HISTORICAL PERSPECTIVE

Why has reversal of the actin-myosin cross-bridge cycle not been observed experimentally?

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Loiselle DS, Tran K, Crampin EJ, Curtin NA. Why has reversal of the actin-myosin cross-bridge cycle not been observed experimentally? J Appl Physiol 108: 1465–1471, 2010. First published February 4, 2010; doi:10.1152/japplphysiol.01198.2009.—We trace the history of attempts to determine whether the experimentally observed diminution of metabolic energy expenditure when muscles lengthen during active contraction is consistent with reversibility of biochemical reactions and, in particular, with the regeneration of ATP. We note that this scientific endeavor has something of a parallel flavor to it, with both early and more recent experiments exploiting both isolated muscle preparations and exercising human subjects. In tracing this history from the late 19th century to the present, it becomes clear that energy can be (at least transiently) stored in a muscle undergoing an eccentric contraction but that this is unlikely to be due to the regeneration of ATP. A recently developed, thermodynamically constrained model of the cross-bridge cycle provides additional insight into this conclusion.

EARLY STUDIES OF EXERCISING HUMAN SUBJECTS

IN 1896, Chauveau (10–13) posed a question with a very modern resonance: “Does descending stairs backwards have the same energetic cost as ascending them forwards?” His reasoning was as follows. Muscles that contract concentrically (i.e., shorten) during ascent (travail positif) must contract eccentrically (i.e., lengthen)—under the same load, thereby performing the same amount of work—during descent (travail négatif). To answer the question, he measured the rates of oxygen consumption under the two exercise protocols. While Chauveau’s reasoning might be questioned, he found, in fact, that the cost of descent was substantially less than the cost of ascent. Might this have been because the gravitational potential energy that had been gained by ascent was reclaimed in the muscles during descent? Translated into modern parlance, might the entire cross-bridge cycle have been driven in reverse, thereby regenerating ATP, during the stretching of actively contracting muscle that attends the backward descent of stairs?

Half a century later, in the early 1950s, Chauveau’s question was readdressed by Abbott and colleagues (3, 4) who constructed back-to-back bicycle ergometers and required that one subject pedal “forward” while the other pedal “backward” to offer resistance. Since a single chain connected the sprockets of the two bicycles (Fig. 1A), the subjects necessarily pedaled at the same velocity—one doing “positive work” while the other performed “negative work.” It transpired that the rate of oxygen consumption (VO2) of the latter was only about one-quarter that of the former. In the 1970s, Bigland-Ritchie and coworkers (6) pursued the issue further following development of a motorized bicycle ergometer (Fig. 1B) especially designed for both “positive” and “negative” work (5). They confirmed that the ratio of energy expenditure was velocity dependent and in excess of four- to sixfold at the highest pedaling speeds. Since the ratios of the integrated electromyograms more or less paralleled the VO2 ratios, further support was provided for their earlier conclusion that, during the performance of negative work, proportionately fewer muscle fibers were active and, since they are operating in the negative region of the force-velocity relation, are developing considerably higher unitary forces (as shown in Fig. 1C).

EARLY THERMOMETRIC STUDIES OF ISOLATED MUSCLE

At the same time that these experiments were being conducted in exercising humans, muscle physiologists had been examining the question of “negative work” but from a more fundamental point of view using isolated muscle. The story commences with Fick (22)1. Making use of Blix’s “myographion,” Fick clearly demonstrated that the heat produced by actively stretched muscle was less than that measured during active shortening. We find the care and exactitude of Fick’s

1An English translation of this article (22) is available, as a Word document, on request from the corresponding author.
experiments sufficiently humbling that we honor his memory by presenting a selection of his tabulated data in graphical form (Fig. 2).

These results were confirmed and extended by Fenn (21), whose equally careful quantification even took into account the cooling that an unstimulated muscle experiences on elongation. Fenn’s conclusion (Ref. 21, p. 387–388) was blunt: “The work done in stretching the muscle does not therefore add itself to the ‘physiological’ heat but . . . replaced energy which would have been liberated by the muscle if it had not been stretched.” In the subsequent decade, A. V. Hill (30) affirmed both the experimental observation and the inference of his protégé:

“The question, therefore—Where does the work done on the muscle in lengthening go to?—is answered. By raising the tension one causes a decrease in the rate of chemical transformation in the muscle. It is not necessary to imagine any actual reversal in the direction of chemical reaction during lengthening: only a slowing of the reaction normally accompanying activity. It has never been found, even with more rapid stretches, that the total energy rate of the muscle is negative: it is less than the isometric energy rate but still positive. Lengthening, therefore, and negative work, do not cause a reversal, but only a slowing, of the processes associated with activity” (p. 163).

