation rate studies, it appears that we have the basis for a practical Li electrode. Indeed, preliminary experiments with practical Li charges yield efficiency values comparable to those achieved with 1.1 C/cm² plates. When combined with suitable cathode materials such as transition metal chalcogenides in general (*16–20*) and TiS_2 (*17, 18*) or a vanadium-transition metal chalcogenide of the form $V_{1-x}M_xS_2$ (M = Fe or Cr) in particular (*19*), electrolytes based on 2-Me-THF may pave the way to the implementation of a practical Li secondary battery (*21*).

V. R. Koch
J. H. Young

EIC Corporation,
Newton, Massachusetts 02158

References and Notes

1. R. J. Jasinski, *High Energy Batteries* (Plenum, New York, 1967).
2. R. Selim and P. Bro, *J. Electrochem. Soc.* **121**, 1467 (1974); J. Jorné and C. W. Tobias, *J. Appl. Electrochem.* **5**, 279 (1975).
3. R. D. Rauh and S. B. Brummer, *Electrochim. Acta* **22**, 75 (1977).
4. V. R. Koch and S. B. Brummer, *ibid.* **23**, 55 (1978).
5. J. Butler, D. Cogley, J. Synnott, *J. Phys. Chem.* **73**, 4026 (1969).
6. V. R. Koch, U.S. patent 4,118,550 (1978).
7. C. Carvajal, J. J. Tölle, J. Smid, M. Szwarc, *J. Am. Chem. Soc.* **87**, 5548 (1965).
8. D. Nicholls, C. Sutphen, M. Szwarc, *J. Phys. Chem.* **72**, 1021 (1968).
9. V. R. Koch and J. H. Young, *J. Electrochem. Soc.* **125**, 1371 (1978).
10. V. R. Koch, *ibid.* **126**, 181 (1979).
11. R. D. Rauh, T. F. Reise, S. B. Brummer, *ibid.* **125**, 186 (1978).
12. F. W. Dampier and S. B. Brummer, *Electrochim. Acta* **22**, 1339 (1977).
13. A. Streitweiser, *Molecular Orbital Theory for Organic Chemists* (Wiley, New York, 1961), pp. 175–176.
14. E. S. Gould, *Mechanism and Structure in Organic Chemistry* (Holt, Rinehart & Winston, New York, 1959), p. 203.
15. R. B. Bates, L. M. Kroposki, D. E. Potter, *J. Org. Chem.* **37**, 560 (1972).
16. J. Broadhead, in *Power Sources 4*, D. H. Collins, Ed. (Oriole, Newcastle-upon-Tyne, England, 1973); U.S. patent 3,791,867 (1974).
17. G. L. Holleck and J. R. Driscoll, *Electrochim. Acta* **22**, 647 (1977).
18. M. S. Whittingham, *Science* **192**, 1126 (1976); U.S. patent 4,009,052 (1977).
19. D. W. Murphy *et al.*, *Mater. Res. Bull.* **12**, 825 (1977); U.S. patent 4,125,687 (1978).
20. For an excellent review of alkali-metal intercalation chemistry, see M. S. Whittingham, *Prog. Solid State Chem.* **12**, 1 (1978).
21. Exxon Enterprises offers a rechargeable Li-Al/TiS_2 button cell (electrolyte unknown). A minimum of five cycles is claimed.
22. The work on THF was supported by NSF-RANN grant AER75-03779 and the Office of Naval Research; the work on 2-Me-THF was supported by the Office of Naval Research.

27 November 1978; revised 5 March 1979

Cement Line Motion in Bone

Abstract. *Compact bovine bone subjected to constant torsional load for long periods of time exhibits large anelastic effects. Displacements occur at the cement lines and are responsible for part or all of the long-term deformation. The absence of an asymptotic creep strain is consistent with an interpretation of the cement line as a viscous interface.*

The present investigation demonstrates that large anelastic deformations occur in specimens of bone loaded in torsion for long periods, that displacements occur at the cement lines, and that these displacements appear to be responsible for much of the time-dependent deformation. The results constitute the first clear experimental evidence of viscous behavior of the cement line.

Anelastic mechanical response has been observed in all biological materials studied thus far with the possible exception of dental enamel and echinoderm skeletons. Anelastic (or viscoelastic) behavior entails dissipation of energy in dynamic loading, conversion of this energy to other forms, and continued deformation (creep) in response to quasi-static loading. In bone such behavior has been

0036-8075/79/0504-0501$00.50/0

hypothesized to function in protecting articular cartilage from impact and vibration which could lead to osteoarthritis (*1*). The loosening of joint-replacement prostheses may result in part from a mismatch between the anelastic properties of the bone and those of the prosthesis (*2, 3*). Bone is sufficiently complex in its structure that many mechanisms will contribute to its anelastic response to stress (*4*). None of the mechanisms dealt with theoretically thus far are capable of explaining the large anelastic effects observed in bone. Slippage at the cement lines, by analogy to the grain boundary effect in metals analyzed by Zener (*5*), could hypothetically result in such large anelastic effects (*6*). Since little is known about the mechanical properties of the ground substance at the cement lines, a theoretical test of the hypothesis is impracticable. We therefore undertook the present experimental study to investigate the role of cement line motion as a possible mechanism for anelasticity and yielding in bone.

