Published in final edited form as:

Anat Rec (Hoboken). 2014 September 1; 297(9): 1543–1547. doi:10.1002/ar.22963.

Memories of Annemarie Weber

Clara Franzini-Armstrong

Dept of Cell and Developmental Biology, University of Pennsylvania, Perelman School of Medicine, Philadelphia PA 19104

Annemarie Weber was born on 9/11/ 1923 in Tübingen, West Germany and grew up in Königsberg (the German enclave), where her father, the well-known muscle physiologist Hans H. Weber, had a position at the University. Starting in school she displayed the independent will power and keen intellect that characterized her whole life and made her highly dominant in the muscle field. She excelled in the subjects of interest. She was a teenager during World War II, a very difficult period during which she was often separated from her family without news. She was in a youth work camp, where she suffered from vitamin deficiency due the restricted diet and saw the beginning of the Russian invasion. She was from a very gifted family. Her brother Jürgen Weber (1928–2007) was a well-known sculptor, author among others of two panels at the Kennedy center in Washington. She was very close to her nephew, Carl Costantin Weber, also a successful sculptor in the Faculty of Architecture at the Academy Anhalt in Dessau; her niece Saskia; and her niece Doina Weber, a successful movie and TV actress currently in Vienna Austria; and to their children, whom she invited to share her summer home in Woods Hole (Fig. 1A). She loved her house in Woods Hole, where she lived a good part of the summer and enjoyed long walks, swims (including the famous long swim across the harbor propelled by tidal current that had been pioneered by Albert Szent-Györgyi) and watching the birds attracted to her feeder.

Annemarie loved exploring the world. She took wonderful trips in the desert with her long time friends Richard J Podolsky, and Vivianne Nachmias and later took me on treks to the White Mountains in New Hampshire and through a wonderful exploration of Crete and the Minoan civilization. Through her life she retained her love of intellectual pursuit and a keen interest in people and places that she shared with her friends. Her extensive library included works on exploration, evolution, and the function of the brain. She introduced me to Scott's expedition to the South Pole, Shackleton expedition, and numerous essays on evolution. She was also a close observer of human nature and loved watching children grow and display their personalities. She was an aunt and great-aunt to young and older children in her immediate German family (Fig. 1A) in addition to innumerable others in the families of friends (Fig. 1B). Her lobster dinners at the house in Woods Hole (Fig. 1C) were the summer highlight for friends, particularly Andrew and Ursula Szent-Györgyi, with whom she maintained a life-long friendship. She was an enthusiastic gardener and was very proud

of having the most vivid flowerbed in her neighborhood (Fig. 1 D). She died in Philadelphia from lung cancer on 7/5/2012.

Hans Weber advised his daughter to pursue a medical career so she took an MD at the University of Tübingen in 1950. However, the research spark had already been activated by discussions with her father and so she never practiced medicine although she maintained a keen interest in disease. With funding from the Rockefeller foundation she had several postdoctoral training experiences, notably with A.V. Hill at University College London (Fig. 2A) and at Harvard University. She also had a formal training period with her father in Heidelberg which probably influenced her most strongly. H. H. Weber had been very much interested in ATP and in the role that the high-energy compound had on muscle contraction. In the course of his *in vitro* studies he developed the philosophical concept that “that if one wants to understand the mechanism of contraction one must understand relaxation” (HH Weber, 1959). It is thus not surprising that both his two most successful trainees, Wilhelm H. Hasselbach and Annemarie Weber, focused on the role of sarcoplasmic reticulum (SR) in relaxation. Her academic career started after she migrated to the United States. She was Research Associate (1954–59) and Lecturer (1959–62) at Columbia University; Associate member, MDA Institute for Muscle Disease, New York (1963–65); Professor of Biochemistry, Washington University in St. Louis (1965–72); Professor and later Professor Emerita of Biochemistry, University of Pennsylvania (1972–2012)

Annemarie was a very intense scientist. In her own work and in that of her trainees and collaborators she expected total attention to details. In her own words, “if you do not remember what you were doing a day ago, you were not paying enough attention”. In her published figures the data follow very precisely the predicted curves. The same precision was expected of others: speakers at scientific meetings had to cross the mine fields of her “look here, sweetie” comments, with which she pointed out the weak points. Collaboration with her involved a strong expectation of corresponding precision. Her interactions at local seminars, Gordon conferences and Cold Spring Harbor Symposia were fully engaged, with intense discussions (Fig. 2B).

