A Fluorescent Lifetime: Reminiscing About Gregorio Weber

David M. Jameson

Abstract During the last few decades, fluorescence spectroscopy has evolved from a narrow, highly specialized technique into an important discipline widely utilized in the biological, chemical, and physical sciences. As in all scientific disciplines, the development of modern fluorescence spectroscopy has benefited from the contributions of many individuals from many countries. However, one individual, *Gregorio Weber*, can be singled out for his outstanding and far-reaching contributions to this field. This chapter will briefly outline aspects of Gregorio Weber's life and times and discuss some of his more important contributions to the fluorescence field. Some of his more important contributions to the field of protein chemistry will also be discussed. In addition to the facts of Weber's life and work, I shall also interject several anecdotes from my personal experience with him, which will serve to illustrate his outstanding personality and character.

Keywords Anecdotes • Awards • Fluorescence • Gregorio Weber • Proteins • Scientific accomplishments

I began my graduate studies in the Chemistry Department at the University of Illinois at Urbana-Champaign (UIUC) in the fall of 1971. After hearing each faculty member discuss the research ongoing in their lab, I chose Gregorio Weber as a faculty advisor. I was particularly attracted by the concept that the interaction of light with matter could provide important information about the nature of biomolecules, especially proteins. During my graduate career I had to synthesize fluorescent probes as well as build the photon-counting instruments I was to use. I also was given the opportunity to work closely with many of the international

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visitors to the lab. Needless to say, this level of training held me in good stead over the rest of my scientific career. In those early graduate student years I was very impressed with Gregorio Weber's huge store of knowledge and his ability to communicate that knowledge to others. With time I learned that he was one of the great pioneers in the fluorescence field. In the remaining pages I shall outline some of the more important aspects of Gregorio Weber's contributions to fluorescence spectroscopy and to protein chemistry.

I once asked Gregorio Weber how he first got interested in science. He told me that he had a very inspiring high school science teacher and that he told this teacher that he was interested to become a scientist. The teacher informed him that support of scientific careers in Argentina at that time (the late 1930s) was rather hit or miss and advised him to pursue a medical degree. In that way, if a scientific career did not work out at least he could support himself seeing patients. Gregorio Weber followed this advice and earned an MD degree from the University of Buenos Aires in 1943. He soon became an assistant to Bernardo Houssay, who was awarded the 1947 Nobel Prize in Physiology and Medicine for his discovery of the role of pituitary hormones in the regulation of glucose in the blood. Houssay was the first Argentine and Latin American to be awarded with a Nobel Prize in some field of the Sciences. Houssay was impressed with Weber's abilities and suggested that he apply for a prestigious British Council Fellowship to support graduate studies toward a PhD at Cambridge University. Gregorio Weber left Argentina for Cambridge England in 1943 and traveled in a convoy which took 44 days to complete the journey, due to precautions taken against the chance of U-boat attacks. Upon reaching England, Weber initially spent 6 months in the laboratory of Eric Rideal, a physical chemist, learning surface chemistry. But he soon became enamored of the work of Malcolm Dixon, the well-known enzymologist. Interestingly, from the point of view of Weber's future career, Malcolm Dixon had carried out some of the early work on the absorption spectrum of cytochrome c. This interest in spectroscopy may be part of the reason that Dixon suggested that Weber investigate the fluorescence of flavins and flavoproteins. As Weber related in 1986 at the first International Weber Symposium in Bocca di Magra, Italy, in honor of his 70th birthday, he knew very little about fluorescence at that time and nothing about flavins [1]. Needless to say, he did not stay ignorant for long! He soon discovered that many of the basic properties of fluorescence, such as lifetimes, quantum yields, and polarizations had been studied by physicists for several decades. The work that interested him the most, however, was that of Francis Perrin. He said that he read the famous paper of Francis Perrin, on the depolarization of fluorescence by Brownian rotations, not once but many times [2]. Interestingly, Weber commented "Argentine secondary education in the first half of the century included French language and literature so that I could not only understand the scientific content but also enjoy the literary quality of the writing. It was written in that transparent, terse style of XVIII century France, which I have tried, perhaps unsuccessfully, to imitate from then onwards." [1]. Throughout his life, Weber often commented that he was also attracted to fluorescence because of the counterpoint of the esthetic and scientific aspects. Specifically, he said that he was impressed by the fact that visual

observation of changes in the color or intensity of fluorescence could immediately be related to a molecular event. Even in those early days, Weber appreciated the need for a true quantitative understanding of the fluorescence phenomenon. In his PhD thesis he wrote "I feel that a knowledge, as deep as possible, of the physical principles concerned is indispensable. Even close collaboration with a physicist cannot spare this task to the biochemist. I am tempted to believe that a biologist having n ideas related to the biological side of the problem and a physicist possessing another n relating to the physical side would result in some 2n useful combinations whereas the same ideas collected in one brain would lead to a number of combinations more like n!" [3].

