A Tribute to Fredric Stewart Fay: June 5, 1943 – March 18, 1997

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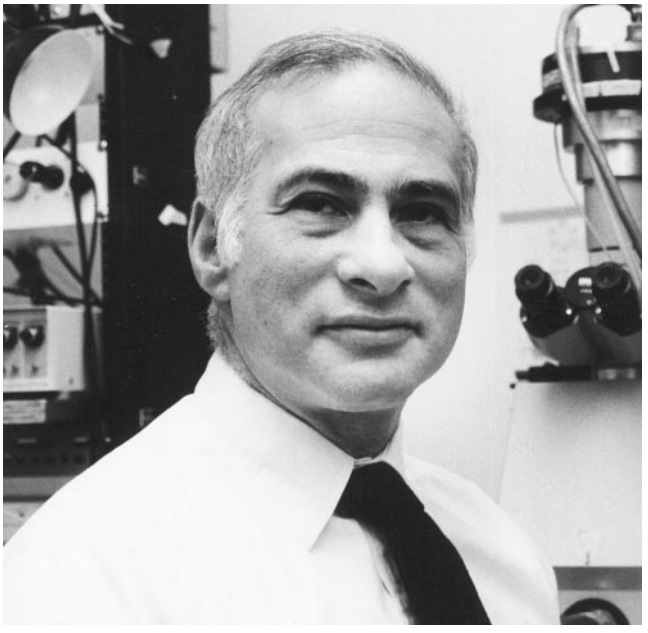
Over a year has passed since Fred Fay suffered a fatal heart attack in Halle, Germany. At the time of his death he was in the laboratory of his host, Dr. Gerrit Isenberg, winding down after a week of presentations and meetings of the German Physiological and Cell Biology Societies. Because Fred could never stay out of touch with progress on his own science for very long, he was on the telephone to his laboratory at the University of Massachusetts when he was stricken. It is of some comfort to consider that, although he died far too young at the peak of an illustrious scientific career, his passing was as he might have chosen: in the afterglow of spirited scientific discourse in the company of respected colleagues. By accounts of some of those present at these meetings, Fred gave his typical dynamic presentations, replete with his customary scientific insight, stunning images, and a video of dynamic events in living cells.

Fred was a long-term member of the Biophysical Society and an enthusiastic participant in its meetings. A developer of significant advances in biophysical methods in his own right, he readily embraced new developments from colleagues and would return from meetings excited over the latest advances. He also was serving on the Editorial Board of the *Biophysical Journal* at the time of his death. To honor his scientific career, the *Journal* dedicates this issue to his memory. Included in this memorial are 18 research papers by some of Fred's many students, collaborators, and colleagues who were profoundly influenced by his science. An Appendix to this tribute lists the papers and their authors.

Fred was born in New York City and according to his sister Louise he demonstrated an early interest in chemistry with a series of experiments in rocketry which culminated in the near destruction of their apartment bathroom. The proximity of water had figured into his experimental plan, an indication of the careful scientist he was to become. He graduated from the Bronx High School of Science in 1961 and attended Cornell University. In his junior year he joined the laboratory of Dr. R. Blake Reeves, with whom he co-authored his first scientific publication. Following his graduation (A.B. in Chemistry), his interest piqued by his undergraduate laboratory experience, he began his studies in physiology at Harvard University, working in the laboratory of Dr. John R. Pappenheimer. His Ph.D. thesis examined the oxygen consumption of the carotid body as a function of flow and oxygen pressure with a view toward understanding the underlying biochemical regulatory mechanisms. When his results demonstrated an insensitivity to oxygen pressure in the oxygen consumption of the carotid body, he turned his attention to the cellular and biochemical mechanisms underlying the contractile response of the ductus arteriosus of the neonatal guinea pig, beginning his long involvement with the physiology of smooth muscle. After earning his Ph.D. in 1969 he spent a postdoctoral year at Harvard continuing his studies on the ductus arteriosus. His creative and meticulous work marked him then as a dedicated and serious research scientist. When in 1970 Dr. H. Maurice Goodman moved to Worcester to chair the Physiology Department at the newly established University of Massachusetts Medical Center, Fred moved with him. In Dr. Goodman's words:



"Fred was recruited as an Assistant Professor of Physiology and was in Worcester to greet the first class of medical students when they arrived in September of 1970. He was intimately involved in all of the planning, organizing, and just plain hard work that goes into starting a new department. Much of the credit for whatever success the Department now enjoys must go to him."