Annemarie’s distinguished research career earned several honors. She became a member of the Deutsche Akademie der Naturforscher Leopoldina (1975) and the American Academy of Arts and Sciences (1976). She was elected a Fellow of the American Association for the Advancement of Science (1978) and of the Biophysical Society (2000).

Her research career can be divided into three distinct stages, each resulting in key publications, of the type that turn a page in history.

1) The Ca^{2+} story

Annemarie provided direct evidence for the role of Ca^{2+} ions as intracellular messengers. Ca^{2+} ions, as controlling agents for contraction were in the air in the late forties. Indeed AV Hill (1949) used Ca^{2+} as a basis for his calculations aimed at showing whether an “activating substance” liberated at the fiber surface and diffusing inward could account for the rapid transition from rest to activity of the entire cross section of a muscle fiber. In two independent experiments small amounts of Ca^{2+} containing solution were introduced into

muscle fibers resulting in localized contractions (Heilbrunn and Wiercenski, 1947; Niedergerke, 1955), an indication that Ca^{2+} could be a physiological activator. However, in vitro experiments with isolated proteins and myofibrils were baffling and gave uncertain results. Before the availability of Ca^{2+} specific chelators, Ca^{2+} was a common contaminator of glassware and chemicals, magnesium added an additional complication and at low concentrations of ATP contraction seemed to be Ca^{2+} independent. So major skepticism remained. Interestingly, HH Weber himself was not convinced that Ca^{2+} could be assigned a specific role in muscle activation. Annemarie however had the right insight. She calculated the effective free Ca^{2+} concentration on various ligands and established that very low concentrations of ionized Ca^{2+} are uniquely necessary and sufficient to activate the contractile machinery of muscle in the presence of physiological (mM) concentrations of MgATP. With this knowledge she interacted with the Japanese scientist Setsuro Ebashi, also a supporter of the Ca^{2+} hypothesis and advised Richard Podolsky at NIH. (see Podolsky and Costantin 1966). Based on initial observations by Annemarie that sensitivity to Ca^{2+} of various myofibrillar preparations varied, Ebashi made the famous discovery of the tropomyosin-troponin complex that controls the state of actomyosin activity in the intact myofibril (Ebashi and Ebashi, 1964; Ebashi et al, 1967).

If Ca^{2+} is an activator, it must be removed from the myofibrils to allow relaxation. Ebashi and Lipmann (1962) identified the vesicular nature of the “Marsh relaxing factor” and its ability to bind Ca^{2+} in the presence of ATP and Hasselbach (1966) demonstrated the ATP dependent Ca pumping action of isolated sarcoplasmic reticulum (SR). However, it was Annemarie again who brought the story to its ultimate conclusion: she demonstrated that SR vesicles could fully account for muscle relaxation by their Ca^{2+} sequestering ability (Weber, 1966; Weber et al., 1963, Fig. 3A). Her experiments and calculations were so compelling that they ended several years of dispute regarding the role of Ca^{2+} and led to the subsequent realization that all cells use Ca^{2+} as an intracellular messenger by virtue of its being kept at low concentration in the cytoplasm. The three main principles of Ca^{2+} action are, first that since it acts at very low concentrations, relatively small movements of ions are necessary for activity; second that compartmentalization and sequestration within the cell are essential for this activity and third that it acts via intermediates with a high affinity for it (troponin C in muscle, and calmodulin in many other cells). Annemarie also clarified the action of caffeine in releasing Ca^{2+} from the sarcoplasmic reticulum (Weber and Herz, 1969). Caffeine is still commonly used in cell biology to test for Ca^{2+} release from internal stores.