Specimens of bovine cortical bone were cut in the wet state into prisms 20 mm long and 3.2 by 3.2 mm in cross section with flared, threaded ends (*7*). The flat lateral surfaces were wet-polished with graded abrasives to permit examination of the specimen by reflected-light microscopy. We then made fiduciary marks by orienting a microtome knife perpendicular to the bone axis and pressing it to the specimen surface. Prior to loading, these marks were verified as straight by examination of the specimen under the reflected-light microscope. Specimens were kept immersed in Ringer solution in equilibrium with bone chips before and during the experiments. Torsional loading was applied within 1 second by means of an arrangement of weights and pulleys; the load was held constant for the duration of the test (*8*). We determined the angular displacement by measuring the linear displacement of an arm by means of a micrometer barrel. Stresses and strains were then calculated from the torque and angular displacement. After loading for periods up to 6 weeks, the specimen was removed from the apparatus and again examined under the reflected-light microscope (*9*).

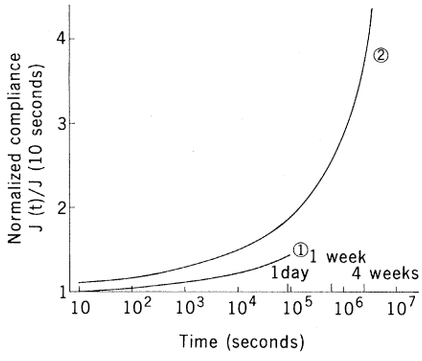


Fig. 1. Torsional creep compliance of bovine bone. The compliance is the strain response to a step stress, divided by the stress; the torsional compliance is proportional to the angular displacement of a specimen subjected to a constant torque. Curve 1, strain at 10 seconds = 0.001; curve 2, strain at 10 seconds = 0.006. The inverse of the compliance at 10 seconds, $J(10\ \text{sec})^{-1} = 4.66 \times 10^9\ \text{N/m}^2$, approximates the stiffness. Cement line motion was observed in this specimen, but the displacements were not as pronounced as those in Fig. 2.

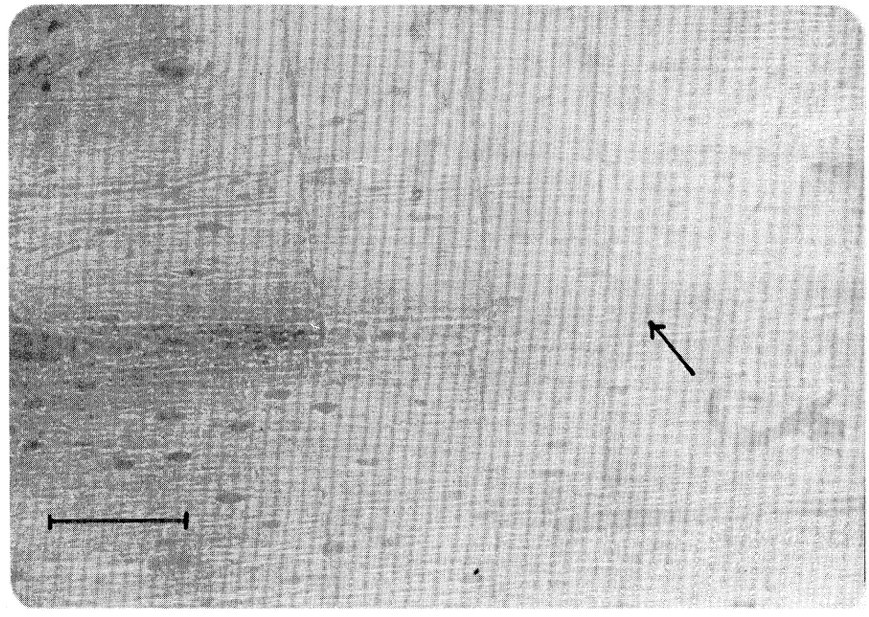


Fig. 2. Reflected-light micrograph of the surface of a specimen of bovine bone with residual strain and displacement at the cement line (the horizontal line marked with an arrow). Vertical lines are fiduciary marks which were applied to the polished surface with a microtome knife; they were straight and unbroken prior to loading. Scale bar, 100 μm.