Fred certainly had the energy and drive required for success in the challenging environment of a new medical school, housed in temporary quarters for its first three and a half years, without a research tradition or infrastructure. He thrived on the challenge of building an institution from the ground up, becoming as expert in the arts of garnering space

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and equipment as he was in asking significant scientific questions. Initially, he continued his studies on the guinea pig ductus arteriosus. The questions raised in his morphological studies with Peter H. Cooke on irreversible ductal closure after birth led him to the studies of the physiology of smooth muscle that are a cornerstone of his scientific reputation. Over time, his efforts resulted in a significant concentration in smooth muscle research at the University of Massachusetts, eventually incorporating the talents of his faculty colleagues Roger Craig, Tom Honeyman, Mitsuo Ikebe, Cheryl Scheid, Josh Singer, John Walsh, and Hiroshi Yamaguchi in addition to the members of his own Biomedical Imaging Group, Walter Carrington, Kevin Fogarty, Lawrence Lifshitz, and Richard Tuft.

In the early 1970s knowledge of smooth muscle contractility was derived from multicellular preparations and was confounded by cell heterogeneity and the mechanical properties of connective tissue. Furthermore, individual smooth muscle cells had none of the regular structural features visible in striated muscle when viewed in the light microscope, so there were few clues to how the contractile apparatus might function. Fred set himself a goal to study contraction in isolated smooth muscle cells and worked to develop a preparation of the intestinal muscle of the salamander *Amphiuma*. Publication during this time by Bagby and colleagues of their preparation of isolated contractile smooth muscle cells from the stomach of the giant toad *Bufo marinus* was the impetus Fred needed to begin a series of experiments spanning more than 25 years. He asked and answered fundamental questions about the organization of the smooth muscle contractile apparatus, its activation, and its regulation, and he was still probing questions on the influence of subcellular organization on cell function at the time of his death.

During the 1970s, a time of great personal scientific accomplishment, Fred was also further developing the organizational and people skills required by the collaborative nature of his science. Working side by side in the lab with medical students, postdoctoral fellows, and collaborating faculty members, Fred relentlessly probed smooth muscle physiology at the cellular and subcellular level. Some highlights of that notable time in his career will give a flavor of the breadth of his activity. With Claudio Delise, he published a scanning electron microscopy study in which he hypothesized that the large "blebs" that appear on the plasma membrane of individual contracted smooth muscle cells arise from the opposition of two forces: the outward hydrostatic pressure of the cytosol and the inward tension of the contractile machinery at specialized regions of the plasma membrane (Fay and Delise, 1973).

Working with Peter Canaday, Fred developed a force transducer capable of measuring the contractile forces of smooth muscle myocytes in micrograms and devised the remarkable technique of "tying" individual cells to the instrument. In his published study (Fay, 1977), Fred showed that the tension of a relaxed smooth muscle cell is virtually independent of its length, in contrast to the findings in smooth muscle tissue. He also found a mean delay of 214 ms between the exciting stimulus and the onset of force generation. The questions these findings raised suggested to Fred that "either the long delay resides in the coupling of transmembrane potential changes to the opening of a Ca^{2+} gate or that there is a considerable delay between the arrival of Ca^{2+} at the contractile proteins and the formation of cross-bridges between myosin and actin" (Fay, 1977). His quest to answer these questions had a major influence on the rest of his career.

Up to this point, Fred had used the light microscope primarily as a useful tool to manipulate and visualize the behavior of individual smooth muscle cells, hypothesizing from these studies on their internal workings. It now became clear that measurements of both intracellular calcium concentration ($[Ca^{2+}]_i$) and the subcellular organization of the contractile machinery would be needed to make further progress. Seen from today's perspective, the microscopic techniques he was instrumental in developing over the next twenty years stand as the second major underpinning of his scientific reputation.

To learn the techniques of $[Ca^{2+}]_i$ imaging current at that time, Fred spent a sabbatical leave with Dr. Stuart Taylor at the Mayo Medical School. Using the luminescent $[Ca^{2+}]$ indicator aequorin, he demonstrated (Fay et al., 1979) that $[Ca^{2+}]_i$ rises rapidly and persistently following stimulation, implying that the rate-limiting step for contraction seemed to be "a slow reaction during which the contractile proteins in smooth muscle are activated." Calcium imaging became a major tool in Fred's investigative arsenal, and he rapidly embraced any advances in the field. When Dr. Roger Tsien developed the ratiometric $[Ca^{2+}]$ indicator Fura-2, Fred rapidly modified his existing equipment to exploit this new development. The resulting publication (Williams et al., 1985) is Fred's most cited paper.