2) Cooperativity in the thin filament

Having identified Ca^{2+} as the intracellular messenger regulating contraction and having established the cellular mechanisms that control its movements in the cell, Annemarie focused on a third essential question: what mechanism regulates the state of actomyosin. Myosin's ATPase activity requires the presence of MgATP as a substrate and is enhanced in the presence of actin. Under physiological concentrations of MgATP (in the mM range) the actin activation occurs only if either purified actin is present (under *in vitro* conditions) or if, in the intact myofibril, Ca^{2+} is bound to troponin C (*in situ and in vitro*). However, it was known that at very low concentrations of MgATP (less than 0.1 mM) the rate of ATP splitting was limited by substrate concentration but independent of Ca^{2+} even in the

complete system. This was one of the observations that had been very puzzling in the early studies with the isolated actomyosin systems. Annemarie explored this question with her acquired knowledge of Ca^{2+} action and knowledge of the tropomyosin-troponin system. She produced observations that greatly influenced thinking about the mechanism of troponin-tropomyosin control of contraction (Bremel and Weber, 1972; Bremel et al., 1972; Weber and Murray, 1973; Murray and Weber, 1974, 1980). It was known that troponin, consisting of three subunits (TnT, TnI and TnC), forms a complex with one tropomyosin (TM) molecule. Two long tropomyosin polymers fit into the two grooves of the actin filament, each tropomyosin covering approximately seven actin monomers (Spudich et al., 1972). Figure 3B graphically illustrates the crux of the experiments. In the presence of the tropomyosin troponin system, the acto-myosin ATPase activity is simply substrate limited at concentrations of ATP below those required for relaxation and is dependent on the ratio of myosin to actin. Under these conditions some myosin cross bridges form rigor complexes with actin filaments. At higher MgATP concentrations the system becomes Ca^{2+} dependent. The experiments were boldly and correctly interpreted to indicate that a rigor cross-bridge, fitting like a foot in a door, can interfere with the inhibitory action of the Tn-TM complex by shifting it to a different position in the groove, thus allowing regulation of a functional unit of seven actin monomers. Cooperativity of the switch activated by Ca^{2+} was also directly confirmed (Murray and Weber, 1980). This mechanical interpretation of the regulatory action of Ca^{2+} fits with currently accepted data, starting with the classical demonstrations of tropomyosin shifts by HE Huxley (1973) and Haselgrove (1973).

3) Actin polymerization and its control

Finally, Annemarie explored questions of actin polymerization. Actin filaments can be either stable or highly labile and their dynamics are of great importance in most cell activities involving movement. They are in a state of balance determined by two different rates of polymerization and depolymerization at the two unequal ends, by the cytoplasmic concentration of free actin available, and by various binding proteins that associate with their ends. Interestingly, in this phase of her career Annemarie extended her interests to a variety of cells through collaborations that encouraged her to explore all possible modes of actin filaments' length regulation. Her extraordinary ability to analyze kinetic data was particularly useful in solving questions of actin nucleation and of polymerization, depolymerization and capping of actin filaments. In a series of papers with Mark S. Mooseker, she defined the complex regulation of actin filaments by villin, finding Ca^{2+} regulation of its capping, cutting and nucleation roles (e.g. Walsh et al., 1984a and b; Northrop et al., 1986; Weber et al., 1987). In collaboration with Lewis G Tilney, she helped to define how the invasive bacterium *Listeria* cleverly exploits host cell actin to form its own cytoskeleton (Tilney et al., 1992). With Vivianne Nachmias she defined the competing effects of thymosin beta 4, profilin, DNase 1 (Weber 1999), and finally, with Velia M. Fowler she demonstrated how tropomodulin action is essential to maintaining muscle thin (actin) filament length (Weber et al., 1999).