Needless to say, at that time, in the 1940s, Weber's fluorescence instrumentation had to be homebuilt. In his original instrument, the light source was a carbon arc, originally developed for use in searchlights during the war. The exciting light was first filtered through a layer of concentrated $NaNO_2$ to remove UV light (<420 nm) and then polarized by a Nicol prism (Fig. 1) (It is interesting to note that during my time as a graduate student in Weber's lab, during the 1970s, we still used these $NaNO_2$ filters routinely, although in our case we used these filters to help in isolating the emission from the exciting light). Weber then used additional glass filters to further remove the exciting light and to isolate the emission. The actual measurement of the polarization of the fluorescence was realized using visual compensation techniques involving observation of interference patterns as a "pile-of-plates" polarizer (the compensator of Arago) was rotated. At that time, photoelectric-based detectors were primitive and could only detect the strongest fluorescence signals, and consequently the eye was the detector of choice. With these visual methods, Weber was able to quantify levels of polarized light reaching

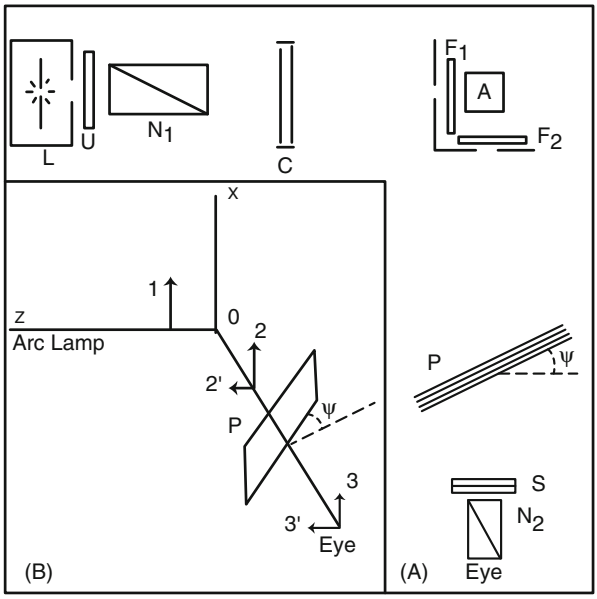

Fig. 1 Original drawing from Gregorio Weber's PhD thesis showing the optical arrangement of the instrument he constructed for polarization measurements

only 1 or 2%. However, he paid a price for these visual observations since, like many of the pioneering spectroscopists, he suffered acute eye ailments in later years as a result of excessive exposure to infrared and ultraviolet light, which led to removal of his lenses, detached retinas, and eventually corneal transplants. As a consequence of the photophobia these eye ailments caused, Weber had to wear sunglasses most of his latter life – those of us who knew him as "The Professor" considered his sunglasses almost as a trademark.

His first publication entitled: *The quenching of fluorescence in liquids by complex formation. Determination of the mean life of the complex* [4] was the first work to demonstrate that fluorescence quenching can take place after formation of molecular complexes of finite duration rather than collisions. (A complete list of Gregorio Weber's publications can be found on the website maintained by the Laboratory for Fluorescence Dynamics at http://www.lfd.uci.edu/weber/publications/). His second publication was entitled *Fluorescence of riboflavin and flavin-adenine dinucleotide* [5], and was the first demonstration of an internal complex in FAD. Some years later he published the first demonstration that NADH also formed an internal complex [6]. He continued to publish important papers on the excited state properties of FAD and NAD in the 1960s and 1970s.

