MILESTONE IN PHYSIOLOGY

JGP 100th Anniversary

The enduring relationship between myosin enzymatic activity and the speed of muscle contraction

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Introduction

There are many notable examples of physiological processes for which current mechanistic understanding seems obvious but in past times were unknown or at best faintly suggested by isolated correlative observations. The systematic determination by Michael Bárány (1967) of the relationship between the speed of muscle contraction and myosin ATPase activity is such a case. His publication of these findings in the *Journal of General Physiology* is a true milestone in our understanding of the principal determinants of speed, force, and power of muscle contraction across the animal kingdom. The work is still cited as the primary historical reference for studies of myosin in the context of its increasingly well-known disparate functional manifestations. As we discuss in this Milestone, Bárány's characterizations of the positive relationships between maximum myosin ATPase activity and the speed of contraction (Fig. 1) or contraction time during a twitch (Table 1) have proven to be both profound and lasting.

Bárány's landmark publication (Bárány, 1967) is representative of its time in terms of both its thoroughness and the arduous methodological approaches that were used. In the 1960s, there were no high-sensitivity fluorescence indicators that are now readily available for time-resolved measurements of ATPase activities of myosins and other ATP-cleaving enzymes. The systematic nature of the study is evident in its inclusion of a wide range of myosins from vertebrate and invertebrate striated muscles, which are voluntarily or reflexively activated in vivo; its inclusion of temperature as an experimental variable; and its inclusion of isolated mammalian smooth muscle but exclusion of cardiac muscle, neither of which were widely studied at that time. The selection of myosins from a variety of muscle types resulted in myosin ATPase activities spanning three orders of magnitude, which was key to establishing causal relationships between contraction speed and myosin enzymatic activity. For the muscles included in the paper, velocities of shortening were available in the literature or via personal communications from other scientists in the field, and in a few instances, contraction times during the twitch were measured in the laboratory. The contributions of other investigators to the work (including the author's scientist wife, Kate Bárány) were generously provided and gratefully acknowledged by the author, but in contrast to current practice, did not result in attributions in the form of coauthorships.

The impact of the results of this study on research in the muscle field has been immeasurable; the work defined a fundamental determinant of contractility and provided an evidence-based conceptual framework for inferring myosin function in nonmuscle systems that are not well suited to measurements of velocity under load. Furthermore, over the past decade, Bárány's finding that myosin ATPase activity is a fundamental driver of contraction speed has influenced investigators to develop small molecule modifiers of myosin turnover kinetics as a means to correct the altered contractility of cardiac muscles due to inherited or acquired myopathies (Green et al., 2016; Mamidi et al., 2018). Following the discovery of intrinsic modulators of the availability of myosin for interaction with actin in skeletal and possibly cardiac muscle (see review by Malcolm Irving, 2017), there is an increasing number of molecular targets for potential therapeutic interventions involving myosin.

An influential study

The number of citations and the persistent presence of the Bárány (1967) publication in the modern literature of muscle biology only begin to suggest the importance of this work to the field. As pointed out by Bárány in that paper, the ATPase activity

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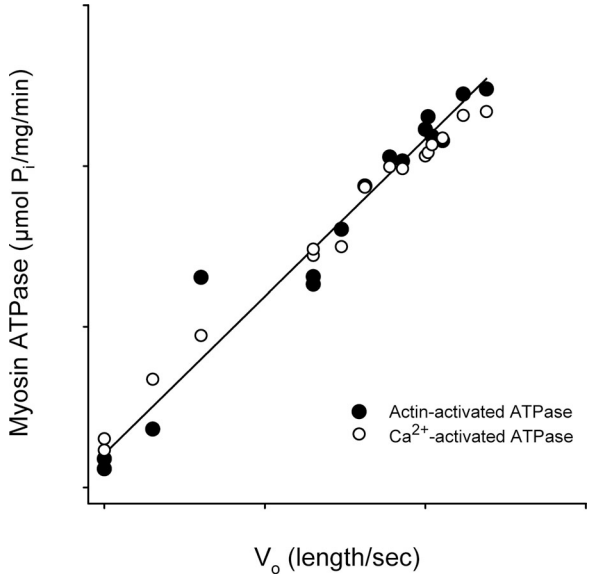

Figure 1. **Relationship between maximal speed of shortening and actin-activated ATPase activities measured in skeletal and smooth muscles from a variety of mammalian species.** The data plotted here were taken from Table IV in article by Bárány (1967).