It is curious that these unequivocal statements, from two figures who dominated the field of muscle energetics at the time, nevertheless generated an extended period of vigorous experimental effort to disprove them. (No doubt the twin seductions of an intriguing hypothesis and the desire to be first to supply evidence in its favor operated as vigorously then as it does now!) It is even more curious that one of the protagonists in this undertaking was A. V. Hill himself. Thus, by 1951, he seemed to have reinterpreted his pre-World War II findings, referring to them as follows (2): “The work which disappeared was presumably absorbed in driving backwards the chemical changes which normally provide mechanical work when a muscle is able to shorten . . .”, although it was cautioned that, on the basis of “rapid stretch” experiments, that interpretation could not be distinguished from his earlier one of 1938. In an accompanying paper (1), his coauthors summarized the findings of their experiments, which involved very slow stretches, as follows: “These results are consistent with reversal of the physical and chemical events associated with shortening . . .”.

Ever-improving technology and ever-refined experimental techniques revealed something of a paradox, however. Hill and Howarth (33) elicited twitches or brief tetani in excised toad muscles and, shortly thereafter, subjected the muscles to stretches of some 10 –20%. Whereas over a complete contraction-relaxation cycle the value of heat minus work (H – W) was always either zero or positive, during the course of a stretch it could take on very large negative values. This apparent paradox was reconciled by Hill and Howarth (33) who invoked the phenomenon of “thermoelastic heat,” whereby a solid body under tension absorbs an amount of heat (Q) given by the product of its coefficient of thermal expansion and the tensile force (P). On relaxation, the identical amount of heat is released. [A thorough explanation of this phenomenon has been provided by Woledge et al. (Ref. 50, p. 237–242).] Hill (31) had previously quantified the relationship between force production and thermoelastic heat generation for shortening contractions and, with some reservations, assumed that the same value obtained during lengthening contractions. To his obvious satisfaction, application of this “correction” eliminated every negative segment that had previously occurred during the time course of heat production, such that the quantity (H – W) became zero, within the limits of experimental error. After ruling out the storage of work in electrical or osmotic forms, Hill and Howarth (33) were left with “chemical storage,” allowing them to posit the following conclusion: “The results described in this paper leave little doubt that the chemical reactions which normally occur during contraction can

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**Fig. 1.** A: back-to-back bicycles connected by a single chain (white arrow) such that pedaling in both “forward” (concentric contractions) and “reverse” (eccentric contractions) directions necessarily took place at the same velocity and, hence, required the same development of force [Reproduced from Abbott et al. (4) with permission from Wiley-Blackwell]. B: a motorized bicycle ergometer in which the reclining posture of the subject constrained activity to the muscles of the legs. In “forward” mode, the subject pedaled against the friction of the belt against the flywheel (B, right inset). In “backward” mode, the subject resisted the torque on the pedals applied by the motor [Reproduced from Bigland-Ritchie et al. (5)]. C: stylized force-velocity relationship (curved solid line) showing disproportionately higher forces for equivalent negative velocities of the same fully active fiber or muscle—i.e., as would occur during eccentric contractions [Reproduced from Bigland-Ritchie and Woods (6) with permission from Wiley-Blackwell].
be reversed by stretch, under the influence of the mechanical work supplied” (p. 189). The same conclusion was promulgated to a wider audience, as the result of Hill’s presentation of the 1959 John R. Murlin Lecture at the University of Rochester and its publication in Science (32).

STUDIES OF ATP SPLITTING BY ISOLATED MUSCLE

At about this time, it was becoming increasingly accepted that ATP hydrolysis represented the energy source for muscle contraction. Furthermore, it had become possible to assay accurately for its intracellular concentration. Thus Infante et al. (38) confidently expected to observe the resynthesis of ATP during stretch. They did not, and bluntly said so, “... thus net resynthesis certainly did not occur,” despite a 50% reduction of ATP hydrolysis. Comparable results were observed by Maréchal (43) who, in addition to measuring ATP, also assayed for creatine phosphate. The former was unchanged by stretch; the latter was consistently found to be elevated vis-à-vis that of control muscles undergoing isometric contractions. Rather than invoking reversal, Maréchal concluded that stretch spares the expenditure of biochemical energy that would have been expended during either an isometric or a concentric contraction, thereby returning to Fenn’s original position (21).