After completing his PhD, awarded in 1947, Weber carried out independent investigations at the Sir William Dunn Institute of Biochemistry at Cambridge, supported by a British Beit Memorial Fellowship, from 1948 to 1952. This fellowship, founded in 1909, was one of the most prestigious and competitive fellowships for postdoctoral or medical degree research in the world. At Cambridge he began to delve more deeply into the theory of fluorescence polarization and also to develop methods which would allow him to study proteins which did not contain an intrinsic fluorophore (intrinsic protein fluorescence from tryptophan and tyrosine had not yet been discovered). He invested considerable time and effort in synthesizing a fluorescent probe which could be covalently attached to proteins and which possessed absorption and emission characteristics appropriate for the instrumentation available in post-war England. For example, as stated earlier, reliable and sensitive photodetectors had not yet been developed and visual observations were the norm, so the emission had to be observable with the eye. The result of 2 years of effort was dimethylaminonaphthalene sulfonyl chloride or dansyl chloride – a probe which is still utilized today. With dansyl chloride and with new instrumentation Weber began to investigate several protein systems, publishing his theory and experimental results in two classic papers published in Biochemical Journal in 1952 [7, 8].

In 1953, Hans Krebs recruited Weber for the new Biochemistry Department at Sheffield University. That same year, Krebs received the Nobel Prize for his elucidation of the metabolic reactions which produce energy in cells – the tricarboxylic acid or Krebs Cycle. David Lloyd, who was an undergraduate student at Sheffield and who was assigned to Gregorio Weber for first year tutorials, told the story (http://www.cf.ac.uk/biosi/staffinfo/lloyd/weber/): "My predecessor as Head of Microbiology in Cardiff, David Hughes, had previously been a member of the Medical Research Council Unit for the Study of Cell Metabolism established for Sir Hans Krebs in Sheffield and later in Oxford. When Gregorio went for interview

there in 1953, David Hughes was given the important duty of showing the already distinguished applicant around and reporting back to 'Prof' as everyone called Krebs. Hughes told me that he felt quite insignificant by comparison to this young genius, and that there was no one whom could question Gregorio's suitability. So his response to Krebs was: Let's not interview, but just appoint."

During his years at Sheffield Weber continued to lay the foundations of modern fluorescence spectroscopy developing both fluorescence theory and instrumentation. One of his significant discoveries during his Sheffield days was that anilinonaphthalene sulfonate (ANS) had a very weak fluorescence in water but this fluorescence increased very dramatically when ANS interacted with bovine serum albumin. Interestingly, more than 60 years after Weber's report (in 1954 with David Lawrence) ANS is still a popular probe and is often used in protein unfolding studies as an indicator of a "molten globular" state. (The emission properties of ANS provide one of my favorite handlamp demonstrations of fluorescence – one which I highly recommend to anyone teaching an introductory class on fluorescence. One simply takes two large test tubes, one containing ANS in water, the other containing BSA in buffer. The exact concentrations are not so important – as long as there is a reasonable amount of probe and protein. Using a UV handlamp to illuminate the samples, one demonstrates that the ANS/water solution exhibits a very weak, yellowish fluorescence, while the BSA exhibits no fluorescence (there is sometimes a weak blue fluorescence from impurities, but it is usually negligible). With the lights out, you then pour the contents of one tube into the other (either way) and the result is a huge increase in fluorescence and a dramatic blue shift, that is, from weak yellow to very bright sky blue (Fig. 2). This demonstration never fails to elicit "Oohs!" and "Aahs!" from the audience.)

During these early years at Sheffield, Weber also began his seminal studies on intrinsic protein fluorescence. Specifically, in 1957 with his postdoctoral fellow F.W. John Teale published the first emission spectra of the aromatic amino acids and the first accurate excitation spectra. Figure 7 from their seminal paper [9] has been reproduced many times and is shown again here in Fig. 3. Weber and Teale published a series of important papers and communications on intrinsic protein fluorescence and the determination of absolute quantum yields. Interestingly, the

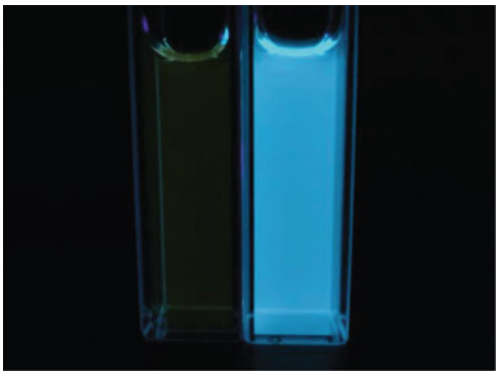

Fig. 2 Solution of ANS in PBS (*left*) and the same concentration of ANS in PBS after addition of bovine serum albumin (*right*). Solutions are illuminated using a UV handlamp set for the long wavelength (365 nm)