4) Annemarie as a teacher

Annemarie was a highly successful and enthusiastic teacher throughout her life and when she was not formally teaching students she passed her knowledge on to her friends and relatives. I still remember her lectures delivered while trekking in the white mountains of New Hampshire.. Students of the famous “Physiology” course of the late fifties at the Marine Biological Laboratory in Woods Hole, which included Hugh E. Huxley and Andrew Szent-Gyorgyi in the teaching staff, still vividly treasure her lessons. She taught comparative physiology at Columbia as well as biochemistry at the medical schools of Washington University in St Louis and University of Pennsylvania. After finally closing her laboratory, at ~75, she entered with renewed vigor into her teaching role, improving, perfecting and teaching her novel biochemistry course for first year medical students. She raised the students’ enthusiasm with a medicine-oriented approach while expecting rigorous understanding of biochemical concepts. She meticulously prepared small group sessions, with well-designed questions and answers and trained the other members of the Department in the task. She made the biochemistry course a showcase that attracted students to the subject and earned her the Provost’s Teaching award in 2001 (Fig. 2C), in addition to the earlier Leonard Berwick Award in 1985. She found great satisfaction in this last role that she maintained, though slowing down, into her eighties.

AMW was a truly inspiring, energetic scientist and teacher.

Acknowledgments

Grant sponsor NIH, HL 48093

References

- Bremel RD, Weber A. Cooperation within actin filament in vertebrate skeletal muscle. *Nature New Biology*. 1972; 238:97–101.
- Bremel RD, Murray JM, Weber A. Manifestations of cooperative behaviour in the regulated actin filament during actin-activated ATP hydrolysis in the presence of calcium. *Cold Spring Harbor Symp. Biol.* 1972; 37:267–275.
- Ebashi S, Ebashi F. A new protein component participating in the supereprecipitation of myosin B. *J Biochem.* 1964; 55:604–613. [PubMed: 14216404]
- Ebashi S, Lipmann F. Adenosine triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. *J. Cell Biol.* 1962; 14:389–393. [PubMed: 19866605]
- Ebashi S, Ebashi F, Kodama A. Troponin as the Ca^{++} -receptive protein in the contractile system. *J Biochem.* 1967; 62:137–138. [PubMed: 6073169]
- Hasselbach W. Structural and enzymatic properties of the calcium transporting membranes of the sarcoplasmic reticulum. *Ann N Y Acad Sci.* 1966; 137:1041–1048. [PubMed: 5229806]
- Haselgrove JC. X-Ray Evidence for a Conformational Change in the Actin-containing Filaments of Vertebrate Striated Muscle. *Cold Spring Harbor Symposia on Quantitative Biology*. 1973; 37:341–352.
- Heilbrunn LV, Wiercenski FJ. The action of various cations on muscle protoplasm. *J Cell Physiol.* 1947; 29:15–32. [PubMed: 20285919]
- Hill AV. The abrupt transition from rest to activity in muscle. *Proc Roy Soc.* 1949; B136:399–420.
- Huxley, HE. Structural Changes in the Actin-and Myosin-containing Filaments during Contraction. *Cold Spring Harbor Symposia on Quantitative Biology*. Cold Spring Harbor Laboratory Press; 1973. p. 361-376.