Throughout the first half of the following decade [perhaps spurred by the knowledge that thermodynamic reversal had been demonstrated for both the sarcolemmal Na\(^+\)-K\(^-\)-ATPase (23, 26, 39) and the sarcoplasmic reticular Ca\(^2+\)-ATPase (27, 28, 41)] a number of groups continued the search. Prominent among these were Curtin, Davies, and colleagues (8, 15–18) who consistently corrected for the amount of ATP hydrolyzed by Ca\(^2+\) cycling and never wavered in their conviction that stretch diminishes, but does not reverse, the rate of ATP hydrolysis of the actomyosin ATPase. More or less simultaneously, Maréchal and colleagues used radioactively labeled H\(_2\)O (44, 45) or inorganic phosphate (24, 25) to seek incorporation of the label into ATP subsequent to stretch. Their 1971 report demonstrated that incorporation of \(^{32}\)P into ATP was linearly related to the tension-time integral developed during the stretch. But the magnitude of incorporation did not differ from that observed during either isometric contractions or contractions with shortening that produced the same tension-time integral, so that stretch did not enjoy any special privilege. Their results did, however, provide an explanation for the apparent reversibility reported by Mannherz (42), using the same techniques. A final mention should go to Ulbrich and Rüegg (48) who interpreted their finding of increased incorporation of \(^{32}\)P into ATP following stretch to indicate the involvement of a high-energy intermediate—possibly actomyosin-ADP-Pi—thereby echoing ideas proffered by Infante et al. (38) a decade earlier.

MATHEMATICAL MODELING

Whereas the test of stretch-induced reversibility of the “ATPase cycle” must ultimately lie within the ambit of experimentalists, we wish to supplement this historical account with additional insight gained from recent mathematical modeling. We stress that this insight arose “incidentally,” as a consequence of extending a comprehensive model of cross-bridge mechanics. In that model (46), a “mean field” description of the cross-bridge cycle was adopted, in contradistinction to the explicit cross-bridge model of Huxley (37). The values of its
various rate constants were selected to allow the model simulations to mimic a very wide range of (cardiac) muscle behaviors reported in the literature. Our contribution (47) has been to incorporate ATP splitting into the Rice model (Fig. 3) in a manner that renders it kinetically and thermodynamically consistent. That is, the possibility of reversal of ATP splitting is formally admitted by insisting that each forward state transition probability \( f^+ \) has a complementary reverse transition probability \( f^- \) in accord with the formalism of T. L. Hill (34–36):

\[
\prod_i f^+ = e^{\Delta G_{ATP}/RT}
\]

where the Gibbs free energy of ATP hydrolysis is given by:

\[
\Delta G_{ATP} = \Delta G_{ATP}^0 + R T \ln \left( \frac{[\text{MgADP}][\text{Pi}][\text{H}^+]}{[\text{MgATP}]} \right)
\]

where \( \Delta G_{ATP}^0 \) is the standard free energy of ATP hydrolysis and \( R \) and \( T \) have their usual meanings. In Eq. 1, the state transition probabilities \( f \) are pseudo-first order (i.e., they depend implicitly on the concentrations of ATP and its hydrolysis products). This dependence is shown in Fig. 3 and presented explicitly in Eq. 3:

\[
k_1^+ \times k_2^-(\varphi) \times k_{\text{ADP}} \times k_3^-(\varphi) \cdot [\text{MgATP}]
\]

\[
\frac{k_1^- (SL) \cdot [\text{Pi}] \times k_2^- \cdot [\text{H}^+] \times [\text{MgADP}] \times k_3^-(SL, \varphi)}{e^{\Delta G_{ATP}/RT}}
\]

where \( SL \) is sarcomere length and \( \varphi \) is the extent of distortion in the cross bridge (47).