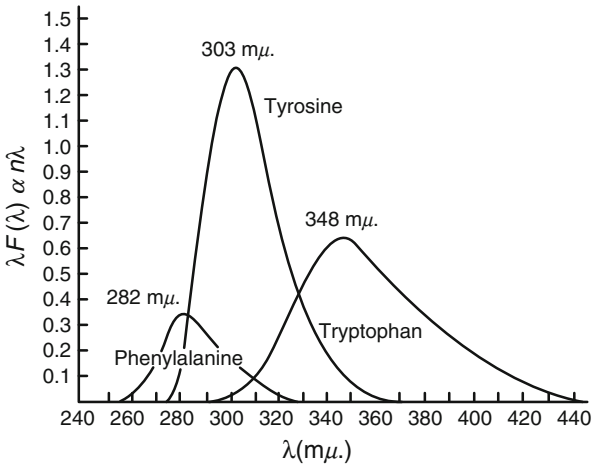

Fig. 7. Fluorescence spectra of the aromatic amino acids in water. Abscissa: wavelength (mμ.). Ordinate: relative number of quanta.

Fig. 3 Figure 7 from [9] giving the first emission spectra of the aromatic amino acids

quantum yield Weber and Teale reported for tryptophan, 0.20, was later found to be somewhat higher than the currently accepted value near 0.13. At the time when Weber and Teale carried out their experiments, the large temperature effect on tryptophan's lifetime and quantum yield was not appreciated. As Weber told me, their work, reported as done at "room temperature," was, in fact, carried out in England in the winter in a Quonset hut without central heating, which caused a marked increase in their tryptophan quantum yield relative to that expected for 25°C. Weber's interest in the photophysics of tryptophan continued over the years, eventually leading to a publication in 1977 of an important and often quoted paper with Bernard Valeur [10] on the ^1L_a and ^1L_b excitation bands of indole and tryptophan. The study of intrinsic protein fluorescence has become one of the most important techniques used in protein research and has been of great importance in establishing the dynamic nature of proteins. This potential was certainly not lost on Weber who presented a classic paper at the "Light and Life" conference held in 1960 and, in a true understatement, summarized his presentation in the Discussion held after the talk by saying "There are many ways in which the properties of the excited state can be utilized to study points of ignorance of the structure and function of proteins" [11]. In fact, in an earlier communication (presented at the annual meeting of the British Biochemical Society on April 3, 1959) Weber estimated that the excited state lifetime of tryptophan in proteins was on the order of 4 ns and commented "These values are too short to permit measurements of fluorescence polarization to be of value in the determination of the rotational relaxation times of proteins in solution, but can give useful information on local conditions about the tryptophan or tyrosine residues." Present day methods of site-directed mutagenesis, which permit the facile removal and/or addition of tryptophan residues to allow the creation of novel single-tryptophan containing

proteins, have led to the full realization of Weber's vision of the utility of intrinsic protein fluorescence.

In 1960, Weber spent a year as a visiting professor at Brandeis University and gave a series of lectures on fluorescence, inspiring several students and postdoctoral fellows with the potential of fluorescence methods. One of these, Ludwig Brand, went on to establish himself as one of the leading researchers in the biological applications of fluorescence spectroscopy. At around this time, I.C. "Gunny" Gunsalus, head of the Biochemistry Division of the Department of Chemistry at the University of Illinois at Urbana-Champaign, recruited Weber away from Sheffield. Gunny related to me the story that while he was convincing his colleagues that Gregorio Weber was an exceptional scientist, someone commented that Weber didn't have as many publications as one might expect from a senior professor. Gunny replied that while this was true, *Weber's ratio of outstanding papers to total papers was unity* and that this ratio – known thereafter as the Weber ratio – was certainly the more important consideration. In fact, when Weber left England several other Sheffield faculty members, who would later go on to establish distinguished careers elsewhere, also left. As related by David Lloyd in his tribute to Gregorio Weber: "In fact I was later to learn that discontent in the Department arose largely because of repeated refusals to promote Gregorio to the research Chair he so evidently deserved. Links with Urbana-Champaign, Illinois were already strong (Gibson, Massey and Weber had spent sabbaticals there; R.E. Hungate, Ralph Wolfe and Woody Hastings had been on sabbaticals in Sheffield. It was therefore no surprise when Gregorio announced his intended departure for that campus. He took with him Jim Longworth (his first research student) and Lorna Young (his technician). Then Vince Massey, Graham Palmer and their research students (Ben Swoboda, and Steve Mayhew who had worked with John Peel in Microbiology) left for Ann Arbor, Michigan; and Rod Bennett went to Dartmouth N.H. Theo Hofmann left for Toronto's Biochemistry Department. Keith Dalziel and Mark Dickinson went to Oxford. Quentin Gibson and Colin Greenwood went to the Johnson Foundation, University of Pennsylvania at Philadelphia." In later years, this exodus became known as the "great Sheffield brain drain."