of myosin predicts not only the maximum velocity of shortening of a muscle but also the curvature of its force–velocity relationship and its maximum power-generating capability and peak energetic efficiency. As a rule of thumb, muscles that generate greater power do so because they express faster isoforms of myosin. As an example, following from Bárány's work, Anthea Rowlerson (Rowlerson et al., 1981; review by Hoh, 2002) was the first to show that mammalian masticatory muscles, which generate high power during chewing, express a "superfast" isoform of myosin, which underlies the remarkable capacity of these muscles to develop high mechanical power at intermediate loads where energetic efficiency is optimal. It was only much later that Pellegrino et al. (2003) showed definitively that the maximum contraction velocity of a muscle fiber is a property of the predominant myosin isoform expressed in the fiber. However, not every muscle expressing fast or superfast isoforms of myosin is meant to develop high power but instead may be designed to contract rapidly against very light load.

The difference between high contraction speed and power generation as design principles in different muscles expressing fast myosin isoforms is dramatically evident in distinct muscles of the Atlantic oyster toadfish. In studies that are both elegant and systematic, Larry Rome (Young and Rome, 2001) showed that the toadfish jaw muscles develop sufficient power to break the shells of oysters to gain access to the nutritious flesh that is the mainstay of the fish's diet. In contrast, the toadfish sonic muscles, which also express a superfast myosin, shorten against the low load of the inflated swim bladder at high speed and in this way generate a brief sound. By contracting at extraordinarily high frequencies, the sonic muscles of the male toadfish evoke a high-pitched buzzing sound due to vibration of the swim bladder. This sound is a critical part of a mating sequence that initially attracts female toad fish (Young and Rome, 2001). Despite the differences in function of the jaw and sonic muscles of the toadfish, each would exhibit a maximum velocity of shortening that is appropriate to the maximum ATPase activity, as predicted by Bárány's results (Fig. 1).

A life of early hardship and ultimate achievement

More important than Michael Bárány's contributions to science, his life is an epic story of the triumph of the human spirit over unimaginable hardships and danger. After graduating from high school in 1939, Bárány was unable to attend university in Hungary, since by that time Jews were not admitted. He therefore took up a trade as a mechanic, which served him well after he was conscripted into military service. With the escalation of the hostilities in World War II, Hungary fell out of favor with Hitler, and Germany occupied Hungary. Michael knew of the extermination of Jews in Czechoslovakia and Poland and managed to save himself by falsifying his identification and going into hiding. Unfortunately, both his parents refused to take this course and subsequently died in Auschwitz. Upon escalation of the war following the Soviet invasion of Hungary, the retreat of the Hungarian–German army, and the relocation of the factory where he worked, Michael was unable to remain in hiding. Michael was arrested and sent to Buchenwald in late 1944 by officers of the Hungarian–German army. Later in life, during his time as a faculty member at the University of Illinois at Chicago, Michael never spoke of this experience, but subsequently posted a vivid account (Bárány and Bárány, 2000) of his experiences and of the horrors he witnessed during his time as a prisoner in the concentration camp (http://www1.chem.umn.edu/groups/baranygp/michaelbarany/). In April 1945, American troops liberated Buchenwald, the war ended, and after hospitalization for tuberculosis, Michael entered medical school at the University of Budapest with financial support from The

Table 1. **Relationship between contraction time and ATPase activity of myosin in cat muscles**

Muscle	Contraction time	Actin-activated ATPase activity	Ca^{2+}-activated ATPase activity
	(ms)	(µmol P_i/mg/min)	(µmol P_i/mg/min)
Flexor hallucis longus	27	0.55	0.36
Vastus intermedius	70	0.24	0.16
Soleus	75	0.17	0.15

Values for contraction time were obtained from Buller et al. (1960). Contraction time was measured at 37°C, whereas both actin-activated and Ca^{2+}-activated ATPase activities were measured at 25°C. Table is modified from Table I in Bárány (1967).

American Jewish Organization. It was here that Michael was exposed to the lectures of Albert Szent-Györgyi, the Nobel Laureate, who provided the inspiration for doing medical research. As Michael commented in his memoir, Szent-Györgyi "convinced him that advances in human medicine will come from biochemical research." Thus, what we now call "translational research" was the objective of Michael's research from the beginning and remained so throughout his career.

After suffering a recurrence of tuberculosis, which interrupted his medical education, Michael joined the laboratory of Bruno Ferenc Straub, who as a young student working with Szent-Györgyi is credited for the discovery of actin. After a prolonged struggle in the laboratory, Michael improved on Straub's method of isolation of pure actin and demonstrated (Bárány et al., 1954) that there was associated with actin polymerization and hydrolysis of ATP that was not attributable to enzymes that were copurified using the earlier method.

