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RECOLLECTIONS

A brief and subjective history of contractility

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Reviewing one's career in order to write for *Recollections* is a very moving experience for an author (rather like what is said to happen in the process of drowning), but interesting a reader in such a review requires adding something. What I have tried to weave into my saga is a personal view of how my field has developed.

Sometime during the course of my university years, I concluded that "achievement" in science consists of studying a significant phenomenon well enough to show in mathematical formalism that what happens is just what one expects from applying the Great Laws to the system under study. After completing graduate school, experiencing a war, and obtaining employment at a great university, beginning my quest seemed to require only the selection of a significant phenomenon. The phenomenon turned out to be biological contractility—in retrospect a good choice, but, at the time, one reached by pure accident. The year was 1946, that is, 48 years before this assessment.

In socio-science, it is erudite to affirm that "everyone stands on the shoulders of someone else," and in this way work back to Babylonian or Mayan authors, but in my quest only two previous contributions really mattered. Seven years before, V.A. Engelhardt and this wife, Lubimova, extracted from muscle the protein, "myosin," and showed that when this protein was drawn into threads and supplied with the enzymatic substrate, ATP, the *mechanical* properties of the threads changed as the ATP was consumed. A few years later, Albert Szent-Gyorgyi and his associate, Straub, showed that "myosin" was a complex of two proteins—real myosin (the ATPase) and an essential partner protein that they named actin. Perhaps I should admit that now we do stand on the shoulders of Engelhardt, Lubimova, Szent-Gyorgyi, and Straub. They isolated everything necessary to study the phenomenon of contraction, and they understood the phenomenon at least in general terms.

Had the research community of the late forties or early fifties pursued more vigorously these basic observations, we might be well ahead of where we are now. Important methods were still under development in those times, but the physics and chemistry of 1946 were ample to cope with any conceptual challenge raised by contractility. Nevertheless, a major change in field direction occurred, for reasons more social than scientific. A new question, "What does the contractile system look like?" was introduced by charismatic new leaders, and it rapidly displaced the earlier question, "How does contraction come about?" The older (and greater) physics/physical chemistry players were running out of gas, and the undiverted younger ones (as the author was then) were proceeding on the wrong premise that actomyosin contracts as typical polymers contract—as mechanically continuous systems shortening in response to ATP. A morphological triumph of 1954 (due to A.F. Huxley and R. Niedergerke, and to H.E. Huxley and J. Hanson), then known as the "the sliding filament theory of muscle contraction," did not *explain* contraction, but did (mercifully) eliminate the plausibility of continuous systems. For the author, this was a painful course correction because in 1948 he had dabbled with something of the true protein arrangement and then turned his back on it. At any rate, to most of us, the morphological "diversion" had at least some benefit. But another early clue, almost universally ignored, was Wallace Fenn's question—Does contraction result from repetitive, impulsive operation? Few noted his remark then; even fewer remember it today.

My research survived the fifties mainly by (unwittingly) using a strategy known in finance as diversification—by taking up various problems having some bearing on contraction but not being themselves the central issue. All of these problems were pursued with friends who were blessed with extensive supplementary talents. Along one line (investigations of ATP) the author met with remarkable serendipity. In the first case (with R.J. Podolsky), we sought the heat of binding of ATP to myosin. We tried to calibrate our calorimeter with the "well-known" heat of ATP hydrolysis, which turned out to be quite wrong. We ended up finding the correct heat of hydrolysis. In the second case (with L. Levintow and A. Meister), we tried to find out why the glutamine synthetase reaction failed to go to completion and ap-

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peared instead to reach equilibrium; it was because it *had* reached equilibrium. From this surprise came the first good estimate of the free energy of hydrolysis. In the third case (with T.L. Hill), we were proposing that the free energy of hydrolysis originated in the coulombic repulsion between the γ and the β,α phosphates; that explanation involved the molecule *as a whole*, so invoking it meant renouncing the "well-established" concept of the "high energy phosphate *bond*." The concept deserved renouncement. A social conclusion may also be drawn: while not exciting, or even palatable to one's peers, thermodynamic results are forever. Along a different line (with J. Botts), the author made some headway in describing the kinetic behavior of connected processes (such as enzyme-catalyzed systems). Such an enterprise may seem odd because kinetic formulation is so old in chemistry, but one has to recognize that old stuff in the physical sciences can be new and exciting when applied in biology. Kinetic formulations later (in the sixties) matured, and in abler hands—those of T.L. Hill in regard to bioenergetics, and of J.Z. Hearon and W.W. Cleveland in regard to general aspects—grew to great prestige; they remain respected grant-getters even today.