In 1962, Gregorio Weber joined the University of Illinois and built a research program that continued actively until his death in 1997. During his early years in Urbana, Weber continued to develop novel fluorescence instrumentation and probes and extended his studies of protein systems. Among the fluorescence probes Weber developed in Urbana were pyrenebutyric acid (which had a lifetime of 100–150 ns and thus extended the polarization method to proteins with molecular weights of 10^6), bis-ANS (which binds to many proteins with much higher affinity than ANS and which also binds to many nucleotide binding sites), IAEDANS (the first sulfhydryl specific fluorescence probe), and PRODAN (2-dimethylamino-6-propionyl-naphthalene; a probe designed by Weber to have an exceptionally large excited state dipole moment and hence to possess an extreme environmental sensitivity). Weber also made derivatives of PRODAN such as LAURDAN, which included a lauric acid tail to render the probe lipid soluble (LAURDAN has been very extensively used in recent years as a probe of membrane dynamics)

Fig. 4 Gregorio Weber with his technician Fay Ferris (circa 1984)

and DANCA, which had a cyclohexanoic group attached that increased the affinity of the probe for heme-binding sites. Most of these probes were actually synthesized by Fay Ferris, Weber's lab technician for many years at UIUC, who acted as his eyes and hands in the lab (Fig. 4)

Much of Gregorio Weber's efforts during the last few decades of his life were focused on development of his ideas on protein dynamics and protein–protein interactions. In this regard, two of the research lines he developed were oxygen quenching of fluorescence and applications of elevated hydrostatic pressure. His initial foray into oxygen quenching was with his student W. M. Vaughan who studied oxygen quenching of pyrenebutyric acid, free in solution and associated with protein [12]. The low solubility of oxygen in aqueous solutions required that the targeted fluorophore had a very long lifetime, which in the case for pyrenebutyric acid was greater than 100 ns. In order to study intrinsic tryptophan fluorescence in proteins, Weber needed to use a cell capable of holding up to 100 atm of oxygen pressure. Joseph Lakowicz was the graduate student who worked on this project and the results showed that oxygen, an uncharged, nonpolar quencher, could reach tryptophan residues in protein interiors [13]. The last paragraph in their seminal paper stated, "The general conclusion to be derived from all the points mentioned above is that the functional properties of protein molecules are not properly represented by rigid molecules that do not include the rapid structural fluctuations necessary to explain the phenomena we have observed. Our experimental findings are fully consistent with the ideas on the character of protein conformation put forward by one of us (Weber, 1972) but not with the often expressed belief that proteins exist in a very small number of permissible conformations. Such models are, in our opinion, inconsistent with the weak forces that determine protein structure." One must appreciate that at this time, in the early 1970s, the popular view of proteins was that of rigid, dense structures that would not allow for small molecules such as oxygen to diffuse into the protein interior.

Weber had for years championed the view that proteins were highly dynamic structures. In his seminal review in Advances in Protein Chemistry in 1975 [14], Weber wrote that proteins were "kicking and screaming stochastic molecules."

In the mid-1970s Weber began to apply the method of elevated hydrostatic pressure, coupled with fluorescence, to the study of molecular complexes and proteins. His appreciation of the possibilities of hydrostatic pressure was no doubt influenced by his friendship with Harry G. Drickamer, a professor in Chemical Engineering at UIUC, whose laboratory was actually in the same building as Weber's lab. Drickamer was arguably one of the great pioneers in high pressure studies in condensed matter – in his life he was awarded 27 major awards for his research including the National Medal of Science awarded by President George H. Bush in 1989. Weber's first work on this topic, published in 1974, was a study of FAD, FMN, and the molecular complex of isoalloxazine and adenine [15]. Over the next three decades Weber applied hydrostatic pressure methods to the study of biomolecules ranging from small complexes to single chain proteins to oligomeric proteins and eventually to viruses. He also applied pressure to biological membranes. Eventually he published 48 articles on pressure effects on biomolecules. His review in 1983 with Drickamer in the Quarterly Review of Biophysics [16] was a landmark paper in the field – in the opening paragraph they stated: "... we concentrate here on the examination of the conceptual framework employed in the interpretation of high pressure experiments and in the critical discussion of our knowledge of selected areas of present interest and likely future significance." Weber's contributions to protein chemistry were recognized by the American Chemical Society in 1986, which named him as the first recipient of Repligen Award for the Chemistry of Biological Processes, whose purpose was "... to acknowledge and encourage outstanding contributions to the understanding of the chemistry of biological processes, with particular emphasis on structure, function, and mechanism."