A typical plot of compliance versus time (creep) is shown in Fig. 1 (*10*). Despite the long period of loading, no evidence was seen in any of the specimens of "leveling off" of the strain to an asymptotic value, even after loading for as long as 42 days. The increase in compliance by a factor of 4.3 is significantly greater than effects reported by others in short-term tests. The final strain of 2.6 percent observed in this test, and of 4 to 8 percent observed in other tests in the present series, considerably exceeds the fracture strain of 1.2 percent reported for wet bovine bone in more rapid torsional tests (*11*). To show that these results are not artifacts caused by prolonged soaking or dissolution of bone mineral, creep tests of 1 day's duration at small load were repeated after 4.7 days and again after 28 days, during which the bone specimen recovered at zero load under Ringer solution. The difference between the compliance measured in the first test and in the last differed by only 6 percent during the first 100 seconds of loading and by less than 2 percent after 100 seconds of loading. The effects of soaking therefore represent a small perturbation on the observed time-dependent mechanical effects (*12*).

The reflected-light micrograph (Fig. 2) shows the appearance of a specimen of bovine plexiform bone in a state of residual strain after loading. The displacements of the fiduciary marks clearly indicate shear slippage at the cement line. Displacements of this type were also observed in other specimens loaded for long periods. Such displacements were not seen in specimens which were fractured rapidly in torsion, even though plastic deformation had resulted in considerable residual strain. Motion at cement lines also was not seen in specimens subjected for short periods to bending deformations approaching those required for fracture.

We conclude that the process of viscous-like slippage at the cement lines is a dominant mechanism responsible for the large creep deformations observed in bone under prolonged, constant stress. The possibility that inhomogeneous motion at the lamellar level also contributes to the deformation cannot be discounted, since the predicted displacements would be comparable to or less than the resolu-

tion of the light microscope (*13*). Indeed, Tischendorf (*14*) has reported changes in the appearance of micrographs of bone at the lamellar level as a result of short-term loading; however, it is difficult to interpret these changes as lamellar slippage.

Composite-theoretical analyses have predicted upper and lower bounds for the elastic moduli of bone on the basis of the elastic properties of two phases, collagen and apatite (*15*). The compliant and viscous-like character of protein-polysaccharides from synovial fluid and from soft connective tissue has led to the hypothesis that polysaccharides in bone, particularly those at the cement lines, must be included as a third phase in any composite theoretical approach to modeling the elastic and anelastic properties of bone (*3, 16*). The results presented here indicate that it is the long-term anelastic behavior of bone in particular that results from viscous-like slip at the cement lines.

Bones adapt to stress in such a way as to become better able to support the stress (Wolff's law). The hypothesis presently accepted by many as the mechanism for this adaptive behavior is that bone cells respond to electric potentials generated by stress by means of a piezoelectric-like effect in the bone matrix (*17*). Cement line motion may constitute a secondary mechanism whereby bone responds to the static portion of the loads applied to it, as follows. Cement line motion over a period of months or years could play a role in the realignment of the osteons to the direction of time-averaged principal stress. The observed alignment of bone's material axes can deviate up to 10° from the axis of a long bone. If, as theoretical study suggests, active remodeling processes are insensitive to torsional stress about bone's material axes (*18*), then only a passive process, such as cement line slippage, could be expected to bring about this realignment of axes.

RODERIC LAKES
Department of Biomedical Engineering, University of Iowa, Iowa City 52242

SUBRATA SAHA
Department of Engineering and Applied Science, Yale University, New Haven, Connecticut 06520