In the sixties and early seventies, morphological and behavioral studies of contractile systems rapidly multiplied, boosted by the realization that muscle occurred not only in rabbits, and contractility not only in muscle. But important mechanistic findings also emerged. Knowingly, or perhaps even *un*knowingly, the field resumed its movement toward a solution. Under the stimulation of F. Oosawa, the structure of F-actin was clarified. H. Mueller and also S. Lowey showed myosin to be duplex, and each strand to be functionally and structurally segmented. The "head" segment (or "S-1 piece") of myosin bore the ATPase site, and, *separately* (an important feature first noted by M. and K. Barany), the sites that interact with actin. First, M.K. Reedy, K. Holmes, and R. Tregear, and later H.E. Huxley, looked hard at their EM pictures of muscle tissue and insightfully suggested that the S-1 pieces of myosins ("crossbridges") might be *impellers* that pushed actin. With that clue, several associates and the author showed that indeed the head segments of myosins have the mechanical requisites to be impellers. Critical findings of another kind emerged in the same era. A. Gordon, A.F. Huxley, and F. Julian, and also E. Benson, showed that the steady force and the steady activated ATPase of active muscle are proportional to the number of heads interacting with actin. Their interpretation would have gratified Fenn because it showed that crossbridges are hydrolyzers and impellers.

In the late sixties, Y. Tonomura began segmenting the ATPase cycle in time, i.e., splitting it into the successive steps that constitute the catalysis, and the task was elegantly completed in the early seventies by D.R. Trentham and C. Bagshaw, and by E.W. Taylor and R. Lymn. The culmination of this work was the identification of the successive enzymatic intermediates in ATPase.

Viewing the mechanical and enzymatic results together, the author saw a long-standing puzzle resolved: mechanical impulses are *repetitively* delivered. That is because, to every intermediate in catalysis (a process that in micro-time is necessarily repetitive), there corresponds a stage in the impulsion. Thus is chemistry "geared" to mechanics. Of course, other puzzles remained. A "stage in the impulsion" is really a "relation" between the S-1 piece and actin—a relation to be defined geometrically and by the forcefield between the proteins. Exactly what are these successive relations? Also, the feature that ATPase and interaction with actin go on at physically separate sites of S-1 leaves open, and complicates, the explanation of how "correspondences" are enforced (more about this later). Still, this pattern of gearing between ATP degradation and work performance (by changing relations at the actin-binding site) seems to the author comparable in stature to the group transfer scheme deduced years ago for ATPase-linked biosynthesis. The remainder of this essay is devoted to the modern problem posed by this abstract scheme for a "working ATPase," but at this point we digress to relate the scheme more specifically to muscle contraction.

At first sight, "impeller" operation seems to require a degree of intelligence rarely attributed to molecules, but, when considered in toto, the muscle situation edges toward plausibility. The contractile machine is an insoluble polymeric system featuring "filaments" of actin, AAAAAA, and myosin, MMMMMM. Individual A–M interactions therefore ensure that the filaments parallel one another, and it is now evident that (in addition to transient forces of other kinds) there is a steady electrostatic A–M attraction that keeps the system together. Perhaps to ensure close packing, the filament axes parallel not only each other, but also the fiber axis. Then, for extensive motion, only the principal axial direction remains. To produce axial motion, the impellers (arranged as appendages extending from M toward A) must move with a major *rotational* component. Accordingly, an individual impeller must "know" how and when to bind to A, to change one or more of its Euler angles, and to unbind. The parallel interfilament arrangement provides for the vectorial addition of the impulses delivered by many impellers. This foregoing description seems very logical to the author (of course!), but many of its components are still under investigation around the world.

There is, for example, the question of how rotation is achieved. The forces between the S-1 pieces of the M's and the A's are short-ranged and local, not central, so it is sensible to speak of forces between patches or sites on S-1 and patches or sites on A, with many patch pairs possible. The experiments of T. Nihei, R.A. Mendelson, and J. Botts indicated that rotation requires the participation of both M–S-1 and A; the rotation could therefore be a transition between one set of interacting patches and another. (Currently there is also a rival hypothesis, which envisions that a shape change inducible in S-1 alone is the same shape change responsible for changing the functional A–M relation.) Even the detection of rotation remains an active subject of investigation. For this detection, the best device is probably a well-placed "orientational probe" (a simple marriage of G. Weber's directional fluorescence and H.M. McConnell's labeling technique that was introduced to contractility by J.F. Aronson, other associates, and the author), but the validation and rigorous interpretation of the probe method is only now being firmly established by T.P. Burghardt.