In 1992, Weber published his book "Protein Interactions" in which he essentially summarized his ideas about proteins [17]. He dedicated this book to "Those who put doubt above belief," in keeping with his lifelong philosophy of wariness in accepting popular scientific theories. Gregorio Weber's scientific achievements were recognized by many honors and awards. These include election to the US National Academy of Sciences, election to the American Academy of Arts and Sciences, election as a corresponding member to the National Academy of Exact Sciences of Argentina, the first National Lecturer of the Biophysical Society, the Rumford Premium of the American Academy of Arts and Sciences, the ISCO Award for Excellence in Biochemical Instrumentation, the first Repligen Award for the Chemistry of Biological Processes, and the first International Jablonski Award for Fluorescence Spectroscopy. It is worth noting that the Rumford Premium is one of the oldest scientific awards given in the USA. It was created by a bequest to the Academy from Benjamin Thompson, Count Rumford, in 1796 – previously awardees include J. Willard Gibbs, A.A. Michelson, Thomas Edison, R.W. Wood, Percy Bridgman, Irving Langmuir, Enrico Fermi, S. Chandrasekhar, Hans Bethe, Lars Onsanger, and other highly original thinkers. The Rumford award

Fig. 5 Gregorio Weber receiving the Rumford Premium. Also receiving awards are Robert L. Mills and Chen Ning Yang

committee recommended that the 1979 award be given to two physicists, Robert L. Mills and Chen Ning Yang, for their joint work on the theory of gauge invariance of the electromagnetic field, and to Gregorio Weber, "Acknowledged to be the person responsible for modern developments in the theory and application of fluorescent techniques to chemistry and biochemistry" (Fig. 5).

In addition to these seminal contributions, Gregorio Weber also trained and inspired generations of spectroscopists and biophysicists who went on to make important contributions to their fields, including both basic research and the commercialization of fluorescence methodologies and their extension into the clinical and biomedical disciplines. Weber is honored today by several awards and meetings including the Gregorio Weber Award for Excellence in Fluorescence Theory and Applications, awarded annually by ISS, Inc (http://www.iss.com/events/weber.html) and the Gregorio Weber International Prize in Biological Fluorescence (Weber Prize) awarded every 3 years for research related to a doctoral (or equivalent) dissertation (http://www.lfd.uci.edu/weber/prize/). Approximately every 3 years (since 1986) an international symposium is held in his honor entitled the International Weber Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine (http://www.lfd.uci.edu/weber/symposium/). These Weber Symposia were held in Italy in 1986 and 1991, and in Hawaii in 1995,

Fig. 6 Group photo for the first International Weber Symposium held in 1986 in Bocca di Magra, Italy

1999, 2002, 2005, 2008, 2011, and 2014. The group picture from the first meeting held in Bocca di Magra, Italy, is shown in Fig. 6 – I shall treat this as a "Where's Waldo" exercise and let the reader locate Gregorio Weber.

An important website, http://www.cf.ac.uk/biosi/staffinfo/lloyd/weber/, was established by David Lloyd at Cardiff University who was actually an undergraduate with Gregorio Weber in Sheffield. This website has short contributions from many of Weber's colleagues from his Cambridge and Sheffield days, including a marvelous and insightful article by David Lloyd, which offer illuminating insights into Weber's personality and his influence on young scientists. For example, one of the interesting anecdotes presented by David Lloyd from his time with Gregorio Weber is "It was a fast-track education to be with Gregorio Weber in those tutorials. He told us of his heroes: James Clark Maxwell, whose unification of the magnetic and electrical forces was perhaps the greatest leap forward in the physics of the 19th century, and the major achievements of the Americans, Willard Gibbs and G.N. Lewis in thermodynamics and solution chemistry. He set us interesting essay topics: 'The dynamics of life', 'The government and administration of cellular metabolism', and one which still puzzles me 'Does nature favour the survival of the fittest (Darwin) or conservation of the mean (Lotka-Volterra)?'". David Lloyd went on to write "As Krebs said of Warburg, so could we say of Gregorio: his influence has spread far and wide. He was an intellectual genius, a colossus who changed everyone he touched. I am told that he did not believe in an afterlife, but rather that we just stop. But as on a snooker table the cue ball that collides sends the others on into their separate trajectories."