In our model (47), the rate of splitting of ATP by the actomyosin ATPase reaches a steady state during the plateau phase of an isometric contraction \((t = 0 \text{ to } 80 \text{ ms}, \text{Fig. 4C})\), thereby mimicking A. V. Hill’s “maintenance heat rate” or any of the experimentally measured heat traces shown in Fig. 5. Imposition of a simulated isovelocity stretch of the muscle, from a sarcomere length of 1.8 to 2.3 \( \mu \text{m} \) (Fig. 4A), generates a brief force transient (Fig. 4B) followed by a steady increase of force, behavior which again mimics experimental observation—especially as seen in Fig. 5C where the stretch was conducted at high velocity. The simulated rate of cross-bridge ATPase activity initially mirrors the force transient before progressively collapsing to become negligible at \( \sim 115 \text{ ms} \). Its rate remains negligible throughout the remainder of the simulation, despite the continued strain imposed on the muscle (Fig. 4A) and the resulting high level of force generation (Fig. 4B), but with no suggestion of reversal. To reemphasize, this result was an unanticipated outcome of the model. That is, no equations were explicitly developed nor parameters varied to achieve it. We simply added to the Rice model (46) the reverse state transition probability \( f_3^- \), which characterizes the transition from unattached cross-bridge state \( U \) to strongly force producing cross-bridge state \( A_3 \) (Fig. 3) as required thermodynamically. The small absolute value of \( k_3^- \), which was imposed by application of Eq. 3, and the requirement for the model...
to fit a wide range of steady-state and kinetic data on force generation [see Tran et al. (47), Supplementary Information], meant that any stretch-induced rate of ATP production would also be small. In fact, no reversal occurred (Fig. 4C). This null result immediately raises the concern that our mathematical model may be incapable of showing reversal under any circumstance. To allay that fear, we optimized conditions for reversibility by increasing [ADP] (from 36 to 40 µM) and [Pi] (from 2 to 20 mM), while greatly decreasing [ATP] (from 5 mM to 10 µM). Under these extreme nonphysiological conditions, a simulated stretch produced a small reversal of the net rate of ATP splitting (Fig. 4F). This was achieved primarily by a decrease in the forward cycling transition probabilities with little change in the corresponding backward ones. In consequence, although reversal was achieved, it occurred at a very low rate. Furthermore, when averaged over the full duration of the stretch, even at these improbable metabolite concentrations, there is still much more ATP consumption than ATP production (Fig. 4F). It would require very sensitive experimental measurements, indeed, to observe anything other than net ATP consumption. Such measurements remain well beneath the detection limit of any current biochemical or biophysical technique.

THE REALITY OF “ENERGY STORAGE”

So there is neither experimental nor convincing modeling evidence to support the notion of stretch-induced reversal of ATP hydrolysis. Yet energy can be stored in the muscle (at least transiently) during a brief stretch. The phenomenon has been repeatedly observed in whole muscles (7, 9, 19, 20, 29) and occurs in isolated single fibers [both mammalian and reptilian (14)] as well and has been labeled “residual force enhancement after stretch.”
The most comprehensive treatment of the energetics of the phenomenon has been provided by Linari et al. (40), who measured heat production and force development simultaneously during isovelocity stretches of single fibers from tibialis anterior muscle of Rana temporaria. The time courses of lengthening, force, heat, work, and energy (heat + work) are shown for three different velocities of stretch in Fig. 5. Clearly shown are the biphasic nature of the force response (a rapid, brief, large increase, followed by a prolonged, slower phase), the accompanying increase of heat production, and the attendant work on the fiber by the motor. Thus, for all velocities except the smallest, the records show net energy gain by the muscle for the period during the stretch per se. It is evident that net energy production continued after completion of the stretch (i.e., despite constancy of fiber length) while stimulation was continued. The magnitude of this poststretch energy liberation exceeded the energy absorption that had occurred during the stretch. The rate of poststretch energy liberation declined with a time constant of ~40 ms (at 0.8–1.8°C). Clearly, the muscle is capable of storing energy for many tens of milliseconds after a stretch. Both the nature and the location of the “storage site” remain a mystery.

CONCLUSION

In summary, despite occasional enthusiastic claims to the contrary, over the better part of a century of experiments, there is no convincing experimental evidence that a stretch applied to an active muscle can lead to the net synthesis of ATP from its hydrolysis products. There is no evidence that during stretching of an actively contracting muscle (as is routinely encountered in downhill running, or in the descent of stairs, for example) the “ATP tank” can be refilled. The in silico model of Tran and colleagues (47) further demonstrates the improbability that such experimental evidence will ever be forthcoming; the extent of reversal is simply too meager to be biochemically detectable.

Nevertheless, it is clear that some form of (non-ATP related) “reversal” occurs (Fig. 5). The storage of energy during stretch of actively contracting muscle is temporary. It seems to us most likely to be of a mechanical or mechanochemical nature such as redistribution of the attached states in the actomyosin cycle, and/or increased strain in the filaments, including titin, or other protein components yet to be positively identified. In this sense, history has come full circle. We no longer expect “reversal” to occur (Fig. 5). The storage of energy during stretch is temporary. It seems to us that such experimental evidence will ever be forthcoming; the extent of reversal is simply too meager to be biochemically detectable.

REFERENCES


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DISCLOSURES

No conflicts of interest (financial or otherwise) are declared by the authors.