bases (tetrahydrofuran, dioxane, triethylamine, diethyl ether), have been used in analogous experiments with consistent results; some individual points are noteworthy.

The polarity of the R_2Li_2·base complexes (*cf.* eq. 1) in hexane is not a function of Lewis base strength. To obtain curves D, C, and E in Fig. 2, Et_3N, Et_2O, and THF (tetrahydrofuran), respectively, were added to equal amounts of *n*-butyllithium. It is seen that the least increase in dielectric constant is with Et_3N, the strongest base; that the greatest increase is with THF suggests that steric requirements by the base are important. This suggestion is supported by the facts that with Et_2O *sec*-butyllithium behaves like the *n*-butyl isomer, but with Et_3N the bulkier *sec*-butyl isomer shows considerably less dielectric change. Apparently the still bulkier *t*-butyllithium dimer cannot associate at all with Et_3N; attempted titration of the *t*-butyl isomer in hexane, where it is known[3c] to exist as $(R_2Li_2)_2$, shows no dielectric change with Et_3N. With THF, a base of low steric requirements, *t*-butyllithium is precipitated from hexane.[6] Even *n*-butyllithium is precipitated from hexane by addition of the "diacidic" base, dioxane.[7]

An interesting question is whether the acidic character of R_2Li_2 which results in eq. 1 should be called Lewis acidity. An ordinary Lewis acid accepts an electron pair with an unoccupied nonbonding atomic orbital (commonly a p-AO); such acidic character is not a consequence of a deficiency of bonding electrons. The electron pair accepted by R_2Li_2 is surely going to fill an unoccupied molecular orbital (probably a π-MO)[8]; such acidic character is one of bonding electron deficiency. It would seem best to describe R_2Li_2 in hexane simply as electrophilic (perhaps akin to description of an organic dienophile as electrophilic) and to say that strucure I is a π-complex. The possible utility of these concepts, the electrophilic character of lithium reagents in hydrocarbons and the neutralization of this character with ethers, is apparent in mechanistic consideration of numerous reactions, *e.g.*, polymerization of olefins by lithium alkyls, which can be stereospecific in hydrocarbons (*ergo* "electrophilic" propagation?) but not when ether is present (*ergo* "anionic" propagation?).

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(6) All titrations of the branched-chain lithium reagents must be carried out at low temperature to avoid the known chemical decomposition of the organometallics by ethers; *cf.* ref. 3b, pp. 7–8. To precipitate *t*-butyllithium from hexane with THF, care must be taken to add no more than the 1 equiv. of base per 2 of organometallic, *i.e.*, just enough to give R_2Li_2·THF, because excess THF redissolves the complex, which then undergoes the exothermic chemical decomposition.

(7) Although the precipitated complex has not yet been satisfactorily analyzed, it is presumably R_2Li_2·dioxane·R_2Li_2.

(8) Almost any theoretical (LCAO) analysis of R_2Li_2 will indicate some low level unoccupied bonding MO for the species. Recently we have found additional experimental evidence for such an MO; these species show specific absorption bands in the ultraviolet region characteristic of π-chromophores.

DEPARTMENT OF CHEMISTRY FRANK A. SETTLE
UNIVERSITY OF TENNESSEE MAUREEN HAGGERTY
KNOXVILLE, TENNESSEE JEROME F. EASTHAM

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Volume Changes Accompanying the Adenosine Triphosphate–Myosin B Interaction

Sir:

Hypothesis[1] as well as experiment[2] suggests that in the interaction between adenosine triphosphate (ATP) and the contractile protein system, myosin B, water molecules are released from a bound state. If so, the interaction may entail a change in the volume of the system. To determine whether this volume change occurs is complicated by the concomitant hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi); any volume change accompanying the hydrolysis superimposes upon and obscures the volume change arising from the structural reaction or "superprecipitation."[1] Because the structural effect occurs only early in the reaction, while the hydrolysis effect continues until ATP is exhausted, it is conceivable that the structural effect may be inferred from a suitable extrapolation to time zero.