In his contribution to this website, Fred Sanger (two-time Nobel Prize winner) wrote:

> I do not feel able to comment on Gregorio's published scientific work as it was in a rather different field from my own interests, but I do believe that his contribution to science was considerably more than has appeared in print. During the time that we were both working in the Cambridge Biochemical Laboratory he would frequently come over to my bench to see what I was doing, discuss my work and make useful suggestions. I found this stimulating and often helpful for my work. Gregorio had a considerably wider knowledge of science than I did, and was a wonderful person.

During the time that I was a graduate student in Weber's laboratory (1971–1977), I overlapped with graduate students, David Kolb, Jim Stewart, Moraima Winkler, Kathy Gibbons, Joe Lakowicz, Alex Paladini, Jr., J. Fenton Williams, John Wehrly, Bob Hall, Wayne Richards, and Tom Li, and with postdoctoral fellows Francisco Barrantes, Roberto Morero, Fumio Tanaka, I. Iweibo, Yueh-hsiu Chien, Louise Slade, Bob Mustacich, Richard Spencer, George Mitchell, Bernard Valeur, Antoine Visser, Bill Mantulin, and Enrico Gratton. Other individuals who spent formative periods in Weber's laboratory include Philippe Wahl, Meir Shinitzky, Sonia Anderson, John Olson, Ken Jacobson, Bob Clegg, Greg Reinhart, and George Fortes. In the 1980s and 1990s Weber's students included, Parkson Chong, Lan King, Catherine Royer, Susana Scarlata, Chris Luddington, Rob Macgregor, Peter Torgerson, and Gerard Marriott, and postdoctoral fellows included Maite Coppey, Frank Kaufman, Mohamed Rholam, Dave Edmundson, Kancheng Ruan, Andre Kasprzak, Gen-Jun Xu, Larry Morrison, Edith Miles, Don Nealon, Leonardo Erijman, Patricio Rodriguez, Suzana Sanchez, Jerson Silva, and Debora Foguel. During my years in Gregorio Weber's laboratory (as a student and later as a postdoc), visitors who came to carry out experiments included Nicole Cittanova, Bill Cramer, Andy Cossins, Pierre Sebban, Serge Pin, Bernard Alpert, Christian Zentz, Patrick Tauc, Maurice Eftink, Tiziana Parasassi, and José Maria Delfino. No doubt I am missing some names and I apologize for my failing memory. Figure 7 is a picture taken at Enrico Gratton's house in the early 1980s where Enrico, Greg Reinhart, and myself are presenting a computerized chess set to Gregorio Weber for his birthday. This chess computer actually was embedded in a full sized chess board that would detect the moves made on the board and indicate its response – a perfect present for "The Professor" who liked to play chess.

As I mentioned already, Weber had many eye problems due to excessive amount of UV and infrared radiation over the years. I was actually visiting him in the early 1990s when the hospital called to say that a cornea transplant was available and so I immediately drove him over. A couple of days later I was visiting him when a doctor came in to remove the bandage from his remaining bandaged eye, which had received a new cornea – the bandage had already been removed from the other eye. Weber's first words were "I have a homogeneous, clear, binocular visual field" – a statement conveying the maximum of information with the minimum of words.

Another memorable comment occurred when he was working on a mathematical solution for resolving multiple lifetime components from phase and modulation data given multiple light modulation frequencies. To accomplish this task Weber

Fig. 7 *Left to right*: Greg Reinhart, David Jameson, Gregorio Weber, and Enrico Gratton

devised a new (for him) mathematical procedure. Later, one of his friends in the mathematics department told him that this approach looked familiar and eventually helped to find a reference in the literature. I remember vividly going with Weber to Altgeld Hall on the UIUC campus, which housed the math library. There, we found the reference to an article by R. de Prony in Volume 1 of the 1795 issue of *J. Ecole Polytech*. When Weber wrote his article on this topic [18], one of the section headings was titled: "Computation of the Component Lifetimes from the Moments by Prony's Method." I asked Weber why he referenced de Prony's article – almost two centuries old – rather than simply state that he had developed the method himself. Weber replied that since de Prony had found the method first he must receive the credit!