Michael received his MD degree in 1951 and was by that time established as a member of the Straub Institute. Hungary was then firmly in the grips of the Communist Party, and again Michael experienced denial of an opportunity, this time for a position as faculty member because his father had been a farmer. Once again, Michael adjusted to a serious challenge and continued work on actin with Ferenc Guba. Guba's name continues to appear around the world on laboratory solution bottles labeled "Guba-Straub" solution for extracting myosin from skeletal muscle. Work with Guba and Kate Bárány, by then Michael's wife and a survivor of Auschwitz, demonstrated another actin purification procedure, which was published in *Nature* (Bárány et al., 1957).

With events that began with the death of Stalin in 1953, the Hungarian revolution came to be fully experienced by the population of Hungary. An attempt to restore democracy was crushed by Soviet troops, and Michael, two-year-old George, and Kate, then five months pregnant with Francis, escaped from Hungary. The trek to Yugoslavia involved terrifying difficulties that are described vividly in their memoir (Bárány and Bárány, 2000).

Eventually, Jewish refugees from Hungary were able to enter Israel, and it was there that Michael joined the Weizmann Institute with the help of Aharon Katchalsky and his brother Ephraim, both of whom were renowned biophysicists. During this time, Michael began studies on the modulation of myosin ATPase activity. Eventually, another leading muscle scientist, H.H. Weber, offered the Báránys positions, but these were in Germany. Overcoming their reluctance to work in Germany, Kate and Michael began studies at the Max Planck Institute in Heidelberg. Work on myosin continued, resulting in seminal work showing that the ATPase and actin binding sites of myosin were distinct (Bárány and Bárány, 1959). It is of some interest that the Báránys' work (Bárány et al., 1960) during this time was on what Weber called "interaction inhibitors," which are now studied as "sarcomere deactivators" as a therapy for the hypercontractility of familial cardiomyopathies (Warren et al., 2015; Rohde et al., 2018).

By 1960, Michael and Kate had moved to the United States, accepting positions at the Institute for Muscle Disease in New York. The focus of the institute was on muscular dystrophy, with research funding from the Muscular Dystrophy Association. A theory of disease in play at that time was that proteolytic enzymes were affecting muscle function and causal in muscular dystrophy. Work by the Báránys during this time established that myosin from dystrophic muscle had the same enzymatic activity as myosin from normal muscle. This finding was an important milestone in the eventual discovery of dystrophin as a causal element in muscular dystrophy. Inspired again by the Szent-Györgyi comment regarding comparative biochemistry of myosin ATPase and by the realization of the existence of fast and slow skeletal muscles, Michael determined that while different muscles all hydrolyze ATP, the rates differed greatly. This realization led to Michael's interaction with a number of laboratories, gathering a wide variety of muscle types and eventual publication of the landmark paper in *JGP* (Bárány, 1967). As far as we now know, Michael's first encounter with the University of Illinois, the university system where he finished his career, was in his interaction with Professor Lad Prosser, an eminent physiologist of that time, who provided help in obtaining the wide variety of muscle types used in the 1967 study.

Figure 2. **Drs. Michael and Kate Bárány upon joining the faculty of the University of Illinois School of Medicine, 1975.** Photo provided by Dr. Francis Bárány.

In 1973, Michael and Kate again faced new challenges with the closing of the Institute for Muscle Diseases, which led them to accept new positions at the University of Illinois at Chicago (UIC) College of Medicine (Fig. 2 is a photo of the Báránys in 1975). The Báránys were attractive candidates not only because of their science but also because of the material fact that the Muscular Dystrophy Association offered 3 yr of salary for each of them, together with a generous yearly research budget. Janos

Figure 3. **Drs. Michael and Kate Bárány operating a Beckman Model E ultracentrifuge to determine protein molecular weights.** Photo provided by Dr. R. John Solaro.