Concurrently with his increasing use of calcium imaging, Fred was using the light microscope to probe subcellular organization in novel and demanding ways. A study of the distribution of α-actinin in single isolated smooth muscle cells (Fay et al., 1983) required the modification of an epifluorescence microscope to permit serial acquisition of successive focal planes 0.5 μm apart through a cell labeled with fluorescent α-actinin. The film images were traced by hand and digitized to provide striking 3-dimensional views of stringlike arrays of fusiform elements containing high concentrations of α-actinin, verifying in part Fred's views of the organization of the contractile machinery in smooth muscle. In this result are seen the beginnings of Fred's involvement in the continuing development of 3-dimensional optical sectioning microscopy. This technique exploits the sensitivity of modern cooled charge coupled device (CCD) cameras and the power of computer workstations to compute a 3-dimensional image of fluorescent staining from a series of images taken at different focal planes (or optical sections) within the cell. By characterizing the imaging properties of the microscope and applying powerful image restoration (or deconvolution) algorithms,

one can compute a view of the cellular fluorescence in which light from out-of-focus planes is correctly attributed to its points of origin. Advances during the mid 1980s that led to the current state of the art in this technology are closely identified with two groups of investigators: Drs. David Agard and John Sedat at the University of California-San Francisco and Fred's Biomedical Imaging Group at the University of Massachusetts Medical Center. The team of technical professionals Fred assembled to realize his vision for the instrument continue to pursue the development and applications of this technology.

From the late 1980s until his death, Fred was involved in extending the spatial and temporal resolution of the optical sectioning microscope. Furthermore, he recognized that the unique tools in his possession were applicable to a broad range of problems in cellular biology and physiology and he did not hesitate to share the resource with those who could put it to good use. The result was a series of studies that broke new ground. For example, one 3-dimensional study demonstrated that the Na^+/K^+ pump and the Na^+/Ca^{2+} exchanger are co-localized in regions of the plasma membrane that are distinct from the anchoring sites of the contractile machinery (Moore et al., 1993). Another high spatial resolution study visualized single RNA transcripts, each labeled with five fluorochromes per molecule (Femino et al., 1998); a third study provided striking images of the organization of pericentrin and γ-tubulin into a lattice structure at the centrosome (Dictenberg et al., 1998). Studies which extended temporal resolution used a high-speed instrument developed in the early 1990s and capable of taking individual focal planes every few milliseconds. One measured changes in the membrane potential of individual mitochondria which were moving at speeds up to 2 μm/s in neuroblastoma cells (Loew et al., 1993). This same instrument was fast enough to permit the study of $[Ca^{2+}]$ gradients in the first 15 ms of systole in cardiac myocytes (Isenberg et al., 1996). Using the high-speed CCD camera and specifically targeted green fluorescent proteins, this system has recently imaged a highly dynamic network of mitochondria in living HeLa cells and has yielded highly detailed 3-dimensional views of the interrelationship of endoplasmic reticulum and mitochondria in the same cells (Rizzuto et al., 1998). The imaging for this study was completed shortly before Fred's death.

Although these later studies emphasize the value and broad applicability of the imaging techniques that Fred was instrumental in developing, it should not be inferred that he lost sight of the problems in smooth muscle that had long intrigued him. He continued to make contributions to the understanding of stimulation-contraction coupling in smooth muscle, especially to the elucidation of the downstream regulatory cascades. He developed a microinjection system to deliver signal transduction modulators directly into single smooth muscle cells. By injecting constitutively active myosin light chain kinase (MLCK), he demonstrated directly that the activation of MLCK is sufficient to initiate contraction (Itoh et al., 1989). Recently he developed caged peptide modulators, such as specific protein kinase inhibitors, which can be injected into cells and functionally activated upon photolysis (Walker et al., 1998). He applied this technique to demonstrate the role of the targeted molecules in the regulation of both smooth muscle contraction and the chemotaxis of white blood cells. He remained interested in the regulation of the contractile apparatus and at the 1997 annual meeting of the Biophysical Society only two weeks before his death, he learned of the acceptance of his study demonstrating the role of calponin, a thin-filament binding protein, in maintaining contractile activity in smooth muscle (Malmqvist et al., 1997).

More detail could be added about his outstanding career, but it would only intensify the loss we still feel at the early passing of one so gifted and energetic. It is better to end by celebrating the life that he led and the contributions he made. The words of his long-time friend and mentor, H. Maurice Goodman, are a fitting closure to this piece:

"Fred will be remembered by his students and colleagues in Worcester and on every continent for more than his science, for he was a very special human being. His home, his heart, and his mind were open for all to share, and his wit and energy brought joy to all who were privileged to know him. He worked hard and played hard. He touched many lives, and left them all better for the contact. He took great pleasure and pride in his wife Madeleine, his children, Andrew, Nicholas, and Isabel and his grandchildren, Sarah, David and Julia, and he was eagerly awaiting the arrival of twin grandsons Oliver and Noah, born after his death. He also derived great joy from the scientific offspring who worked in his lab. The list includes seven medical students, five graduate students, twenty-three postdoctoral fellows, and six senior scholars who spent their sabbatical leaves with him. Many of his former students and fellows have gone on to build impressive careers for themselves, and their science will always bear Fred's indelible imprint. Fred died far too soon, but we can take some comfort in the knowledge that he packed a great deal into his all too short lifetime. In all he published over 125 papers and reviews in the most highly critical and respected journals. His creativity resulted in the award of eight patents, and his technological innovations in optical methods paved the way for him and future generations to 'boldly go where no one has gone before.'"