In the present note we report an attempt to resolve these effects, using conventional Linderstrom–Lang dilatometers[3] immersed in an accurately controlled constant temperature bath. Figure 1 shows the time course of the volume change resulting from the addition of 3 ml. of 0.030 *M* ATP to 14 ml. of a 1% suspension of myosin B when both constituents are in 0.06 *M* KCl + 0.04 *M* tris(hydroxymethyl)aminomethane–maleic acid buffer, pH 7.00, at 26°. (Fig. 1 has been corrected for the volume change accompanying dilution

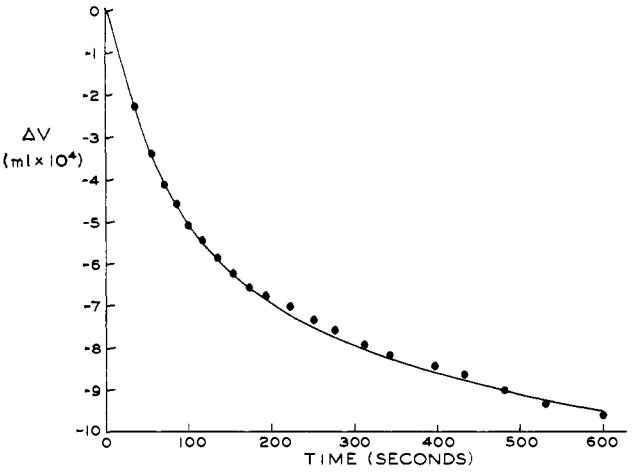

Figure 1.

of ATP; the volume change accompanying dilution of the protein is negligible). Mixing was achieved by magnetic stirring, and the plug due to superprecipitation was clearly formed within the first 2 min. of reaction. When P_i liberation is measured under the same conditions it is found that the ATP is about 95% dephosphorylated in 10 min. If the volume increment is plotted against the [P_i] increment (Fig. 2), the slope of the graph indicates that ΔV_{obsd} is 10.9 ml./mole of ATP split. Since[4,5]

$$\Delta V_{dephosph} = \Delta V_{obsd} + \phi \sum_i R_i \Delta V_i$$

(1) A. Szent-Gyorgyi, "Chemistry of Muscular Contraction," Academic Press, Inc., New York, N. Y., 1947, p. 38.

(2) R. V. Rice, H. Asai, and M. F. Morales, *Proc. Natl. Acad. Sci.*, **50**, 549 (1963).

(3) J. Rasper and W. Kauzmann, *J. Am. Chem. Soc.*, **84**, 1771 (1962).

(4) R. J. Podolsky and M. F. Morales, *J. Biol. Chem.*, **218**, 945 (1956).

(5) S. A. Bernhard, *ibid.*, **218**, 961 (1956).

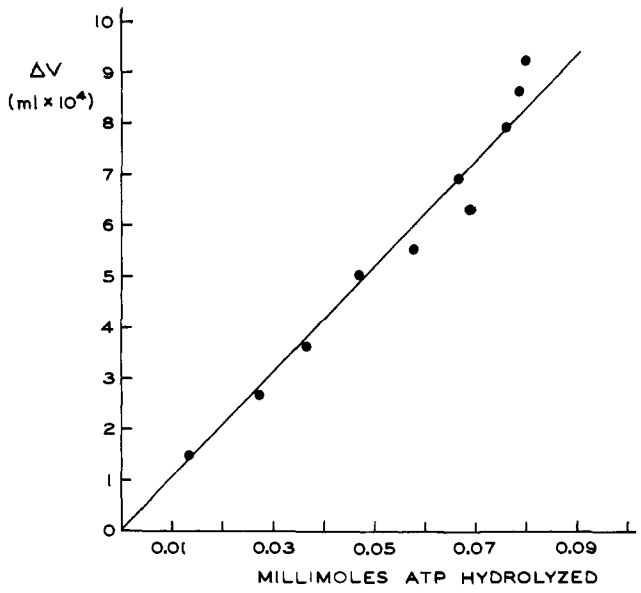

Figure 2.