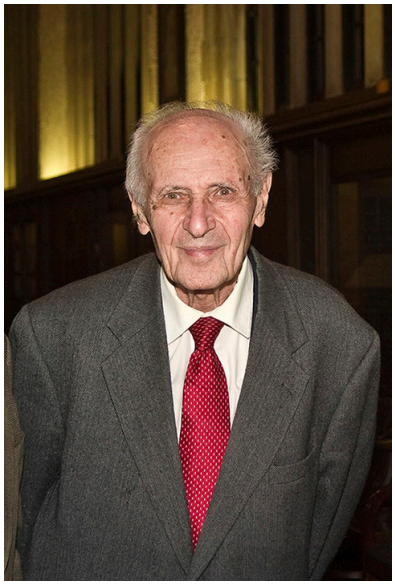

Figure 4. **Dr. Michael Bárány as Emeritus Professor at UIC.** Photo provided by Dr. R. John Solaro.

Molnar and Harold Feinberg, professors at UIC, recognized the great things that would be possible if the Báránys joined UIC. In their years at UIC, Michael and Kate made great strides in the understanding of the regulation of smooth muscle contraction using analytical biochemistry and gel electrophoresis. Michael also was among the very first scientists to recognize the value of naturally abundant isotopes including ^{31}P and ^{13}C in NMR. Taking advantage of instrumentation available in the UIC Research Resources Center, Michael was productive. A PubMed search of "Bárány M, NMR" yields 25 citations from 1978 to 1992. Among many talented mentees during the late 1970s and early 1980s, Michael trained Joseph Chalovich, who has gone on to be a highly respected university professor and investigator. Joe tells great stories of the work ethic in the Bárány laboratory and the long hours spent with the instrumentation (Fig. 3 shows Michael and Kate with a Beckman Model E ultracentrifuge used by Kate to determine protein molecular weights). While in the laboratory, Chalovich published papers following up on Michael's continued interest in finding modifications in muscular dystrophy apart from changes in myosin (Chalovich et al., 1979; Chalovich and Bárány, 1980).

Personal remembrances of the Báránys

One of us (Solaro) has recollections of several close collegial interactions with Michael and Kate. I met the Báránys in the late 1970s at the Biophysical Society Meeting during the meeting of the now renamed Contractility Sub-Group. Kate asked me about our method to isolate the 18,000-kD myosin light chain from skeletal muscle. She was skeptical that we could do this even after showing her some gels, so I invited Kate and Michael to isolate the protein themselves in my laboratory. They gladly accepted the invitation and came to my laboratory at the University of Cincinnati College of Medicine. It was a great privilege to work closely with Kate and Michael and to gain appreciation for how meticulous they were in their planning and execution of experiments. My contribution was generously acknowledged in a paper published in 1979 (Bárány et al., 1979).

By the mid-1980s, the Báránys got wind of the fact that I was interviewing for positions as department head. Michael generously made strong overtures to the search committee to consider me, and I sent in my application. While working in England at University College London, I got a call from the chair of the search committee, inviting me to interview in Chicago. On my arrival, Kate and Michael invited me to dinner. As far as I knew, they did not drink wine but had purchased a bottle for this occasion. My son, Chris, a student at Northwestern, joined in the dinner. Michael and Kate had math puzzles for Chris, an engineering student at the time, and they were quite impressed when he could solve them. When it came to opening the wine, no corkscrew was available, and Kate appeared with a crescent wrench. Fortunately, Chris was quick to ask if they knew their neighbors, who came to the rescue. Kate and Michael had prepared a stack of 3 × 5 cards with questions I might be asked by the Dean, and questions I should be thinking about to ask the Dean. This was daunting, but extremely touching. I enjoyed a 27-year tenure as Department Head at UIC, and the Báránys played no small part in starting me on that adventure.

The Bárány laboratories were in the Department of Physiology and Biophysics, although Michael had his office in Biochemistry, where he held his professorial appointment. I believe anyone who interacted with Michael would agree that his presence (Fig. 4 is a photo of Michael late in life) is still felt in the department and the college, which give awards carrying the Bárány name to deserving students. I can still picture Michael walking down the corridor carrying protected flasks and buckets of acid used to wash pipettes. It was typical that the work was "hands-on," often with jerry-rigged apparatus. The days of everything disposable never came to the Bárány laboratory, as far as I can tell!

One of the last challenges for Michael at UIC was having to deal with mandatory retirement, now a bygone rule in Illinois. He was among the very last of his age group to have to retire, which was a blow to him that we discussed often. In the end, though, he accepted his fate and in retirement made scientific contributions and mentored faculty, trainees, and students alike. In 1996, the Department of Biochemistry and the Department of Physiology and Biophysics at the UIC College of Medicine celebrated the 50th anniversary of the Báránys' entry into science. The program can be viewed on the Bárány website, which also includes biographical material for both Michael and Kate.

Olaf S. Andersen served as editor.

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