References and Notes

1. E. L. Radin, H. G. Parker, J. W. Pugh, R. S. Steinberg, I. L. Paul, R. M. Rose, *J. Biomech.* **6**, 51 (1973).
2. A. A. Lugassy and E. Korostoff, *Research in Dental and Medical Materials* (Plenum, New York, 1969).
3. R. S. Lakes and J. L. Katz, *J. Biomech.* **7**, 259 (1974).
4. ——, *ibid.*, in press.
5. C. Zener, *Phys. Rev.* **60**, 906 (1941).
6. R. S. Lakes, thesis, Rensselaer Polytechnic Institute (1975).
7. Bovine bone specimens were obtained from a slaughterhouse.
8. A "shoulder" at least 7 mm in diameter was provided at the ends of the gauge section of the specimen to prevent it from screwing further into the threaded socket in the testing apparatus.
9. During the 5 to 15 minutes required to take the photomicrographs, the specimen recovered a portion of the residual strain. Since most of the creep deformation occurred over a long period (of the order of weeks), the portion of strain recovered in 15 minutes was small. See, for example, J. D. Ferry, *Viscoelastic Properties of Polymers* (Wiley, New York, ed. 2, 1970).
10. Creep refers to an experiment in which a step stress is applied and the strain is measured as a function of time. The creep compliance is defined as the strain divided by the (constant) stress.
11. H. Yamada, in *Strength of Biological Materials*, F. G. Evans, Ed. (Krieger, Huntington, N.Y., 1973).
12. A bacteriostatic additive was placed in the Ringer solution to prevent decay of the bone specimen. Evidence for effects due to soaking alone on the mechanical properties of compact bone is equivocal at this point. For example, R. C. Tennyson, R. Ewert, and V. Niranjan [*Exp. Mech.* **12**, 502 (1972)] noted a 33 percent decrease in the compressional stiffness of bovine bone after immersion for 20 days in plain water and repeated impacts exceeding the yield point. Smaller decreases in stiffness were observed in human cancellous bone after immersion for 45 hours in distilled water, according to E. R. Fitzgerald [*Biorheology* **12**, 397 (1975)].
13. The scanning electron microscope would provide higher resolution; however, the required drying of the bone would render the specimen more brittle and alter its anelastic behavior.
14. F. Tischendorf, *Arch. Entwicklungsmech. Org.* **145**, 318 (1951).
15. J. L. Katz, *J. Biomech.* **4**, 455 (1971).
16. ——, *Isr. J. Med. Sci.* **7**, 439 (1971).
17. C. A. L. Bassett and R. O. Becker, *Science* **137**, 1063 (1962).
18. D. H. Hegedus and S. C. Cowin, *J. Elasticity* **6**, 337 (1976).

1 December 1978; revised 19 January 1979

Chicken Gizzard: Relation Between Calcium-Activated Phosphorylation and Contraction

Abstract. *Of the proteins in mechanically disrupted chicken gizzard fibers (no functional sarcolemma) only the 20,000-dalton light chains of myosin underwent large Ca^{2+}-and Sr^{2+}-dependent changes in phosphorylation. Phosphorylation closely corresponded with the Ca^{2+}- and Sr^{2+}-activated tensions. Adenosine 5'-O (3'-thiotriphosphate) only in the presence of Ca^{2+} induced irreversible Ca^{2+}-insensitive activation of tension and thiophosphorylation of the 20,000-dalton light chains, and blocked incorporation of ^{32}P from [γ-^{32}P]adenosine triphosphate into the myosin light chains.*

Since the discovery of a Ca^{2+}-sensitive myosin control system in the chicken gizzard (smooth muscle) (*1*), considerable effort has been devoted to elucidating the mechanism of activation of contraction. Further work has suggested that phosphorylation of the myosin (*2*) occurs over the same range of Ca^{2+} concentrations as the adenosinetriphosphatase activity of the actomyosin (*3*).

The best evidence that phosphorylation plays a role in the Ca^{2+}-activation of chicken gizzard (smooth muscle) has come from studies on the Ca^{2+}-dependent light-chain kinase isolated from chicken gizzard (*3-5*). This kinase phosphorylates the light chains of the myosin (*4*) and has a 105,000- and a 17,000-dalton component (*4*). The 17,000-dalton component appears to be the Ca^{2+} receptor site and to have molecular weight, amino acid composition, and biological activity similar to those of the modulator protein (*5*). In the presence of the light-chain kinase, phosphorylation of the 20,000-dalton light chain, the heavier of the two types of myosin light chains, and adenosinetriphosphatase activity are triggered in gizzard muscle actomyosin in the same range of Ca^{2+} concentrations. Other investigators studying smooth muscle present strong evidence that phosphorylation of the 20,000-dalton light chains of myosin is a requirement for actomyosin adenosinetriphosphatase (*6*). Support for the hypothesis that phosphorylation of the 20,000-dalton light chains alone could cause the activation of this enzyme comes from studies with platelet or myoblast myosin (*7*). All of these data are circumstantial, however, since they relate phosphorylation to enzyme activity, a biochemical measure of contraction, and not to the physiological measure, tension; therefore, we conducted the present study. Using functionally skinned chicken gizzard fibers, we identified the protein phosphorylation which was dependent on intracellular Ca^{2+} and determined the relationships between intracellular Ca^{2+}, phosphorylation, and activated tension.

Mechanically disrupted bundles of chicken gizzard muscle fibers were prepared by light homogenization as described by Kerrick and Krasner for mammalian skeletal muscle (*8*). These fiber bundles were freely permeable to ions physiologically and biochemically, as indicated by their graded tension response to changing concentrations of Ca^{2+} or Sr^{2+} (Fig. 1) in the presence of adenosine triphosphate (ATP). A rigor state, produced by the removal of ATP

0036-8075/79/0504-0503$00.50