Another issue among contractile events is timing. After writing reaction schemes employing rate constants, there is a natural reluctance to admit that these schemes only describe (extremely large) *population* behavior. The impeller appendages in MMMMMM are attached by swivel joints, and because there is no evidence of coordination among the impellers, one has to accept that individual impellers will behave stochastically. This physical circumstance has totally defeated attempts to deduce impeller trajectories by improving (within practical limits) observational time resolution. More reasonable have been attempts at entrainment, either by imposing "delta-function" changes in conditions (R.J. Podolsky, and especially A.F. Huxley and R.M. Simmons), or by imposing sinusoidal changes (M. Kawai) and

deducing limited information (the spectrum of the constituent Fourier frequencies) by observing the relaxation from the changes. Using a slight variant of this approach—observing relaxation from naturally occurring delta-function changes ("fluctuations")—J. Borejdo, S.V. Putnam, and the author found, from watching a limited population of impellers in an active muscle fiber, that the frequency spectra are similar for tension, impeller orientation, and ATPase. Later, technologically superior experiments by T. Yanagida yielded the same conclusion for tension and ATPase. Very recently, S. Block, and also J.T. Finer (and their respective associates), using appropriate stochastic analysis, have produced dazzling evidence that the average spatial "throw" in contracting systems is also what one would expect from rotating impellers. The author feels that experimental results like these are as close as we will get to proving repetitive impulsion. However, "cross-correlation" between orientation or tension and the concentration of an intermediate has yet to be established.

Assuming that ATPase-driven contractile systems operate by repetitive impulsion, that the impulsive cycle is a succession of changing relations between two proteins, and that each of these relations corresponds to the particular intermediate bound at the time, we come finally to prospects for a first principles explanation of how the correspondence is enforced.

In his present role of amateur historian, the author sees the research community (and its funding apparatus!) as largely preoccupied with enterprises that, while interesting, are not aimed at the central problem and are not likely to assist solution except by lucky accident. However, he also sees bright prospects. First, the central question can, to some degree, be formulated, albeit on incomplete experimentation. Second, critically important data have appeared—the actin structure of Kabsch and Holmes and the myosin S-1 structure of Rayment and Holden. Third, if the final phase of the problem plays out in the domain of proteins, skillful new players may be drawn into the game because, in the protein domain, the Great Laws are directly applicable.

A component of the central issue—that the free energy of binding ATP (or its daughter intermediates) is later used to bring about the structural changes constituting work performance—was sensed early in contractility (even though the specific work to be performed [viz., polymer shortening] was at the time mistaken), and also in the mitochondrial context (by P.D. Boyer). The rest of the problem formulation has come later. After constructing a primitive acto-S-1 structure from Forster transfer distance measurements, and using the chemical work of many, J. Botts, J.F. Thomason, and the author reported that, when nucleotide was added to one site of S-1, mechanical effects were transmitted to an acto-S-1 interface almost 5 nm away. It was left as a presumption that the transition observed was indeed the one that accompanies mechanical work performance. Much the same circumstance was reported by G. Inesi for sarcoplasmic reticulum, wherein the ATP-binding site is also about 5 nm away from the site where electrical work is done. The interpretation in these cases is that chemical transitions between bound intermediates enforce distant, "corresponding" structural changes, the effective forces being transmitted through the structure of the ATPase. As among hemoglobinologists, there is an alternative conceptual view. The idea that, in the relevant temperature range, there coexist conformations corresponding to functional states in, say, thrust, and that the binding of intermediates stabilizes the end state was introduced by J. Shriver and by B. Sykes, and has since been supported by experiments of the former. Further, the relation of this notion to the "Feynman ratchet" has been discussed by G. Oster. We are at present at an early stage of deciding between these two formulations, but for now it is more important that finally there *are* formulations testable in straightforward physical ways. What is so important about the advent of actin and myosin crystal structures is that they provide the arena for this testing. Of course, these structures will first be used in less challenging ways—to locate sites, to interpret reactions, and so on—but ultimately we must think of them as structures subject to *mechanical analysis*, like man-made bridges and engines. What will someday come is foreshadowed by C. Chothia's studies of domain hinging, by the mechanical simplifications (helices as rigid cylinders, loops are random coils, etc.) proposed by D.J. Thomas, and by the normal mode analyses of M. Tirion and D. ben-Avraham, etc.

Associated with closing this essay are many emotions, but only two deserve airing. If the earlier definition of "achievement" is correct (I still think it is), then I turned out to be one who tried hard, but neither I, nor as yet the field as a whole, reached the required denouement. The other emotion has to do with indebtedness and attribution. Although it is for reasons of style and space, it is nonetheless discomforting to shuffle so many ideas and facts without giving specific citations. Had these been given, many would have been to my 55 or so gifted, one-time colleagues. An indebtedness that cannot be anonymous, however, is to Jean Botts and Terrell Hill, whose careers often intersected with mine in a manner that always benefited me.

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