For this retrospective on Fred Fay's career we owe a strong debt of gratitude to Dr. David Warshaw, one of Fred's early postdoctoral fellows, who marvelously recapitulated Fred's career for the 1997 FASEB Smooth Muscle Summer Research Conference, and to Dr. H. Maurice Goodman, Fred's long-time department chairman, who added his warm personal reminiscences. We also thank the many authors, reviewers, editors, and staff of the *Biophysical Journal* for their enthusiastic cooperation in preparing this special memorial. The outstanding science represented in the following papers in this issue is the best form of tribute to Fred and one that he would have certainly enjoyed and appreciated.

APPENDIX: Fay Memorial Papers

Two-dimensional Determination of the Cellular Ca^{2+} Binding in Bovine Chromaffin Cells, Mohammad Naraghi, Thomas H. Müller, and Erwin Neher

Role of Mitochondria in Calcium Regulation of Spontaneously Contracting Cardiac Muscle Cells, David N. Bowser, Tetsuhiro Minamikawa, Phillip Nagley, and David A. Williams

Local Measurements of Viscoelastic Parameters of Adherent Cell Surfaces by Magnetic Bead Microrheometry, Andreas R. Bausch, Florian Ziemann, Alexei A. Boulbitch, Ken Jacobson, and Erich Sackmann

Chloride Channel Blockers Inhibit Ca^{2+} Uptake by the Smooth Muscle Sarcoplasmic Reticulum, N. S. Pollock, M. E. Kargacin, and G. J. Kargacin

Ca^{2+} Removal Mechanisms in Rat Cerebral Resistance Size Arteries, Tomoko Kamishima and John G. McCarron

Multifocal Excitation Provides Optical Sections from Deeper within Scattering Specimens than Confocal Imaging, Victoria E. Centonze and John G. White

Time Course of the Initial $[Ca^{2+}]_i$ Response to Extracellular ATP in Smooth Muscle Depends on $[Ca^{2+}]e$ and ATP Concentration, Mỹ G. Mahoney, Linda L. Slakey, Christopher D. Benham, and David J. Gross

In Situ Characterization of the Ca^{2+} Sensitivity of Large Conductance Ca^{2+}-Activated K^+ Channels: implications for their Use as Near-Membrane Ca^{2+} Indicators in Smooth Muscle Cells, Alvaro Muñoz, Lucía García, and Agustin Guerrero-Hernández

How Microtubules Get Fluorescent Speckles, Clare M. Waterman-Storer and E. D. Salmon

Calcium Transients and the Effect of a Photolytically Released Calcium Chelator During Electrically Induced Contractions in Rabbit Rectococcygeus Smooth Muscle, Anders Arner, Ulf Malmqvist, and Rudolf Rigler

Intracellular Fluorescent Probe Concentrations by Confocal Microscopy, Charles Fink, Frank Morgan, and Leslie M. Loew

Calcium Waves Induced by Large Voltage Pulses in Fish Keratocytes, Ingrid Brust-Mascher and Watt W. Webb

A Simple Method for High Temporal Resolution Calcium Imaging with Dual Excitation Dyes, Luc Leybaert, James Sneyd, and Michael J. Sanderson

4Pi-confocal Imaging in Fixed Biological Specimens, Martin Schrader, Karsten Bahlmann, Günter Giese, and Stefan W. Hell

Characterization of the Sperm-Induced Calcium Wave in Xenopus Eggs Using Confocal Microscopy, Ray A. Fontanilla and Richard Nuccitelli

Simulation of the Fertilization Ca^{2+} Wave in Xenopus laevis Eggs, John Wagner, Yue-Xian Li, John Pearson, and Joel Keizer

Cholesterol Distribution in Living Cells: Fluorescence Imaging Using Dehydroergosterol as a Fluorescent Cholesterol Analog, Sushmita Mukherjee, Xiaohui Zha, Ira Tabas, and Frederick R. Maxfield

MgADP Promotes a Catch-Like State Developed Through Force-Calcium Hysteresis in Tonic Smooth Muscle, Alexander Khromov, Avril V. Somlyo, and Andrew P. Somlyo

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