- Murray JM, Weber A. The cooperative action of muscle proteins. *Scientific American*. 1974; 230:58–64. [PubMed: 4810515]
- Murray JM, Weber A. Cooperativity of the calcium switch of regulated rabbit actomyosin system. *Mol. Cell. Biochem*. 1980; 35:11–15. [PubMed: 6452574]
- Niedergerke R. Local muscular shortening by intracellular applied calcium. *J Physiol*. 1955; 128:12–13.
- Northrop J, Weber A, Mooseker MS, Franzini-Armstrong C, Bishop MF, Dubyak GR, Tucker M, Walsh P. Different calcium dependence of the capping and cutting activities of villin. *J. Biol. Chem*. 1986; 261:9274–9281. [PubMed: 3087992]
- Podolsky RJ, Costantin LL. The internal membrane system and muscle activation. *Ann NY Acad Sci*. 1966; 137:1038–1040. [PubMed: 5229805]
- Spudich JA, Huxley HE, Finch JT. Regulation of Skeletal Muscle Contraction II. Structural Studies of the Interaction of the Tropomyosin-Troponin Complex with Actin. *J. Mol. Biol*. 1972; 72:619–632. [PubMed: 4349760]
- Tilney LG, DeRosier DJ, Weber A, Tilney MS. How *Listeria* exploits host cell actin to form its own cytoskeleton. II. Nucleation, actin filament polarity, filament assembly, and evidence for a pointed end capper. *J. Cell Biol*. 1992; 118:83–93. [PubMed: 1618909]
- Walsh TP, Weber A, Higgins J, Bonder EM, Mooseker MS. Effect of villin on the kinetics of actin polymerization. *Biochemistry*. 1984a; 23:2613–2621. [PubMed: 6432033]
- Walsh TP, Weber A, Davis K, Bonder EM, Mooseker MS. Calcium dependence of villin-induced actin depolymerization. *Biochemistry*. 1984b; 23:6099–6102. [PubMed: 6525347]
- Weber A. Energized calcium transport and relaxing factors. *Current topics in Bioenergetics*. 1966; 1:203–254.
- Weber A. Actin binding proteins that change extent and rate of actin monomer-polymer distribution by different mechanisms. *Mol. Cell. Biochem*. 1999; 190:67–74. [PubMed: 10098971]
- Weber A, Herz R. The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. *J Gen Physiol*. 1969; 52:750–759. [PubMed: 5688082]
- Weber A, Murray JM. Molecular control mechanisms in muscle contraction. *Physiol Revs*. 1973; 53:612–673. [PubMed: 4577547]
- Weber A, Herz R, Reiss I. On the mechanism of the relaxing effect of fragmented sarcoplasmic reticulum. *J gen Physiol*. 1963; 46:679–702. [PubMed: 13999359]
- Weber A, Northrop J, Bishop MF, Ferrone FA, Mooseker MS. Nucleation of actin polymerization by villin and elongation at subcritical monomer concentration. *Biochemistry*. 1987; 26:2528–2536. [PubMed: 3607030]
- Weber A, Pennise CR, Fowler VM. Tropomodulin increases the critical concentration of barbed end-capped actin filaments by converting ADP.P(i)-actin to ADP-actin at all pointed filament ends. *J. Biol. Chem*. 1999; 274:34637–34645. [PubMed: 10574928]
- Weber HH. The relaxation of the contracted actomyosin system. *Ann NY Acad Sci*. 1959; 81:400–421.

Figure 1.
Annemarie Weber as a great-aunt taught and entertained the children, while advising the parents (A with some of her German family during a party for her birthday; B with two Armstrong grand children). C) Her friends enjoyed a lobster dinner in her house in Woods Hole. D) Her flower-filled garden gave her great joy.

A **B** **C** **Figure 2.**

A) A.V. Hill and Annemarie on the roof of University College London in 1951, photograph by D. R. Wilkie (1985). B) An animated discussion between Annemarie and Hugh E. Huxley (seated) at a Conference. Andrew F. Huxley is caught in the image. With the recent deaths of the three figures in this image, a major chapter of muscle research is closed. D) Annemarie receives the Provost award (the highest honor at Penn) for her teaching, in 2001.

Figure 3.

Figures from Annemarie's works that constitute historical landmarks. A) A demonstration that the relaxing effect of the isolated SR (relaxing factor) is due to its sequestration of Ca^{2+} . Abscissa, relaxing factor concentration. Curve 1, filled circles: exchangeable Ca^{2+} bound to myofibrils (left ordinate), Filled squares are in the presence of 2 mM EGTA; curve 2, open circle myofibrillar ATPase (right ordinate). Double circle is in the presence of 2mM EGTA. Open triangles: superprecipitation (equivalent to contraction, inset ordinate on right). Inverted triangle in the presence of 2 mM EGTA. (Reprinted from Fig. 1 of Weber A., Herz

R. and Reiss I., 1963 Originally published in J gen Physiol 46, 679–702 by permission of publishers). B) Evidence for cooperativity in actin filament regulation. Actin activated ATP hydrolysis by S1 (the isolated myosin “head”) in the presence of reconstituted actin filaments containing the tropomyosin-troponin complex. The rate of hydrolysis is substrate dependent but independent of Ca^{2+} at MgATP concentrations below those required for dissociating all rigor complexes (circles), but becomes Ca^{2+} dependent at higher MgATP concentrations (crosses) The experiments at right was done at a lower S1/actin ratio. (Reprinted from Fig. 3 of Bremel, R.D. and Weber, A. 1972. Originally published in Nature New Biology 238:97–101 by permission of publisher).