An anecdote which demonstrates Weber's nurturing attitude toward students was given in my book "Introduction to Fluorescence" [19]. Namely: "When I was a graduate student, I was trying to improve the sensitivity of my measurements and I hit upon the idea of having two adjacent sides of a fluorescence cuvette coated with a mirror finish. My idea was that the excitation beam would then be reflected from the back side through the solution again, and the fluorescence reaching the side facing away from the detector would be reflected toward the detector. In fact, this arrangement improved my signal about threefold. I was, naturally, proud of this accomplishment and demonstrated it to Gregorio Weber who politely praised my ingenuity and then proceeded to show me an old article he had written (from the 1950s) in which he had also used mirror coatings on his cuvette. In that article, he also acknowledged that he was following the idea of Francis Perrin who published the same approach in the 1920s!" So Weber first praised me for my ingenuity and initiative – raising my self-confidence – but then later educated me by pointing out

that others, starting with Francis Perrin in 1929, had hit upon the same approach. Surely this is the manner in which professors should treat students and colleagues!

Another incident I well remember was when I was attending a NATO conference with Weber in the early 1980s in Sicily. At the cocktail party preceding the opening of the conference I was standing next to Weber when a young woman came up to him and asked if he was Meir Shinitzky (who had actually been a postdoc in Weber's lab years earlier and who was certainly a very distinguished scientist at the time of this incident). Weber replied "No, I am Gregorio Weber." The young woman next asked if he knew anything about fluorescence! Weber paused and clearly considered carefully his reply – which was "I know some things, but not everything." The young woman replied, "Well then, I had better go find Meir Shinitzky." I loved Weber's statement since it epitomized his intellectually honesty in his approach to science and to life in general. He might have replied, for example, that he probably knew more about fluorescence than anyone else on earth, but his actual reply captured simultaneously his humility and his honest appraisal that no matter how much he knew there was always a vastly greater amount that he did not know. Continuing with the theme of humility, I am reminded when Weber gave the final talk at the end of the 1986 Bocca di Magra meeting. Of course when he finished there was loud and unrelenting applause. Finally Weber said loudly "Please stop – you are celebrating the birthday of Gregorio Weber, not Josef Stalin!"

Modern students take the internet for granted and are accustomed to being able to retrieve information on just about any topic rapidly while at their desks. I have previously written that in my graduate student and postdoctoral days we did not have the internet or Google – but rather we had Weber. The difference being that Weber always gave us the *correct* answer. My point was that all of the people who knew Weber in those days considered him an authority, not only on fluorescence but also on all matters relating to science in general. His knowledge on a wide range of topics, including the scientific literature, was simply astonishing and saved many of us countless hours in the library that we would have spent digging out the information we needed – I may also add that it also settled many bets in the lab among the students!

I hope this chapter has given the reader some concept of the scientific insights of Gregorio Weber and his important and original contributions both to fluorescence and to protein chemistry. Those of us lucky enough to have known and worked with Gregorio Weber, however, can attest to his other qualities, including his humanity and simplicity. He inspired generations of young biophysicists from around the world, demonstrating by example how scientists ought to interact with each other, namely with courtesy, respect, selflessness, good humor, and generosity. Throughout his life Weber shared his resources, both professional and personal, with all. He became Professor Emeritus in 1986, at the age of 70. Although there was no mandatory retirement age at the university, Weber said that he wanted to retire to free up the faculty position for others just starting out. He was given a smaller lab and office and continued to work on his own – and with the occasional visitor – up until his death. I can attest to the fact that he maintained his scientific curiosity and intellectual honesty to the end of his life. Although he was too sick – from leukemia

Fig. 8 David Jameson and Gregorio Weber in Hawaii (circa 1990)

– to leave his hospice bed he still enjoyed discussing life and science with his visitors. While bed-ridden and in his final days he was reading a book on the history of the French Revolution – in French! I asked him why he was reading that particular book and he replied that it was because the French Revolution was so interesting.

As a final anecdote, I vividly recall a conversation I had with Weber in Hawaii in the early 1990s (Fig. 8). Weber was in his 70s at the time and he turned to me one day and said "You know David, when I was much younger an older colleague said to me that when I passed the age of 60 I would begin to notice that my students had more ideas than me and better ideas than me." To this statement I replied "Gee – really Professor?" After a rather long pause he said "I have not found this to be the case."

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