where ϕ is the number of protons released per mole of P_i generated, R_i is the fraction of ϕ neutralized by buffer i, and ΔV_i is the molar volume change of ionization of said buffer, we have to evaluate also the contributions of the buffers. The buffer systems are tris(hydroxymethyl)aminomethane, maleate, and protein (roughly equivalent to 3 mmoles of histidine), and, in principle, the contribution from each is calculable. The buffer correction may be obtained *in toto*, however, by measuring ϕ and by measuring separately the volume change which occurs when ϕ moles of H^+ are added to the buffer mixture. From the simultaneous measurement of H^+ generated (pH stat) and P_i generated (colorimetric assay) under the aforesaid conditions we estimate $\phi = 0.60$. Under rather similar experimental conditions Green and Mommaerts[6] also obtained 0.6; ϕ calculated[4] from published ionization constants is about 0.67. By measuring the volume change when HCl is added to the buffer mixture (the protein buffer makes little difference in this measurement) we obtain $\sum_i R_i \Delta V_i = 12.8$ ml./mole of H^+. Therefore, under our ionic strength and pH conditions

$$\Delta V_{\text{dephosph}} = 10.9 \text{ ml./mole} + 0.6 \times 12.8 \text{ ml./mole} = 18.6 \text{ ml./mole of ATP split}$$

This value is not unreasonable, considering the ionic character of the dephosphorylation.[7] The only previous measurements[8,9] of this quantity we have found give a value of 10.5 ml./mole, a value close to our apparent (uncorrected) value. Evidently no buffer corrections were made on these earlier results which, in addition, were carried out with relatively crude enzyme preparations.

If the volume change due to the structural effect occurred instantaneously upon tilting the dilatometer (at $t = 0$), curves of Fig. 1 extrapolated to $t = 0$ should give an intercept which should be the "ΔV of superprecipitation."

With our technique the earliest measurement was made at 35 sec., so we cannot be sure of the instantaneous character of the reaction. However, when we make the extrapolation, the intercept found is not significantly different from zero.

The result of our experiments is a reasonable estimate of the ΔV of dephosphorylation. At face value our results indicate that the "ΔV of superprecipitation" might be zero; we conclude, however, that conventional dilatometry is probably inadequate to measure the ΔV of superprecipitation. The basic difficulty is that under the ionic conditions of superprecipitation myosin B is a viscous gel, so that if mixing with ATP is to be achieved within a minute or two, concentrations in excess of 1% are impractical. The minimum volume change detectable in these experiments was about 1×10^{-4} ml. This amounts to a volume change of about 350 ml./mole of protein [assuming a 1% suspension of protein having a molecular weight of 5×10^5 g. (myosin)]. To bring about a volume change of this magnitude entirely through the interaction of charges would require the neutralization of about 20 charges per mole of protein [assuming a ΔV/charge of 17 ml./mole[3]], which is probably a much larger charge effect than can be expected. Extensive conformational changes, however, could easily bring about a volume change of such a magnitude, and our results are an indication, though not a proof, that extensive conformational changes do not occur during superprecipitation.

The difficulties in measuring the ΔV of superprecipitation might be circumvented if much greater quantities of protein were used, but the use of large volumes introduces new experimental difficulties.

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(10) San Francisco Heart Association Fellow.
(11) Postdoctoral trainee, U. S. Public Health Service.

CARDIOVASCULAR RESEARCH INSTITUTE
UNIVERSITY OF CALIFORNIA
SAN FRANCISCO, CALIFORNIA
HAJIME NOGUCHI[10]
DONALD KASARDA[11]
PATRICIA RAINFORD

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Xenic Acid Reactions with *vic*-Diols

Sir:

Preparation of xenic acid by the hydrolysis of xenon tetrafluoride or hexafluoride as reported by Dudley, *et al.*,[1] and Williamson and Koch[2] has led to the investigation of physical properties and chemical reactivity of a hitherto unknown acid. In many respects the behavior of xenic acid is expected to resemble that of the neighboring periodic acid. In fact, Pauling,[3] years before the discovery of xenic acid, predicted that the structure of the then hypothetical xenic acid should be analogous to that of orthotelluric acid, H_6TeO_6. The oxidation potential of xenic acid has been estimated by Appelman and Malm[4] to be in the same order

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